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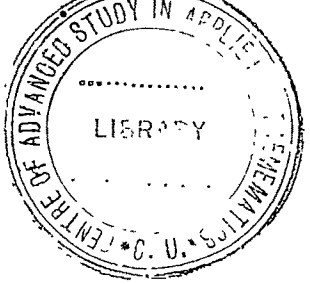
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REDRESSING THE BALANCE

PERSUADING young people to go in for science and technology is an essential part of the management of a modern economy. For what shall it profit a nation if it has a sound external balance of trade, a steady growth of industrial production and stable prices, but lacks assurance that the future will be sustained by plenty of scientists and engineers? This is the train of thought which led the British Government to take fright when it became known a year ago that the places available at universities were not being filled for lack of would-be students of good quality. One immediate result was the committee under Dr. F. S. Dainton to enquire into the "Flow of Candidates in Science and Technology into Higher Education". The committee has now issued an interim report (Cmnd. 2893, H.M.S.O., 3*s*.) mapping out a programme of research for several years ahead. In time, it is now plain, there will be much better statistical information on which to base decisions about the strategy of higher education. On present form, however, it is less certain that the committee will provide compelling answers to the most pressing of the questions it has been asked.

An objective study of what is meant by vacancies at British universities is nevertheless a good beginning. If the universities could have found room for 1,000 or so extra students in science and technology in each of the past two years, this is in part, at least, a measure of the speed at which they have been growing. Between 1962 and 1965 the recruitment of undergraduates in science and technology increased sharply, in response to the Robbins Report. If the vacancies at the universities were the only signs of discontent, it might not be surprising that demand for undergraduates outstripped supply. Indeed, there is something to be said for reminding the universities that good undergraduates are an asset to be sought for and then treasured. Already the talk of vacancies may have stimulated some university departments to make themselves more attractive to good potential students, which is an unexpected gain.

The vacancies at the universities are unfortunately only part of the proof that something is wrong. Departments in the humanities have also grown quickly, and yet have had to turn away good students. Ironically, a programme of university expansion intended in part to provide more scientific and technological skill has first of all succeeded in producing more humanists. Similar tendencies are apparent in the schools. It is true that there is still a steady growth of the numbers following courses in science and mathematics in the years immediately before going on to higher education, but these subjects occupy a steadily diminishing proportion of the efforts of the sixth forms in British schools. As a proportion of the total, passes in science subjects in the advanced level examinations have decreased steadily since 1958. By contrast, there has been a rapid growth of interest in studies such as economics. Within the traditional school science curriculum, biology has prospered but the number in chemistry at advanced level has absolutely decreased. In the competition to attract the growing intellectual interests of young people in Britain, the humanists (and the economists) have the edge.

The statistics in the interim report have mostly been collected from familiar sources, and thus do little to clarify the causes for the shift of interest among young people. That is no surprise, for it is too soon to expect a thorough study. What matters is the programme of research now promised. By studying the transition from school to university over a longer period, the committee will help to decide whether the present disenchantment with science and technology is a delayed response to the acute shortage of university places in these subjects only a few years ago. More information about the academic qualifications and the professional training of teachers in schools may suggest ways of recruiting good teachers in the sciences, though it is likely that the demands made by industry for scientists and technologists will always make it hard to recruit as many highly qualified people as the schools would like. (By the same token, British schools have scarcely begun to exploit the tendency of industrial scientists and engineers to look for other jobs after a decade or so at a laboratory bench.)

The committee also plans a number of more difficult qualitative studies. It will, for example, study what is known about the selection of students by universities and the choice of specialties by students. The examining authorities, recently much maligned, are likely to be further harassed by statistics which suggest that it is harder, and sometimes more like a lottery, to pass an examination in science than in some other subjects. The interim report declares that the committee will study selection procedures so as to see "that individuals with the necessary qualities of character, imagination and originality for careers in science and technology are not excluded on purely academic criteria" and, in the same spirit, it is to analyse "mathematical attainments in the population and their possible relation to premature and irrevocable decisions against science and technology". Obviously there will be fur flying among the custodians of university entrance requirements. Finally, to round off this ambitious programme of research, the committee will study the qualities needed to make a good teacher, the importance of advice about careers to students leaving school, and the influence of schemes for curriculum development (and reform) in making science seem more (or less) attractive as a career. There is plenty of meat in these proposals. The final report is plainly and understandably some way off.

What, in the meantime, is to be done? Happily, there are signs of progress on issues which the Dainton Committee plans to study. The importance of recruiting good teachers and the need to help the old hands keep up to date (at no cost to themselves) is being recognized. Curriculum development may yet do wonders, especially by making science seem to young people still at school the modern equivalent of what the humanities were to the Rennaissance. There is even talk of liberalizing

entrance requirements to universities, and the promise of some progress. All this will help, but it may not—unfortunately be enough. For it is possible that the causes of the drift from science in the schools are closer to the foundations of the British education system, and lie in the unique pattern of specialization in the years before university. It may be significant that science holds its own with the humanities in the early years at school, and that it loses ground when young people are expected—at 16 or 17—to make an almost final choice. May not some potential scientists be protesting that they would first like to speak French more fluently, and to learn what Keynes was all about? And may they not be right? In other words, a proper study of the disaffection of potential scientists demands a study of the balance of the curriculum—at schools and, to some extent, at university. Even if such a study should raise the prospect of a four-year undergraduate course, the Dainton committee should not flinch from adding this to its already overcrowded brief.

BY RAIL TO FRANCE?

MR. STANLEY RAYMOND, the chairman of British Rail, may have spoken more wisely than he knew when he said, on March 14, that if the digging of a tunnel beneath the English Channel did not begin this year, it had better be abandoned altogether. For the case for the Channel Tunnel is no longer overwhelming. If the tunnel could have been built at the end of the nineteenth century, it would no doubt have been a great boon to Britain and to France. But now, as Mr. Raymond seems to feel, the passage of time is against it. Other ways of crossing this narrow strip of water have become progressively more efficient and attractive, while the consequences of the existence of a tunnel have become more disconcerting. Hovercraft may be cheap, fast and flexible to use. The claims of the bridge-builders strengthen as the years go by. Then in Kent and in the Pas de Calais town and traffic planners are already wondering how they will manage if a tunnel should be built. The problems of driving trains through narrow apertures at the speeds now being dreamt of have yet to be faced. And there is no knowing that a single tunnel could accommodate the growth of travel across the English Channel since the last traffic survey, and the extrapolation based on it, nearly a decade ago.

These, of course, are not the doubts that worry Mr. Raymond. He is faced with the need to provide for the potential travellers and for the freight which seems to increase alarmingly in volume whenever the prospects of Britain joining the European Economic Community brighten, however fleetingly. Should British Rail put its faith in a tunnel, or should it buy new ships or hovercraft instead? That, on the face of it, is a commercial decision to be made. In reality, however, the problem is more complicated. In spite of all the surveys which have been carried out, the tunnel remains something of an unknown quantity. How much would it really cost? How effectively would the undoubted virtues of a through journey by rail persuade users to fight their way to the loading points? And, if it were successful, what would be the social costs in congestion and in disturbance of funnelling a large proportion of an important traffic through a single route? Matters like these have not yet been studied with the care that they deserve. The case for yet another survey is overwhelming. It is true that repeated surveys are frequently no more than means of postponing important decisions, but they also share some of the rare benefits of the trials by ordeal popular in the Middle Ages. If, indeed, the delay of one more year should be intolerable, the Channel Tunnel had better be abandoned now, before it is begun.

PHYSICS—GROWTH OR DECAY?

THE threat of unaccustomed lack of funds for research in physics in the United States is one of the spectres to emerge from a report now published by the Physics Survey Committee of the U.S. National Academy of Sciences*. "For the first time in the history of physics in the United States, budgetary limitations threaten our ability even to sustain productive continuing activity in many segments of physics." Almost literally, professional physicists are wondering where the next accelerator is to come from.

The Physics Survey Committee is one of several committees set up two years ago by the Committee on Science and Public Policy of the National Academy. The intention is to make a comprehensive and systematic survey of the condition of science and its need of support. (The committee on chemistry has already reported (*Nature*, 209, 541; 1966).)

The sense of discontent in what the report has to say about federal support for physics springs chiefly from recent fluctuations in the money available and from changes in policies for spending it. "When sharp variations occur—either upward or downward—from a steady growth rate in financial support for physics, serious difficulties can occur."

The Committee says that when funds suddenly become scarce, the first research to suffer tends to be the comparatively unspectacular work at universities by post-graduate students in training. The "high visibility" laboratories, as they are called, "tend to pass a disproportionately large share of the cut-back on to smaller projects, thus striking a disproportionately large blow at the training of new scientists". The Committee also argues that at times of sudden shortage, new researchers suffer more than established men; according to figures from the U.S. National Science Foundation, only 7 per cent of the proposals for research by new investigators in 1963–65 were granted. The committee also protests at the danger that unexpected surpluses can lead to the construction of facilities which cannot be fully used, and at the imbalances that can arise when government agencies impose external (and sometimes arbitrary) limits on the proportions of available funds to be spent on services and on materials.

But how much money should there be? The Committee acknowledges that any answer to this question must be hedged with qualifications. It also proclaims that "physics does not seek to grow without limit. . . . Science is clearly becoming a more extensive part of modern life, but science in general and physics in particular do not threaten to consume the gross national product". Briefly, however, the Committee would like to see an annual growth rate of 16 per cent between 1963 and the end of this decade—a figure which it modestly compares with a rate of growth of between 20 and 30 per cent in the late fifties and early 'sixties.

* *Report on the Present State of U.S. Physics and its Requirements for Future Growth*, National Academy of Sciences, Washington, D.C. 5[illegible]

Reality has already fallen short of expectation. The Committee says that between 1963 and 1964 the increase of funds for physics (excluding certain amounts spent by the National Aeronautics and Space Administration on astronomy) was roughly 7 per cent. The National Science Foundation bears out this estimate for university research, and also suggests a similarly modest increase between 1964 and 1965. Hopefully, the Physics Survey Committee has calculated that in the remaining four years of the decade, the annual growth of funds for research in physics should be 21 per cent.

Arriving at these estimates of future needs has involved a study of current research in separate branches of physics which is itself of absorbing interest. Each of six sub-committees was given a part of the subject to investigate, while a seventh was made responsible for theoretical physics of all kinds. The terms of reference asked for an assessment of the then level of activity in each sub-field of physics, in money and in men. The committees were also asked to study the production of doctorates and the balance of activity between government, industry and universities. Finally, they were presented with a group of questions intended to identify the intellectual goals of current and impending research, and to estimate the resources needed to attain these ends.

The following are the chief recommendations of the panels:

Astrophysics, Space Physics, Cosmic Rays and Gravitation Physics

The sub-committee asks for a recognition that "the development of new techniques does not render the old ones obsolete", which is intended to imply that vigorous space research must be accompanied by a commensurate programme on the ground. Space telescopes (in all regions of the spectrum) are expensive, so that ground-based instruments should be used whenever the conditions of seeing permit. The sub-committee also points to the way in which radioastronomy has placed a strain on the few optical instruments capable [illegible] objects possibly associat[illegible] sub-committee endo[illegible] Whitford Comm[illegible]

ators, and the sub-committee has made a valiant attempt to show some of the predictable ways in which such machines could be used. Thus it would like to see detailed quantitative studies of the new short-lived resonance states of the elementary particles, in the hope of finding new symmetries or new quantum numbers. The study of weak interactions by inverse β-decay, using beams of neutrinos produced by accelerators of high intensity operating at energies in the GeV range, is an important need. Because a substantially more powerful accelerator could not be in operation until 1973, the sub-committee commends the devices of storage rings for atomic particles, the improvement of existing machines, and the building of a 'meson factory' (also supported by the nuclear physicists) for producing beams of mesons at high intensity by means of protons accelerated to between 500 MeV and 800 MeV.

This sub-committee also argues that high-energy physics is a valuable means of training Ph.D. graduates, now being produced at a rate of 140 a year. The total annual cost of high-energy physics works out at 150,000 dollars a year for each post-doctoral physicist in the field. Expenditure increased from 125 million dollars in 1963 to 147 million dollars in 1964. If the sub-committee has its way, the total cost of high-energy physics will be 500 million dollars in 1975. Among the new projects the sub-committee would like to see are the new 200-GeV proton accelerator, on which a decision is expected in the coming U.S. financial year; the improvement of the synchrotrons at the Argonne and the Brookhaven National Laboratories, and an improvement programme for the linear accelerator at Stanford; the building of magnetic rings for storing electrons and positrons; and a design study for a proton accelerator in the energy range 600 GeV–1,000 GeV.

Nuclear Physics

The sub-committee directs attention to the uses that may be made of mu-mesons in defining the nucleon structure [illegible] nuclei once sufficiently energetic and intense beams [illegible] available. It also boasts of the usefulness of such

question with the phrase "the borderline between science and technology".) The sub-committee expects that expenditure on solid-state research will double between 1963 and 1969, with the U.S. Government and private sources (industrial and university) sharing the cost almost equally.

Theoretical Physics

This sub-committee is clearly pleased to be able to imply that theoretical studies—as such—are cheap. Government support for theoretical research in the United States is estimated to have doubled between 1959 and 1964, when it reached 8·45 million dollars. The sub-committee is excited by the possibilities in the physics of elementary particles, in many-body systems (including plasmas), and in solid-state and low-temperature physics.

Classical Physics

This sub-committee has a worthy tale to tell, particularly in statistical mechanics and mechanics—particularly hydrodynamics and the like. It remains a surprise that 6 per cent of physicists in the United States work in acoustics. Optics has been enormously stimulated by space research.

The outcome of the separate studies (together with that of the committee on applied physics) is summarized in Table 1.

These figures will undoubtedly be the basis for discussions between physicists and government agencies in the United States on the financing of future work. In reality, however, they are more than a rapacious shopping-list. The Physics Survey Committee has also made recommendations to professional physicists and to universities and other institutions of higher education. Thus members of the profession are asked to pay more attention to the problems which the Committee itself has done much to define. The implications of this study for universities in the United States are understandably far-reaching.

Table 1. UNITED STATES EFFORT IN BASIC PHYSICS, PRIVATE AND FEDERAL GOVERNMENT COMBINED

	New Ph.D. graduates 1963	Total spending 1963 (millions of dollars)	Estimated spending 1969 (millions of dollars)
Astrophysics, etc.	50	59	105
Atomic, etc.	118	17	52
Elementary particles	110	125	330
Nuclear physics	154	69	99
Plasma physics	35	50	100
Solid-state	226	173	380
Theoretical, etc.	*	4	8
Totals.	693	497	1,074†

* Ph.D. graduates assigned to other categories.

† 55 million dollars has also been estimated for 'intermediate' energy physics, which would bring the total to 1,129 million dollars.

The Committee is principally concerned with the demand for university teachers and researchers because of the continued expansion of the universities, and because post-graduate education is being more widely regarded as a supplement to a first degree. Based on a model of the balance between different layers in the educational pyramid, the Committee looks forward to—and asks for—a sustained growth of university physics in the years ahead.

Only time, of course, will show how all this turns out. At some stage the National Academy's Committee on Science and Public Policy is going to have to put the claims of the several subject committees alongside each other, and then go through the motions of harmonizing them. The outcome of that quasi-judicial procedure will be an important precedent even if the Federal Government does not take all the advice that it is being offered on the rate at which expenditure should grow.

ELECTRON SPECTROSCO[illegible] BINDING
[illegible]NG

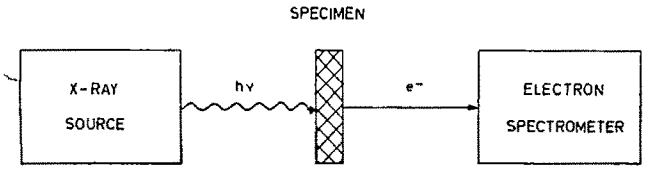


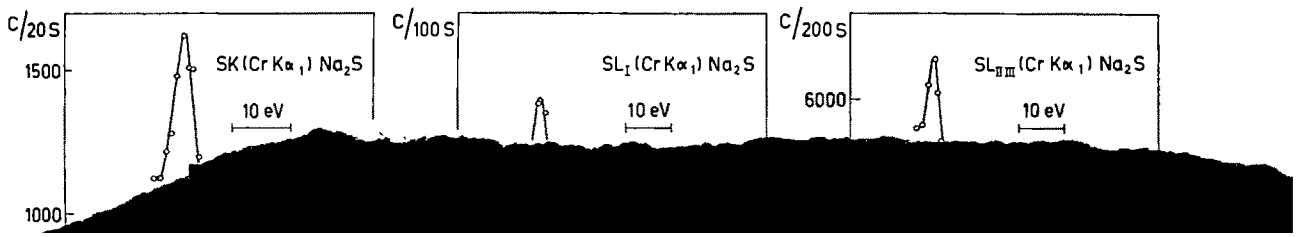
Fig. 1. The principle of the ESCA-method. Photo and Auger electrons obtained from an irradiated specimen are analysed in an electron spectrometer

where E_b is the electron binding energy. E_b assumes different values for different electronic shells and it is not solely a property of the atom for it is also to some extent dependent on the chemical state of the atom, that is, on the arrangement of neighbouring atoms. Therefore, the energies of expelled photo electrons may be slightly shifted as the chemical state is changed. Inasmuch as present-day techniques in electron spectroscopy make it possible to observe and measure these shifts of X-ray-produced photo electron lines, the new method makes it possible to throw some light on the finer details of the electronic structure that are connected with chemical binding. Previous attempts in this direction, made in the 1930's by H. R. Robinson and his colleagues[6], have met with only limited success. Fig. 1 shows in schematic form the experimental arrangement that we have used. Two high-precision electron spectrometers are utilized, one spectrometer of the iron-free double-focusing type and one semi-circular iron-cored spectrograph. The X-ray source used with this latter instrument is equipped with a bent crystal monochromator and detection is accomplished by counting the electron tracks in a photographic emulsion. The instruments are described in detail in refs. 7 and 8.

In the experiments reported here we extended our earlier measurements for the element sulphur[3]. The procedure was first to find a relationship between the spectral position of a photo electron line and the oxidation state of the atom from which photo electrons had been expelled and then to use this information for determining the oxidation state of the same element in some other chemical compounds.

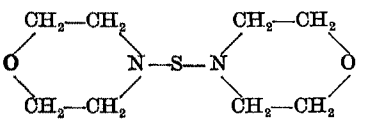
Two sets of sulphur compounds were chosen in which the oxidation number of sulphur varied from 2− to 6+, namely, the sulphide, sulphite and sulphate of sodium and potassium respectively. The zero state was represented by sublimed sulphur. Photo electron lines were recorded from the K, L_{I} and $L_{\mathrm{II,III}}$ shells of sulphur in the different compounds. Copper and chromium $K\alpha_1$ radiation was used for the production of photo electrons. Electron spectra for sodium sulphide and sodium sulphate are shown in Fig. 2. The lines of these electron spectra are very sharp (the half-width being about 5 eV) and the peaks can be located to within a fraction of an eV. On the low-energy side there is a distribution of electrons that have suffered energy losses on emerging from the source. The losses occur in discrete amounts, typically of the order of 10 eV. With the high resolution achieved in the electron spectrometer the undisturbed peak may be separated from the distribution of energy-degraded electrons. The lines of Fig. 2 were recorded with the iron-free double-focusing instrument and the intensity of the electron lines is high for the K shell with a net counting rate at the peak of the strongest line of 6,000 counts per min.

As seen in Fig. 2, the electron lines are shifted by several eV when going from the 2− to the 6+ oxidation state of sulphur. Many spectra were recorded from each compound and sources were prepared in different ways and on different backings. All the substances are poor electric conductors. One may therefore expect the sources to become charged, especially if insulating backing materials are used. Such effects have been observed for β-ray sources in nuclear spectroscopy[9]. In our experiments



when the source was continuously exposed to X-radiation the situation was quite different. Even in our first experiment with an insulating tape as a backing material[4] the charging of the source was found to be negligibly small. A possible small effect of this kind of about 1 V was in some cases observed if the thickness of the sample was more than a few tenths of a millimetre and with insulating backing material. Most sources were, therefore, made by dissolving the compounds and letting a droplet of the solution evaporate to dryness *in vacuo* on an aluminium backing. For such thin sources on a metallic backing no charging was observed.

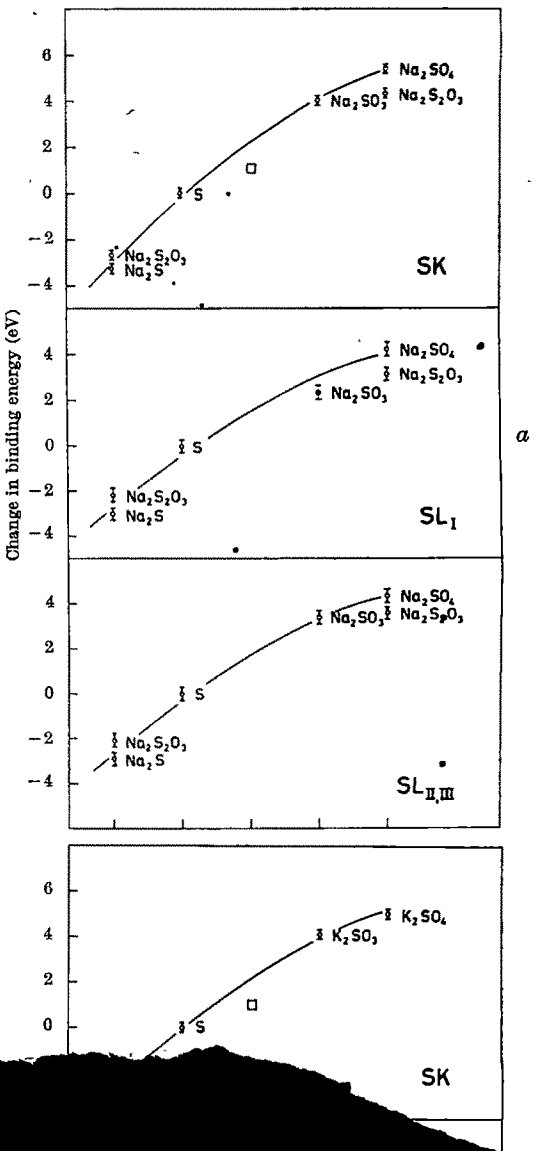
Fig. 3 shows the shifts of the binding energies obtained for the K, L_{I} and $L_{II,III}$ electrons of sulphur for different oxidation numbers. Fig. 3*a* shows the results obtained for compounds with sodium as a cation and Fig. 3*b* with potassium as a cation. Error bars indicate reproducibility errors and lines have been fitted to the experimental points as an eye guide. In the diagrams for the K shell we have, somewhat arbitrarily, included a result obtained for di-(4-morpholino)-sulphide:



which may be taken to represent the 2+ state of sulphur. Some interesting details contained in the diagrams may be noted:

(1) The electron binding energies of inner shells increase with increasing oxidation number. (2) All three levels K, L_{I} and $L_{II,III}$ have an energy shift of about 1 eV per degree of oxidation, that is, on a relative scale the shifts are larger for the L shells than for the K shell. (3) The shifts are somewhat larger for the K-level than for the L-levels. (4) The shifts are about the same for the set of compounds with potassium as a cation as for the compounds with sodium as a cation. The sulphide shifts, however, differ by as much as 1 eV.

These observations can be compared with those made on the shifts of X-ray emission lines (see, for example, ref. 10). It is then found that X-ray data are consistent with the electron spectroscopic data, but it must be borne



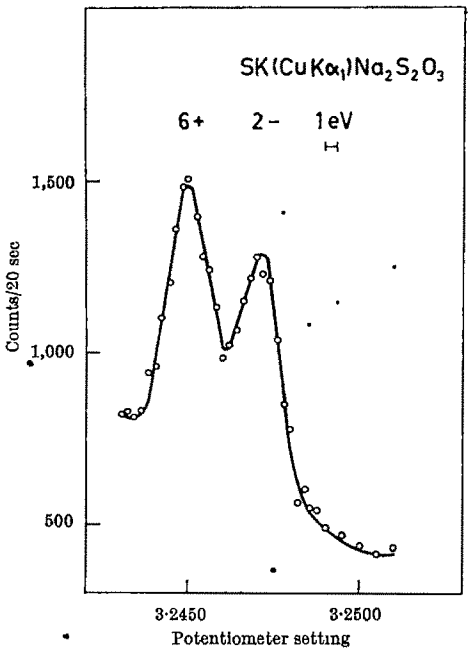


Fig. 4. Photo electron spectrum of the K shell of sulphur in sodium-thio-sulphate, $Na_2S_2O_3$. Two lines are obtained corresponding to the oxidation numbers 2− and 6+

equation (2) equal to the mean radius of the $3p$ orbital in sulphur we obtain $r = 1{\cdot}0$ Å from the wave functions calculated by Herman and Skillman[11] and an energy shift of the inner levels of 14 eV per degree of ionization. The observed shifts are about 1 eV per oxidation number and a change in oxidation number of one unit would then correspond to a charge in atomic change of about 0·07 units, that is, would correspond to 0·07 electrons removed to infinity. More refined calculations of the energy shifts of inner levels due to removal or addition of valence electrons have recently been made by I. Lindgren with a modified Hartree–Fock–Slater technique[12]. For low degrees of ionization he obtains an energy shift of 12 eV per degree of ionization, and also finds a somewhat larger shift for the K shell than for the L shells. Based on the very simple free-ion model the theory is, of course, still far from being complete.

Electron binding energies determined with the electron spectroscopic method have the Fermi-level as a reference level[13]. A possible explanation of the observed shifts could be that the Fermi-level is shifted to different positions in the forbidden gap between the valence and conduction bands or that all levels except the Fermi-level are shifted near the surface as a result of the formation of surface states, the shifts being different in different compounds. This explanation may be rejected if one finds different energies of an electron line from atoms of an element in different molecular positions. In fact, this observation has been made. One example is given in Fig. 4 which shows the photo electron spectrum of the

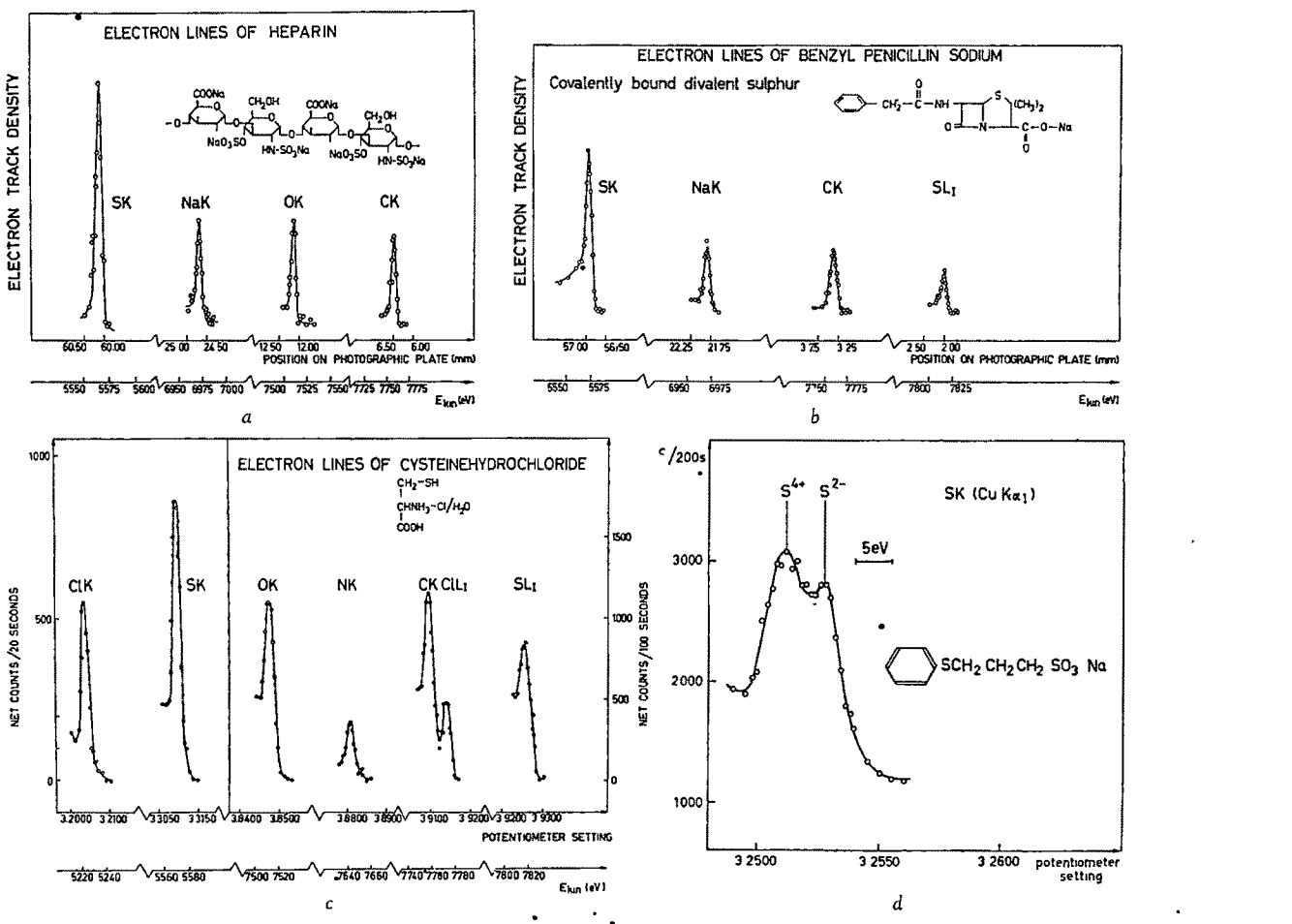
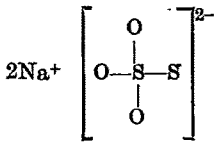


Fig. 5. Electron spectra from complex organic compounds. (*a*) Electron spectrum of heparin with lines from sulphur, sodium, oxygen, and carbon. The K shell binding energy for sulphur corresponds to an oxidation number of 6+. (*b*) Electron spectrum from benzyl penicillin sodium with covalently bound divalent sulphur. Lines are also obtained from sodium and carbon. (*c*) Photo electron lines from an amino-acid, cysteinehydrochloride. Fine signals are obtained from oxygen, nitrogen, carbon, chlorine, and sulphur. (*d*) Electron spectrum of the K shell of sulphur in sodium-thio-sulphonate with two lines corresponding to the oxidation numbers 2− and 4+

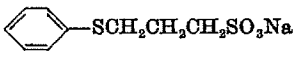
sulphur K shell in sodium-thio-sulphate with copper $K\alpha_1$ radiation. Sodium-thio-sulphate, $Na_2S_2O_3$, has the following structure:



with two sulphur atoms in different chemical positions with oxidation numbers 2− and 6+. Two lines are obtained in the electron spectrum. The peak positions of the thio-sulphate are consistent with the graphs of Fig. 3 which give relationships between the spectral positions of photo electron lines and the oxidation number of the sulphur atom.

Oxygen was present in the crystal water, as an adsorbed layer on the surface, and as a constituent of the sulphite, sulphate and thio-sulphate ions. Conceivably, the oxygen atoms in these different positions could have slightly different electron binding energies and one could expect a broadening or even a splitting of the photo electron lines of oxygen. No such effect was observed and the oxygen K photo electron line could therefore be used as an internal reference line in each sample. Thus, further evidence was obtained that the observed shifts of the sulphur-levels do not result from shifts of the Fermi-level or from the formation of surface states.

We have also made measurements on complex organic molecules. Fig. 5 shows electron spectra obtained from the organic compounds heparin, benzyl penicillin sodium, cysteinehydrochloride and sodium 3-phenylthiopropane-sulphonate, all of which contain sulphur as a constituent. The heparin and penicillin spectra of Fig. 5*a* and 5*b* were obtained with the semi-circular spectrograph and electron track counting technique, and the spectra of Fig. 5*c* and 5*d* were recorded in the double-focusing instrument. Not only the sulphur lines were recorded but also electron lines from other elements in the compounds. Most of these elements have low atomic numbers and cannot readily be seen in X-ray spectra. With the aid of the graphs of Fig. 3 the oxidation number of the sulphur was estimated at 6+ for heparin, 2− for penicillin, and 2− for the amino-acid cysteinehydrochloride. The phenylthio-sulphonate is another example of a compound with sulphur in different chemical positions, and the electron spectrum of Fig. 5*d* has two lines from the sulphur K shell. They correspond to the oxidation numbers 2− and 4+ as one could in this case expect from the structural formula:



Many molecules are known to decompose under prolonged X-irradiation. No such effect was observed as the time of exposure was kept relatively short.

Electrons are emitted from an X-irradiated sample not only by photo electric effect but also in the Auger process. The Auger process is the radiationless alternative to X-ray emission for the de-excitation of atoms with inner shell vacancies. In a measurement of the KLL Auger spectrum of sulphur we have found that also the Auger transition energies depend on the chemical state of the atom[14]. This is illustrated in Fig. 6 for the sulphur $KL_2L_3(^1D_2)$ Auger transition in sodium-thio-sulphate. Again two lines are obtained, corresponding to the two different chemical states of the sulphur atoms. Auger spectra yield additional and complementary information to the photo electron spectra. Thus, the observed separation, 4·3 eV, of the double Auger line in Fig. 6 confirms our previous result that the K-level shift is somewhat larger than the L-level shift (see Fig. 3).

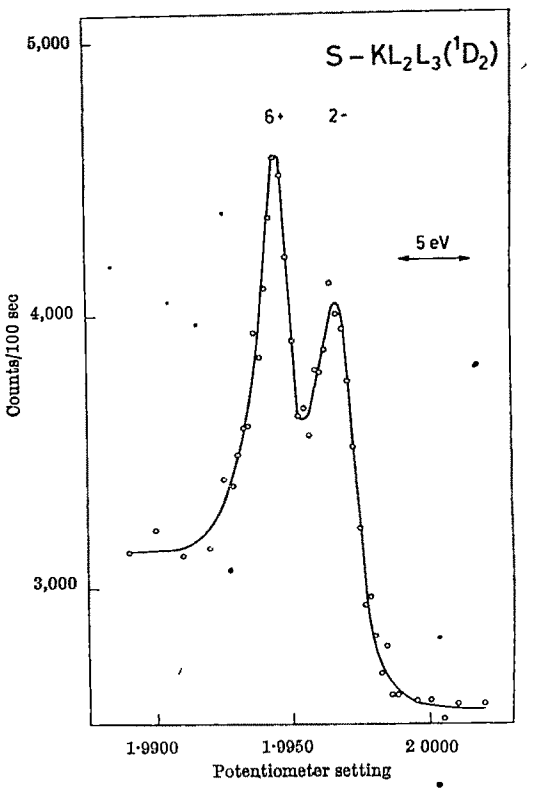


Fig. 6. The energy of an Auger transition depends on the chemical state of the emitting atom. This is illustrated in the figure by the Auger line $KL_2L_2(^1D_2)$ from sulphur in sodium-thio-sulphate. The line has two components, one for the 2− and one for the 6+ state of oxidation, and the energy separation is in agreement with the photo electron data of Fig. 3*a* for the individual levels. Finer details about chemical effects on the inner electronic structure can be obtained from Auger spectra

In addition to the element sulphur we have found chemical shifts in other elements such as phosphorus, chlorine and iodine. These shifts are also of the order of several electron volts. The results obtained so far strongly encourage further exploration of the electron spectroscopic approach to an improved understanding of the electronic structure of atoms and solids and the nature of the chemical bond.

This work has been supported in part by the U.S. Air Force, through the European Office of Aerospace Research and by the U.S. Department of Army through its European Research Office. We thank Dr. Bernt Lindberg, Pharmacia, Uppsala, for providing most of the organic compounds. We also thank F. M. Gunilla Axelson, F. M. Robert Carlsson and F. M. Ulf Ericson for valuable help with the measurements.

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NEUTRON ACTIVATION ANALYSIS AND ARCHAEOLOGY

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ARCHAEOLOGISTS have long applied scientific techniques in their study of man's past. The first of these techniques was borrowed from geology, and is that of stratigraphy—the ordered sequence of cultural deposits. This was first noticed by the British archaeologist, Stukely, who in 1723 published the section of a barrow which he had excavated near Stonehenge. On the American side of the Atlantic, the first person to notice the phenomenon of archaeological stratification was the statesman Thomas Jefferson, also an amateur archaeologist, who in his *Notes on Virginia*, published in 1784, indicated the stratigraphical stages in the construction of a mound which he had examined.

The archaeologists of this period were ahead of their time, however, and it is only in the past 30 years or so that there has been a birth of truly 'scientific' archaeology as opposed to the indiscriminate collecting of artefacts. The onset of modern methods in British excavation came with the work at Caerleon, Monmouthshire, in 1924, directed by Dr. (now Sir) Mortimer Wheeler, while perhaps the most important contribution of science to archaeology has been the application of radiocarbon dating by W. F. Libby. Even in the past few years, there has been a marked increase in the contribution of science to archaeology, as exemplified by the recent investigations of Aitken[1] and Biek[2], and the volumes edited by Brothwell and Higgs[3], Heizer and Cook[4], and by Pyddoke[5].

Such studies can best be considered under three headings, namely, site location, dating, and artefact analysis. The first category includes aerial photography, magnetic location, resistivity prospecting, the use of the mine detector and proton and rubidium magnetometers, and the new technique of location by infra-red radiation. Dating includes the well-known method of radiocarbon dating which is now so common that there is some abuse of the technique in archaeological interpretation, and also dendrochronology, dating by magnetism, thermoluminescence, fluorine–uranium–nitrogen ratios, and also newer radioisotope decay methods. Finally, there is the field of analysis of artefacts, but this has not received so much consideration as the other two categories. The paper by Caley[6] explains the reasons for chemical analysis of archaeological materials, and this present article examines one method of analysis, that of neutron activation. It is interesting, however, first to explain the reasons for the need to analyse archaeological finds, and secondly to show why neutron activation is in many instances the best of several methods.

The most obvious reason for analysis is simply to find out what an object is made of, with all that this implies. Very often, appearances can be deceiving, and, for example, weapons have been assigned to the Bronze Age simply because they were supposedly manufactured from bronze. Recently, analysis has shown that some of them are, in fact, copper—resulting in the need for a reappraisal of this period of British prehistory. Again, even an archaeologist with expert knowledge of metallurgy would have difficulty, given a silver coin, to tell; on the basis of appearance alone, whether it contained a moderate proportion of alloy or whether it was fine silver. This is of importance in showing either the existence of refining techniques, or else in the deliberate debasement of the coinage. Similarly, from the composition it is possible to determine, especially in metals, the method of manufacture, and consequently the degree of technical competence of the culture that produced it. Metals can, for example, only be spun, cast or wrought within certain well-known compositional limits.

Carrying this one step further, the scientist can state where metallic ores occur, but it is the archaeologist who then determines the conditions of contact and commerce that brought them to the area of manufacture. It is often possible to find trace elements in an analysis that act as a 'fingerprint' which identifies the source of supply. The presence of impurities in the clay of one area, coupled with the same impurities found in the pottery of another area, would be a strong reason for postulating contacts between the two areas. More recently, it has been found that particularly for glass there was a change in composition not from one area to another, but from one period to another. Thus, simple chemical analysis can possibly be used as a method of dating[7,8]. Similarly, in Britain, as has already been mentioned, it has been found that in the Bronze Age there are several chronologically significant innovations in metallurgy, with the later period being marked by the addition of a small percentage of lead, used as a wetting agent to improve the casting.

There are many different methods of chemical analysis, but the majority of them can be disregarded, because they are destructive, and even nowadays many archaeologists are reluctant to have their finds destroyed, even though by this destruction they can learn more about them. They tend to forget, however, that the process of archaeological excavation itself, by which the objects were uncovered, is also a destructive 'experiment' that cannot be repeated.

Spectrometry has the disadvantage that it is not possible to identify elements of an atomic number lower than 12, unless the work is carried out *in vacuo*. Also the analysis relates only to a thin surface layer of the specimen which would often not be representative of the whole composition. Diffraction analysis also suffers from the limitation that it is not always possible to obtain a representative sample from an object such as coarse-textured pottery. Neutron activation analysis, however, is rapid, can be non-destructive, and is extremely accurate; however, it has the one drawback of needing the presence of a neutron source, which is usually a nuclear reactor, but which could also be a neutron howitzer. This is more than compensated for by the ability to work with very small quantities (5 mg or less) which would permit the unobtrusive removal of small samples for laboratory analysis from a museum exhibit.

The basic principles of activation analysis can be broken down into three elementary steps: the activation, detec-

tion, and final analysis. The sample to be analysed is first activated by exposure to a source of thermal energy neutrons. Being a non-charged particle, the neutron penetrates the entire sample with relative ease, and induces activity for observation not only on the surface, but throughout the artefact. If one has a large object to irradiate, and only wishes to induce activity in a small portion, and removal of any fraction of the artefact is not feasible, one may shield the majority of the sample in cadmium to prevent activation. Because the process is a nuclear phenomenon and involves only very few atoms, there is no detectable change in the appearance or chemical characteristics of the artefacts involved.

The amount of activity produced is a function of the number of neutrons that penetrate the sample (flux), the amount of each element which can be activated, and the cross-section of the element (the measure of the probability of a neutron interacting with an atom). Each element which is active emits radiation (α, β, γ) in a unique pattern. Since one is interested in all active elements, even inside the artefact, γ-rays are chosen as the type of radiation to be detected.

Basically, a γ-ray is emitted from the sample and impinges on a detector (usually sodium iodide activated with thallium) which absorbs the ray and produces a light pulse proportional to the energy absorbed. This light pulse is then picked up by a photomultiplier tube which amplifies the signal further, which then goes through additional amplification, and then to a pulse height analyser. This 'black box' contains a sorting and storage mechanism whereby it can discriminate between the different incoming pulses and store each different pulse in a separate memory bank. At the end, the memory is read and the number of counts in each bank or channel indicates the number of γ-rays of a certain energy that were detected, and thus gives an indication of how much of each element was present. There are usually 100 or more channels for storage, and by previously calibrating these with γ-rays of a known energy, not only can the amount of each element be determined, but the different energies can be identified, and thus it is possible to identify the particular element emitting the ray.

Since there are variable mechanisms by which a γ-ray can interact and efficiency may not be 100 per cent, and, since there is always inherent electrical distortion of the signals, a broad spectrum of energies is obtained, but the main peaks correspond to the γ-rays of interest. These spectra then have to be interpreted, and to facilitate the identification of elements, tables of standard spectra have been compiled. Often for quantitative measurements known standards can be run for comparison. When many elements are present the spectra may become complex, and to handle this, computer programmes have been written to resolve the components.

Because of the inherent sensitivity of activation analysis (in many instances, elements in the parts per billion region have been detected), and because of the possibility of using small samples, and due to the rapidity of the technique, with many complete analyses in an hour or less, the archaeologist now has a very powerful tool at his disposal for artefact analysis, especially since many of these analyses are completely non-destructive.

The technique of neutron activation analysis has only recently been applied to archaeology and its problems, but it is a research tool that holds much promise for the future. Several pilot studies of this method as applied to the analysis of archaeological materials have been made in the United States, Britain, France and The Netherlands. The earliest application of the method was in 1952, in France, where Ambrosino and Pindrus[9] studied coins from the collections of the Louvre. This was followed by work in Japan, where Emoto[10] used a cyclotron to detect silver in the lead tiles of the gate of Kanazawa Castle and also in the covered gold plate found at Changuyong in Korea.

At the suggestion of Dr. R. Oppenheimer, a conference of chemists and archaeologists was held at the Institute of Advanced Studies, Princeton, in 1956, to examine the possibility of the use of nuclear analysis to help solve archaeological problems. Rising out of this meeting, work was undertaken in the United States at Brookhaven National Laboratory, and in Britain at the Research Laboratory for Archaeology and the History of Art of the University of Oxford. Sayre made the initial study of the method as applied to Mediterranean archaeology, and he irradiated sherds and fragments of figurines from Tarsus, Rhodes, Knidos, Boestia and Arezzo[11]. In an evaluation of this work it was decided that the results were encouraging, and that specific questions of archaeological analysis could be answered by activation analysis. This was followed[12] by a study of Mayan bowls and sherds from Piedras Negras and Kixpek in Guatemala, which were found to bear a strong chemical resemblance to each other. This type of pottery is found only rarely at Piedras Negras, but is extremely common at Kixpek. This analysis therefore lent strong support to the archaeological theory that the fine orange ware originated at Kixpek and was traded to Piedras Megras. In the same study, *terra sigillata* of the Roman period in Europe was analysed, and stamped Arretine sherds known to have been from bowls made at Arretium were compared with similar ware of uncertain origin found in other regions. Some pieces could be recognized by the analysis as having come from Arretium, while others which were suspected on typological grounds as being ancient forgeries (bearing the stamp 'ARRET' to obtain a higher market value) were found to differ in composition from the genuine Arretine pieces. Sayre has recently embarked[7,8] on a study of ancient glass; some work (as yet unpublished) has also been carried out at the Argonne National Laboratory on the analysis of both copper and obsidian. Most recently, there has been a programme of archaeological activation analysis undertaken by the Museum of Anthropology and the Chemistry Department at the University of Michigan, in Ann Arbor[13].

In Britain, the work on the archaeological use of neutron activation analysis has been as varied as in the United States, and not only has pottery been studied but also metals. The pottery studies are mainly the work of Emeleus, who pioneered the technique in Britain[14,15] and applied it particularly to Samian ware, *terra sigillata*[16–18]. Other workers concentrated on trace elements in coinage, especially gold and copper in early Greek silver coins[19–22]. More recently, the technique has detected silver in some late Roman coins[23] and has been used to analyse the famous Moulsford torc to show that it was of gold alloyed, either deliberately or naturally, with silver and copper[24].

In The Netherlands, an analysis has been made of electrum coins[25] and also a determination of the silver content of Greek coins[26]. This particular research has a novel feature in so far as a Van de Graaff generator was used as a source of neutrons for irradiation instead of a nuclear reactor.

We thank Dr. J. B. Griffin and Dr. A. Gordus for advice, during the course of our work while at the University of Michigan, and acknowledge the financial assistance given by the University of Michigan, Michigan Memorial Phoenix Project.

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OBITUARIES

Prof. H. J. Bhabha

PROF. HOMI JEHANGIR BHABHA, chairman of the Indian Atomic Energy Commission, was killed with 116 others in an air-crash on Mont Blanc on January 24. He was on his way to Vienna to attend a meeting of the Scientific Advisory Committee of the International Atomic Energy Agency. He was a member of the Advisory Committee, and also of the Agency. Bhabha was a leader of science of the highest distinction, and his untimely death under poignant circumstances, at the height of his powers and influence, is a stunning blow to Indian science. It has left a void which will be difficult to fill. He was a theoretical physicist of world fame and one of the most gifted of scientific administrators to be found anywhere. The phenomenal progress made by India in atomic energy research and development will ever remain a lasting monument to Bhabha's vision, drive and dedication. He was a pioneer in several ways; and his great example will continue to be a source of inspiration for many years to come.

Homi Bhabha was born in Bombay on October 30, 1909, into a wealthy Parsee family related to the House of Tatas—he was a nephew of Lady Dorab Tata. He received his early education at the Cathedral and John Connon High School, Elphinstone College, and the Institute of Science, Bombay. After a brilliant academic record in India he joined Gonville and Caius College at Cambridge. He took the Mechanical Sciences Tripos in 1930. From 1932 until 1934 he held the Rouse Ball travelling studentship in mathematics, and worked with W. Pauli in Zurich and E. Fermi in Rome. He was awarded an Isaac Newton studentship in 1934, and a senior studentship of the Exhibition of 1851 in 1936. In 1940 he accepted a readership in theoretical physics, specially created for him, at the Indian Institute of Science, Bangalore. He was appointed professor in 1942. In 1945 he moved to Bombay to assume the directorship of the Tata Institute of Fundamental Research. The Institute, established at Bhabha's initiative, was made possible by a donation from the Sir Dorab Tata Trust. The Institute now has an academic staff of more than 200 and is almost entirely supported by the Atomic Energy Commission. It is interesting to recall that in 1944 Bhabha, in making his proposal to the Tata Trust, stated that his aim was "to build in the course of time a school of physics comparable to the best anywhere", and he continued, "when nuclear energy has been successfully applied for power production in say a couple of decades from now, India will not have to look abroad for its experts but will find them ready at hand". That all this has come about is a tribute as much to his vision as to his determination and single-mindedness in the pursuit of the goal that he had set for himself.

The Atomic Energy Act of India was passed in 1948. Under the Act the Atomic Energy Commission was set up with Bhabha as its chairman; he was also appointed secretary to the Department of Atomic Energy directly under the Prime Minister, in addition to the directorship of the Tata Institute of Fundamental Research and the Atomic Energy Research Establishment at Trombay. He enjoyed in full measure from the very beginning the support and confidence of the late Prime Minister Nehru, and this enabled Bhabha to lift the atomic energy programme to a level and carry it out with a speed which otherwise would scarcely have been possible.

The name of Bhabha will always remain associated with the beautiful theory (based on radiative energy loss by electrons, and pair-creation by photons), formulated jointly with W. Heitler in 1937, which has proved of fundamental importance in the understanding of the phenomenon of electron showers in cosmic rays. The theory was put forward simultaneously by J. F. Carlson and J. R. Oppenheimer. The mathematical aspects of the theory were developed further by Bhabha and S. K. Chakrabarty in 1943.

Bhabha was one of the earliest research workers to investigate the problem of scattering of mesons by nucleons and obtained (1938) an expression analogous to the Thomson scattering for light. He was the first to predict the lengthening with increasing energy, as required by relativity, of the decay-time of cosmic ray mesons.

Bhabha was elected a Fellow of the Royal Society of London in 1941. In 1943 he was awarded the Adams Prize by the University of Cambridge for his work on cosmic rays, and in 1948 the Hopkins Prize of the Cambridge Philosophical Society. In 1963 he was elected foreign associate of the U.S. National Academy of Sciences, and an honorary life member of the New York Academy of Sciences. In 1964 he was made foreign corresponding academician of the Royal Academy of Sciences, Madrid. From 1960 until 1963 he was president of the International Union of Pure and Applied Physics. Bhabha was president of the historic International Conference on the Peaceful Uses of Atomic Energy, held under the auspices of the United Nations, at Geneva in August 1955.

Bhabha was president of the National Institute of Sciences of India in 1963, and president of the Indian Science Congress Association in 1951. He was from the very beginning a member (and later chairman) of the

Scientific Advisory Committee to the Indian Cabinet—this Committee also acts as the National Committee for the International Council of Scientific Unions. He was a member of the Governing Body of the Indian Council of Scientific and Industrial Research, and of several committees concerned with planning and development of science. He was awarded the title of *Padma Bhushan* by the Indian Government in 1954. He received honorary doctorates from several universities in India and abroad, including London and Cambridge.

Bhabha's interests covered a wide range. He was fond of music, and was no mean musician and painter himself. He had a keen aesthetic sense and sophisticated tastes: the Tata Institute at Bombay is not only a great centre of vigorous research but it is also one of India's 'prestige buildings'.

He was a man of great personal charm. He was always eager to seek outstanding talent (even in fields remote from his own interests) and give it every possible encouragement. He had travelled extensively, and had wide international contacts. He will be sorely missed by his numerous friends in and outside India.

He is survived by his mother and a younger brother.

D. S. KOTHARI

Prof. T. J. Jenkin

PROF. THOMAS JAMES JENKIN, who died on November 7, 1965, was professor of agricultural botany at the University College of Wales, Aberystwyth, and director of the Welsh Plant Breeding Station from 1942 until he retired in 1950. He was an agricultural scientist of the highest repute, and his pioneer work on the breeding of improved varieties of grasses earned for him world-wide recognition.

As a young man, Jenkin could not have envisaged a scientific career, for this only became possible later by taking advantage of the few and unexpected opportunities that came his way.

Born in 1885 on a small isolated farm in North Pembrokeshire, his youthful ambition was to become a successful farmer, and for this purpose he attended, in 1907, a short course of one academic term in the University Department of Agriculture at Aberystwyth. However, in the following year he was seriously injured on the farm. This accident, which made him less fit for manual work, was probably the turning-point in his career, because it led him to further academic study. Following a continuation course of another term at Aberystwyth he was advised and encouraged to enter the Old College School at Carmarthen, where he achieved the distinction of obtaining university matriculation in one year. In 1910 he returned to Aberystwyth to read for a degree in rural science, where he graduated with first-class honours in botany in 1914.

After a year as agricultural organizer for the Counties of Brecon and Radnor, Jenkin was appointed adviser in agricultural botany at the University College of North Wales, Bangor, where he remained until his return to Aberystwyth to occupy a similar post in 1919. This gave him the opportunity to study the botanical composition and productivity of sown pastures, when he showed that the varieties of herbage plants then available lacked persistency in the sward and were soon replaced by indigenous grasses. His work attracted the attention of Prof. (later Sir) R. G. Stapledon, and he joined the newly established Welsh Plant Breeding Station as senior research assistant in 1920. This was the beginning of a long and fruitful association between two great agricultural scientists who, although differing widely in personality and in their scientific approach and methods, made outstanding contributions to grassland improvement and productivity.

Jenkin's extensive collection of indigenous grasses, which showed considerable genetic variation, formed the basis for the deliberate breeding of improved varieties. The techniques for controlled hybridization had to be developed, but even after adequate testing of the progeny as single plants he was confronted with the problem of assessing the value of a combination of favourable genotypes when grown in a sward under highly competitive conditions. Without modern knowledge of population genetics he was extremely successful in producing a series of improved varieties which maintained their identity and performance after several generations of seed production. His variety *S*23 of perennial ryegrass has probably contributed more to the improvement of both hill and lowland pastures in Britain than a whole series of commercial seed mixtures.

Although the greater part of his work involved the production of new varieties for use on the farm, Prof. Jenkin was able to conduct pioneer research on interspecific and inter-generic relationships of the grasses, particularly *Lolium*, *Festuca* and *Phalaris*. He was awarded the degree of D.Sc. for these investigations.

As professor and director he guided the work of the Department of Agricultural Botany and the Welsh Plant Breeding Station with distinction throughout the difficult war and immediate post-war years, and when he retired in 1950 he was made a C.B.E. He was later awarded the Gold Medal of the Royal Welsh Agricultural Society and made an Honorary Member of the Swedish Seed Association for his outstanding contribution to agriculture.

Thomas James Jenkin belonged to that unique band of pioneers who have contributed so much towards the advancement of agriculture, and future generations will acknowledge their debt to the young Pembrokeshire farmer who became an outstanding agricultural scientist.

P. T. THOMAS

Prof. Ferdinand Herčík

PROF. F. HERČÍK died on January 20 at the age of sixty. He was director of the Institute of Biophysics of the Czechoslovak Academy of Sciences at Brno since its inception in 1955. One of the foremost contemporary research workers of Czechoslovakia, his work was widely known throughout the world and his friendship was cherished by many of us who had the good fortune to know him well. His activities in connexion with the affairs of the United Nations Organization brought him into extensive touch with Western scientists. Being a member of the United Nations Scientific Committee on the Effects of Atomic Radiation and a governor and vice-chairman of the International Atomic Energy Agency made it possible for him to travel and to meet colleagues freely at a time when contacts between Eastern and Western scientists were much more restricted than they are now. When chairman of the United Nations Scientific Committee, his tact, perseverance and unquestionable integrity made it possible for this Committee to publish a balanced and unanimous report, though there was then no test-ban treaty and the question of the hazard from fall-out was in the arena of emotion and politics. At the International Atomic Energy Agency, Prof. Herčík was tireless in his support for fundamental investigations on the biological effects of radiation, which he considered an essential part of any research programme designed to further the peaceful uses of atomic energy. His unique status enabled him to ensure that investigations into the problems of health and safety were not too narrowly based and he emphasized the importance of studying chemical agents which modified the degree of biological damage produced by a given dose of radiation.

These achievements as a scientific statesman required more than technical competence, and as soon as one met him one was struck by his extremely rich personality. He was a talented and very active painter, had a passion

for mountaineering and a full and very happy family life. An abiding impression of a visit to his Institute was the intense affection and admiration in which he was held by members of his staff. He defended and protected their scientific integrity under conditions which were not always easy and his reward was that the Institute he established had in the surprisingly short time of ten years acquired a world reputation. The work of the Institute is of a very high quality and shows a marked originality of approach which may, in part, be the outcome of struggling with shortages and difficulties in obtaining the most modern equipment.

Prof. Herčík's own researches were varied; being trained both in medicine and in physics, he held at different times chairs in both schools of medicine and of science. After his thesis, taken in Brno, which dealt with surface tension in relation to biology, he worked for a time at both the Pasteur Institute in Paris under Lecomte du Nouy and in the Rockefeller Institute in New York under R. W. G. Wyckoff. He was among the first to appreciate the importance of electron microscopy in biology and medicine, but he is perhaps best remembered for his researches into radiation effects at the cellular and sub-cellular level, where he made full use of viruses and micro-organisms to elucidate basic mechanism. He brought to biophysics the extensive knowledge and breadth of interest which a successful pursuit of this complex subject demands. PETER ALEXANDER

NEWS AND VIEWS

New Fellows of the Royal Society

AT a meeting of the Royal Society on March 17, the following were elected to fellowship of the society: PROF. A. R. BATTERSBY, professor of organic chemistry in the University of Liverpool; DR. T. BROOKE BENJAMIN, assistant director of research, Department of Engineering and Department of Applied Mathematics and Theoretical Physics in the University of Cambridge; DR. K. G. BUDDEN, lecturer in physics at the Cavendish Laboratory, in the University of Cambridge; PROF. R. E. DAVIES, professor of biochemistry and chairman, Department of Animal Biology in the University of Pennsylvania, School of Veterinary Medicine, Philadelphia; DR. W. R. S. DOLL, director of the Medical Research Council Statistical Research Unit and lecturer in Medical Statistics and epidemiology at University College Hospital Medical School in the University of London; PROF. S. F. EDWARDS, professor of theoretical physics in the University of Manchester; DR. J. S. Forrest, director of the Central Electricity Research Laboratories, Leatherhead, Surrey; DR. F. C. FRASER, keeper of zoology and deputy chief scientific officer at the British Museum (Natural History), London; PROF. H. HARRIS, Galton professor of human genetics, head of Department, and director of the Galton Laboratory at University College, in the University of London; PROF. D. O. HEBB, professor of experimental psychology in McGill University, Montreal; SIR WILLIAM HUTCHISON, deputy chairman, Gas Council, London; DR. A. ISAACS, member of scientific staff, National Institute for Medical Research, London; DR. B. KASSANIS, senior principal scientific officer at the Department of Plant Pathology, Rothamsted Experimental Station, Harpenden, Herts; DR. R. A. KEKWICK, reader in chemical biophysics and head of the Department at the Lister Institute of Preventive Medicine, in the University of London; DR. P. E. KENT, chief geologist, British Petroleum Co. Ltd., London; MR. D. G. KING-HELE, senior principal scientific officer, Royal Aircraft Establishment, Farnborough, Hants; SIR FRANCIS KNOWLES, reader in comparative endocrinology at the Medical School, University of Birmingham; PROF. G. KREISEL, professor of mathematics in the University of Paris; DR. C. E. LUCAS, director of Fisheries Research for Scotland (Department of Agriculture and Fisheries for Scotland) and director of the Marine Laboratory, Aberdeen; PROF. J. D. MCGEE, professor of applied physics, at the Imperial College of Science and Technology, in the University of London; DR. J. W. MENTER, director of research and development, Tube Investments, Ltd., Hinxton, Cambridge; DR. A. E. MOURANT, director, Medical Research Council Serological Population Genetics Unit, at St. Bartholomew's Hospital, London; PROF. E. S. PEARSON, emeritus professor of statistics at University College, in the University of London; PROF. D. H. PERKINS, professor of elementary particle physics at the Nuclear Physics Laboratory in the University of Oxford; DR. LILLIAN M. PICKFORD, reader in physiology in the University of Edinburgh; PROF. H. O. SCHILD, professor of pharmacology at University College in the University of London; DR. H. M. STANLEY, director and controller of Research and Development Division, The Distillers Co. Ltd., London; PROF. B. A. D. STOCKER, professor of medical microbiology in Stanford University, California; PROF. J. SUTTON, professor of geology and head of the department at the Imperial College of Science and Technology, in the University of London; PROF. M. SZWARC, research professor and director of polymer research at the State University College of Forestry, in Syracuse University, N.Y.; DR. D. H. WHIFFEN, deputy chief scientific officer, Basic Physics Division at the National Physical Laboratory, Teddington, Middlesex; SIR FREDERICK WHITE, chairman of the Commonwealth Scientific and Industrial Research Organisation, Canberra.

Structural Chemistry in the Bradford Institute of Technology : Prof. D. A. Long

Dr. D. A. LONG, who has been appointed to the new chair of structural chemistry in the Bradford Institute of Technology (proposed University of Bradford), was educated at Sir Thomas Rich's School, Gloucester, and Jesus College, Oxford, where he graduated in 1947 with first-class honours. Dr. Long then carried out postgraduate research with Dr. L. A. Woodward and Mr. R. P. Bell. After completing the degree of D.Phil. in 1949, he spent a year with Prof. B. L. Crawford in the University of Minnesota and then returned to Oxford as Pressed Steel Research Fellow in spectroscopy from 1950 until 1955. During this period he continued his studies in spectroscopy, concentrating particularly on intensities of vibrational Raman spectra. He was appointed lecturer in chemistry at the University College of Swansea in 1956 and was promoted to senior lecturer in 1958 and to reader in 1963. During his period in Swansea, Dr. Long continued work on Raman intensities and in the application of computer techniques to the study of force constants. More recently he has investigated the use of lasers in both Raman and the stimulated Raman effect. He has also undertaken studies of the kinetics of hydrolysis of peptides. In 1964 Dr. Long was awarded an Organization for Economic Co-operation and Development senior visiting fellowship of the Department of Scientific and Industrial Research, to enable him to visit a number of centres of laser research in the United States and Canada.

Port Development

IN replying for the Government in a short debate on port development in the House of Commons on March 9, the Joint Parliamentary Secretary to the Ministry of

Transport, Mr. S. Swingler, said that planned capital investment in the ports in 1965–66 was £29·9 million compared with £18 million in 1964–65, and under the National Plan would rise to £51 million in 1966–67 and to £63 million in 1967–68. Work on the Tilbury development scheme of the Port of London Authority had been put in hand to the extent of £10 million. Improvements to the Royal Edward Dock at Avonmouth had been authorized to the extent of £750,000. Work was about to start on the new lock at Grangemouth under a £7 million scheme. Work was also in hand at Leith which represented an investment of £6 million. The Portbury scheme put forward by the Port of Bristol Authority was an imaginative scheme of great merit and was being very carefully considered, but Mr. Swingler was not able to announce a decision.

Finance for Research and Development

IN written answers in the House of Commons on March 9, the Minister of State, Department of Education and Science, Mr. R. E. Prentice, stated in 1964–65 research and development in the United Kingdom financed from Government sources was provisionally estimated at £427 million. Of this, £177 million was in Government research establishments, £49 million in universities and technical colleges; and £187 million in industry; for 1955–56, the corresponding figures were £222·2 million; £78·3 million; £11·8 million; and £128·4 million, respectively; in 1958–59, £316·1 million; £134·9 million; £18·7 million; and £155·9 million; and in 1961–62, £385 million; £163·1 million; £27·6 million; and £180·5 million, respectively. It was estimated that in 1964 about 7,000 scientists and engineers emigrated from Britain and there were about 4,500 immigrants.

British Research and Development Reports

THE first number, for January 1966, of the bulletin, *British Research and Development Reports*, issued by the National Lending Library for Science and Technology, opens a series intended both to direct attention to the existence of such reports and to increase their flow. The reports are treated as part of the collection of the Library and will be available through its normal services. They include reports from defence or industrial organizations which have no security or commercial limitations on availability and reports from university laboratories containing so much numerical data that they are too large for normal publication. The subjects covered include science, technology (including agriculture and medicine) and the social sciences. The subject headings used are deliberately broad and in the first issue entries are predominantly under engineering.

The National Plan

"THE National Plan—Its Contribution to Growth" is the title of a broadsheet by Geoffrey Denton (*Planning*, 31, No. 493, 331. London: Political and Economic Planning, 1965. 6*s*.). Mr. Denton examines the nature of the Plan adopted by the past Government and tries to suggest how planning might be developed to the advantage of the British economy. Mr. Denton deals first with the Plan as a forecast and then as a target and a feasibility study. He goes on to consider the Plan as a directive and deals finally with the value of such a plan and with the type of plan which is desirable. Mr. Denton thinks that the Plan cannot be regarded as an alternative to the major political changes which may be required, and he feels that the need to ensure appropriate democratic control over the planning process is a further argument for considering the implications of alternative rates of growth. The method by which an indicative target plan of this type can promote faster growth has not adequately been explained, and it may well be an uncertain basis for this operation. While showing awareness of the relation between targets and the policy measures necessary to fulfil them, the Plan fails to indicate convincingly how the present balance of payment figures can be integrated with policy in a manner which can fill the gap between forecast and plan. National plans can nevertheless contribute much to the successful management of the economy. The broadsheet indicates one way in which the Plan could possibly be adapted to provide a faster rate of economic growth.

University News: School of Pharmacy

THE Council of the School of Pharmacy, University of London, has conferred Honorary Fellowship of the School on Sir Harry Jephcott, in recognition of his great services to the School over many years. Sir Harry Jephcott has been chairman of the School Council since its formation in 1948.

Manchester

DR. F. R. JEVONS, at present lecturer in biological chemistry, has been appointed to a newly established chair of liberal studies in science.

Strathclyde

D. S. BUTLER, reader in numerical analysis in the Department of Mathematics at the University of Strathclyde, has been appointed professor of numerical analysis as from April 1.

Sussex

PROF. G. A. SIM, at present professor of physical chemistry in the University of Illinois, has been appointed professor of chemistry.

Appointments

DR. J. L. GODDARD is to be commissioner of the Food and Drug Administration of the U.S. Department of Health, Education and Welfare. He was previously chief of the Communicable Disease Center, Public Health Service.

G. R. W. DE SILVA has been appointed director of the Division of Conference and General Services of the International Atomic Energy Agency in succession to D. G. Sullivan.

H. E. RILEY, formerly chief statistician at the International Labour Office, has been appointed head of the Office of Economic and Manpower Studies of the U.S. National Science Foundation.

Announcements

THE spring meeting of the British Biophysical Society, on "Excitable Tissues", will be held in the University of Oxford during April 14–15. Further information can be obtained from Dr. A. R. Peacocke, St. Peter's College, Oxford.

A SYMPOSIUM on "Aspects of Surface and Colloid Chemistry" will be held at the John Dalton College of Technology on April 15. Further information can be obtained from Dr. A. Powell, Department of Chemistry, John Dalton College of Technology, Chester Street, Manchester 1.

A MEETING of the Thermal Analysis Group of the Society for Analytical Chemistry will be held at the Royal College of Advanced Technology, Salford, during April 21–22. Further information can be obtained from the Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

A CONFERENCE on "Phonons", arranged by the Institute of Physics and the Physical Society, will be held in the University of Edinburgh during April 6–7. Further information can be obtained from the Meetings Officer, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

CHEMICAL CARCINOGENESIS

A SERIES of six lectures entitled "Chemical Carcinogenesis" was given at the Northern Polytechnic, London, between October 7 and November 14, 1965. Their purpose was to publicize the carcinogenic hazards of chemicals, particularly to those engaged in the production and marketing of chemical, rubber and pharmaceutical products. The course was inspired by an article by Prof. A. Haddow (*New Scientist*, 25, 348; 1965) entitled "Control of Cancer-inducing Chemicals", and Prof. Haddow very kindly helped to plan the course in its initial stages. The need for these lectures was shown by the high level of attendance at the meetings, and by letters from people expressing regret at not being able to attend and asking for abstracts. The lecturers, who kindly supplied abstracts for inclusion in this report, were: Prof. A. Haddow, director of the Chester Beatty Research Institute, London; Prof. E. Boyland and Dr. F. J. C. Roe (Chester Beatty Research Institute); Dr. P. N. Magee (Toxicology Research Unit Medical Research Council Laboratories, Carshalton, Surrey); and Miss Joan Davies (The Royal Marsden Hospital, London).

The meetings began with a lecture by Dr. P. N. Magee on carcinogenesis by nitroso compounds.

A large number of *N*-nitroso compounds are known to be capable of inducing cancer in experimental animals[1–5]. These compounds are among the most potent of all known carcinogenic substances[6,7]. The simplest member of the dialkylnitrosamine series, dimethylnitrosamine, is a powerful liver poison, causing necrosis of the liver in all the common species of laboratory animal[8]. When continuously fed to rats, mice and hamsters it induces malignant tumours of the liver in high incidence[1]. When given to young adult rats for shorter periods at higher levels, and even in survivors of single large doses, kidney tumours are induced but no liver tumours[2]. Injection into newborn rats is followed after about a year by kidney and liver tumours[9]. Dimethylnitrosamine is rapidly metabolized[10] and a large part of the carbon of the injected dose is excreted in the expired carbon dioxide[11,12]. The liver appears to be the main organ concerned with metabolism which is enzymatically mediated[13,14]. The kidney and probably some other organs in the rat are also capable of metabolism of dimethylnitrosamine but much less actively than the liver. One metabolic pathway appears to involve oxidative removal of a methyl group with the formation of the very unstable monomethylnitrosamine which decomposes spontaneously to yield an active alkylating intermediate, possibly the corresponding diazoalkane[15,16]. There are certainly other metabolic pathways of dimethylnitrosamine but these have been less thoroughly investigated.

A hypothesis to explain the toxic, mutagenic and carcinogenic action of the nitroso carcinogens suggests that the active agent concerned is the alkylating intermediate. Evidence in support of this hypothesis comes from examinations of carcinogenic activity in relation to chemical structure with a large number of nitrosamines[4], although certain discrepant features remain unexplained. Direct evidence of alkylation of tissue constituents has been obtained with the demonstration that proteins and nucleic acids become alkylated in animals injected with dimethyl and other nitrosamines[15,16]. Alkylation, mainly on the 7-position of guanine, occurs in the DNA and RNA of liver and kidney in the treated animals[16]. The methyl group in both the methylated DNA and RNA is unstable and rapidly disappears from the nucleic acids *in vivo* at a rate which cannot be explained by normal metabolic turnover[17]. This alkylation reaction is the same, chemically, as that which is suggested to underlie the mutagenic action of the biological alkylating agents. Whether it underlies the carcinogenic action of the nitroso carcinogens remains to be established.

Prof. E. Boyland devoted two lectures to discussing the biochemistry of a wide range of chemical compounds.

As most cancer is caused by chemical substances in the environment, study of the biochemistry of chemical carcinogens is of interest because it should lead to the prevention of the disease. Some carcinogenic compounds act without previous metabolic change and are considered to be direct carcinogens. Alkylating agents, which include mustard gas, nitrogen mustards, methylsulphonyl esters, lactones and epoxides, are probably direct carcinogens of this type. They react with the purine, guanine residues of nucleic acid. The nucleic acid is thus changed so that mutations and cancer result.

On the other hand, the aromatic amines, 2-naphthylamine, benzidine, and 4-aminobiphenyl, which induce cancer of the bladder, are carcinogenic after metabolic activation and are therefore indirect carcinogens. The active metabolites are derivatives of arylhydroxyamines and *ortho* aminophenols which can induce cancer of the bladder directly.

Most chemical carcinogens have been first recognized through accidental industrial exposure. An early example of this was the recognition of scrotal cancer in chimney sweeps as a consequence of exposure to the soot of chimneys (Percival Potts, 1775). The observation that soot and coal tar were carcinogenic led to the isolation of the active polycyclic hydrocarbon, 3,4-benzpyrene. Following this, a number of related polycyclic hydrocarbons were synthesized and tested by Cook and Kennaway, and the relationship of chemical structure and biological activity investigated. It is not known whether these hydrocarbons require metabolic change before exerting their biological action.

Urethane, which was used as an anaesthetic, as a solvent for drugs and in the treatment of leukaemia and myeloma, is also carcinogenic. Urethane is converted by mammals to *N*-hydroxyurethane, which has many biological activities that urethane does not possess. *N*-hydroxyurethane and some related hydroxylamine derivatives are carcinogenic and also inactivate the Shope fibroma virus and other viruses *in vitro*. The mechanism of action of *N*-hydroxyurethane and its analogues is probably due to reaction with the pyrimidine base, cytosine, of nucleic acids.

Many of the compounds used in the chemotherapy of cancer (such as nitrogen mustards, urethane and oestrogens) are carcinogenic. Knowledge of the mode of action of these substances is therefore of value both in prevention and treatment of cancer.

Prof. A. Haddow spoke on the heterogeneity of carcinogens and questions of the mechanism of action. An outstanding feature in carcinogenesis was the great number and variety of causative agents, whether chemical, physical or viral. This made it certain that such agents operate by biochemical routes which, initially at least, must be correspondingly different. However, it could by no means be excluded that they could lead to a key cellular event or alteration, similar, or perhaps even identical, in the different cases. Hence there was a prospect of the emergence, from a picture of great complexity, of a more synthetic comprehension of the carcinogenic process, with all that this could mean towards elucidating the essential biochemical differences between normal and cancer cells, and perhaps in turn, to rational

methods of control and even prevention. Prof. Haddow illustrated this theme by accounts of the carcinogenic hydrocarbons, aminostilbenes, aromatic amines, the biological alkylating agents, metals and metal–carbohydrate complexes, and of such miscellaneous examples as asbestos, the cycads, and the recently discovered aflatoxins. It was remarkable that, even now, so little was known with any precision as to the mode of action of the carcinogenic hydrocarbons. Their molecular planarity had suggested a possible interaction with the nucleotide plates, but this view might require to be modified or abandoned in view of the more recently recognized non-planarity of the potent 9 : 10-dimethyl-1 : 2-benzanthracene. However, other collateral evidence (for example, solubilization of the polycyclic hydrocarbons by purines and nucleic acids) still suggested some type of interaction with DNA, and a special case being widely investigated was that of the possible intercalation of hydrocarbon molecules between adjacent base-pair plates.

Of all the classes of chemical carcinogen, it was perhaps in the field of the alkylating agents (such as nitrogen and sulphur mustards, *bis*-epoxides, polyethyleneimines, dimesyl compounds, lactones and alkylnitrosamines) that most progress had been made in the past 15 years—largely as a result of the relative simplicity and higher chemical reactivity of these agents. Most remarkable was the demonstration by Brookes and Lawley of alkylation in guanylic acid at position 7, and of *bis*-guanyl and similar products almost certainly derived from inter-chain cross-linking of the DNA helix. That many of the DNA-viruses might act by modification of the genome similar to the cytogenetical changes induced by chemical agents was one of the considerations prompting hopes of an eventual synthetic understanding.

Miss Joan Davies spoke about occupational bladder tumours. More than 2,000 men die with bladder tumours every year in England and Wales, and some of these tumours are of occupational origin. If a bladder tumour is diagnosed at an early stage, treatment may be simple and effective, but often a tumour does not cause any symptoms until it is comparatively advanced, and treatment is then more difficult. This is why early diagnosis by means of cytological screening (regular microscopic examination of urine samples) is important for groups of workers who run an enhanced risk of developing the disease—groups such as certain chemical workers for whom the Association of British Chemical Manufacturers set up a screening service in 1956.

In 1954 a high risk of bladder tumours among men working in firms manufacturing alpha-naphthylamine, beta-naphthylamine and benzidine had been demonstrated[18]. Between 1921 and 1952, 243 out of 2,500 workers had developed the disease and half these had died as a result (at an average age of about 55). Another 250 would probably develop tumours in due course, for some 18 years usually elapsed between starting work with the chemicals and the onset of the disease. Beta-naphthylamine was the most dangerous chemical. The longer a man worked with these substances, the more likely he was to be affected, but even brief and indirect contact could cause tumours.

Until 1949 the rubber industry made extensive use of antioxidants containing alpha- and beta-naphthylamine, and in 1954 Case and Hosker[19] directed attention to an excess risk of death from bladder tumours among skilled rubber workers. However, no full survey has ever been made to determine the size of the risk or the range of workers affected. In 1956–57 the Rubber Manufacturing Employers' Association set up a screening service, but by no means all member firms made use of this service and many exposed rubber workers were not screened until very recently.

The same anti-oxidants were used until 1949 in insulating rubber in the electric cable industry, but here the risk apparently went unheeded until recent publicity following the inquest on a former cable worker[20]. The severity of the risk can be gauged by the finding[24] that in the rubber mill of one cable factory thirty out of 139 men had worked for 10 or more years in contact with such an anti-oxidant, and six of the thirty have so far developed bladder tumours (four of these dying of the disease). Apparently no cable workers were screened until after the inquest mentioned. As well as definitively identifying the carcinogens responsible, a full survey of the rubber and cable industries might identify the workers running the greatest risk of developing the disease, and so enable screening priorities to be established.

In 1953, bladder tumour was made a prescribed industrial disease under the Industrial Injuries Acts, but through a general lack of awareness many rubber and cable workers with the disease (or their widows) have never been advised to claim the extra financial benefits to which they are entitled.

The final lecture was given by Dr. F. J. C. Roe, who reviewed the evidence that some metals, minerals and pharmaceutical preparations are capable, under certain conditions, of inducing cancer in laboratory animals or man. Among the metals, clear evidence of tumour induction has been obtained in the cases of arsenic, beryllium, cadmium, chromium, cobalt, lead, nickel and zinc. Arsenic is undoubtedly carcinogenic for man but it has not been shown to be so for other species. Some of the other metals, such as lead and zinc, have not yet been shown to be carcinogenic in man. Asbestos has recently been found to be the principal cause of a rare type of human tumour, the mesothelioma. Also, it is now clear that the association between lung cancer and asbestosis is both definite and strong.

A wide variety of pharmaceutical preparations have been shown to be carcinogenic in laboratory animals. A few have been shown to induce cancer in man. Where the use of such preparations is life-saving, it may be necessary to accept a risk that cancer will be induced in a proportion of patients. But where non-carcinogenic alternatives exist, or where the benefits to be expected from the use of a drug are small, the continued prescribing of carcinogenic agents cannot be justified. A full report of the preparations considered is due to appear shortly elsewhere[22].

Of special importance in relation to the carcinogenic hazard from drugs is the difficulty of extrapolation from animal experiments to man. Whereas potent carcinogens are frequently active in almost all species, weaker agents may not be so. Thus, a weak effect observed in one animal species may be unmatched by a similar response in another. It is important, therefore, to have information on tests in more than one species. An experiment on animals is more likely to be relevant to the human situation if the dosage used is realistic and the route and mode of administration the same, or comparable. One of the most contentious problems is knowing how to interpret the induction of cancers in animals by the parenteral administration of a substance normally given orally to man. In general, the need for greatest concern relates to drugs which, if they are taken at all, tend to be taken in large doses over long periods, and to drugs freely available without medical prescription. The real risk of cancer induction in man by such substances should be ascertained by epidemiological methods, though this may be impossible where a drug can be bought without prescription. Epidemiological surveys are expensive and often slow to yield results. If a new drug were introduced to-day, it may be more than 20 years before its carcinogenic action in man could be detected by epidemiological means, because of the long latent periods generally required for the induction of human cancer. In the meantime, the best use must be made of experimental findings to guide prescribing practice.

The importance of the subject was underlined by Dr. Roe, who suggested that as much as 75 per cent of human

cancer is attributable to exposure to carcinogenic agents in the environment and therefore potentially preventable. Potent carcinogens tend to reveal their activity sooner or later, even if no careful investigation is made. The real difficulty relates to weaker agents, none of which by themselves has a marked effect on cancer incidence, but which, together, may very materially increase the cancer burden.

R. G. LUCKCOCK

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RECENT ADVANCES IN THE MICRO-IRRADIATION OF CELLS

RESEARCH workers who are interested in the effects of microbeams of radiation of a variety of biological materials met recently to discuss problems which are currently important in the development of this subject. The meeting was arranged by Dr. M. Bessis and was held in the Laboratoire de Pathologie Cellulaire, le Kremlin-Bicêtre, Paris.

Dr. C. L. Smith (Cambridge) was chairman for the first session, which was concerned with recent advances in the design and construction of microbeams and opened with some comments by H. Glubrecht (Hannover) on the development of an ultra-violet microbeam coupled with an ultra-violet microspectrophotometer of the Caspersson type. He emphasized that the dose of ultra-violet radiation which was used for microspectrophotometric measurements on living cells should be not more than one-thousandth of that which had been used in the microbeam to irradiate the cells. In answer to L. Trapp (Zeiss, Oberkochen) he said that ultra-violet absorption measurements had been made over areas varying from 0·7μ to 12μ and dose-rates had been obtained by taking into consideration the reflexion and transmission coefficients of all the relevant surfaces. G. Nomarski (Paris) indicated the importance of increasing the ultra-violet reflecting power of aluminized mirrors above the normal figure of 85 per cent, particularly in arrangements where there could be as many as ten to sixteen reflexions.

Nomarski then gave details of developments in laser techniques. It is now possible to produce a beam which is constant in intensity over an area approximately equal to that of a single cell, and dose reproducibility has been improved to about 10 per cent by surrounding the laser with a 0·5-mm thick water jacket. The available wavelengths may soon be extended by harmonic generation from two fundamental wave-lengths at 6940 Å and 10600 Å with the aid of a new crystal and, in the near future, repeatable pulses varying in length from 50 μsec to 6 msec at 0·1-sec intervals may be available. Glubrecht suggested that when this became possible dose-rate effects would be very interesting, and drew an analogy with pulse radiolysis.

There was then a discussion on the importance of localization of the radiation delivered by a microbeam system, and Nomarski indicated that, for ultra-violet light, precision of the order of 0·15μ was possible for a thin target. P. P. Dendy (Cambridge) and A. Forer (Copenhagen) both supported the importance of precise physical location but drew attention to biological difficulties which include the unavoidable irradiation of membranes and other cell parts in addition to the target, and the ease with which biochemical changes can be transferred from the site of the primary lesion.

M. Ernst (Hannover) described a recently constructed X-ray microbeam which uses 5-keV X-rays to irradiate an area approximately 4·5μ in diameter. The whole apparatus is contained in a constant-temperature box which can be sterilized. In this way, uncovered biological specimens can be used, and this, in turn, allows the use of lower-voltage X-rays which give a better localization of energy. The apparatus was compared with the earlier models of Seidel and Buchholtz[1] and Seed[2].

A discussion on the production of small holes followed and Nomarski indicated that 1μ-diameter holes in 10μ-thick tungsten could be produced by laser radiation. H. Mel (Paris) told the meeting about a relatively new procedure in which small areas of a surface can be weakened by the deposition of certain elements followed by neutron irradiation. Suitable chemical treatment leads to the production of very small regularly shaped holes at the weakened sites.

Smith gave a brief outline of a new electron microbeam which is being built in Cambridge. It is hoped ultimately to obtain a 3μ spot of 50-keV electrons, and incident illumination coupled with phase contrast will be used to view the biological material and control the site of irradiation.

The session ended with a description by Forer of a modified reflecting objective which permits the observation of a cell in polarized light under phase contrast conditions during ultra-violet microbeam irradiation. Nomarski pointed out the advantage of circularly polarized light for this type of work, particularly at high numerical apertures.

In the second session, the results of some recent experiments were presented. Prof. H. Glubrecht (Hannover) took the chair and suggested that the speakers should try to show how their experiments had extended ancillary techniques and quantitative methods for assaying the biological effect, both of which are strictly limited in microbeam work. Bessis described some work in which mitochondria in *KB* cells had been stained with various vital stains. At staining concentrations which were twenty times lower than the limit of visibility, mitochondria became highly sensitive to laser microbeam irradiations. An electron microscope study of the irradiated cell showed that mitochondria which had been hit were extensively damaged. Certain mitochondria which were not in the irradiated region showed structural changes 2–3 h after irradiation but they had recovered by 24 h.

A discussion followed on valid criteria for deciding if a cell is alive, and Bessis stated that, in their work, the ability to take up nucleic acid precursors and the activity of enzymes had been used as criteria. This point was taken up by the next speaker when Dendy discussed some experiments which had been performed at Cambridge. He referred briefly to earlier micro-irradiation

experiments in which effects had only been investigated for a few hours and it had been realized that an important factor in the cell viability problem was the ability or otherwise of irradiated cells to develop into colonies. The most recent experiments had therefore been designed to study the development of single cells for 7 days after irradiation with a microbeam of heterochromatic ultra-violet light. At the end of this time, several irradiated cells had formed small colonies which contained a number of morphologically normal cells. A study of the DNA metabolism of individual cells in these colonies at 7 days showed many anomalies, even in the morphologically normal cells.

Forer gave a brief account of the use of polarizing microscopy coupled with ultra-violet microbeam irradiation to study the effects on chromosome separation when a small volume of the spindle fibres is irradiated, and Glubrecht presented some results which were obtained when moss cells were irradiated with a monochromatic ultra-violet microbeam. In the latter experiments, cell killing was most efficient when radiation at $\lambda = 2650$ Å was directed at the nucleus but $\lambda = 2800$ Å was most efficient when directed at the cytoplasm.

The meeting ended with a tour of the new Laboratoire de Pathologie Cellulaire, and the Chairman thanked Prof. Bessis for making all the arrangements for the meeting and thanked the representative of NATO, Ing. Gen. Giboin, for financial support. P. P. DENDY

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IODINE-LABELLED PROTEINS AND HORMONES

ENCOURAGED by the success of a symposium in 1964 on the preparation and biomedical application of labelled molecules Euratom has sponsored another more specialized meeting on problems connected with the preparation and use of labelled proteins. This was held at the University of Pisa in January 1966 and attracted some 150 participants. As might be anticipated from a survey of the literature, nearly all the communications were concerned with proteins labelled with iodine isotopes, the 3-day conference being broadly equally divided into sessions on hormones and non-hormonal plasma proteins.

Hughes (Boston) reviewed the chemistry of iodination and the particular chemical requirements for satisfactory trace-labelling of proteins. For most purposes the protein should retain its biological activity after labelling. He considered that iodotyrosines might conceivably be used *in vivo* for protein synthesis but the opportunity seldom presented for this to happen because of the widespread distribution and rapidity of action of tissue deiodinases. Rosa (Saluggia) emphasized the importance of achieving a truly random distribution of isotopic iodine throughout the population of protein molecules and produced further details of the electrolytic method of doing this which he has elaborated in collaboration with Donato and his group at Pisa. It remained for Freeman (London) to discuss biological methods of verifying that the labelled protein is a reliable tracer since it is generally accepted that only *in vivo* tests are sufficiently sensitive. He mainly favoured measurements of retention in the livers of rats, of catabolic rates in rabbits (if necessary rendered tolerant to human proteins), and of iodide activities in the urine and body water in the first 24 h in man. These and other speakers emphasized the crucial importance of using only proteins which have been circumspectly handled before labelling, and of taking steps afterwards to minimize self-irradiation damage.

In introducing a session on metabolic studies with labelled plasma proteins McFarlane (London) recommended that speakers should refer specifically to absolute or fractional rates of turnover as the case might be. Since iodine isotopes are not used for protein synthesis, conclusions about synthesis can only be derived indirectly from slopes of plasma specific activity curves of animals in metabolic equilibrium. These are proportional to fractions of the total body pool synthesized in unit time whereas currently used procedures for measuring catabolic rates give them as fractions of the plasma pool. It was important therefore also to specify the pool. Bianchi (Pisa) and Sorin reviewed the use of iodine-labelled albumin in metabolic studies, Andersen (Copenhagen) did the same for γ-globulin and Regoeczi (London) for fibrinogen. These were mainly straightforward communications, but controversial questions arose when subsequent speakers discussed mechanisms for regulating plasma protein turnover. Rothschild (New York) reported results of experiments in which low albumin concentrations were produced in rabbits by infusing γ-globulin or dextran and in which little or no change was observed in the mass of albumin catabolized per day. Fractional synthesis rates in these experiments were unrelated to fractional or absolute catabolic rates, to albumin pool sizes or to concentrations and in fact were sub-normal. This apparent paradox could be resolved by postulating a control mechanism based on the colloid osmotic pressure of hepatic interstitial albumin which is assumed to be increased by injections of γ-globulin or dextran. Dykes (Birmingham), on the other hand, found greatly reduced absolute catabolic rates in cirrhotic patients with low albumin concentrations both of which were restored to normal by infusions of albumin. He visualized a second-order relationship between albumin concentration and absolute catabolic rate.

Included in a variety of shorter papers were descriptions of the preparation and use of tritium-labelled plasma proteins by Gostonyi *et al.* (Budapest) and by Gerber *et al.* (Mol, Belgium). Gordon and Jacques (London) reported on the distribution of iodine-labelled proteins in subcellular particles of the rat liver. Høedt-Rasmussen (Copenhagen) reported on a 10–20 per cent extravascular catabolism of parenterally administered albumin, and Milhaud (Paris) on the effects of ligaturing the thoracic duct on ^{131}I-albumin distribution and metabolism in rats.

Most fittingly, a culminating session to this part of the conference consisted of a round-table discussion of mathematical methods of analysing results. Experts on the panel comprised Donato (Pisa), Matthews (London), Nosslin (Malmö), Segrè (Camerino), and Vitek (Prague). The more recently published methods of Nosslin, which are largely independent of compartmental considerations, received most attention. While Nosslin agreed that his second method, which is based on integrating areas under plasma and total body activity curves, might be less vulnerable to errors associated with identifying terminal slopes of these curves, he considered it was not altogether free of them, and indeed that the accuracy of all methods was to some extent limited by this factor. The deconvolution method of Vitek and Donato was also the subject of some discussion, as was the use of analogue computers by Segrè.

In the sessions on hormone labelling it was soon apparent that a greater degree of protein modification is permissible and altogether higher specific activities are required. Some seventeen hormones can now be estimated by radioimmunoassay, and Yalow (New York) considered the

general principles underlying the procedures used, drawing on the extensive experience of the New York group using insulin, growth hormone, ACTH and parathyroid hormone. Requirements of the labelled hormones were regarded as less stringent than is widely supposed, the essential one being that the plasma hormone and a standard preparation should be equivalent in their capacity to inhibit the reaction between labelled hormone and antiserum. It follows that neither the animal species from which the standard preparation, or the preparation for iodination, is obtained nor, within limits, the degree of damage suffered during iodination is critical. There was general agreement, however, that some purification of the labelled hormone is essential and methods for doing this were described. The possibility that metabolic fragments of endogenous hormones which are immunologically but not hormonally active might be present in plasma was also discussed. The general problem of damage to hormones was included in a round-table session organized by Milhaud (Paris).

Some fifteen successful assays using the chloramine *T* method have been carried out in various laboratories, but Greenwood (London) referred to unsatisfactory experiences with iodine-131 obtained since 1964. Results with the method in the United States had been more consistent than those obtained elsewhere and this seemed to depend mainly on higher specific activities of the available iodine-131. Michel (Paris), Quabbe (Berlin), Brauman (Brussels) and Franchimont (Liège) had broadly shared Greenwood's experience. The determination of ^{131}I-specific activities was outlined by Yalow. In view of the lack of knowledge of iodine and radioiodine chemistry emphasized by Hughes any improvement in the precision of micro-iodine estimations must be welcomed.

The availability of standard preparations of human growth hormone and antisera was discussed by various speakers including Coates (London) and Stewart (Frankfurt). Rosa (Pisa) and Ooms (Brussels) presented short communications on the immunological and biological properties of radioiodinated insulins, while Melani (Frankfurt) reported a procedure for assaying adrenocorticotrophic hormone (ACTH) which depends on the use of an ion exchange resin. The sensitivity of his assay was equivalent to that of the first ACTH one reported by Berson *et al.*, and this has since been greatly increased. Crosignani and Polvani (Milan) and Freedlender (Boston) reported on assays for human chorionic gonadotrophin and growth hormone respectively which differed in detail from previous assays using the double antibody method. Franchimont (Liège) outlined assays both for human follicle-stimulating hormone and luteinizing hormone, and reported millimicrogram levels during the normal menstrual cycle with two peaks of luteinizing hormone and one of follicle-stimulating hormone. A full report of his procedure is awaited with considerable interest.

In 1963 groups in New York and Washington jointly reported an assay for parathyroid hormone, the procedure having since been improved in sensitivity and applied to humans and animals. Yalow summarized the present position of the New York group and gave unpublished results of human plasma measurements from which it was evident that clinical application of the method is still hindered by its lack of sensitivity. Potts, on behalf of the Washington group, showed data bearing on the control of animal secretion of the hormone. In the course of this work an identical reaction between crude human parathyroid hormone and purified bovine parathyroid hormone was demonstrated. Availability of purified bovine hormone and antiserum was also discussed.

The clinical and physiological applications of radioimmunoassays were only outlined for insulin by Samols (London) and for glucagon by Weinges (Homburg-Saar) representing Unger's group. Both communications served *inter alia* to emphasize the usefulness of procedures which can be carried out at rates of up to 200 measurements per week. A discrepancy was noted in the levels of glucagon found in plasma by Samols and Unger, and this may explain the differences in their results after oral administration of glucose.

Abstracts of the papers presented are available (*J. Labelled Comp.*, 1, No. 4, 311) and a full report in book form will appear in due course.

A. S. McFarlane
F. C. Greenwood

DOMESTIC FOOD CONSUMPTION AND EXPENDITURE

DOMESTIC Food Consumption and Expenditure, 1963, is the fourteenth annual report produced by the National Food Survey Committee*. The 1962 report reviewed the findings of surveys conducted during the years 1950–60. Over such a period it is easier to observe changing trends in consumption patterns and the quantities of food consumed or purchased than from one year to the next. Nevertheless, interesting facts do emerge from the present report.

Survey methods remained unchanged, but for reasons of economy the sample in the 1963 survey was reduced by 18 per cent as compared with the 1962 sample, the effective size being 7,532 households. There was no change in the average number of persons per household, which again was least in London (2·95) and highest in rural areas (3·41). Because of random sampling, rural areas were over-represented in the 1963 survey, and in order to correct this bias the national averages in this report have been obtained by weighting the separate averages for the different types of urban and rural areas by the estimated population resident in those areas. The composition of the population is interesting. In social class *A* there were only eight households with four or more children and only very cautious conclusions can be drawn from this sample. The representation of old-age pensioners, of whom two-thirds were women, further increased. It appears that the highest proportion of elderly men live in the south-west region. The average number of earners per household tended to vary inversely with the income of heads of households including a number of children, and adult earners tended to be fewer in Class *D* than in any other earning classes because of the smaller average number of adults per household. The highest number of earners was in families containing adolescents.

The exceptional severity of the 1963 winter adversely affected the supply and distribution of certain foods and this in turn affected prices during the first quarter of the year. Over the year, and for the country as a whole, the average total expenditure was 32*s*. 4*d*. per week, a slight increase over 1962, which can be explained partly by a rise in food prices and partly by an increase in the quantity of food purchases. Expenditure on 'convenience foods' rose to 6*s*. 1*d*. per head per week as compared with 5*s*. 11*d*. in 1962, but remained at about 19 per cent of the household food budget. Consumption of dairy products and eggs increased, while the consumption of fish fell. Consumption of poultry increased, though at a slower rate than previously, probably at the expense of meat, the

* Ministry of Agriculture, Fisheries and Food *Domestic Food Consumption and Expenditure. 1963—Annual Report of the National Food Survey Committee.* Pp. ix+131. (London H M S O, 1965.) 12*s*. 6*d* net.

consumption of which showed a slight decrease. The demand for tea remains inelastic, in spite of changes in price or income, but the demand for coffee has risen, particularly for 'instant coffee', purchases of which were nearly 80 per cent higher than in 1960.

The tables showing the expenditure on and consumption of food are informative. Expenditure per week was highest (50*s*. 3*d*.) in class *A* for families containing two adults under 55, falling to 27*s*. 4*d*. for a family with three children; it was lowest in classes *C* and *D*, where 19*s*. 9*d*. was spent by the family with four or more children. The lowest consumption per head per week of a high proportion of foods was most frequently recorded in families with four or more children; for bread, vegetables and beverages, such as tea, however, families with two or three children occupied this position. For all foods except tea taken by families of two adults over 55, the highest consumers consisted of families of two adults under 55. With respect to differences in social classes, class *A* were, as might be expected, the highest consumers of the more expensive foods such as dairy produce, eggs, meat, chicken, fish and coffee, whereas old-age pensioners led with tea and sugar, and class *C* with vegetables. The cheaper foods, bread and cereals, sugar and tea, were taken in the smallest quantity by class *A*, whereas classes *C* and *D* consumed less of all the other food groups, being joined by the old-age pensioners in the low consumption of meat and fruit.

The energy value of the diet of the average person remains much the same as in 1962, with class *A* and old-age pensioners having the highest intake of calories. The percentage of calories provided by protein remains unchanged at 11·5 per cent. The level of intake of other nutrients in families appeared satisfactory when expressed as a percentage of recommended requirements, but this may not apply to all individuals. The way in which food is distributed within the family is unknown, and possibly some members of some families were abundantly fed at the expense of others. MARGARET E. CAMERON

ABUNDANCE ANALYSIS OF THE PECULIAR STAR *HD* 101065

By DR. A. PRZYBYLSKI
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THE eighth magnitude star *HD* 101065 ($\alpha = 11h\ 35{\cdot}2m$, $\delta = -46°\ 26'$, 1950·0) has a unique and extremely peculiar spectrum[1-4]. While spectral peculiarities are superimposed on a more or less normal spectrum in other abnormal stars, there are scarcely any normal features in the spectrum of this star. Most striking is the absence of the elements usually seen in the spectra of normal stars such as iron, titanium, chromium, manganese and sodium, and the great strength of lines of barium and the rare earths. Hydrogen, calcium, barium and possibly strontium and lithium are the only elements which can be seen in the spectrum besides the ionized rare earths. The hydrogen lines are of normal width but they are much shallower than in a normal star of similar temperature. The calcium *H* and *K* lines are sharp and are weaker than many strong lines of the rare earths. The *K* line, with its equivalent width of 0·3 Å, is about sixty times weaker than in the solar spectrum even though we are dealing with a star of similar temperature.

Counting each local minimum of intensity as a separate line, about 3,000 lines have been measured between λ3600 and λ4950 Å on plates with a dispersion of 6·7 Å/mm. The effect of mutual blending of lines is very large and the true continuum is probably not seen at all in the whole spectral region below 5000 Å.

Normally the number of lines decreases with the increasing wave-length and for this reason the red region of the spectrum should be easier to analyse. Unfortunately, high dispersion spectra of *HD* 101065 in the red region are beyond the reach of existing southern telescopes. Only one spectrum of the visual region with a dispersion of 20 Å/mm has been obtained at Mount Stromlo Observatory, but it is of poor quality. For this reason only limited use of this spectrum is made in the present article. It is hoped that in the near future better spectra will be obtained in the visual range. The present results are based mostly on the best spectrum obtained in the photographic region. Its width is 0·35 mm and its dispersion 6·7 Å/mm. The projected slit width is 28μ.

The effective temperature of *HD* 101065 derived from *B*, *G*, *R*, *I* colours of the six-colour photometry by G. E. Kron and K. C. Gordon[2] is 6,350° K. However, if it is taken into account that the two other colours *U* and *V* show the largest known ultra-violet deficiency of 0·4 magnitudes, this temperature should be lowered by about 400 degrees. In the present investigations it has been assumed that the effective temperature of *HD* 101065 is identical with that of the Sun (5,900° K).

The parallax of the star is unknown but a fairly high proper motion of 0·054″ per annum, coupled with the low radial velocity of the star (+10 km/sec), seems to indicate that the star is not far from the main sequence. It is unlikely that it is a supergiant although it could possibly be a giant of absolute magnitude +1. However, it is most likely a dwarf on the main sequence and therefore physical parameters of the solar atmosphere used by L. H. Aller[5] have been assumed in the analysis (the reciprocal temperature $\theta = 0{\cdot}9$ and the logarithm of the electron pressure $\log p_e = 0{\cdot}9$). No estimate of the electron pressure from the spectrum is possible since no element is seen in two stages of ionization.

In view of the fact that *HD* 101065 is an abnormal star, the possibility that some unknown sources of opacity influence its atmosphere cannot be excluded. On the other hand, the agreement of its *B*, *G*, *R* and *I* colours with those of normal late *F* type dwarfs suggests that its main source of opacity is the negative hydrogen ion. This has been assumed in the present investigations, since scarcely any other assumptions could properly be justified.

Because of severe mutual blending, very few spectral lines are suitable for an analysis of the spectrum. Only 350 lines of fifteen elements could be used for the abundance analysis. It should be emphasized that almost all equivalent width determinations are only crude estimates which would be disregarded in the analysis of a normal star. However, in view of the importance of *HD* 101065 for the theory of stellar evolution, even an analysis of the chemical composition of its atmosphere based on poor data is certainly better than pure guesswork or complete uncertainty.

In spite of the difficulties in the measurement of the equivalent widths, the individual points on the curve of growth diagrams do not show an obviously larger scatter than on diagrams for normal stars. This may give a certain confidence in the results of the analysis, but does not exclude the possibility of systematic errors in the measurements of the equivalent widths. These errors may be due partly to the error in the continuum (which has been drawn through the highest intensity peaks), partly to the contribution of lines which have not yet been catalogued, and partly to the method used in order to eliminate the effect of blending by neighbouring lines.

Neighbouring lines have been separated by drawing straight lines perpendicular to the dispersion on the tracing through the intensity peaks. In all probability the equivalent width of faint lines has been overestimated.

A coarse analysis of the star was carried out using Wrubel's curve of growth. The most reliable curve has been obtained for cerium for which the largest number of lines was available. This curve gave the best determination of the turbulent velocity of 5 km/sec which was afterwards used for all other elements. This fixing of the turbulent velocity permitted a better determination of abundances since it removed one source of uncertainty in the position of the theoretical curve of growth superimposed on the stellar curve of growth diagram.

The values of the oscillator strength were taken from Corliss and Bozman's monograph[6]. Unfortunately no data are available for holmium and terbium. However, it was possible to evaluate at least approximately the spectroscopic data entering into the abscissae of the curve of growth from the intensities of lines tabulated by Meggers, Corliss and Scribner[7]. The additional error caused by this approximate procedure is probably less than 0·1 in the logarithm of the abundances. The same method was also used for erbium and thulium, since oscillator strengths were available only for a small number of lines of these elements. The determination of the abundance of thulium from lines with no known *gf*-values leads to practically the same result as the determination from lines with known oscillator strengths.

The results of the analysis are shown in Table 1. They are normalized to the logarithm of hydrogen abundance 12·0. The last column of Table 1 shows the over- or under-abundance of elements in comparison with Suess and Urey's[8] cosmic abundances.

Table 1

Element	Log abundance	Δ Log abundance
Hydrogen	12·0	—
Calcium	2·4	−3·8
Strontium	3·4	+0·6
Barium	3·6	+1·5
Lanthanum	3·7	+1·9
Cerium	4·0	+2·1
Praseodymium	4·0	+2·9
Neodymium	4·1	+2·4
Samarium	4·0	+2·7
Europium	4·0	+3·2
Gadolinium	4·0	+2·7
Terbium	3·5	+3 0
Dysprosium	3·5	+2·2
Holmium	3·8	+3·2
Erbium	3·7	+2·7
Thulium	3·6	+3·6

The abundance of calcium is based on only one line (*K* line at λ3933 Å) since the *H* line at λ3968 Å is blended with a strong line of dysprosium. However, the determination of calcium seems to be quite reliable. The abundance of strontium is based on two lines at λ4215 Å and λ4161 Å, but the possibility cannot be excluded that these lines may be wrongly identified. The strontium line at λ4077 Å is in a wide blend and cannot be used even for a positive confirmation of the presence of this element in the atmosphere of *HD* 101065. Strontium is included in Table 1 because the line at λ4215·42 Å (differing by 0·1 Å from the laboratory value) cannot be identified as a known line of a rare-earth element.

The over-abundances of barium and lanthanum seem to be higher than in peculiar *A* stars of Cr–Eu type but they are lower than the over-abundances of the rare earths following lanthanum. The spectrum of *HD* 101065 shows very strong lines of holmium for which no abundance determination in stars is yet available. However, holmium lines seem to be a common feature in Cr–Eu stars.

No conclusive evidence for the presence of the two heaviest rare earth elements (ytterbium and lutetium) in the atmosphere of the star is available. A good spectrogram in the red region would certainly solve this question for lutetium, which has a number of strong lines in this part of the spectrum, but for ytterbium the answer may be more difficult.

The spectrum of the star in the visual range seems to indicate the presence of a lithium line at λ6707 Å, but this spectrum is of poor quality and does not permit precise determination of the wavelength of this line. Anyhow this line would be blended with a fairly strong line of samarium. It is hoped that future observations in the red region will definitely settle the question of whether lithium is present in the spectrum or not. This is an extremely important question since the presence of lithium would mean that the disappearance of many elements in the spectrum of the star may be due to the destruction of these elements as the result of surface activity. Lithium and other light elements are the debris of the destruction of heavy elements through bombardment by high-energy protons.

A very faint line at λ4057·8 Å could possibly be due to neutral lead. If this identification is correct the logarithm of the abundance of neutral lead would be −0·6 on the scale adopted in Table 1. Adding the abundance of ionized lead obtained from Saha's equation we obtain 1·1 as the logarithm of total abundance of this element. However, the identification of the line of λ4057·8 as coming from PbI is rather doubtful.

No lines of iron could be identified in the spectrum of the star. This could be due to the fact that sometimes strong lines of the rare earths coincide with iron lines. However, the absence of two neutral lines at λ3820·43 Å and λ4045·82 Å, which do not coincide with known rare-earth lines, is a well-established fact. Assuming that any possible iron line at λ4045·82 Å has an equivalent width of less than 20 mÅ we obtain +1·1 as the highest possible logarithmic abundance of neutral iron. The total logarithmic abundance of iron on the scale of Table 1 would then be 2·5, which is 4·8 less than the cosmic abundance of iron according to Suess and Urey[8]. If Aller's abundances are taken for comparison the under-abundance of iron would be lower (Δlog abundance = −4·1), but other elements listed in Table 1 would show no significant differences in their over- or under-abundances.

Adding all the abundances of metals shown in Table 1 we obtain log $N = 5{\cdot}0$ as the total abundance of metals which are represented by lines in the spectrum of the star. This is 2·3 less than Suess and Urey's[8] logarithmic abundance of iron. *HD* 101065 may therefore be a metal-poor star in spite of its extremely high blanketing effect and its highest known Strömgren metallic line index for any *F* or *G* star (B. Westerlund, private communication). Unfortunately a definitive determination of the metal content in the atmosphere of the star would require the knowledge of the abundances of elements which are not represented in the Fraunhofer spectrum of the star.

If *HD* 101065 is a metal-poor star, the adopted value of the logarithm of electron pressure may be too high, which would also mean that the opacity used in the analysis is too high. The results shown in Table 1 should be reduced by 0·3 if the star is as poor in metals as the sub-dwarf *HD* 140283. They should be lowered still more by about 0·5 if *HD* 101065 is a metal-poor giant. Unfortunately the spectrum of the star contains no features which could be used for the determination of the electron pressure in its atmosphere. It should be borne in mind that, although the absolute abundances shown in Table 1 are valid only for the adopted value of electron pressure, the relative abundances of the rare earths should be quite reliable within the limits of accuracy of the curve of growth method.

The present analysis was made during my stay at the Royal Greenwich Observatory and later at Mt. Wilson and Palomar Observatories. I thank the Astronomer Royal, Sir Richard v.d. R. Woolley, and Dr. H. W. Babcock for their hospitality. I thank Prof. B. J. Bok,

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Drs. W. Buscombe, D. J. Faulkner, S. C. B. Gascoigne, A. R. Hogg, H. Hyland, A. W. Rodgers and B. E. Westerlund for their advice.

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POSITIONS OF RADIO SOURCES MEASURED WITH THE ONE-MILE RADIO TELESCOPE

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THE one-mile radio telescope[1] at the Mullard Radio Astronomy Observatory, Cambridge, is being used in a continuing programme to study radio galaxies[2] and other sources. This article reports measurements of the positions of twenty-two lesser-known sources of which all but two are unresolved by the 23″ beam of the telescope at 1,407 Mc/s. Observations of extended and double sources will be discussed elsewhere[3]. The positions have been examined on the prints of the Palomar *Sky Survey* and for many of the sources there are coincident optical objects. The accuracy of the radio positions is such that these objects are almost certainly associated with the radio emission.

The measurements involve continuous observation of each source for a 12-h period using three 60-ft. paraboloidal reflectors on an east–west axis. Four simultaneous outputs are obtained, corresponding to interferometers of two different spacings and at two wave-lengths, 73 cm and 21·3 cm, but in general the most accurate positions for unresolved sources are those measured with the largest available spacing, 1·6 km. at the shortest wavelength, 21·3 cm.

To reduce the observations a position of the source is assumed and the expected variation of phase difference of the signals arriving at the two aerials of each interferometer pair is calculated for the 12-h period. This is then

Table 1

	Radio position	Optical position	Notes
3C 14	00 h 33 m 29·46 s 18° 21′ 32·4″		A faint diffuse object, visible only on the red print, lies within a few sec of arc of the source
3C 16	00 h 35 m 08 53 s 13° 08′ 34·1″		Nothing is visible within 13″ of the source
3C 19	00 h 38 m 13·88 s 32° 53′ 42·9″		There is no object brighter than 20 m within 10″ of the source. See also Adgie and Gent (ref. 5)
3C 20	00 h 40 m 20·30 s 51° 47′ 10·2″	00 h 40 m 20 46 s ±0·25 s 51° 47′ 08″ ±3″	A pair of 19 m red galaxies separated by 6″ lies close to the source, which is slightly extended. The optical position (Parker) is that of the S. following member. An almost stellar 18·5 m object follows this by 8″. See also Adgie and Gent (ref. 5)
3C 93·1	03 h 45 m 35·76 s 33° 44′ 08·5″	03 h 45 m 35·95 s ±0·25 s 33° 44′ 07″ ±3″	The optical position (Parker) is that of a 19·5 m red galaxy in a cluster. The source scintillates (ref. 6), indicating structure <1″
3C 153	06 h 05 m 44·56 s 48° 04′ 52·8″	06 h 05 m 44·55 s ±0·15 s 48° 04′ 49·0″ ±1·0″	The optical position (Parker) is that of the diffuse red 18·5 m object noted by Longair (ref. 4). See also Adgie and Gent (ref. 5)
3C 173	06 h 58 m 56·41 s 38° 01′ 46·0″		There is nothing visible in the position of the radio source which lies 11″ S. following a 14 m star
3C 213·1	08 h 58 m 05·02 s 29° 13′ 35·5″	08 h 58 m 05·3 s ±0·3 s 29° 13′ 38″ ±4″	The optical position (Parker) is that of a 17·5 m object, probably a compact galaxy. The red image is almost stellar but on the blue print the object is more diffuse and fainter.
3C 241	10 h 19 m 09·36 s 22° 14′ 44·2″		Nothing is visible within 50″ of the source which scintillates (ref 7), indicating structure <1″. See also Adgie and Gent (ref. 5)
3C 256	11 h 18 m 04·22 s 23° 44′ 23·2″		Nothing is visible within 20″ of the source which follows the galaxy 'a' in Longair's finding chart (ref. 4) by 29″. The source scintillates (ref. 6), indicating structure <1″. See also Adgie and Gent (ref 5)
3C 258	11 h 22 m 06·23 s 19° 36′ 05·4″	11 h 22 m 06·4 s ±0·3 s 19° 35′ 59″ ±4″	The optical position (Parker) is that of a 19 m object. Its red image is irregular in outline but the blue image is brighter and more star-like. The radio source may be slightly extended
4C 31 38	11 h 53 m 44·27 s 31° 44′ 43·8″	11 h 53 m 44 22 s ±0 15 s 31° 44′ 44″ ±2″	The optical position (Parker) is that of an 18 m blue object. It appears stellar on the blue print but fainter and diffuse on the red print. Nothing else is visible within 30″
3C 268 3	12 h 03 m 54·19 s 64° 30′ 18·2″		A very faint diffuse object lies at the radio position with others within 10″, probably a distant cluster of galaxies
3C 268·4	12 h 06 m 41·90 s 43° 56′ 00·0″	12 h 06 m 42·1 s ±0·1 s 43° 56′ 02″ ±2″	The optical position (Parker) is that ot an 18 m stellar object; equally bright on the red and blue prints. Some faint nebulosity extends about 10″ N from it See also Adgie and Gent (ref. 5)
3C 270·1	12 h 18 m 03·98 s 33° 59′ 49·2″	12 h 18 m 04 00 s 33° 59′ 50·0″	The optical position (Veron (ref. 8)) is that of a quasi-stellar source (refs. 2, 8)
3C 277·1	12 h 50 m 15·35 s 56° 50′ 36·3″	12 h 50 m 15·31 s 56° 50′ 37·0″	The optical position (Veron (ref. 8)) is that of a quasi-stellar source (refs. 2, 8) According to Sandage *et al.* the photo-electric colours do not eliminate the possibility that the optical object is a blue star, but the positional agreement found here strongly suggests that it is nevertheless the radio source
3C 288 1	13 h 40 m 29·98 s 60° 36′ 47·9″	13 h 40 m 30·2 s ±0·2 s 60° 36′ 50″ ±3″	The optical position (Parker) is that of an 18 m blue stellar object noted previously (ref. 9). Faint blue nebulosity extends about 5″ E. from the image
3C 309·1	14 h 58 m 57·62 s 71° 52′ 11·3″	14 h 58 m 57·8 s ±0·15 s 71° 52′ 12·0″ ±1″	The optical position (Parker) is that of a 17 m blue stellar object (ref. 2), probably a quasi-stellar radio source
3C 318	15 h 17 m 50 68 s 20° 26′ 51·2″	15 h 17 m 50·5 s ±0 2 s 20° 26′ 52″ ±3″	The optical position (Parker) is that of a 19·5 m object, equally bright in red and blue. It is shown on a finding-chart by Longair (ref. 4), immediately following and S. of the pair of objects 'a'. Other faint red objects are nearby. The source scintillates (ref 6), indicating structure <1″. See also Adgie and Gent (ref. 5)
3C 454	22 h 49 m 07·85 s 18° 32′ 49·9″	22 h 49 m 07·77 s ±0 1 s 18° 32′ 46·1″ ±1″	The optical position (Parker) is that of an 18 m blue stellar object (refs. 2, 5). Schmidt (ref. 10) has obtained a spectrum showing it to be a quasi-stellar radio source with a very large red-shift. The source scintillates (ref. 6), indicating structure <1″. See also Adgie and Gent (ref. 5)
3C 454 3	22 h 51 m 29·54 s 15° 52′ 58·5″	22 h 51 m 29·45 s ±0·1 s 15° 52′ 53·1″ ±1″	The optical position (Parker) is that of a 16 m stellar object, equally bright on the red and blue prints. A similar object with a faint companion lies 12″ N. The source scintillates (ref. 6), indicating structure <1″
3C 460	23 h 18 m 59·77 s 23° 30′ 24·7″	23 h 18 m 59 8 s ±0 1 s 23° 30′ 21″ ±1·5″	The optical position (Parker) is that of an 18 m red galaxy, object 'a' of Wills and Parker (ref. 9). See also Adgie and Gent (ref. 5)

compared in a computer with the observed phase variation and the difference between the two, together with the observed intensity of the source, is automatically plotted out as a function of time for the 12-h run. From these curves it is possible to determine independently the difference in R.A. and declination between the true and assumed positions, as will be explained in detail elsewhere. Small corrections are necessary for ionospheric refraction.

If the relative positions of the aerials are known sufficiently accurately by surveying, then the source positions may be determined absolutely with an accuracy limited only by the uncertainties in these values, by the effects of system noise, and by deflexions caused by other confusing sources within the reception pattern. In practice, however, it has been found that there is a systematic discrepancy of a few seconds of arc between the accurate positions of well-known optical objects and the corresponding radio source positions derived absolutely. The cause of this discrepancy is not yet clear and the positions listed in this article are based on the geometry of the instrument derived from this comparison of radio and optical positions rather than that found from surveying. The probable errors of the radio positions are determined by the uncertainties in this calibration procedure and are $\pm 0{\cdot}1$s in right ascension and $\pm 1'' \times \operatorname{cosec} \delta$ in declination. Quite apart from any real differences which there may be between optical and radio positions, these errors are generally larger than those of which the instrument is now capable. This is because the observations have been spread over the past 18 months, during which time there have been considerable improvements in the phase stability of preamplifiers and temperature compensation of radio-frequency cables.

The field of each source has been examined on *Sky Survey* prints making use of a transparent overlay[4] to locate the radio position.

Notes on the associated optical objects and, in many cases, their positions measured by means of a travelling microscope are given in Table 1. Since only the *Sky Survey* prints were available to us these optical positions are not so accurate as they might be had the original plates been used.

When this work was nearly completed positions of some of the sources with a comparable accuracy were sent to us privately by Adgie and Gent[5]. In nearly every case these agree well with the positions in Table 1. Some of the objects have been identified independently by Veron (ref. Adgie and Gent[5]). J. D. Wyndham (private communication) and T. A. Matthews (private communication) have also noted some as possible or probable identifications on the basis of less accurate radio positions.

We thank Prof. Martin Ryle for his advice and G. Macdonald and other colleagues for assistance.

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ETHYLENE PRODUCTION FROM LINOLENIC ACID

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LIEBERMAN and Mapson[1] have shown that linolenic acid may serve as a precursor of ethylene. The possibility that the ethylene that is evolved from plants arises from linolenic acid was investigated by determining the relationship between ethylene evolution and linolenic acid levels from a number of plants.

Ethylene production from linolenic acid was measured in a manner similar to one described earlier[2]. The fatty acid peroxides were measured according to the methods of Dahle *et al.*[3]. Extraction and measurement of fatty acids from plants followed the method of Huston and Albro[4]. Ethylene evolution from intact plants was measured by placing material into 3-l. containers and withdrawing samples of gas after 20 h. Measurement of ethylene by gas chromatography has also been described earlier[5]. Etiolated beans (*Phaseolus vulgaris* L. var. red kidney) and peas (*Pisum sativum* L. var. 'Alaska') were grown in the dark for a week at 25° C and 80 per cent relative humidity. Apples (*Malus pumila* Mill. var. 'Eastern Delicious') were obtained from local markets.

Fig. 1 is a representative experiment showing the production of ethylene from peroxidized methyl linolenate. Other hydrocarbon gases such as ethane and propane, and a number of unidentified peaks were also produced. The presence or absence of a methyl group on the fatty acid has no effect on ethylene production. Under the conditions shown in Fig. 1, the amount of ethylene evolved after 24 h represents a 0·2 per cent yield on a mole of ethylene per mole of peroxidized linolenate basis. The effect of ascorbate as a catalyst could be duplicated by 0·1 mg of a crude pea protein fraction described earlier[2] or similar amounts of lipoxidase. In these cases the activity of the protein was destroyed by heating. Addition of 1 μmole of potassium cyanide and 0·25 μmole of ethylenediamine tetraacetic acid inhibited gas production. A *p*H optimum between 7 and 8 was obtained with phosphate buffer. Other buffers, such as pyrophosphate, *tris* hydroxy methyl amino methane, and glycylglycine lowered the rate of gas production.

The possibility that ethylene production from peroxidized linolenate was mediated by means of a free radical mechanism was tested by the addition of 10 μmoles of catechol of iodine. Both these substances are known to quench free radical reactions by preferentially trapping the unpaired electrons. Both catechol and iodine inhibited ethylene production from pea protein and ascorbate-catalysed reactions. Reaction inhibition was about 70 per cent for the pea protein and about 85 per cent for the ascorbate.

Other fatty acids were examined for their ability to serve as a substrate for this reaction. Stearic, oleic, linoleic, and arachidonic were found to be inactive. However, a C_{22} fatty acid from herring oil, clupanodonic acid, was found to be active. The similarity between this compound and linolenic acid is the presence of a terminal CH_3—CH_2—CH= group at the methyl carbon end of the chain. On the premise that this particular end-group accounts for the activity of linolenic acid, other compounds having the same structure were examined. 2-Pentenoic acid, 3-hexenoic acid, and 3-heptene were also active as a source of ethylene. Under conditions identical to those in Fig. 1, ethylene production from these compounds was in the order of 1 nl. ethylene after 30 min.

Levels of linolenic acid and rates of ethylene production from apples were determined to explore the suggestion of Lieberman and Mapson[1] that ethylene production from

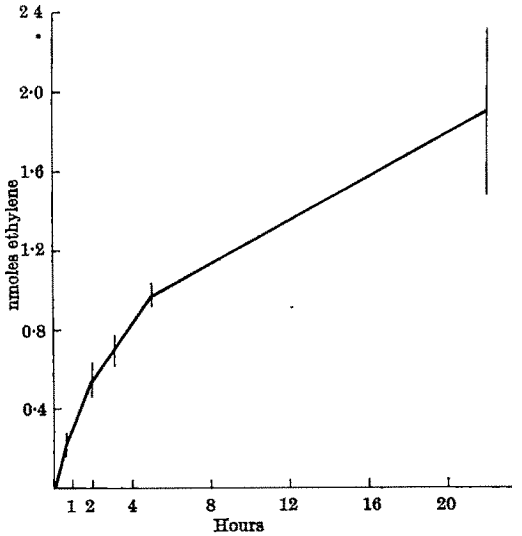


Fig. 1. Ethylene production from peroxidized linolenic acid. 0·88 μmole peroxidized (0·06 mole oxygen/mole fatty acid) linolenic acid, 50 μmoles sodium ascorbate, 125 μmoles potassium phosphate buffer, *p*H 7·3, liquid vol. 2·5 ml., helium gas phase, 25° C. Vertical lines indicate extent of standard error

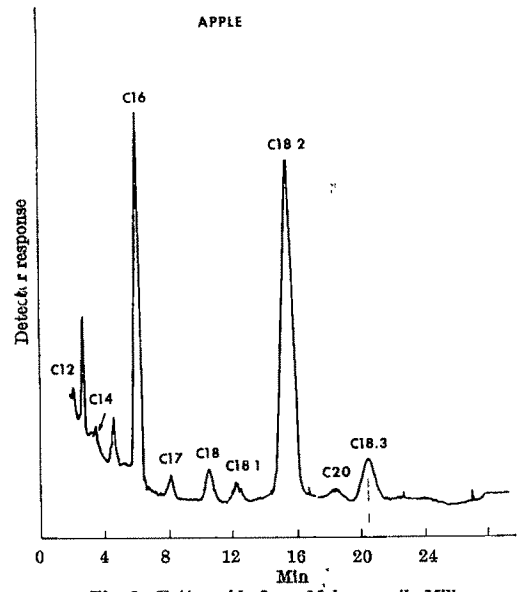


Fig. 2. Fatty acids from *Malus pumila* Mill

apples may arise from linolenic acid. Market apples were peeled, cored, and sliced into quarter sections. Two slices were stored in a refrigerator and the other two placed in a container and the ethylene evolution measured after 20 h. A representative chromatogram of the fatty acids extracted from apples is shown in Fig 2. The data from two experiments in Table 1 indicate that the linolenic acid content of apples remains approximately constant during this 24-h period. The gas produced during this time is equivalent to about 20 per cent of the linolenic acid content. If linolenic acid is the source of ethylene in this tissue then the reaction is far more efficient in apples than *in vitro*. The data also indicate that the amount of linolenate does not change greatly during the period of time ethylene production was measured and also that a C_{18} fatty acid with two double bonds is not present in the

Table 1. COMPARISON OF ETHYLENE PRODUCTION AND LINOLENIC ACID LEVELS FROM *Malus pumila* MILL. VAR 'EASTERN DELICIOUS'

Experiment No.	nmoles linolenic acid		nmoles ethylene 20 h
	g fresh weight		g fresh weight
	0 h	24 h	
1	50	33	8 8
2	32	48	15

fatty acids extracted from the apple. If ethylene does arise from linolenic acid it does so from a small pool of activated (peroxidized) acid, the level of which is maintained by a rapid turnover of the activated fatty acid.

Naphthaleneacetic acid (NAA) is known to stimulate ethylene production from peas and beans[5]. It was of interest, then, to determine if increased rates of ethylene production would have any effect on linolenic acid levels from plants treated with NAA. Week-old etiolated pea and bean (cotyledons removed) plants were sprayed with 2×10^{-3} M NAA until run off. Treated plants and controls were placed in containers for 20 h, after which time ethylene evolution was measured and fatty acids were extracted. Representative chromatograms of the fatty acids of these plants are shown in Figs. 2 and 3. No significant change was noted in the levels of any particular fatty acid between treated and control plants. Although care was taken to ensure that the extraction of fatty acids was quantitative, it was not possible to tell if changes in ethylene production between treated and controls were reflected in levels of linolenic acid (Table 2).

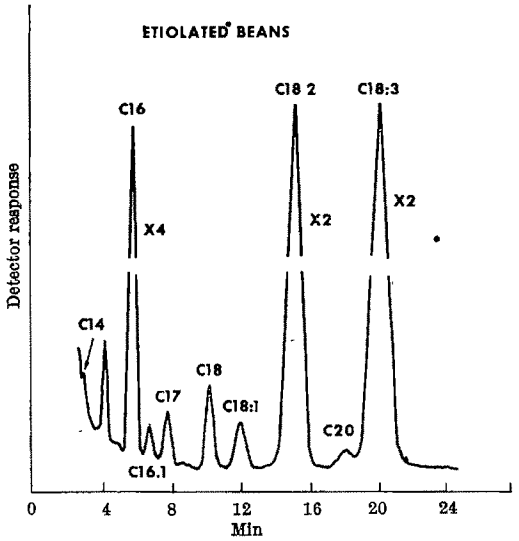


Fig 3. Fatty acids from etiolated *Phaseolus vulgaris* L.

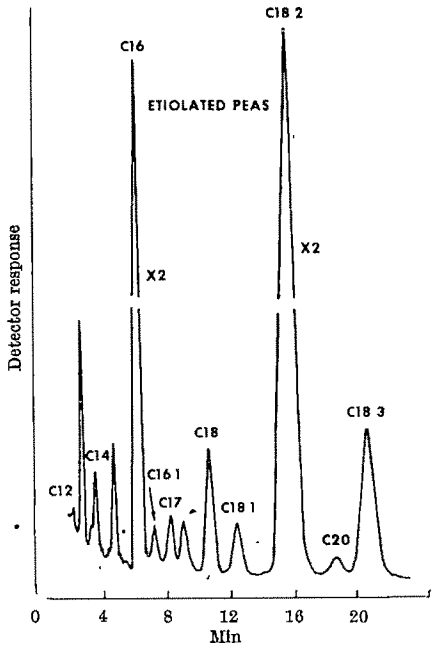


Fig. 4. Fatty acids from etiolated *Pisum sativum* L.

Table 2. COMPARISON OF LINOLENIC ACID LEVELS AND ETHYLENE PRODUCTION FROM NAA-TREATED PLANTS

Experimental material	nmoles linolenic acid		nmoles ethylene 20 h	
	g fresh weight		g fresh weight	
	Control	2×10^{-5} M NAA	Control	2×10^{-5} M NAA
Pisum sativum L.	340	130	0·05	7·1
Pisum sativum L.	140	260	0·05	2 2
Phaseolus vulgaris L.	570	480	0·18	14

The results with clupanodonic acid and other compounds containing terminal $CH_3—CH_2—CH=$ groups indicated that the activity of linolenic acid is due only to this particular configuration. The possibility that ethylene may arise from this particular type of structure in Nature remains open. Observing that a variety of other compounds such as ethylene oxide, diethyl ether, ethanol, and methionine have been reported[1] to produce ethylene suggests other alternatives. Any scheme that postulates linolenic acid as a precursor of ethylene in apples is unattractive because, first, the *in vivo* reaction is inefficient (0·02 per cent after 20 h) and the ratio of ethylene production to linolenic acid levels in apple is high (20 per cent after 20 h), and second, no C_{16} fatty acid with two double bonds occurs in apple tissue. The fact that linolenic acid must be peroxidized[1] before it serves as a source of ethylene raises the question of whether fatty acid peroxides occur in normal tissues. Desai and Tappel[6] have shown that enzymes and subcellular particles are damaged by peroxidized fatty acids. However, there are numerous reports that peroxidized fats do occur *in vivo*[7].

In conclusion, the evidence indicates that linolenic acid does not serve as a precursor of ethylene. Final proof of this depends on measurements of rates of linolenic acid turnover and the extent to which peroxidized linolenic acid occurs in Nature.

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ROLE OF THE AMINO-ACID 'CODE' AND OF SELECTION FOR CONFORMATION IN THE EVOLUTION OF PROTEINS

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I WAS able to show in a previous communication[1] that homologous positions in a series of evolutionarily related proteins (the same protein in different species, or proteins resulting from duplicated genes in the same or related species) tend to be occupied by amino-acids which are related by the nature of their side-chains. Certain sites in an amino-acid sequence are usually occupied by polar amino-acids, while others are generally occupied by non-polar amino-acids. This type of distribution is readily understood if it is assumed, first, that the folded (tertiary) structure of the molecule is determined by its amino-acid sequence[2], and, second, that hydrophobic groups reside predominantly in the interior of the molecule while polar groups are on the outside. The latter assumption was initially based on the predictions of Kauzmann[3] and on the X-ray diffraction studies of myoglobin by Kendrew[4]. Detailed analysis of the conformations and sequences of several haemoglobins and myoglobins have recently been published by Perutz[5] and by Perutz, Kendrew and Watson[6]. Their results again demonstrate that the interiors of these molecules are almost completely hydrophobic in character and that virtually all polar groups are at the surfaces. Hydrophobic groups are also found at or near the surface, although these residues are quite often alanine. In several instances, surface hydrophobic groups appear to be involved in interchain bonds, a situation predicted by Fisher[7]. Investigation of the three-dimensional structure of egg-white lysozyme has indicated that the separation between the interior and the exterior of a molecule may not be so definite as the structures of the haem proteins suggested[8]. However, as a working rule it seems reasonable to assign surface positions to the polar residues and interior positions to at least a large number of the hydrophobic residues of a molecule (glycine and alanine do not appear to prefer either location).

On the basis of initial comparisons, the hypothesis was advanced that the necessity to preserve correct three-dimensional structures by the maintenance of particular distributions of hydrophobic and polar residues in their amino-acid sequences is an important factor in guiding the 'evolution' of protein sequences. What was not known were the relative importance of the amino-acid code itself, independent of selection, and of subsequent natural selection in preserving these distributions of residues. It had already been pointed out that related amino-acids (related in terms of the nature of their side-chains or of their biosynthetic precursors) often had similar RNA codons[9], but only with the development of an ordered code could the full significance of this observation be determined. The sixty-four codon triplets listed by Nirenberg *et al.*[10] can be conveniently represented in the three-dimensional matrix schematized in Fig. 1. A single-step mutation results in movement from one position to another along one of the three orthogonal axes—diagonals are not allowed. Two-step mutations involve movement within a horizontal or vertical plane; three-step mutations can, of course, result in movement between any two places in the matrix. As is readily apparent from the interrelationships depicted in Fig. 1, and as has already been noted by others[11–13], there are several groups of related amino-acids which can change from one to another by single-step mutations. For example, phenylalanine, leucine, isoleucine and valine can be interconverted by single mutations in the first nucleotide of the triplet, as can, likewise, leucine, methionine and valine; tyrosine, histidine, asparagine and aspartic acid; and glutamine, lysine and glutamic acid. Changes in the third nucleotide can accomplish shifts from asparagine to lysine, aspartic acid to glutamic acid, and histidine to glutamine, while shifts in the second can produce such interchanges as phenylalanine and tyrosine, alanine and glycine, and histidine and arginine. On the other hand, certain interchanges that might on *a priori* grounds be expected to be readily accomplished—such as of the basic amino-acids, lysine and histidine, or of the aromatic residues, tryptophan and tyrosine—cannot be accounted for by single-step mutations.

If it is assumed that all triplets (except those for 'termination') will code for an amino-acid and that mutations at any position in the triplet and from any one

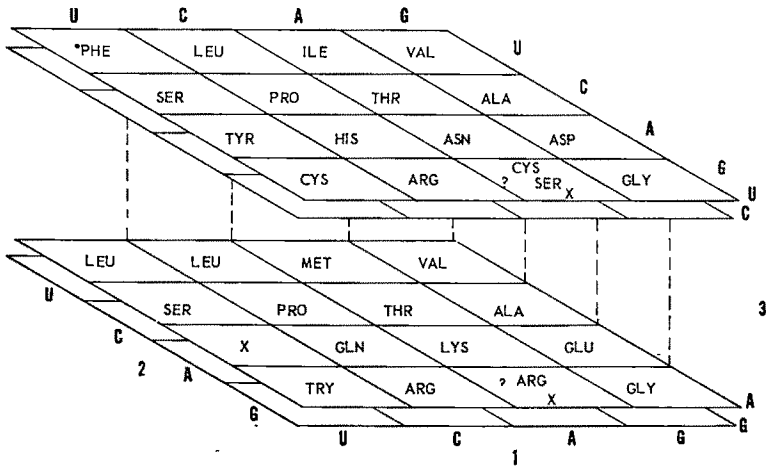


Fig. 1. The amino-acid 'code', adapted from the table of Brimacombe *et al.* (ref. 10). Each axis of the 'grid' represents a different base in the codon 'triplet'. Since there appears to be degeneracy for either the purine or pyrimidine bases occupying the third position in the triplet, the vertical axis for this position has been suitably condensed. '*X*' indicates codons thought to cause chain termination; '?' indicates uncertain assignments

nucleotide base to another are equally likely, it is possible to calculate the expected outcomes of random one- and two-step mutations. A single triplet can give rise to nine others by a one-step mutation and to eighty-one (including itself) by two-step changes. As in the previous communication[1], the amino-acid interchanges can be divided into three gross categories: (I) polar to polar or hydrophobic to hydrophobic; (II) between polar and glycine or alanine; (III) between polar and hydrophobic. While type I changes would appear to be favourable and type III unfavourable, those of type II could be either, depending on their location. A type II change affecting the surface would not be expected to be unfavourable; that affecting the interior of the molecule (and usually glycine or alanine→polar) would probably be unfavourable. The assignment of the amino-acids to the groups of polar and non-polar is also as before, and is based on the distribution of residues observed by Kendrew in myoglobin[4]. The frequency distribution of the various outcomes is shown in Table 1. It is soon apparent that, in nearly every case, type I mutations predominate over type III. Moreover, between 0·11 and 0·33 of the mutations would result in the same amino-acid and would not, therefore, be recognizable. If the frequencies are averaged over all amino-acids, weighting each triplet equally, the distributions shown at the bottom of Table 1 are obtained. If only observable mutations (mutations not leading to the same amino-acid) are counted and mutations leading to uncertain outcomes are eliminated, the results are I : II : III = 0·56 : 0·12 : 0·31 for one-step mutations and 0·52 : 0·14 : 0·34 for two-step mutations. Deriving distributions in this way may be misleading, since rare amino-acids are weighted as heavily as common ones. Therefore, frequencies have been calculated for a protein with the composition of the β-chain of human haemoglobin[14]. The results, which represent the expected observable outcomes of random nucleotide substitutions in the β-chain gene, are 0·62 : 0·14 : 0·25 and 0·53 : 0·16 : 0·32, for one- and two-step mutations, respectively. The weighted frequencies, which are not greatly different from those obtained without weighting, indicate that not more than one-quarter to one-third of observable random mutations will be of type III. These figures are considerably lower than the value of 0·40 for type III mutations obtained in the absence of information about the code and used in the previous analysis.

When such estimates of the outcomes of random mutations are applied to amino-acid substitutions found in actual proteins, certain difficulties immediately become apparent. Chief among these is knowing in which direction a given mutation has occurred. For example, if proline and glutamine (a type III combination) are found in homologous positions, the expectation of a type III outcome is either 0·67 or 0·29, depending on whether glutamine was substituted for proline or vice versa. Furthermore, the finding that two amino-acids are interconvertible by a single-step mutation does not ensure that one is derived from the other. Both may be derived from a third amino-acid. Likewise, amino-acids two mutational steps away from each other may both be derived, by single-step mutations, from a third common precursor. Therefore, in order to make meaningful estimates of how random a group of observed mutations may be, it is important to know the order in which such mutations have occurred. There are only a few instances in which such information is available, and two of these will now be examined.

More than thirty single-step mutations involving either the α-, β-, γ- or δ-chains of human haemoglobin or the evolution of the δ- from the β-chain are known and have been summarized by Beale and Lehmann[15]. If only those

Table 1

	One-step mutations								Two-step mutations							
	Total					Recognizable			Total					Recognizable		
	I	II	III	Self	Termination or uncertain	I	II	III	I	II	III	Self	Termination or uncertain	I	II	III
Phe	0·67		0·22	0·11		0·75		0·25	0·47		0·32	0·14	0·07	0 60		0·40
Leu	0·41		0 22	0·33	0·04	0 64		0·36	0·41		0 34	0·20	0·05	0 55		0·45
Ile	0·56		0·22	0·11	0·11	0·71		0·29	0·44		0·32	0·14	0·10	0 58		0·42
Val	0·44		0·22	0·33		0·67		0 33	0·52		0·26	0 19	0 06	0 67		0·33
Met	0·56		0·22	0·11	0·11	0 71		0 29	0·42		0·32	0 14	0 12	0·57		0·43
Pro	0·22		0·44	0·33		0 33		0 67	0 31		0·47	0 19	0·04	0 40		0 60
Cys	0·22		0·56	0·11	0·11	0·29		0·71	0 37		0·35	0 14	0·15	0 51		0·49
Tyr	0·44		0·22	0·11	0 22	0 67		0·33	0·54	0·05	0·20	0 14	0·07	0 68	0 07	0·25
Try	0 22	0·11	0·33	0·11	0·22	0·33	0·17	0·50	0·35	0·12	0·24	0·14	0 15	0·49	0·17	0·34
His	0 67		0·22	0·11		0 75		0·25	0·44	0·05	0 30	0·14	0·07	0·56	0 06	0 38
Thr	0·22	0·11	0·22	0 33	0 11	0 40	0·20	0·40	0·30	0·12	0·28	0·19	0·11	0·43	0·17	0·40
Ser	0·22	0·11	0 28	0·33	0·06	0 36	0·18	0·45	0·30	0 12	0·32	0·19	0 07	0 41	0·16	0·43
Gly	0·28	0·28		0·33	0 11	0 50	0·50		0·35	0·36		0·19	0·11	0 49	0·51	
Ala	0·33	0·33		0·33		0 50	0·50		0·40	0·38		0 19	0 04	0 51	0·49	
Glu	0·44	0·22	0·11	0·11	0·11	0·57	0·29	0 14	0·40	0·20	0·20	0 14	0 07	0·50	0 25	0·25
Gln	0·56		0·22	0·11	0·11	0·71		0·29	0·47	0·05	0·27	0·14	0 07	0·60	0·06	0·34
Asp	0·56	0·22	0·11	0·11		0·63	0·25	0·12	0·40	0·20	0·22	0·14	0 05	0·49	0·25	0·26
Asn	0·67		0·11	0·11	0·11	0·86		0·14	0·44	0·05	0·22	0 14	0·15	0 62	0·06	0 31
Lys	0·56		0·11	0·11	0·22	0·83		0·17	0·47	0 05	0·20	0·14	0·15	0·65	0 07	0·28
Arg	0·17	0·11	0·28	0·33	0 11	0·30	0 20	0·50	0 26	0·12	0·32	0 19	0·11	0·37	0 17	0 46
Means	0·39	0·08	0·22	0·24	0·07	0·57	0 12	0·31	0 39	0·10	0·26	0·17	0 08	0·52	0·14	0·34

Outcomes of one- and two-step mutations involving the codons for the various amino-acids. The proportions of recognizable mutations were calculated by eliminating all self, termination, and uncertain outcomes, and by assuming that all other triplets would actually code for their respective amino-acids.

cases in which the direction of mutation is known are considered (all the single-site changes and some of the β→δ substitutions), and if allowance is made for the fact that most of these mutations were recognized by differences in electrical charge (the *M* mutations, which are recognized in a special way, are omitted), the expected ratios of outcomes of observable random mutations is calculated to be I : II : III—20·8 : 5·6 : 4·7 (for a total of thirty-one mutations). The observed ratios are 20 : 10 : 1, and although there is a deficit of observed type III and an excess of type II changes (in this case, all involving surface residues) the small numbers would not allow the differences between the observed and expected ratios (considering I + II to be favourable, and III unfavourable) to be significant. It is conceivable, although probably not likely, that the results might ultimately be compatible with complete randomness of mutation. These mutations are relatively late in appearance and, except for rare cases such as haemoglobin S, have probably not yet been strongly selected for or against. Furthermore, in most instances a single non-permissible (type III) substitution, especially one involving a surface residue, would probably not seriously interfere with the proper folding of the molecule.

On the other hand, when two proteins which have evolved over a long period of time (such as the β- and γ-chains of haemoglobin) differ in more than a quarter of their residues, selective forces, if operative, should become apparent. If the evolutionary scheme proposed by Ingram[16] is accepted—with the α- and γ-chains having a common precursor, and the β-chain diverging from the γ later (that is, [α+β+γ] precursor→α-chain+[β+γ] precursor; [β+γ] precursor→β+γ-chains), it is possible to ascertain the direction of mutation during the divergence of the β- and γ-chains. Thus, if the α- and β-chains have a common amino-acid at a homologous location and the γ-chain a different one at the same site, the mutation can be considered as having occurred in the β→γ direction. Conversely, if α and γ have the common amino-acid, the mutation should have occurred in the γ→β direction. The β- and γ-chains differ at thirty-nine residues[14,17], and the direction of the mutation can be determined for twenty. Of these twenty mutations (sixteen of which involve exterior residues, four interior residues), the ratio of types I : II : III is 13 : 6 : 1; fourteen are one-step, four two-step, and two may be either one- or two-step depending on whether an uncertain set of codons for arginine (AGA, AGG) is allowed. The expected outcomes of random mutations involving the same group of starting amino-acids are in the ratio 10·9 : 4·7 : 4·4 (or 11·3 : 4·5 : 4·2 if the uncertain codons for arginine are used). There are fewer type III and more type I changes than expected, but the differences between observed and expected ratios could not, again, be significant because of small numbers. For the present, these results must be considered more in the nature of an illustration than as a definitive test of the code versus selection question. The latter will require the examination of a much larger number of mutations to allow for any valid conclusions. Nevertheless, the tendency in both sets of data is toward a deficiency of type III mutations. If the data are combined and the observed and expected ratios of I + II ('favourable') to III ('unfavourable') compared, the differences are statistically significant ($P < 0{\cdot}05$).

The limited data under consideration also provide some information about the kinds of mutations that have taken place. If each interchange is characterized in terms of the specific member of the triplet that has been altered, the results are as follows : for the haemoglobin single-site mutations and β–δ-chain conversions—first nucleotide base, 15; second, 14; third, 2; for haemoglobin β- and γ-chain comparisons—first, 13; second, 8; third, 4; first or third, 2. In both instances, the number of mutations involving the first or second base in a triplet are approximately equal. The numbers involving the third base are much lower. This is understandable, since, in general, a third of all third-place mutations would not be recognizable (for example, when U and C or A and G are interchanged). Furthermore, for half the triplets, none of the mutations at the third position would be recognized (for example, valine, alanine, proline) since the identity of these amino-acids is fully specified by the first two bases in the codon. As a result, no more than a third of the mutations at the third position in the triplet would be detected. For the single-site mutations, which depend principally on charge differences for detection, the expected ratios of detectable (by charge) changes involving the three nucleotide positions would be about 4 : 5 : 1. For the two groups of amino-acid exchanges under consideration, the observed ratios are close to what would be expected, and there does not seem to be any predilection for mutation in a specific position in the RNA codon.

Nucleotide base substitutions may be characterized as either transitions (pyrimine ↔ pyrimidine or purine ↔ purine) or transversions (pyrimidine ↔ purine). Theoretically, any nucleotide base may, by single-step mutations, undergo two transversions and one transition. However, of the three transitions available to a triplet, that involving the third base would not be detectable. Conversely, of six transversions which a triplet may undergo, the two involving the third base (or a third) would not be detectable in half the triplets (leucine, valine, alanine, etc.), or, in other words, a sixth of all transversions would not be detectable. Therefore, if mutations are random, the expected ratio of observed transitions to transversions should be approximately $(3 \times \frac{2}{3}) : (6 \times \frac{1}{6}) = 2 : 5$. Even if detection of mutants by charge differences has occurred, the ratio should be very similar. The ratios observed are 9 : 15 with two mutations indeterminate (or 11 : 11 if uncertain codons are assumed) for the haemoglobin β- and γ-chain comparisons, and 19 : 12 for the single-site mutants. There does appear, especially in the latter instance, to be an excess of transitions relative to transversions, although not of the degree suggested by Freese and Yoshida[18] on the basis of their work in bacteria. However, in line with the work of these authors, transitions from GC to AT pairs are favoured over the reverse direction, 20 versus 8. Further investigation of these points will be necessary since they will give an indication of the degree of randomness of the mutation process itself and may have to be taken into account in future calculations.

What, then, can be said about the initial question of the effect of the code versus natural selection in determining the sequences of proteins during the course of their evolution? It must first be realized that certain amino-acid substitutions, or at least substitutions at specific sites in the sequences of proteins, may not occur at all. This has been well documented by Margoliash and Smith[19] in the cytochrome c series in which many sites are invariant, that is, have apparently not been altered by mutation. Likewise, as has already been mentioned, the interiors of various globins virtually never contain polar residues, although several different hydrophobic groups may occupy the same position[6]. Presumably, these 'invulnerable' amino-acid residues are essential for specific aspects of the structure or activity of the protein, and their alteration would have grave consequences at a molecular level. For this group of residues, selection for conformation must clearly be present. Undoubtedly, there are also substitutions which, for reasons that may not be readily apparent, result in a distinct biological advantage or disadvantage to the host and will be strongly selected for or against. However, alterations of many of the residues of a protein during evolution probably do not have such gross effects, and it is with this group that we have been primarily concerned. On the basis of the studies of Perutz *et al.*[6], and of the analyses of properties of the code itself and two limited examples from the human haemo-

globins, it seems likely that we are observing a combination of two factors—natural selection, especially for amino-acid residues in the interiors of the proteins, superimposed on a high degree of conservatism (on a polarity basis) imposed by the amino-acid code itself. The conservatism inherent in the code may be sufficient to maintain the integrity of the surface residues which, as a group, are probably capable of greater variability than was initially appreciated. It must again be pointed out that if all mutations, rather than just those which are observable as changes in sequences, are considered, between one-sixth (for two-step changes) and one-quarter (for one-step changes) will not result in an amino-acid alteration—another element of conservatism inherent in the code.

Since the amino-acid code does favour mutations which would, in general, be expected to produce the least deleterious effects on structure and, since it is itself non-random in the assignment of codons, it is reasonable to enquire into the genesis of the code itself. Recent writers[11–13] on the subject have discussed two major possibilities—either there is a special chemical affinity between the amino-acids and the nucleotide bases in the so-called 'anticodons' (the triplet in the transfer RNA that is supposed to recognize the codon in the messenger RNA) or the code has itself evolved to its high degree of order by natural selection. Most authors appear to favour the former possibility, although there is not, at present, any chemical evidence to support it. Their hypothesis would require a specific inter-relationship between an amino-acid and the anti-codon part of its transfer RNA, a situation which cannot be examined until the tertiary structure of the transfer RNA molecule is known.

The simple evolution of the code to its present state has been rejected because of the seeming statistical impossibility of arriving at such an ordered set of triplets by natural selection alone[11,13]. Such an argument cannot be accepted, since it could be made to apply with equal force to virtually any set of complex processes or even morphological characteristics in which the role of evolution is unquestioned. Certain suppressor mutations (non-structural gene mutations which allow for the synthesis of proteins with mutations in their structural genes) have been thought to involve an alteration in the specificity of transfer-RNAs for specific amino-acids or codons[20]. It is easy to visualize that similar mutations could, over a long period of time, allow for the reshuffling of the codon assignments until a favourable set is obtained. As Sonneborn[21] has pointed out, the driving force for such assignments would be the minimization of the deleterious effects on organisms which would result from mutation. Preservation of the tertiary structure of proteins could act in such a way, and it may be suggested, therefore, that the codon assignments have been influenced, at least to a significant extent, by the need to ensure the proper folding of proteins.

Note added in proof. In a paper published after submission of this manuscript, Woese[22] has suggested that the code evolved to reduce translational errors (during protein synthesis) rather than mutational errors (during gene replication) as is proposed here. Also, a representation of the code analogous to that in Fig. 1 has recently appeared[23].

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LEUCOCYTE ANTIGENS IN MAN: A COMPARISON OF LYMPHOCYTOTOXIC AND AGGLUTINATION ASSAYS FOR THEIR DETECTION

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THE need for *in vitro* histocompatibility tests for the matching of donors and recipients for transplantation is now generally appreciated. There is evidence to show that histocompatibility antigens occur on the leucocytes. This, together with the recent success in the establishment of human leucocyte isoantigenic groups, underlines the importance of using leucocytes for histocompatibility typing.

The first genetic polymorphisms involving leucocyte isoantigen systems in man were delineated by agglutination methods[1–3]. The question arises as to whether the same systems can also be detected by lymphocyte cytotoxic methods. Cytotoxicity assays have been used successfully in the identification of antigenic differences between leucocytes in mouse[4,5] and man[6,7]. Although no leucocyte antigen systems have as yet been identified in humans using the cytotoxicity assays, a comparison of these two methods has some practical interest.

As an initial approach, sera known to possess leuko-agglutinins produced by multiparous women were tested

for cytotoxic activity. A comparison of the frequency of reactions when tested on a panel of random donors was first made. Secondly, the sera shown by Payne *et al.*[2] to have LA1 and LA2 and 4a and 4b specificities were studied to determine whether they could be classified into the same groups when assayed for cytotoxic activity.

The agglutination tests were performed at Stanford University School of Medicine and tests for cytotoxic activity at the University of California School of Medicine at Los Angeles. The leucocyte agglutination test used by Payne has been described in *Methods in Medical Research*, 1964[8], and the lymphocyte cytotoxicity test has been described in detail by Terasaki and McClelland[9].

The first study involved twenty-four agglutinating antisera, including representatives of LA1, LA2, 4a and 4b types, some monospecific and some multispecific. The lymphocytes from seven members of a leucocyte donor panel, repeatedly typed by agglutination tests, were examined by the cytotoxicity assay. The antigen profile of the seven panel members had been well established with respect to some of the known systems, as well as the unclassified sera used in this study. A summary of the comparison between the two tests using this panel is shown in Table 1.

Table 1. COMPARISON OF THE AGGLUTINATION AND CYTOTOXICITY TESTS USING TWENTY-FOUR LEUCOCYTE ANTISERA WITH A SEVEN-MEMBER LEUCOCYTE PANEL

Agg.	Cyto.	No. of tests
+	+	61
+	−	17
−	+	0
−	−	68
		146
		$\chi^2 = 85{\cdot}3$

There is a reasonably high degree of agreement between the tests by the two methods. Out of a total of 168 comparisons there were seventeen (10 per cent) cases of a definite discrepancy between the two methods, all of which were agglutination positive – cytotoxicity negative. There were also twenty-two comparisons which could not be satisfactorily evaluated owing to conflicting results in one or the other method on repeated tests. Leaving out these latter comparisons, the χ^2 testing the association between the two methods is 85·3 for 1 d.f., which is highly significant. It seems worth noting that six of the definite discrepancies involved the monospecific 4b type sera, Nos. 13, 18 and 26, none of which gave a single positive result in the cytotoxicity test with this panel. There was no clear indication of a relation between discrepant results using the two assays and the reproducibility of a given serum based on the agglutination assay.

The next study consisted of a much more extensive series of cytotoxicity tests run with more than 200 lymphocyte donors using the same twenty-four antisera. The aim was to compare frequencies of reaction in the two methods and to see whether the cytotoxicity assay would detect two known leucocyte antigen systems, LA and group 4[1,2]. Table 2 compares the frequency of reaction by the two methods. Column 5 shows the difference between the percentage frequencies obtained by agglutination and cytotoxicity assays and Column 6 the χ^2 testing the significance of this difference. Agglutination frequency exceeded that obtained by cytotoxicity in all except two cases (sera No. 3 and No. 22). Nine of the antisera had a frequency difference which was less than 10 per cent, and not significant at the 5 per cent level. The remaining fifteen antisera all had frequencies more than 10 per cent lower by cytotoxicity than by agglutination, which differences were significant at least at a 5 per cent level. The nine sera which reacted with similar frequency in the two methods included a monospecific LA1, No. 14, a monospecific LA2, No. 28, and a 'limited' 4a serum No. 27. ('Limited' serum refers to one which reacts with some but not all 4a cells.) The high proportion of cases in which the cytotoxicity assay gives a significantly lower frequency of reaction than the agglutination assay, as shown in Table 2, suggests that it may be possible for sera to contain one, two or more agglutinating antibodies, none or not all of which have cytotoxic activity. This could be the situation, for example, in the case of serum No. 25 containing anti-LA1 and an anti-4b type antibody, the frequency of which by the cytotoxicity method (36 per cent) was considerably lower than that given by the agglutination method (65 per cent) and corresponds to the expected population frequency of LA1. It seems from these data that anti-LA1, and anti-LA2 and 4a type antibodies in most of these sera are detected satisfactorily by this cytotoxicity method whereas anti-4b type antibodies are not.

The results of a two-way analysis of these cytotoxicity data for purposes of grouping following Payne *et al.*[2] are shown in Table 3. The values in the body of the tables are the 2×2 χ^2 with an asterisk denoting a negative association. The top part of Table 3 relates to antisera detecting antigens of groups LA1 and LA2, and the

Table 2. COMPARISON OF FREQUENCY OF REACTION OF ANTISERA USING AGGLUTINATION AND CYTOTOXICITY METHODS

	Serum No.	1 Total No of tests Cytotox	2 Total No of tests Agglut.	3 Frequency of reaction (per cent) Cytotox.	4 Frequency of reaction (per cent) Agglut.	5 Difference in per cent frequency (Col. 4 − Col. 3)	6 χ^2
LA1							
	14	217	175	31	33	+ 2	0 08
	8	112	209	20	44	+24	12·73*
	25	204	131	36	65	+29	26·18*
	30	200	85	16	41	+25	19 99*
LA2							
	28	208	135	42	50	+ 8	2·14
	6	84	189	4	52	+48	53 08*
	22	196	143	71	65	− 6	1 84
	31	151	96	70	71	+ 1	0 00
	29	113	107	51	69	+18	7·28†
4a							
	4	163	200	77	90	+13	9·96†
	33	165	60	17	30	+13	4·11‡
	27	120	127	37	39	+ 2	0·03
4b							
	10	121	179	34	74	+40	46·55*
	26	215	125	33	49	+16	7·29†
	3	193	185	95	92	− 3	1·39
	18	12	135	8	64	+56	14·44*
Others	13	118	176	17	31	+14	6·67†
	38	121	41	45	82	+37	17·34*
	36	110	44	10	13	+ 3	0 23
	37	108	40	23	82	+59	43 15*
	34	115	52	53	54	+ 1	0 00
	35	64	48	9	25	+16	4·96†
	17	114	160	29	71	+42	45 99*
	1	128	180	97	99	+ 2	0·71

* Significant at 0·1 per cent level.
† Significant at 1 per cent level.
‡ Significant at 5 per cent level.

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Table 3. χ^2 RELATIONSHIPS AMONG LA ANTISERA AND AMONG GROUP 4 ANTISERA USING THE CYTOTOXICITY ASSAY

	Serum No.	14								
	25	34								
LA1	30	23	9							
	8	11	6	2						
	6	0·1*	0·2*	0·2	0·1*					
	22	4	0·4*	0·0	0·8*	0·9				
LA2	28	0 3*	5*	1*	2*	4	40			
	29	0·3*	4*	0·0	1*	2	24	35		
	31	0 0*	0·0*	0·9	1*	0·4	15	34	14	
	Serum No.	14	25	30	8	6	22	28	29	31
				LA1				LA2		

	Serum No.	4								
4a	27	0·4								
	33	6	28							
	36	1	6	13						
	3	1*	0·4	1	0·1					
	10	2*	3	0·0*	2*	0·1				
4b	13	0·5*	0·5*	0 0*	0 2*	0·3	2			
	26	0·1	1*	8*	0·1*	1	0·0*	9 0*		
	18	0·5*	0·0	0·1*	0·0	0·0	0·1*	0·0	0 6*	
	Serum No.	4	27	33	36	3	10	13	26	18
				4a				4b		

* Negative association.
– Significant at 5 per cent level.

Table 4. LA PHENOTYPE AND GENE FREQUENCIES

Phenotype		Observed cases		Total
		cytotox.	agglut.*	
LA1	LA2			
+	+	25	11	36
+	–	40	29	69
–	+	58	54	112
–	–	74	41	115
		197	135	332

Heterogeneity $\chi^2_3 = 5{\cdot}42$ ($P > 10$ per cent).
The χ^2_1 for the LA1 – LA2 negative association on the combined data is 5·99 ($P \sim 1{\cdot}5$ per cent).

	Gene frequencies (± *S.E*)	
	Cytotoxicity	Agglutination*
*LA*1	0·1786 ± 0 020	0·1617 ± 0·023
*LA*2	0·2355 ± 0·023	0·2811 ± 0·030
*LA*3	0·5859 ± 0·027	0·5572 ± 0·033

χ^2_1 testing goodness of fit of expected phenotype frequencies for the cytotoxicity is 7·51 ($P < 1$ per cent) of which 4 25 is due to excess of observed over expected LA1 + LA2 + cases.
* Data from Payne *et al.* (ref. 2).

bottom part to antisera detecting antigens of the 4 system. The LA1 antisera show significant positive associations with each other, indicating that they share a common antibody. Similarly, the LA2 antisera, with the exception of No. 6, show highly significant positive associations. As pointed out by Payne *et al.*[2], allelism of LA1 and LA2 would be indicated by a negative association between pairs of sera, one from each group. Fifteen out of the twenty associations between the LA1 and LA2 antisera are negative, although only two of these are significant at the 5 per cent level. Using the agglutination assay a larger proportion of the negative associations between the LA1 and LA2 sera were significant. This apparent discrepancy may be due to the inclusion of some familial data in this analysis. Familial data were specifically excluded from the agglutination data analysis. Thus it appears that the cytotoxicity assay identifies the LA1 and LA2 groups although the allelic relationship between them is not so clearly demonstrated. In the case of the 4a, 4b classification, the 4a antisera reacted well and there is a significant positive association between them. The 4b antisera, however, showed no significant positive associations and three of them (Nos. 10, 13 and 18), as already pointed out, reacted with significantly lower frequencies than are obtained with the agglutination assay (Table 2). So far as the allelic relationship between 4a and 4b is concerned, there is only one significant association between a 'limited' 4a antiserum, No. 33, and a 'limited' 4b, No. 26. There are no consistent significant associations in the data on cytotoxicity between the LA sera and the 4 sera, nor are there any consistent associations between these groups and the sera which do not fall into either of these systems.

Table 4 compares the population frequencies of the LA1 and LA2 types and derived *LA*1, *LA*2 and *LA*3 gene frequencies obtained in the present investigation by cytotoxicity with those reported by Payne *et al.*[2].

Neither population phenotype nor gene frequencies showed significant differences. However, for the cytotoxicity data, the observed frequency of *LA*1 and *LA*2 heterozygotes is significantly in excess of that expected on the basis of the estimated gene frequencies. This again, as has already been discussed, is probably due to the inclusion of some familial data. In spite of this discrepancy there is good correspondence between the *LA*1, *LA*2 and *LA*3 gene frequencies obtained from these two independent bodies of data.

Considering the data as a whole, good agreement between cytotoxicity and agglutination assays has been demonstrated. In approximately a third of the cases tested, sera reacted with comparable frequency in the two methods. These cytotoxicity assays thus detected many of the same leucocyte antigens identified by the agglutination assay. Where discrepancies exist, they are in the direction of more positive agglutination reactions. It is of interest to note that when two sera, No. 33 and No. 28, the agglutinating antibody of which had been completely absorbed out, were tested by cytotoxicity with cells which in a previous cytotoxicity test had been positive with the unabsorbed serum, no cytotoxic activity was found. This indicated that a quantity of cells sufficient to absorb all agglutinating antibody also removed all cytotoxic activity.

There are two basic differences between the tests, namely: (1) The agglutination test utilizes a cell suspension containing granulocytes and lymphocytes, whereas in the cytotoxicity test only lymphocytes are present. (2) The cytotoxicity assay depends on the ability of an antibody to fix added complement. For the sera tested here, it has been shown that the agglutinating activity can be absorbed out by lymphocytes, which indicates that the antigens that these sera detect are not specific for granulocytes[2]. The first point, therefore, cannot be a factor contributing to the lower frequency by cytotoxicity in these data. EDTA is not used in the agglutination assay and a small amount of complement is always present. Nevertheless, the existence of some non-complement binding antibodies in these sera may have been a factor leading to greater positive reactions by agglutination than cytotoxicity. Among the technical variables which could account for the differences are: (1) the sera for this study had been selected on the basis of their agglutinating ability, whereas sera selected by cytotoxicity were not investigated; (2) cytotoxicity was assayed with a maximum of 0·004 ml. of antisera, and use of larger volumes might have produced a higher percentage of positive results; (3) cytotoxic activity which declines on prolonged storage and heat inactivation may have deteriorated, whereas the agglutinating activity has not. The inactivation of all the sera tested here by heating at 56° C for 30 min may thus have affected the cytotoxic assay, particularly in those sera the cytotoxicity of which is weakest. Any assessment of the relative importance of the foregoing factors in the explanation of the differences observed will require considerable further work.

There is much evidence accumulating which favours the hypothesis that some of the leucocyte antigens may be important histocompatibility factors[10–14]. Preliminary investigations undertaken in an attempt to correlate survival of skin grafts with compatibility for the 4, 5 and 6 systems have given negative results[15,16]. The correlation between survival of kidney transplants of the Denver series and compatibility in the LA system as determined by the cytotoxicity assay is now being investigated. It is interesting to note that Simonsen[17] has suggested, on the basis of survival of kidney transplants and the frequency of graft-versus-host reactions in the lymphocyte transfer test, that if there is just one

major histo-compatibility locus in man, this may have only three alleles. Although the assumption of a single strong locus may be questioned, this calculation emphasizes the importance of correlating compatibility for each new leucocyte group by more than one assay with survival of homotransplants.

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EFFECTS OF TOTAL-BODY X-IRRADIATION IN SINGLE AND FRACTIONATED DOSES ON DEVELOPING CEREBRAL CORTEX IN RAT FOETUS

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INTEREST in dose fractionation procedures in radiological studies has arisen in part from a consideration of the clinical advantages to be gained through these procedures[1,2] as well as from an interest in the resilience or recovery ability of irradiated cells and tissues[3,4]. While there is considerable evidence indicating that a single exposure at a given dose-level results in more severe effects and/or a higher incidence of effects than do fractionated exposures producing the same cumulative dose[5–8], there is some evidence to the contrary[9–11]. It has become apparent that the end result of any given dose fractionation regimen depends on a number of variables. Among the more important of these are number of fractions, size of each fraction, intervals between fractions. total exposure time, dose-rate, changing sensitivities of cells throughout a given period of irradiation, degree of oxygenation of tissues and the time of exposure in relation to the various phases of the cell replication cycle.

One of the aims in the investigations we have recently initiated[12] on dose fractionation is to determine the relative importance of such variables on fractionated exposures in developing nervous tissues *in vivo*. This article reports results of preliminary investigations of histological effects of single and fractionated doses of whole-body X-irradiation in developing cerebral cortex in the foetal rat.

The foetuses used in this study were taken from Sprague–Dawley female rats the pregnancies of which were timed. Three series of animals were collected, namely, control, 150 r. single exposure and 150 r. fractionated exposure. The single exposure was given at 50 r./min for 3 min and the fractionated exposures were given at 50 r./min in individual 1-min doses at 9-h intervals. The morning of the sperm-positive determinations was considered day zero. Irradiation was initiated on gestation day 13 at 10 a.m. The foetuses were taken at 24-h intervals thereafter. A minimum of four foetuses was used for study of each foetal day for each treatment.

The irradiation was given by placing the females in a snug-fitting 'Lucite' box allowing dorsal exposure. The dose was determined by means of a Victoreen dosimeter with the probe placed inside a paraffin block within the 'Lucite' box. Irradiation was administered with a Westinghouse 'Quandrocondex' unit operated at 250 kVp. at 15 m.amp with a filtration factor of 1/2 mm copper and 1 mm aluminium.

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On the proper gestation day each rat was anaesthetized with sodium pentobarbital and the foetuses removed by Caesarian section. The calvaria were removed and the brains fixed by immersion in Bouin's solution, embedded in paraffin, sectioned at 10μ, mounted and stained with iron haematoxylin and acid fuchsin. The region of the brain studied in the foetuses corresponds to area 2 as described by Kreig[13] for the adult rat brain. The following descriptions apply only to this area except where otherwise noted.

The 14-day control animals present a ventricular wall with a dark-staining ependyma and a lighter-staining mantle layer, which generally extends dorsally only as far as the apex of the hemisphere, having a solitary ependymal layer extending down the medial wall. The cells in the ependymal layer are arranged in longitudinal rows or columns extending from the inner (luminal) border of the hemisphere wall outward toward the mantle layer. A single row of mitotic figures characterizes the inner row of ependymal cells, while other scattered mitotic figures are seen in the remainder of the ependyma and the mantle layer.

In the 14-day-old single-dose animals the luminal wall of the hemisphere is highly irregular in contour, and the mantle layer is seen only in the low lateral area. The ependyma has been severely disrupted and in many sites is not recognizable as a distinct layer. Rosettes are clearly seen throughout the hemisphere, and large evaginations of the ventricular wall protrude into the ventricle. Numerous sloughed pyknotic nuclei are observed within the cavity, and large macrophages are commonly seen in this cellular debris with whole and fragmented nuclei within their cytoplasm.

Both the cells forming the rosettes and the surrounding cells have staining characteristics that resemble normal ependyma. Many pyknotic nuclei interspersed with cells exhibiting mitotic nuclei are found in both the ependyma and mantle layer, and mitotic figures are also numerous in the rosettes.

In the brains from 14-day-old foetuses which received the fractionated dose, the luminal wall is generally intact, and there are no sloughed neural elements or phagocytic cells in the ventricles. The wall of the hemisphere is smooth without gross deformities and is thicker than the wall in 14-day-old control animals. The ependymal layer is distinct, although its outer margin often has a frayed or indefinite appearance, and the mantle

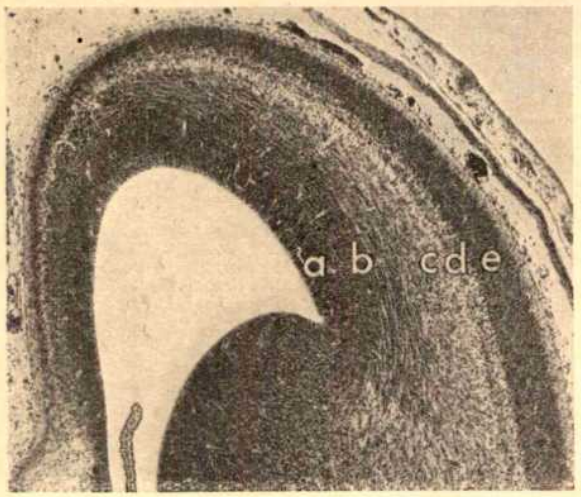


Fig. 1. Transverse section of cerebral hemisphere of normal 17-day-old foetus. Stained with haematoxylin and acid fuchsin (× 45). (*a*) Ependymal layer. (*b*) Subependymal zone of mantle layer. (*c*) Intermediate zone of mantle layer. (*d*) Secondary migratory lamination. (*e*) Primordial cortical layer

layer extends over the dorsal wall and down the medial wall. Mitotic cells are numerous in the ependymal and mantle layers, and a large number of pyknotic cells are seen in the mantle layer. These cells appear to be as numerous as in the single-dose series. It is particularly noteworthy that no rosettes are seen in these brains.

In the 17-day-old control animals, the mantle layer has taken on a somewhat striated appearance (Fig. 1) with the cells oriented in laminae more or less parallel to the pial surface and running ventrically toward the corpus striatum. Within the mantle layer two well-defined zones may be distinguished: an inner sub-ependymal zone (Fig. 1*b*) and an outer intermediate zone (Fig. 1*c*). The latter is characterized by a thinning out of cells (decreased cell packing density) as compared with the other layers at this stage of development.

The primordial cortical layer (Fig. 1*e*) which forms the primary migratory lamination[14] in the lateral wall on the fifteenth day of gestation (sixteenth day of Tilney[14]) now constitutes a very definite layer many cells in thickness extending over the roof and down the medial walls. There is also evidence of a secondary migratory lamination (Fig. 1*d*) which, in our preparations, is first seen at 16 days. This layer is observed to extend as far as the dorsal wall at this stage. The mean value for depth of the primordial cortical layer is about 150μ in these preparations, while depth of ependymal and mantle layers combined is approximately 400μ.

The brains from 17-day-old animals which received a single exposure appear to be markedly retarded in development (Fig. 2), and rosettes are still evident in dorsal and lateral walls. The primordial cerebral cortex (Fig. 2*c*) has not reached the medial wall but is evident on the lateral and dorsal walls. No evidence of a secondary migratory lamination beneath the primordial cortical layer is seen. The ependyma and sub-ependymal layers (Fig. 2*a*) are not easily differentiated but can be clearly distinguished from the primordial cortex, which appears more distinct and uniform and measures about 70μ in depth. The combined ependymal and mantle layers measure about 265μ in these animals.

In the 17-day-old foetuses which received the fractionated dose the ependyma and sub-ependyma are similar to the controls (Fig. 3), but the primordial cortical layer (Fig. 3*e*) is not so well developed and is poorly distinguished on the medial wall. Some evidence of a secondary migratory lamination is seen in the lower lateral wall (Fig. 3*d*) extending nearly to the apex. The primordial cortex is about 120μ in depth and the combined ependymal and mantle layers measure approximately 412μ.

The brains from 20-day-old control animals exhibit a relatively deep cortical layer blending indefinitely into the mantle layer, with the cells still oriented in vertical columns perpendicular to the plane of the pial surface. The distinction between the ependymal and sub-ependymal tissues and the intermediate zone is now quite apparent. The latter zone exhibits decreased cellularity as compared with earlier stages, but some strands of cells parallel to the cortical layer, apparently constituting part of the secondary migratory lamination, are seen deep to the cortical layer. At this stage of development the cortical layer is about 330μ in depth while the combined ependymal and mantle layers measure 570μ.

In the 20-day-old single-dose animals the lateral ventricles are clear but the luminal wall remains irregular. The ependymal and sub-ependymal areas are not well defined and the cells and their processes are arranged in a random pattern, appearing to be more widely spaced than in controls. The rosettes are still numerous but have lost their clear spherical centre and radiating character and

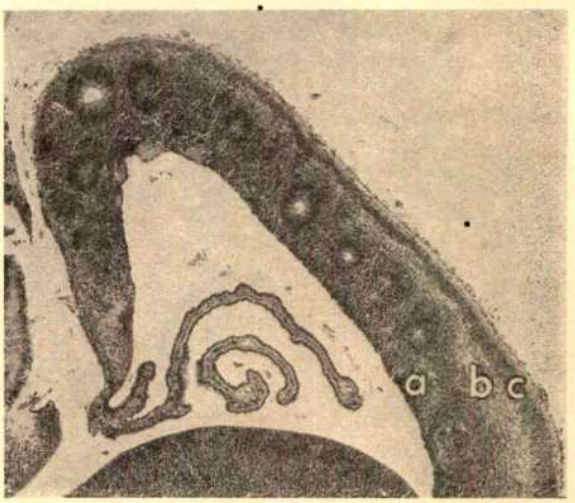


Fig. 2. Transverse section of cerebral hemisphere of 17-day-old foetus given a single dose of 150 r. on thirteenth gestation day. Stained with haematoxylin and acid fuchsin (× 45). (*a*) Ependymal and subependymal layers. (*b*) Mantle layer. (*c*) Primordial cortical layer

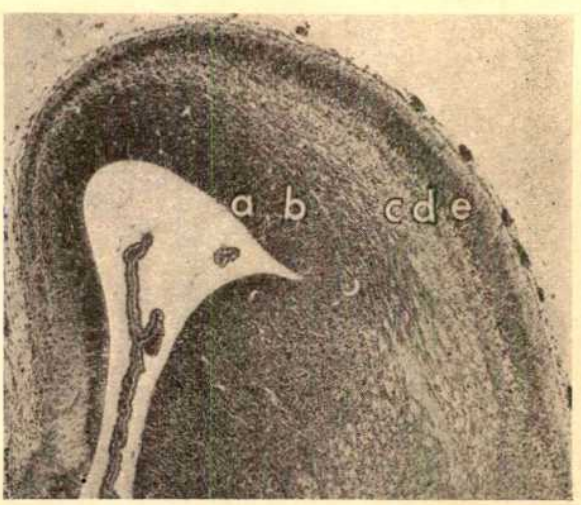


Fig. 3. Transverse section of cerebral hemisphere of 17-day-old foetus given fractionated dose of 150 r. on thirteenth gestation day. Stained with haematoxylin and acid fuchsin (× 45). (*a*) Ependymal layer. (*b*) Subependymal zone of mantle layer. (*c*) Intermediate zone of mantle layer. (*d*) Secondary migratory lamination. (*e*) Primordial cortical layer

now appear as a mass of densely packed, dark-staining, unorganized tissue. The primordial cerebral cortex is present and extends to the medial wall, but it appears that the secondary migratory lamination has not yet formed. The ependymal, sub-ependymal and mantle layers all appear very disorganized and exhibit numerous whorls of fibres and masses of interdigitating fibres. The cortical depth in these brains averages 230μ and the ependymal and mantle layers combined measure about 640μ in thickness.

The brains of 20-day-old fractionated dose animals resemble the brains of the controls with the exception that they are much smaller and the primordial cortex is thinner. The latter measures about 270μ, but the combined depth of the ependymal and mantle layers is about equal to that in the control brains.

In summary, the results observed in this preliminary histological study are in general agreement with the findings of many workers involved in dose fractionation studies[5–8] in that more severe effects are observed with the single exposure than with fractionated doses. Whether the differences between single and fractional exposures may be explained entirely on the basis of cell recovery between fractions[3,4] or partly by other factors (for example, a relatively smaller effect or a disproportional inefficiency of the individual fractions[7]) cannot be determined from the data at present available.

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EFFECT OF AGENTS SIMULATING THE ABNORMALITIES OF THE GLUCOSE-6-PHOSPHATE DEHYDROGENASE-DEFICIENT RED CELL ON *Plasmodium berghei* MALARIA

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INDIVIDUALS whose red cells are deficient in the enzyme glucose-6-phosphate dehydrogenase (G6PD) appear to be more resistant to infection with *Plasmodium falciparum* malaria[1,2]. Associated with the deficiency of G6PD in red cells are (1) a relatively inefficient apparatus for reducing methaemoglobin[3] and (2) a relative paucity of reduced glutathione (GSH)[4]. It seemed possible that one or other of these could be responsible for rendering the G6PD-deficient red cell less suitable for the plasmodium.

If methaemoglobin accumulation or GSH deficiency were responsible for inhibiting growth of the malaria parasites, then agents which cause these abnormalities might alter the course of malaria infection. Sodium nitrite, a methaemoglobin producing agent[5], and alloxan and phenylhydrazine hydrochloride (PH), which have been shown to deplete red cells of GSH[6–9], were therefore tested in *Plasmodium berghei* infection.

The mice used were a Walter Reed derivative of the Bagg Swiss mouse, weighing 15–25 g. They were infected with varying doses of parasitized red blood cells by intraperitoneal injection.

The effect of therapy on survival of the treated and untreated animals was compared using the Mann-Whitney test[10], which compares medians and does not depend on a normal distribution for its validity. Preliminary investigation had indicated that the distribution of deaths in infected mice was not normal, for there was considerable skew to the right in the graph of mortality against time.

The percentage of parasitized red blood cells was determined on thin smears stained with Leishman's stain and counter-stained with Giemsa's stain[11]. Methaemoglobin was determined by the method of Evelyn and Malloy[12]. Red cell GSH was determined by the 5-5′ dithiobis (2-nitrobenzoic acid) method of Beutler *et al.*[13]. Reticulocyte counts were performed using brilliant cresyl blue[14]. The *P. berghei*, obtained from the laboratory of Dr. R. Coatney of the National Institutes of Health, was a derivative of the *NYU*–2 strain. Saline solutions of PH and alloxan were made immediately prior to use because of their instability[7,15].

A single dose of 3 mg sodium nitrite produced a moderate methaemoglobinaemia for 2–3 h. Twice-daily doses of 3 mg of sodium nitrite for seven consecutive days had no cumulative effect on the methaemoglobinaemia, was not lethal to mice, and failed to prolong survival (Table 1).

The effect of various daily intraperitoneal doses of PH is shown in Table 2. Small doses of PH significantly prolonged survival. The effect of a single dose of 2 mg of PH on the parasitaemia of infected mice is shown in Fig. 1: the developing parasitaemia was retarded for 3

Table 1. Effect of Sodium Nitrite on *P. berghei* in the Mouse

	Median day death	Day of death range
Control (9)*	10	(9–22)
Treated (9)*	8	(8–9)

Control and treated mice were infected with 10^5 parasitized red blood cells. 24 h after infection a single dose of 4 mg sodium nitrite was given by intraperitoneal injection to the treated group. Because this dose of sodium nitrite was lethal to one mouse, subsequent doses were 3 mg each. The mouse killed with sodium nitrite was excluded from the experiment. Methaemoglobinaemia reached a peak of 50 per cent between 60 and 90 min after injection of sodium nitrite; 3 h after sodium nitrite methaemoglobinaemia was less than 2 per cent.

* The numbers in parentheses are the numbers of animals in each group.

Table 2. Effect of Phenylhydrazine Hydrochloride on *P. berghei* in the Mouse

		Median day death	Day of death range	*P*
Untreated	(10)	8·5	(7–14)	
0·1 mg	(10)	15·5	(13–18)	< 0·002
0·5 mg	(8)	12	(10–17)	< 0·002
1·0 mg	(10)	14	(7–16)	< 0·002
1·5 mg	(9)	7	(7–12)	
Saline	(10)	8	(7–10)	

Mice were infected with 10^8 parasitized red cells. Phenylhydrazine hydrochloride in the indicated dose was given daily by intraperitoneal injection beginning on the day after the infection.

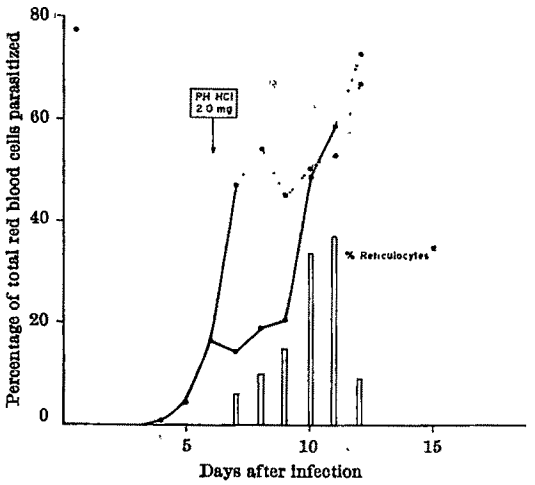


Fig. 1. Parasitaemia of *P. berghei* infected mice; response to a single dose of phenylhydrazine hydrochloride. Ten mice were infected with 10^5 parasitized red blood cells. 6 days after infection they were randomized into two groups. The treated group was given 2 mg phenylhydrazine hydrochloride by intraperitoneal injection. The control group was given an equal volume (0·2 ml.) of normal saline. The mean parasite count of each group is plotted. The broken line is used where the first mouse in a group has died. Parasitaemia was suppressed in the treated group for 3 days (lower of the two solid lines). The reticulocytosis resulting from a 2 mg dose of phenylhydrazine hydrochloride to a group of normal mice is shown in the bar graph*

days. The action of PH in retarding the development of parasitaemia and in prolonging survival of infected mice occurred despite the haemolysis and reticulocytosis that the drug produces. *P. berghei* infects reticulocytes preferentially, and animals treated to produce reticulocytosis ordinarily have an accelerated course with *P. berghei* infection[16].

One possible explanation of the antimalarial action of PH, which damages older red blood cells preferentially and leads to their selective sequestration in the spleen[17], is that it has the same effect on parasitized red blood cells. If PH were antimalarial solely by virtue of preferential sequestration of parasitized red blood cells in the spleen, then splenectomy would presumably obliterate its therapeutic effect. But PH exerted a significant therapeutic effect despite splenectomy (Table 3).

Table 3. INFLUENCE OF SPLENECTOMY ON THE EFFECT OF PHENYLHYDRAZINE HYDROCHLORIDE

		Median day death	Day of death range	P
Control	(9)	9·5	(8–15)	<0·02
Splenectomized +PH	(10)	14	(13–17)	

Splenectomy was performed one day before infecting mice with 10^5 parasitized red blood cells. A daily dose of 0·1 mg phenylhydrazine hydrochloride was given by intraperitoneal injection beginning on the day after the mice were infected. Control mice received 10^5 parasitized red blood cells only.

Table 4. EFFECT OF ALLOXAN ON *P. berghei* IN THE MOUSE

		Median day death	Day of death range	P
Control	(12)	10	(9–26)	<0·002
Alloxan-treated	(10)	19·5	(18–23)	

Mice were infected with 10^5 parasitized red blood cells. 3 mg of alloxan was given daily by intraperitoneal injection beginning on the day after the mice were infected.

Alloxan was tried next. Three mg of alloxan daily had a highly significant effect in prolonging survival (Table 4). Larger doses of alloxan proved too toxic for daily administration. The effect of a single dose of alloxan on parasitaemia is shown in Fig. 2. This dose of alloxan retarded developing parasitaemia for about a week; no anaemia or reticulocytosis resulted from this dose in normal mice.

Alloxan and PH had unmistakable antimalarial action against *P. berghei*, but their effect on red cell GSH in the normal, uninfected mouse was minimal (Table 5).

Table 5. RED CELL GLUTHATHION IN ALLOXAN- AND PHENYLHYDRAZINE HYDROCHLORIDE-TREATED MICE

		GSH		SD
Control*	(10)	65·1	±	4·8
Alloxan—2 h	(8)	67·8	±	7·3
Alloxan—6 h	(10)	69·5	±	6·6
Control	(8)	70·7	±	6·1
Phenylhydrazine 30 min	(9)	52 0	±	9·4
Phenylhydrazine 2 h	(9)	70·6	±	9·4

* Mice were randomized into three groups. The alloxan-treated mice received 7·5 mg alloxan by intraperitoneal injection and were killed 2 h and 6 h later.

† Mice were randomized into three groups. The treated mice were given 2 mg phenylhydrazine hydrochloride by intraperitoneal injection and killed 30 min and 2 h later.

Mice with parasitaemias of varying severity were also dosed with PH and alloxan and the GSH in their red blood cells determined; no consistent difference from the response of the normal mice to these agents was demonstrable.

Any discussion of the mechanism of action of these drugs is necessarily speculative. GSH depletion does not suffice as an explanation of their anti-malarial effect. Alloxan and PH have both been shown capable of inhibiting or destroying a variety of biological systems[21–29]. One property they share is the capacity to function as redox intermediates, causing the oxidation of substrates which ordinarily are not in ready equilibrium with oxygen. The action of PH in oxidizing haemoglobin is one example of this[30]; that of alloxan and dialuric acid in accelerating oxygen uptake in tissue homogenates is another[31].

The G6PD-deficient red cell is uniquely sensitive to oxidative haemolytic agents and is readily destroyed by them. It may be considered a 'defective' cell in this sense. The *Plasmodium* is also a 'defective' cell in the sense that it is an obligate intracellular parasite and therefore lacks at least one system essential to extracellular survival.

The susceptibility of both the *Plasmodium* and the G6PD-deficient cell to oxidative agents suggests that their metabolic defects may be similar. The relative insusceptibility of the G6PD-deficient red cell to *P. falciparum* is consistent with this hypothesis: the plasmodium does not prosper in the G6PD-deficient red blood cell because it needs what the host red blood cell possesses only in short supply. Tracer studies of plasmodial metabolism are consistent with this hypothesis: free *P. berghei* produced almost no $^{14}CO_2$ from ^{14}C-glucose labelled in the '1' position[32], suggesting that the pentose phosphate shunt in the parasite is either absent or incomplete.

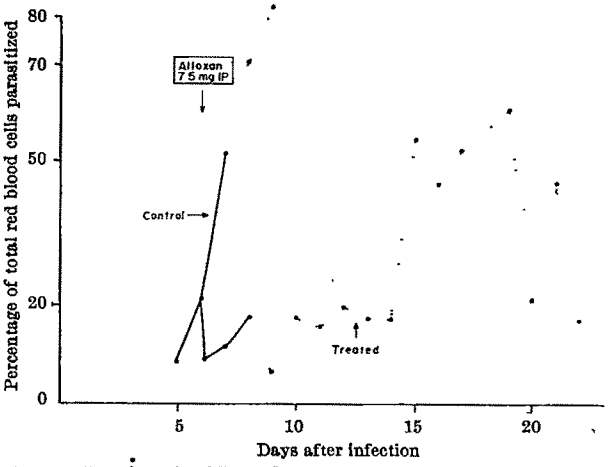


Fig 2. Parasitaemia of *P. berghei* infected mice; response to single dose of alloxan. Ten mice were infected with 10^5 parasitized red blood cells. 6 days after infection they were randomized into two groups. The treated group was given 7·5 mg alloxan by intraperitoneal injection. The mean parasite count of each group is plotted. The broken line is used after the first mouse in a group has died. Parasitaemia is suppressed in the treated group for 9 days. The early deaths in the treated group are attributable to alloxan toxicity. Median day death alloxan-treated mice: 21. Median day death control mice: 10

Finally, G6PD was not detected in the free parasites of *P. knowlesi*[33]. The consequence of a defect in the pentose phosphate pathway would extend beyond energy metabolism; a paucity of TPNH and of pentose sugars would affect lipid synthesis and nucleic acid synthesis. Separately or together, these could potently inhibit parasite growth and multiplication.

An antiplasmodial action of PH has also been noted by Ridgon[18]. An antimalarial effect of alloxan is apparent in the data of Tolbert[20].

Extrapolation of results with *P. berghei* to the metabolism or host–parasite relationships of *P. falciparum* is unwarranted. These studies are, at best, only suggestive of the state of affairs in human infections.

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INCORPORATION OF CARBOXYL GROUPS INTO MELANIN BY SKIN TYROSINASE

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IN the classic Raper–Mason[1] metabolic pathway of melanogenesis, the enzymatic conversion of tyrosine into melanin is accompanied by the cyclization and decarboxylation of intermediates. However, a series of recent investigations have indicated that carboxyl groups may be present in melanin derived from various sources. Magnesium and calcium salts of the carboxyl group are present in sepiomelanin[2]. Further, in sepiomelanin and in certain synthetic melanins, Piatelli *et al.*[3] have reported the presence of indole units with a carboxyl group at position 2 and pyrrolic acid units. Rat melanoma and human melanins also contain carboxyl groups, but in contrast to sepiomelanin, the mammalian pigment contains units of uncyclized carboxylated intermediates in the polymer[4]. Using carbon-14 carboxyl-labelled tyrosine and dihydroxyphenylalanine (DOPA) in the investigation of melanin formation by mushroom tyrosinase, the incorporation of the carboxyl group was obtained[5]. In experiments using 2-(3,4-dihydroxyphenyl)-ethylamine-^{14}C, with the label at various side-chain or ring positions, Swan and Wright[6] found that the oxidative fission of the indole intermediate into pyrrolic acid takes place during melanogenesis. Therefore, the carboxyl group in the melanin polymer may be derived from the breakdown of the indole benzonoid ring. However, Swan[7], with the aid of α- and β-deuterated 3,4-dihydroxyphenethylamine and 3,4-dihydroxyphenylalanine, showed the retention of deuterium in melanin formed either enzymatically or autoxidatively from the former substrate or autoxidatively from the latter substrate. Thus the substrates or their intermediates, without cyclization and decarboxylation, may be incorporated into the pigment. It is possible, therefore, that carboxyl incorporation may occur during melanogenesis under natural conditions. In the present article, the *in vitro* incorporation of the tyrosine or DOPA carboxyl group into melanin by fish skin tyrosinase is demonstrated.

Eight sexually inactive black moor goldfish (*Carassius auratus* L.), 3·79 ± 0·18 g in weight, were utilized in this investigation. The animals were maintained under constant conditions of temperature (25° C), photoperiod (12 h) and diet. All animals were acclimatized to laboratory conditions for a period of not less than 3 weeks prior to use. The fish were individually captured, immediately decapitated and the body skin with scales and fins removed without delay. The skin was immediately placed in a tared chilled beaker, weighed, and frozen (−27° C) for at least 5 h. The substrates used were L-tyrosine-1-^{14}C, DL-tyrosine-2-^{14}C, uniformly labelled L-tyrosine-^{14}C, DL-DOPA-1-^{14}C and DL-DOPA-2-^{14}C. The specific activities utilized are indicated in the appropriate tables. The details of the preparative procedures and assay method have previously been described[8]. All assays and controls were performed in duplicate.

In a comparison of uniformly labelled L-tyrosine with L-tyrosine-1-^{14}C, the DOPA-dependent incorporation of the carboxyl group of tyrosine into melanin is demonstrated. This is shown in Table 1. It is reasonable, therefore, to consider the incorporation of the carboxyl group into melanin as part of the action of tyrosinase.

Considering both carboxyl incorporation and decarboxylation, a correction factor for tyrosinase activity assayed with uniformly labelled L-tyrosine is necessary. This has been evaluated in the comparison of uniformly labelled L-tyrosine-^{14}C and DL-tyrosine-2-^{14}C as substrates assuming that D-form is not utilized (Table 2). With

Table 1. COMPARISON OF TYROSINASE ACTIVITY OF SKIN HOMOGENATES UTILIZING L-TYROSINE-1-^{14}C AND UNIFORMLY LABELLED L-TYROSINE-^{14}C AS SUBSTRATES. SPECIFIC ACTIVITY OF EITHER SUBSTRATE: 0·329 MC./MMOLE. MEAN ACTIVITY OF ASSAY EXPRESSED IN C.P.M.

Enzyme preparation	Absence of DOPA: Carboxyl label	Absence of DOPA: Uniform label	Presence of DOPA: Carboxyl label	Presence of DOPA: Uniform label	Carboxyl: Uniform Ratio
1	0	16	11	146	0 0753
2	0	55	52	476	0·1092
3	3	29	27	242	0 1116
4	3	16	17	139	0 1223
				Mean:	0·1046 (10·46%)

Table 2. COMPARISON OF TYROSINASE ACTIVITY OF SKIN HOMOGENATES UTILIZING L-TYROSINE-2-^{14}C AND UNIFORMLY LABELLED L-TYROSINE-^{14}C AS SUBSTRATES

Enzyme preparation	Specific activity* (mc./mmole)		Tyrosinase activity (c.p.m.)		Ratio of activity†		Decrease (per cent)
	DLT2	ULT	DLT2	ULT	Calculated	Experimental	ULT
1	0·173	0·658	142	523	3·8	3·68	3·16
2	0·173	0·658	252	830	3·8	3·29	13·42
3	0·173	0·329	135	242	1·9	1·79	5·78
4	0·173	0·329	588	951	1·9	1·62	14·74
							Mean: 9·28

* Specific activity of L-form in the substrate. DLT2, DL-tyrosine-2-^{14}C; ULT, uniformly labelled L-tyrosine-^{14}C.
† Calculated ratio: ratio of specific activity of substrates.
Experimental ratio: ratio of tyrosinase activities.

Table 3. COMPARISON OF TYROSINASE ACTIVITY OF A SKIN HOMOGENATE UTILIZING DL-DOPA-1-^{14}C, DL-DOPA-2-^{14}C AND UNIFORMLY LABELLED L-TYROSINE-^{14}C AS SUBSTRATES*

Group	DL-DOPA (μg)	DL-DOPA-1-^{14}C (μg)	DL-DOPA-2-C^{14} (μg)	L-Tyrosine (μg)	ULT-^{14}C (μg)	Tyrosinase activity (c.p.m.)
1	4	—	—	—	40	163
2	—	4	—	40	—	48
3	—	—	4	40	—	164

* Specific activity of labelled substrates: 0 329 mc./mmole. Specific activity of L-form in DL-DOPA-2-^{14}C and DL-DOPA-1-^{14}C. 0·165 mc./mmole.

uniformly labelled L-tyrosine, an average loss of 9·28 per cent of the activity is incurred. However, with complete decarboxylation, one labelled carbon of the nine present in a molecule of uniformly labelled L-tyrosine should be lost. The difference between such theoretical loss (11·11 per cent) and the actual loss (9·28 per cent) is 1·83 per cent. The increase in radioactivity is produced by the incorporation of the carboxyl group of the substrate into the end-product. The radioactivity obtained with uniformly labelled L-tyrosine is 90·72 per cent of the radioactivity obtained with L-tyrosine-2-^{14}C. Therefore, the tyrosine activity assayed with uniformly labelled L-tyrosine can be corrected to the L-tyrosine-2-^{14}C value. Using such corrected uniformly labelled L-tyrosine values, the mean incorporation of radio-tyrosine without decarboxylation is no longer 10·46 per cent (Table 1) but 9·50 per cent. The incorporation of radio-tyrosine without decarboxylation can be determined directly by comparing L-tyrosine-1-^{14}C and DL-tyrosine-2-^{14}C as substrates, assuming that the D-form is not utilized. In both Tables 1 and 2, enzyme preparation 3 was the same. This calculated value is 10·51 per cent, which approximates to the calculated mean value, 9·50 per cent.

Although the incorporation of tyrosine without decarboxylation is the same for all three types of labelled tyrosine utilized, the radioactivity due to the incorporation of the carboxyl group is quite different. The gains in radioactivity are 0 per cent, 9·50 per cent and 1·06 per cent (1/9 × 9·50 per cent) using L-tyrosine-2-^{14}C, L-tyrosine-1-^{14}C and uniformly labelled L-tyrosine-^{14}C, respectively. The increased radioactivity due to the incomplete decarboxylation of uniformly labelled L-tyrosine should be equal to the increased radioactivity due to the incorporation of carboxyl group. The difference between 1·83 per cent and 1·06 per cent is probably due to experimental error. In any case, as the increase in radioactivity resulting from the incorporation of the carboxyl group of uniformly labelled L-tyrosine is small, it may be considered negligible. Thus, the results of the assay utilizing uniformly labelled L-tyrosine-^{14}C may be corrected for the radiocarboxyl loss by simply dividing by 90 per cent or multiplying by 1·11.

The incorporation of the carboxyl group of DOPA into melanin was also demonstrated (see Table 3). In a previous communication[8], 4 μg DL-DOPA (2 μg L-DOPA)/ml. incubation mixture has been found to be the saturated substrate level for tyrosinase, and has been utilized both as catalyst and substrate in the assay. Thus, this quantity of labelled DOPA was used as the substrate in the presence of unlabelled tyrosine. However, the tyrosinase activity obtained with DL-DOPA must be multiplied by 2, as the specific activity of the L-form in DL-DOPA is only half of that of the L-tyrosine used. Comparing DL-DOPA-1-^{14}C and DL-DOPA-2-^{14}C (Table 3), the incorporation of DOPA without decarboxylation into melanin is 28·65 per cent. The total activity obtained with DL-DOPA-2-^{14}C and uniformly labelled L-tyrosine-^{14}C was the same. Thus, the enzymatic rate of DOPA oxidation is about twice that of tyrosine and the incorporation of the DOPA carboxyl group is about three times that of tyrosine. Moreover, only the incorporation of radioactive tyrosine, but not unlabelled DOPA, into melanin is visualized when tyrosine alone is labelled. Conversely, the incorporation of radioactive DOPA but not unlabelled tyrosine into melanin is measured when DOPA alone is labelled. Therefore, the overall activity in c.p.m. using 40 μg L-tyrosine and 2 μg L-DOPA should be (166) (1·11) + (163) (2) = 508, which is about three times that of uniformly labelled L-tyrosine. This has been demonstrated previously[8]. In reality, the incorporation of tyrosine and DOPA without decarboxylation is neither 9·50 per cent (radioactive tyrosine) nor 28·65 per cent (radioactive DOPA) but 22·31 per cent, as obtained by the following calculation (Table 3):

Tyrosinase activity with uniformly labelled L-tyrosine:	163 c.p.m.
Tyrosinase activity with DL-DOPA-1-^{14}C:	48 c.p.m.
Tyrosinase activity with DL-DOPA-2-^{14}C:	164 c.p.m.
Tyrosinase activity estimated as L-DOPA-1-^{14}C:	(48) (2) c.p.m.
Tyrosinase activity estimated as L-DOPA-2-^{14}C:	(164) (2) c.p.m.

Using the correction factor 1·11 for utilizing uniformly labelled L-tyrosine-^{14}C and the estimated incorporation of tyrosine without decarboxylation, 9·50 per cent from Tables 1 and 2, the incorporation of tyrosine and DOPA without decarboxylation under the system investigated will be:

$$\frac{(163)\,(1{\cdot}11)\,(0{\cdot}095) + (48)\,(2)}{(163)\,(1{\cdot}11) + (164)\,(2)} \times 100 = 22{\cdot}31 \text{ per cent}$$

Carboxyl incorporation does not appear to result from the attraction between the carboxyl group of DOPA or tyrosine and the basic ring nitrogen of melanin, as little or no incorporation of other 14-labelled aliphatic amino-acids into the end-product was observed[8]. However, it is possible that DOPA, tyrosine or their metabolites with a carboxyl group may be attracted by melanin or a precursor by way of the carboxyl and phenol groups due to structural similarity and polarity. This may support the attraction hypothesis[8].

Melanin is formed by polymerization of indole-5,6-quinone, a metabolite from tyrosine or DOPA, by repeated oxidative condensation involving the anionoid centre of the pyrrole ring of one molecule and the cationoid centre of the benzonoid part of another[9,10]. The indole-5,6-quinone-2-carboxylic acid may be co-polymerized with

indole-5,6-quinone in acid solution although it is not oxidizable itself into melanin. In addition, direct incorporation of other potentially anionoid substances such as tyrosine or DOPA may also be expected during generation of the indole-5,6-quinone. Considering the basic principle of polymerization analogous to indole–quinone condensation, incorporation of the carboxyl group of tyrosine or DOPA into the melanin polymer[1–5,7] may result from copolymerization of these substances and their intermediates with melanin or precursors prior to decarboxylation. However, the carboxyl groups of the pyrrolic acid units in the melanin polymer originate from the breakdown of the indole benzonoid ring of the polymer[6], and thus are not derived from the carboxyl groups of tyrosine and DOPA.

In melanin formation, the incorporation of radio-DOPA without decarboxylation is much higher than that of radio-tyrosine without decarboxylation. The attraction hypothesis may explain this difference. As the degree of tyrosine or DOPA incorporation into melanin, using fish skin homogenates, is small[8], the amount of radio-DOPA and intermediates present at any time during incubation is also small. The effective concentration of radio-DOPA and intermediates will be much higher at any given time in a system of 40 μg L-tyrosine and 2 μg L-DOPA-^{14}C than in one with 40 μg L-tyrosine-^{14}C and 2 μg L-DOPA. At the beginning of incubation, the effective concentration of L-DOPA-^{14}C is 2 μg/ml. in the former system while it is zero in the latter system. During incubation, the probability that the concentration of radio-DOPA in the latter system does not attain the level present in the former is high for several reasons. The radioactive DOPA concentration in the latter system is reduced by the slow conversion rate of tyrosine to DOPA and, perhaps, partial loss of monophenolase activity in the enzyme preparation[11–13]. The polarity and structure of DOPA is more like that of the diphenolic compounds (or their quinone form) than tyrosine. Therefore, DOPA and its melanogenic intermediates may be more easily copolymerized than tyrosine. An intermediate which may be readily copolymerized is indole-5,6-quinone-2-carboxylic acid, which has been demonstrated in sepiomelanin[2–4]. Since the effective concentration of radio-DOPA is higher in a system utilizing labelled DOPA rather than labelled tyrosine the incorporation of the label by copolymerization will be proportionally higher in the former system.

From the foregoing discussion together with other available information[1–5,7–13], the following conclusions may be reached: (1) Only DOPA or its intermediates may readily be converted into a melanin precursor (indole-5,6-quinone) for polymerization. (2) Only DOPA or its intermediates may easily be attracted by melanin or a melanin precursor for copolymerization. (3) With the catalytic reaction of DOPA in tyrosine–DOPA conversion, enzymatically and/or autoxidatively the incorporation of tyrosine into melanin by polymerization and copolymerization may take place. (4) The direct incorporation of tyrosine into melanin with indole-5,6-quinone or into melanin by copolymerization is not excluded. (5) Both polymerization and copolymerization may be considered, primarily, to result from the attraction of the intermediates during melanogenesis.

Electron spin resonance studies have demonstrated the irregularity of the melanin polymer[14] as first suggested by Swan[7]. A portion of this irregularity may result from copolymerization. Further, during polymerization, cross-linkage may also be responsible for the irregularity[1,7,14] and this may also be produced by attraction at different sites of the same monomer. Thus, the attraction hypothesis may explain the observed irregularity of the melanin molecule by both polymerization and copolymerization. Under these circumstances, it is obvious that the molecular environment in the cell will directly affect the structure of the melanin polymer during melanogenesis. As the intra-cellular environment is in constant flux, the melanogenic end-product will also vary.

In summary. with the use of carboxyl-labelled tyrosine and DOPA, the incorporation of tyrosine and DOPA without decarboxylation into melanin by skin homogenates of black moor goldfish has been demonstrated. Comparison of the results obtained with L-tyrosine-1-^{14}C, DL-tyrosine-2-^{14}C, uniformly labelled L-tyrosine-^{14}C, DL-DOPA-1-^{14}C and DL-DOPA-2-^{14}C, the incorporation of radio-tyrosine without decarboxylation into melanin in the presence of unlabelled DOPA was 9·50 per cent of the total radio-tyrosine converted; the incorporation of radio-DOPA without decarboxylation into melanin in the presence of unlabelled tyrosine was 28·65 per cent of total radio-DOPA converted; the calculated incorporation of tyrosine and DOPA without decarboxylation in utilizing both radio-tyrosine and radio-DOPA was 22·31 per cent. The incorporation of tyrosine and DOPA carboxyl groups into melanin is probably due to the copolymerization of tyrosine, DOPA or/and their intermediates with indole-5,6-quinone or that unit of melanin primarily contributed by the attraction between the intermediates. This supports the attraction hypothesis. Furthermore, the greater incorporation of the DOPA carboxyl group than that of the tyrosine carboxyl group can be explained by the attraction hypothesis in addition to the tyrosine-DOPA conversion limiting factor(s).

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HABITUATION AT THE SYNAPTIC LEVEL IN *Aplysia*

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A DIMINUTION of compound excitatory post-synaptic potentials (EPSP) has been observed in the giant cell in the abdominal ganglion of *Aplysia* on repeated mechanical stimulation applied to the skin of the animal[1] or on repeated electrical stimulation of the afferent fibres[2]. Similar diminution has been observed in the giant cell of the left pleural ganglion[3]. In the present investigation an attempt was made to analyse the mech-

indole-5,6-quinone in acid solution although it is not oxidizable itself into melanin. In addition, direct incorporation of other potentially anionoid substances such as tyrosine or DOPA may also be expected during generation of the indole-5,6-quinone. Considering the basic principle of polymerization analogous to indole–quinone condensation, incorporation of the carboxyl group of tyrosine or DOPA into the melanin polymer[1-5,7] may result from copolymerization of these substances and their intermediates with melanin or precursors prior to decarboxylation. However, the carboxyl groups of the pyrrolic acid units in the melanin polymer originate from the breakdown of the indole benzonoid ring of the polymer[6], and thus are not derived from the carboxyl groups of tyrosine and DOPA.

In melanin formation, the incorporation of radio-DOPA without decarboxylation is much higher than that of radio-tyrosine without decarboxylation. The attraction hypothesis may explain this difference. As the degree of tyrosine or DOPA incorporation into melanin, using fish skin homogenates, is small[8], the amount of radio-DOPA and intermediates present at any time during incubation is also small. The effective concentration of radio-DOPA and intermediates will be much higher at any given time in a system of 40 μg L-tyrosine and 2 μg L-DOPA-^{14}C than in one with 40 μg L-tyrosine-^{14}C and 2 μg L-DOPA. At the beginning of incubation, the effective concentration of L-DOPA-^{14}C is 2 μg/ml. in the former system while it is zero in the latter system. During incubation, the probability that the concentration of radio-DOPA in the latter system does not attain the level present in the former is high for several reasons. The radioactive DOPA concentration in the latter system is reduced by the slow conversion rate of tyrosine to DOPA and, perhaps, partial loss of monophenolase activity in the enzyme preparation[11-13]. The polarity and structure of DOPA is more like that of the diphenolic compounds (or their quinone form) than tyrosine. Therefore, DOPA and its melanogenic intermediates may be more easily copolymerized than tyrosine. An intermediate which may be readily copolymerized is indole-5,6-quinone-2-carboxylic acid, which has been demonstrated in sepiomelanin[2-4]. Since the effective concentration of radio-DOPA is higher in a system utilizing labelled DOPA rather than labelled tyrosine the incorporation of the label by copolymerization will be proportionally higher in the former system.

From the foregoing discussion together with other available information[1-5,7-13], the following conclusions may be reached: (1) Only DOPA or its intermediates may readily be converted into a melanin precursor (indole-5,6-quinone) for polymerization. (2) Only DOPA or its intermediates may easily be attracted by melanin or a melanin precursor for copolymerization. (3) With the catalytic reaction of DOPA in tyrosine–DOPA conversion, enzymatically and/or autoxidatively the incorporation of tyrosine into melanin by polymerization and copolymerization may take place. (4) The direct incorporation of tyrosine into melanin with indole-5,6-quinone or into melanin by copolymerization is not excluded. (5) Both polymerization and copolymerization may be considered, primarily, to result from the attraction of the intermediates during melanogenesis.

Electron spin resonance studies have demonstrated the irregularity of the melanin polymer[14] as first suggested by Swan[7]. A portion of this irregularity may result from copolymerization. Further, during polymerization, cross-linkage may also be responsible for the irregularity[1,7,14] and this may also be produced by attraction at different sites of the same monomer. Thus, the attraction hypothesis may explain the observed irregularity of the melanin molecule by both polymerization and copolymerization. Under these circumstances, it is obvious that the molecular environment in the cell will directly affect the structure of the melanin polymer during melanogenesis. As the intra-cellular environment is in constant flux, the melanogenic end-product will also vary.

In summary, with the use of carboxyl-labelled tyrosine and DOPA, the incorporation of tyrosine and DOPA without decarboxylation into melanin by skin homogenates of black moor goldfish has been demonstrated. Comparison of the results obtained with L-tyrosine-1-^{14}C, DL-tyrosine-2-^{14}C, uniformly labelled L-tyrosine-^{14}C, DL-DOPA-1-^{14}C and DL-DOPA-2-^{14}C, the incorporation of radio-tyrosine without decarboxylation into melanin in the presence of unlabelled DOPA was 9·50 per cent of the total radio-tyrosine converted; the incorporation of radio-DOPA without decarboxylation into melanin in the presence of unlabelled tyrosine was 28·65 per cent of total radio-DOPA converted; the calculated incorporation of tyrosine and DOPA without decarboxylation in utilizing both radio-tyrosine and radio-DOPA was 22·31 per cent. The incorporation of tyrosine and DOPA carboxyl groups into melanin is probably due to the copolymerization of tyrosine, DOPA or/and their intermediates with indole-5,6-quinone or that unit of melanin primarily contributed by the attraction between the intermediates. This supports the attraction hypothesis. Furthermore, the greater incorporation of the DOPA carboxyl group than that of the tyrosine carboxyl group can be explained by the attraction hypothesis in addition to the tyrosine-DOPA conversion limiting factor(s).

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HABITUATION AT THE SYNAPTIC LEVEL IN *Aplysia*

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A DIMINUTION of compound excitatory post-synaptic potentials (EPSP) has been observed in the giant cell in the abdominal ganglion of *Aplysia* on repeated mechanical stimulation applied to the skin of the animal[1] or on repeated electrical stimulation of the afferent fibres[2]. Similar diminution has been observed in the giant cell of the left pleural ganglion[3]. In the present investigation an attempt was made to analyse the mech-

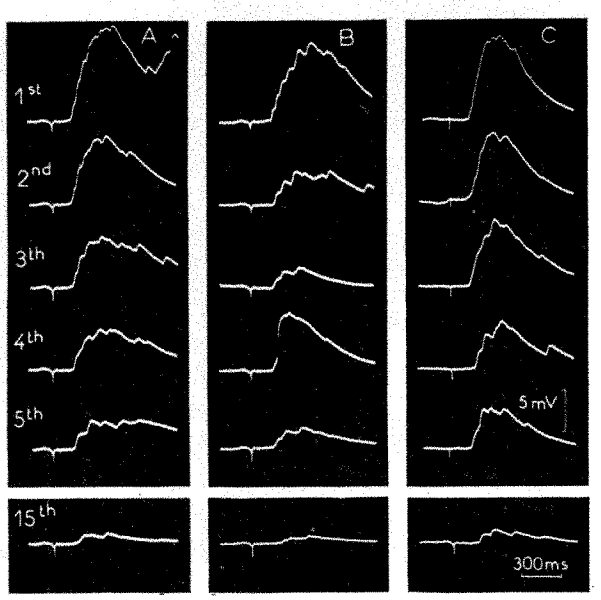


Fig. 1. Modifications of compound EPSP during habituation and dishabituation produced by drops applied at 10-sec intervals on the head near the left anterior tentacle. *A*, First series of fifteen stimuli. *B*, Second series of stimuli showing restoration of the EPSP after 10-min rest. *C*, Continuation of series *B* without break in stimulation frequency but following a 2-sec repetitive stimulation (5 per sec) of the left posterior pedal nerve. Note the restoration of the initial amplitude of the EPSP

anisms of the previously described diminution of EPSP and to relate it to the phenomenon of behavioural habituation demonstrated long ago in other gastropods[4,5].

The experiments were performed on *Aplysia depilans* and *Aplysia punctata*. Part of the central nervous system consisting of the perioesophagial ganglia together with most of the nerves was isolated from the animal body, but in some preparations the head of the animal with the most anterior part of the foot was also excised and the nervous connexions between the cerebral ganglia and the head (designated as cerebral nerves) were maintained. These preparations were mounted in a 'Plexiglass' chamber filled with sea water. The mechanical stimulation was provided by drops of sea water falling from approximately 30 cm on a restricted area of the head, which was kept continuously moist. Intracellular recordings by means of microelectrodes filled with 2·5 M potassium chloride were taken from the giant cell of the left pleural ganglion[6]. The preparations, maintained at room temperature (20°–22° C), showed no detectable deterioration during the 6 to 7 h they were studied.

A drop of sea water applied on the head produced a withdrawal of one or both tentacles. Simultaneously, a 10–15 mV compound EPSP was observed in the giant cell (Fig. 1*A*). Such an EPSP seldom produced a spike. The latency of the tentacular contraction was the same or greater than that of the EPSP. Consequently it can be concluded that the synaptic potentials were not driven by the muscular contraction; nor was the withdrawal of the tentacles dependent on spike activity in the giant cell.

When the drops of sea water were applied at regular intervals (10 sec or more), there was a progressive diminution of both the tentacular response and the compound EPSP (Fig. 1*A*). After fifteen stimulations the EPSP was reduced to 15 per cent or less of its original amplitude. Both responses could be restored by a period of several minutes without stimulation (Fig. 1*B*). The magnitude of recovery depended on the number of stimuli previously administered as well as on the length of the pause. For example, a 10-min rest was nearly sufficient after a series of 10 stimuli. However, when the series was repeated a number of times, with each stimulus series separated by a 10-min rest, it was noticed that the pause became progressively less effective in restoring the original amplitude. After four series of this type, a pause of 50 min restored the EPSP to 85 per cent of its original amplitude. Later series differed from the initial one in two additional ways: the rate of decline of the EPSP was faster, and the terminal EPSP smaller. The tentacular response followed similar kinetics of decrease. The amplitude of the attenuated EPSP could be restored without any rest period either by scratching the skin of the head or electrically stimulating (5–10 per sec for 2–4 sec) one of the nerves or connectives (Fig. 1*C*). The amplitude of the tentacular response could also be restored by scratching the skin, but the tetanization of the nerves was not effective unless the stimulation was prolonged.

The restoration of the compound EPSP by extraneous stimulation suggested the central origin of EPSP 'habituation'. Consequently, it was not surprising to find that the kinetics of diminution and restoration resulting from direct nerve stimulation were similar to those obtained by mechanical stimulation of the skin. Thereafter, this similarity permitted us to use the isolated nervous system for analysis of the mechanism of the EPSP 'habituation', applying the same pattern of stimuli and rest directly to one of the cerebral nerves.

The diminution of the compound EPSP on such a stimulation series was mainly due to an increase in latency and/or the disappearance of the unitary EPSPs which were often distinguishable in the compound potential. It was also noted that simultaneously the amplitude of some of these unitary EPSPs decreased on repetition. It was possible to observe the modification of these unitary potentials in isolation by stimulating the cerebral nerves just at the threshold, resulting in the activation of only one presynaptic interneurone[7]. The fact that the latency of these unitary potentials changed under stimulation at one or two per second, and that they failed to follow at high frequencies, suggest non-monosynaptic connexions.

The changes in the unitary EPSP that occurred under repeated stimulation were very similar to those shown by the compound EPSP. As for the compound potentials, the unitary potentials showed a diminution in amplitude

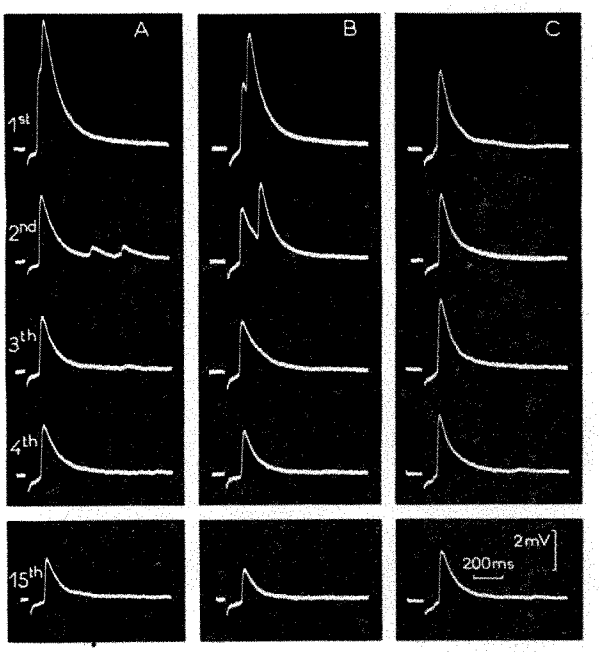


Fig. 2. Modifications of unitary non-monosynaptic EPSP produced by electrical stimuli at 10-sec intervals, applied to one of the left cerebral nerves (small tegumentary nerve). *A*, First series of fifteen stimuli. At the beginning of this and the next series other EPSPs superimposed first unitary EPSP. *B*, Restoration of the unitary EPSP after 10-min rest. *C*, Restoration of the unitary EPSP produced by 3-sec repetitive stimulation (10 per sec) of another left cerebral nerve (posterior tentacular nerve)

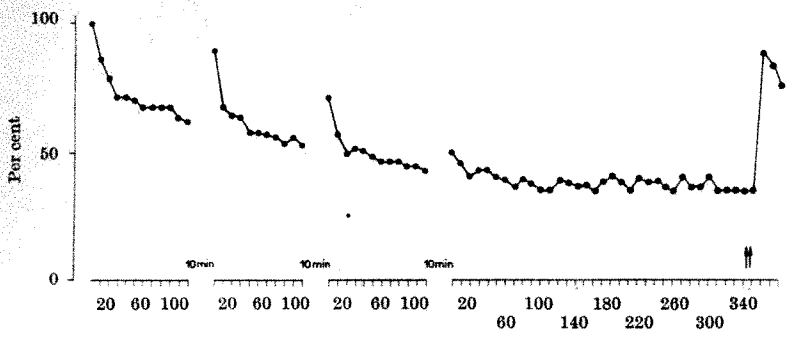


Fig. 3. Accumulation of effects of stimulation (at 10-sec intervals) on recovery process observed in a unitary monosynaptic EPSP. Ordinate: Percentage of amplitude of initial EPSP. Abscissa: Time (in sec and min). Arrows in final series indicate occurrence of 3 sec tetanization of left posterior pedal nerve; note slightly delayed recovery of EPSP

within a stimulus series (Fig. 2*A*). Moreover, as before, the amplitude of the attenuated potential could be restored either by rest or by electrical stimulation of one of the nerves or connectives (Fig. 2*B* and 2*C*). Successive rest periods were increasingly ineffective in restoring the original EPSP amplitude.

Unitary potentials which appeared to be monosynaptic were obtained by stimulation of the left pleuro-visceral connective[8]; and these EPSPs showed identical changes to those already described (Fig. 3).

Such modifications of unitary EPSPs on the level of interneurones would explain the inability of the latter to give a propagated spike after a few stimuli, which in turn would result in the increase in latency and the disappearance of some individual elements in the compound EPSP (see Fig. 2). But it should be noted that the described properties of EPSPs were observed only in some cells. The EPSPs of other *Aplysia* cells do not show a decrease on repeated stimulation at low frequency[9].

The mechanism of this depression of unitary EPSPs is obscure. It is difficult to attribute a post-synaptic character to this phenomenon; indeed, the physical constants of the post-synaptic membrane were demonstrated to remain unchanged during repetitive stimulation of the afferent fibres. Moreover, the hypothesis of a possible desensitization of subsynaptic receptors[10,11] would be incompatible with the recovery of the EPSP after brief tetanization of the same input. Presynaptic inhibition, which was also demonstrated in *Aplysia*, seems not to be responsible for the 'habituation' of the EPSP, as the corresponding effects of pre-synaptic inhibition are less and less effective[9], contrary to the effects of accumulation observed in these experiments. It seems more likely that the decrease is due to a diminution of transmitter release, probably caused by the depletion of the transmitter in the terminals and its slow mobilization. We have frequently observed that, after one stimulus, a diminution of the subsequent EPSP amplitude could be detected even after 3–4 min. In some respects the phenomenon is similar to the depression observed in some other synapses and especially in neuromuscular synapses[12–14], but it differs in its much longer time course as well as in the long lasting dependence on previous stimulation.

The restoration of the EPSP by a brief tetanization of the same or different nerve seems to be provoked either by post-tetanic potentiation[15] or by heterosynaptic facilitation observed in a group of cells in the abdominal ganglion of *Aplysia*[8].

Hence the modifications of EPSPs of the giant cell observed during habituation to a repeated mechanical stimulus seem to be due to long-lasting changes in synaptic efficacy. Despite the fact that the giant cell is not situated in the reflex arc of the tentacular response, the similar kinetics of the amplitude modifications of the contraction and of the EPSPs suggest that this synaptic mechanism may be one of the elements of behavioural habituation.

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FORMATION OF NEW SITES OF ACETYLCHOLINESTERASE ACTIVITY IN DENERVATED MUSCLES OF YOUNG RATS

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AND
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When an adult mammalian skeletal muscle is denervated, the activity of acetylcholinesterase at its motor end-plates gradually decreases[1] whereas that of muscle–tendon junctions remains unchanged[2,3].

If the denervation is carried out in the rat at birth or shortly afterwards, the fall of activity at the end-plates is more abrupt[4,5] and, contrary to what was observed after denervation in the adult, no acetylcholinesterase (AChE) activity is seen at the myotendinous junctions[6]. The changes in the acetylcholinesterase activity of the muscle, however, are not confined to these loci. About 2 weeks after denervation, new spots of fairly intense acetylcholinesterase activity make their appearance. They are scattered over the muscle some distance from the line of end-plates (Fig. 1).

Although the number and spatial dispersion of these spots make it unlikely that they correspond to incipient end-plates produced by an uncontrolled nerve regeneration such a possibility was discarded by experiments in which a permanent motor denervation of limb muscles was

Table 1. COMPARISON OF MUSCLE FIBRES FROM THE TIBIALIS ANTERIOR OF RATS DENERVATED AT BIRTH WITH NORMAL TIBIALIS FIBRES AT SIMILAR AGES*

Days after birth	Muscle	Length of muscle (mm)	Fibre diameter (μ)	Length of end-plate (μ)	Ratio: end-plate length to fibre diameter	No. of additional active spots on a fibre	Length of active spots (μ)
0	normal	4·1	5·9 ± 0·2	18·4 ± 0·6	3·10	0	—
17	normal	12	13·5 ± 0·5	26·2 ± 0·9	1·95	0	—
	denervated	8·6	10·5 ± 0·2	72·0 ± 3·4	6·90	3·5 ± 0·8	27·2 ± 1·1
21	normal	13	14·7 ± 0·5	24·4 ± 0·7	1·65	0	—
	denervated	10	10·7 ± 0·3	79·7 ± 2·9	7·50	3·4 ± 0·5	26·6 ± 0·9
32	normal	14·5	22·8 ± 1·1	29·7 ± 1·6	1·30	0	—
	denervated	12·7	15·2 ± 0·6	100·4 ± 3·6	6·60	3·5 ± 0·4	40·5 ± 1·5

* About seventy isolated fibres from two animals were measured at each age. The diameters of fibres were measured in their cylindrical part, far from the terminal taperings. The dimensions of end plates are those of their AChE-stained portions.

obtained by removal at birth of the lumbar and sacral parts of the spinal cord[6]. The resulting irreversible de-efferentation produced an outcrop of new spots of AChE activity similar to those observed after section of the nerve.

The distribution and appearance of AChE-active sites was studied in individual fibres teased after incubation of the whole muscle with acetylthiocholine by a slightly modified Silver[7] method.

In the denervated muscle the diameter of fibres increases more slowly with age than that of normal muscle fibres. Striking changes occur at the end-plate, where AChE activity falls considerably, and the sole becomes straight and about three times longer than a normal end-plate at this age (Fig. 2*a*, *b*). At various distances from the end-plate, small cytoplasmic elevations bulge over the surface of the fibre. Some of these show much stronger cholinesterase activity than does the denervated end-plate (Fig. 2*c*, *d*). Structural details in the active spots are obscured by the large amount of stained granules, but sometimes a nucleus may be discerned inside the cytoplasmic hillock or nearby. These spots are always sharply outlined and sometimes seem to adhere to the surface of the fibre rather than to protrude from the inside. Although the spots are usually separated by large distances (0·5–3 mm), two spots occasionally appear in close proximity (Fig. 2*d*).

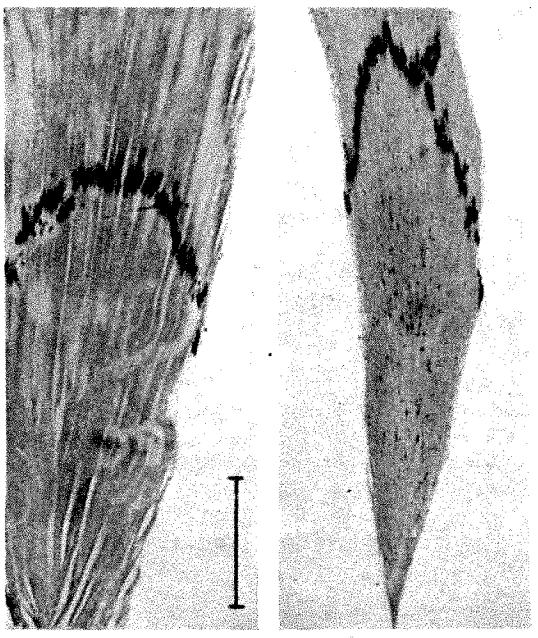


Fig. 1. Longitudinal sections of the tibialis anterior muscles of 21-day old rats. Left, normal. Right, denervated at birth. Stained for AChE by a modified Koelle and Friedenwald (ref. 18) method, after pre-incubation with 3×10^{-7} M di-isopropylfluorophosphate. Additional spots of activity are scattered in the denervated muscle some distance from the line of end-plate. Scale, 2 mm

The zones of muscle fibres in which the additional spots of AChE activity are mainly distributed are shown in Fig. 3. These zones do not seem to vary with age within the period investigated, except for the elongation due to growth. The number of additional spots in muscle fibres does not vary significantly with age between 17 and 32 days.

The ability to form new foci of AChE activity seems to be connected with a certain stage of development of the muscle. Denervation in the adult does not produce these foci. It seemed valuable, therefore, to characterize certain features of muscle fibres at birth, at the moment of denervation, and to compare the further development of denervated fibres producing additional spots of activity with normal muscle fibres at similar ages. The results of measurements made on fibres from the tibialis anterior muscle are summarized in Table 1.

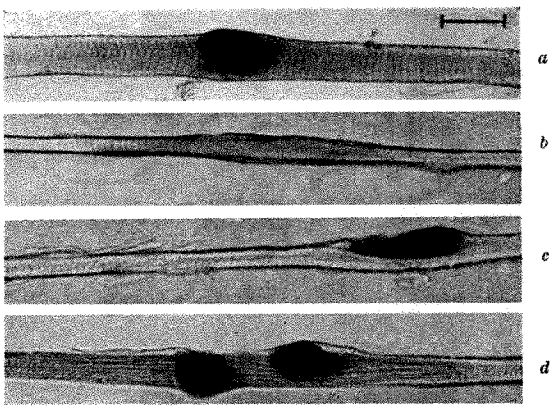


Fig. 2. Parts of isolated muscle fibres from the tibialis anterior of 21-day old rat. *a*, Normal end-plate; *b*, denervated end-plate; *c* and *d*, additiona spots of AChE activity in denervated fibres. Stained for AChE by acetylthiocholine method. Incubation times and photographic processing identical in all fibres. Scale 25μ

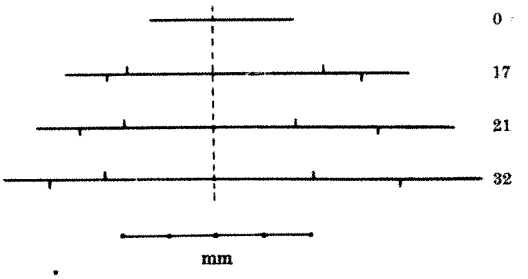


Fig. 3. Diagram showing the average length of fibres from the tibial anterior muscle at birth and at 17, 21 and 32 days after denervation. The vertical line indicates the position of the end-plates which are not in the middle of fibres in this muscle. The upward dashes indicate the average distance of the nearest spot from the end-plate, the downward ones that of the farthest spot. Twenty to thirty fibres were measured in each muscle

It is difficult to interpret the formation of new spots of AChE activity in young denervated muscles. No focal accumulations of AChE were found in embryonic muscles before innervation[8] or in chick muscle in tissue culture even at an advanced degree of differentiation of muscle fibres[9]. Rat muscles do not seem to have been investigated under these conditions. However, as the first effect of denervation of a developing rat muscle is the dispersal of AChE at the end-plates and at myo-tendinous junctions, the formation at later stages in other parts of muscle fibres of new foci of AChE activity caused by the same factor is surprising. It is not clear whether the AChE activity is actually concentrated in sarcoplasmic bulges or in the protoplasm of some cells closely adhering to the surface of muscle fibres. Light microscope investigations of whole teased fibres, as well as of muscle sections, were technically inadequate to decide this question.

The fact that the additional spots appear only if the muscle is denervated at a certain stage of its development and that they are clustered only in certain regions of the muscle suggests that it is the denervated muscle fibre itself that produces focal accumulations of AChE in its sarcoplasm. Other possibilities are, however, not excluded. A possible non-myogenic source of the active spots might be from the Schwann cells of the degenerating intramuscular nerve branches. As is well known[10,11] the Schwann cells become migratory after the breakdown of their axons and it is possible that they could creep along the surface of muscle fibres, closely applied to them. Although in normal nerves the Schwann cells are devoid of AChE activity[12,13] the ability to produce AChE may appear some time after denervation. This is suggested by the observations that the intra-muscular branches of degenerating nerves show positive staining for AChE several weeks after denervation whereas normal nerves are not stained under similar conditions[14,15].

It is of interest in this connexion that the possibility of acquisition by the Schwann cells of a mechanism for synthesis and destruction of acetylcholine has been invoked to explain various phenomena observed in the late stages of denervation, such as the reappearance of miniature end-plate potentials after a silent period in the frog[16], or the increase of choline-acetylase activity in denervated salivary glands[17] that follows an initial fall of activity of this enzyme.

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ROLE OF MITOSIS AND MITOTIC INHIBITION IN THE IMMUNOSUPPRESSIVE ACTION OF THIOGUANINE

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IT is well known that antibody-forming cells undergo mitosis during the early stages of the immune response[1] and that many immunosuppressive agents are inhibitors of mitosis[2]. These facts have led to the common assumptions (*a*) that the increase in antibody-levels after antigenic stimulation may be accounted for quantitatively by the rapidly repeated divisions of antibody-forming cells, and (*b*) that the effects of immunosuppressive agents may similarly be accounted for by their ability to prevent mitosis. This article suggests that one or both of these propositions is incorrect.

Conventional measurements of levels of immunity, that is, antibody titres, intensities of hypersensitivity reactions or rates of homograft rejection, reflect the cellular events of the immune response only with a considerable delay and insensitivity. This gives rise to great difficulty in analysing the effects of immunosuppressive agents. For example, the characteristic delayed appearance, reduced rate of growth and reduced peak of the partly suppressed response could be caused by a variety of cellular effects. These include cell destruction, overall slowing of the response (due, for example, to mitotic inhibition) and a delay in initiating the response with one or more of its components undergoing a natural decay. However, measurements of levels of immunity have not so far made it possible to indicate with confidence which, if any, of these mechanisms is responsible for suppression of the response.

Techniques devised[3,4] for counting the numbers of antibody-forming cells in various tissues seem to offer a possible method of examining more directly the effects of immunosuppressive agents on the cells concerned.

Male *A2G* mice, weighing 15–21 g at the beginning of each experiment, were injected intraperitoneally with 0·2 ml. of 10 per cent formolized sheep red cells in saline. At intervals after the injection, groups of mice were killed and the number of haemolysin-producing cells in their spleens counted by Jerne's agar-plating technique[5]. Plates were incubated at 37° C for 1 h, 10 per cent guinea-pig serum in saline added and the haemolysed plaques counted after a further 30-min incubation.

The combined results of several experiments are shown in Figs. 1 and 2, the counts on individual spleens in the former and the geometric means of the counts in the latter. Unimmunized mice had about fifty plaque-forming cells per spleen. The number rose slightly to about ninety 1 day after immunization and then rapidly to a peak of 30,000 on the fourth day. The doubling-times were 9·2 h between days 1 and 2, 6·8 h between days 2 and 3 and 9·8 h between days 3 and 4. After the fifth day there was a fall that slowed progressively and the number of haemolysin-forming cells dropped to 150–300 by the third week.

Thioguanine was selected for investigation as an immunosuppressive agent as it is highly effective in inhibiting antibody production in relatively non-toxic doses[5] and does not cause gross destruction of lymphoid tissue which might make interpretation of results difficult[6]. The agent was ground in 0·5 per cent carboxymethyl-cellulose in normal saline and injected subcutaneously in doses of 10–240 mg/kg body-weight. Injections were

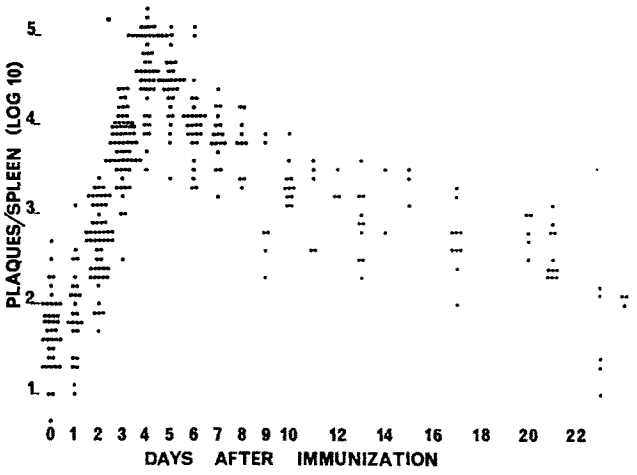


Fig. 1. Number of haemolytic plaques per mouse spleen at intervals after intraperitoneal injection of 0·2 ml. of formolized sheep red cells, 10 per cent suspension. Each point represents the count on one spleen

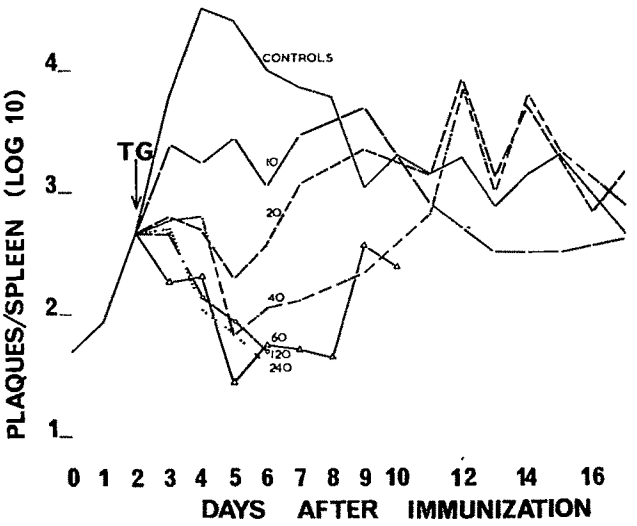


Fig. 2. Effects of various doses of 6-thioguanine, given subcutaneously on day 2, on the number of haemolytic plaques per spleen. ——, Controls; — — —, 10 mg/kg; — — — — — —, 20 mg/kg; —·—·—·—·—, 40 mg/kg; ——Δ——Δ——, 60 mg/kg; O——O——O, 120 mg/kg; ····· , 240 mg/kg. Curves show the geometric means of each group of counts. Numbers of counts: controls as in Fig. 1, 10 mg/kg—three mice/day, 20, 40, 60, and 120 mg/kg—five to nine mice/day at each dose level, 240 mg/kg—twenty or twenty-one mice/day

given 48 h after the antigen, as this is the optimum time for inhibiting antibody production with a single dose of agent[7]. Doses of 20 mg/kg or more caused a fall in the number of haemolysin-producing cells that reached its lowest point within 3–4 days and was followed by a slow rise toward normal levels (Fig. 2). A maximum rate of fall was produced by doses of 60 mg/kg or more.

The results of four experiments relevant to the assumptions outlined above are shown in Fig. 3. In these experiments, administration of 60–240 mg/kg thioguanine was followed by a fall in the number of antibody-producing cells, the halving time varying from 7·8 to 13·6 h. Arrest of mitosis cannot of itself have caused this fall unless the cells underwent a natural wastage due to death, cessation of antibody-synthesis or migration elsewhere. If, however, this wastage was natural and not caused by the thioguanine it must also have occurred during the normal, unsuppressed response. Accordingly, the increase in antibody-forming cells after antigenic stimulation was achieved in spite of a natural wastage and their generation time must have been even shorter than that suggested by their observed rate of increase.

It has been suggested that a major factor limiting the rapidity of cell multiplication is the time needed for the cell to synthesize a full complement of DNA[11]. The minimum times quoted for this process are 5·5 h in mouse thymus large and medium lymphocytes[8], 6 h in mouse lymphoma cells[8], 6·5 h in mouse trophoblast[9] and 6–7·5 h in various normal tissues and tumours of the adult mouse[10,11]. As the rest of the cell-cycle, including mitosis, takes at least an hour, it would appear that the shortest possible generation time for mammalian cells is 6–7 h. In fact, the generation time of rapidly dividing mammalian cells is more usually 12–18 h[11], but a few shorter times have been recorded, for instance 6·8 h for mouse thymus large lymphocytes[8], 7·6 h for mouse lymphoma cells[8], 8·2 h for mouse thymus medium lymphocytes[8] and 9·6 h for mouse trophoblast[9]. The rate of increase of haemolysin-producing cells between days 2 and 3 would therefore appear to be about the fastest that is compatible with cell-division.

Allowance for natural wastage may be made as follows. If, in antigenically stimulated animals, the number of antibody-forming cells rises exponentially from A to B in time t, the observed doubling time being OT_2, and, in such animals after administration of an immunosuppressive agent, falls from C to D over the same period, the observed halving time being $OT_{\frac{1}{2}}$, and if it is assumed that this fall is due only to a natural wastage that is uncovered when the agent stops cell division, it can be shown that the true doubling time of the cells, T_2, is given by

$$T_2 = \frac{OT_2 . OT_{\frac{1}{2}}}{OT_2 + OT_{\frac{1}{2}}}$$

$$\text{and } T_2 = \frac{t . \log 2}{\log B + \log C - \log A - \log D}$$

When the start of t is at the time of administration of the agent, then $A = C$ and

$$T_2 = \frac{t . \log 2}{\log B - \log D}$$

In this case it is necessary to perform cell counts only at the end of the treatment period; the starting level is irrelevant.

The values of the observed doubling and halving times for each of the four experiments are noted in Fig. 3. The estimated true doubling times, given the assumptions stated, vary from 5·5 h down to 3·6 h. In the light of

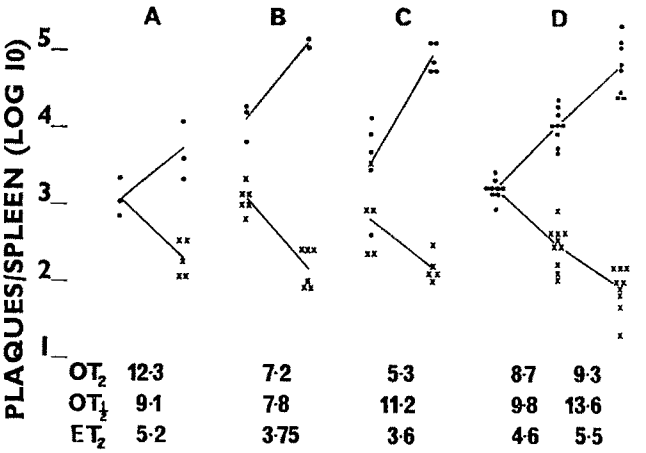


Fig. 3. Reduction in number of haemolytic plaques per spleen after administration of 6-thioguanine on day 2, compared with the increase in untreated mice. Each point represents the count on one spleen; the curves are drawn through the geometric means of each group. ●, Control mice, X, treated mice. Doses—60 mg/kg in experiment A, 240 mg/kg in experiments B, C and D. Counts performed on days 2 and 3 in experiment A, days 3 and 4 in experiments B and C and days 2, 3 and 4 in experiment D. OT_2—observed doubling time of haemolysin-producing cells in control mice; $OT_{\frac{1}{2}}$—observed halving time of haemolysin-producing cells in thioguanine-treated mice; ET_2—estimated true doubling time (see text)

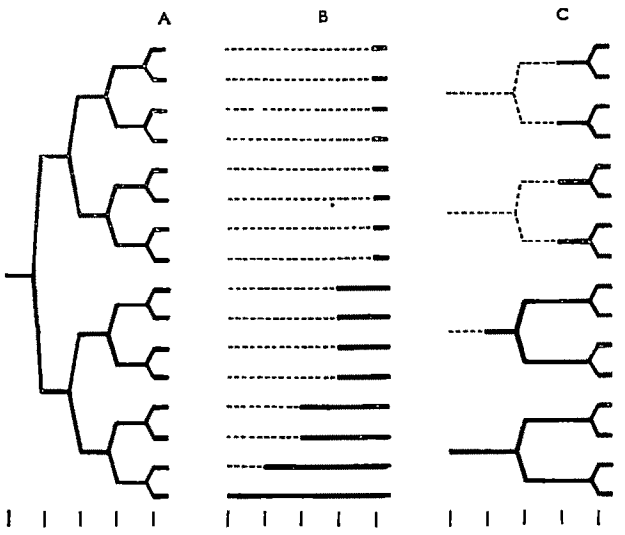


Fig. 4. Three possible ways in which the number of haemolysin-forming cells could increase, the observed rate of increase being the same in each case. — — — — — —, Cell not producing antibody, ———, cell producing antibody. In *A* there are four consecutive divisions in the period shown and one antibody-producing cell multiplies to form 16. In *B* antibody production begins at different times in 16 non-dividing cells. In *C* there are two consecutive divisions in the period shown, four cells multiplying to form sixteen, and antibody production begins at different times in the four dividing cell lines

present knowledge of the minimum times required by mammalian cells to synthesize DNA and proliferate, such doubling times cannot be ascribed to cell division alone.

It follows that one or both of the original assumptions must be wrong. If the increase in antibody-forming cells is due entirely to cell-division, the decrease after administration of thioguanine cannot be due to a natural wastage that becomes observable only when mitosis is stopped but must be due to destruction of cells or impairment of their ability to produce antibody. Conversely, if thioguanine acts only by stopping mitosis, the rapid increase in antibody-forming cells in the early stages of the immune response cannot be due entirely to cell-division.

Three possible modes of increase of antibody-forming cells are shown in Fig. 4. All give the same observed doubling time but in *A* the increase is due entirely to cell division, in *B* entirely to induction of antibody-formation at different times in initially non-producing cells[3] and in *C* to a combination of both mechanisms. For clarity these models do not take cell wastage into account, but they may readily be adjusted to allow for this factor. As has already been shown, model *A* is credible only if immunosuppressive agents, whatever their effects on mitosis, also destroy cells or prevent them from synthesizing antibody. In model *B* there is no cell-division and the effects of immunosuppressive agents must be ascribed to cell destruction (either alone or combined with natural wastage) or to destruction and/or wastage combined with inhibition of induction or antibody synthesis. This model, however, is unacceptable in its simple form, as antibody-forming cells undoubtedly multiply by the conventional processes of DNA synthesis and mitosis[1]. In model *C*, the cell generation time may be assigned acceptable values, for example 7–12 h, and the shorter observed doubling time of antibody-forming cells achieved by the induction of antibody synthesis at different times during the proliferation of initially non-producing cells.

In this model, the effects of immunosuppressive agents could be due to any of the mechanisms postulated for model *B* and also, under some circumstances, to mitotic inhibition alone. If, for example, induction were mitosis-dependent, simple mitotic arrest would stop the appearance of new antibody-producers and their numbers would then fall by natural wastage. If induction were mitosis-independent, simple mitotic arrest could still cause the number of antibody-producers to fall if the induction that continued in the population of non-dividing cells did not keep pace with natural wastage. In the latter case, however, by a similar argument to that used above, it would follow that the fall in number of antibody-producing cells after administration of thioguanine occurs in spite of continued induction of new antibody-producers and that the true half-life of these cells is shorter than the halving times observed.

The results reported here do not disprove the hypothesis that immune processes are accounted for quantitatively by a wave of cell division that follows antigenic stimulation, nor that which ascribes the effects of immunosuppressive agents to mitotic inhibition. They do show, however, that these hypotheses are incompatible with each other.

I thank the Medical Research Council and the British Empire Cancer Campaign for Research for financial support.

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LABORATORY REARING OF THE PARASITIC STAGES OF *Boophilus decoloratus* (KOCH, 1844) ON THE EUROPEAN RABBIT (*Oryctolagus cuniculus*)

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WHILE endeavouring to breed *B. decoloratus* (Koch, 1844) on its principal host, the ox, I experienced great difficulty in obtaining information on the durations of development of the various parasitic stages of the life-history of this tick species. Therefore, both the guinea-pig and the rabbit were tried as experimental hosts. The larvae of *B. decoloratus* were able to become established on the rabbit, but not on the guinea-pig. The latter was therefore discarded in preference for the rabbit.

The larvae used for the infestation of the rabbit were bred from eggs that were incubated in the laboratory at a temperature ranging between 76° and 88° F (ref. 1). The humidity in the incubation chambers (that is, glass specimen tubes), in which the laying female tick species

were individually placed, was maintained by moistening cotton-wool stoppers at convenient intervals. The larvae were starved for about 2 weeks once their cuticle had hardened and then fed on the rabbit.

The rabbit was infested by the following technique. The experimental host was placed in a cylindrical glass jar with a lid. The larvae, which had been warmed in their tubes to ordinary laboratory temperature, following a period of inactivation in the refrigerator at 59°–68° F, were transferred to the body of the rabbit. Any larvae crawling on the inner surface of the jar were picked up with a fine camel hair brush and placed on to the rabbit. Airing of the glass jar occurred simultaneously. Constant observations were made until almost all the larvae had become attached to the body of the host. Three hours later, the rabbit was transferred to a small glass container inside a box made of mosquito-proof wire mesh. The host was fed mostly with pelleted roughage and water. Ticks which fell off the host were collected before any debris was removed from the cage. On the fourth day after infestation, when the engorged larvae were ready to detach from the host, the latter was transferred into a ventilated rectangular glass container from which the larvae were collected as they dropped off the host. The replete larvae were stored in the laboratory and the newly moulted nymphs resulting from them were also fed on the rabbit, using the same technique.

When the larvae of *B. decoloratus* became established on the rabbit the results showed that the tick behaved rather unusually as a three-host and as a two-host tick species. These behaviour patterns are discussed below.

The larvae became fully engorged, dropped and moulted off the host's body. It has been shown in Table 1 (*a*) that at the recorded mean laboratory temperatures, which appeared to be fairly similar, the average feeding period for 2,296 larvae was 4·2 days (range 3–5 days). Most of the larvae, that is 1,543, became fully engorged in 4 days and dropped off; 296 engorged fully and dropped off in 5 days, while the rest, namely 457, engorged and dropped off in 3 days.

Table 1. LARVAL STAGE: DURATION OF DEVELOPMENT OF IMMATURE STAGES OF *Boophilus decoloratus* (KOCH, 1844)

(*a*) Feeding on rabbit

No. of mother tick	Date of infesting host	Date engorged larvae dropped off the host	No. of engorged larvae dropping off	Laboratory temperature (° F) Range	Mean	Duration of feeding of larvae (days)
		22.6	846			4
1	19.6	23.6	124	72–86	79·7	5
	19.6	3 8	457			3
5	1.8	4.8	697	72–84	77·5	4
	1.8	5.8	172			5
		Total	(2,296)			(21); Av. 4·2

(*b*) Moulting off rabbit

No. of mother tick	Date engorged larvae dropped off the host	Laboratory temperature (° F) Range	Mean	Date of larval moult into nymph	No. of larvae moulting	Pre-moulting period of larvae (days)
1	22.6	72–86	79·7	6.7	22	15
	22.6	72–86	79·5	7.7	19	16
	22.6	72–86	79·5	8.7	235	17
	22.6	72–86	79·4	9.7	109	18
	22.6	72–86	79·5	10.7	31	19
	22.6	72–86	79.8	11.7	160	20
	22.6	72–86	79·1	13.7	61	22
5	3.8	72–84	77·5	17.7	250	15
	3.8	72–84	77·6	18.7	344	16
	3.8	72–84	77·4	19.7	560	17
				Totals	(1,791)	(175); Av. 17·5

Table 1 (*b*) shows that during an average period of 17·5 days (range 15–22 days), 1,791 engorged larvae metamorphosed into nymphs off the host's body at the average laboratory temperatures of from 77·4° to 79·8° F. Most of the larvae, 795, metamorphosed into nymphs within 17 days; 363 moulted within 16 days, and the third largest number, 272, moulted in 15 days.

As already stated, such newly moulted nymphs, having hardened within 3–6 days, were also experimentally fed on the same rabbit. They engorged fully and fell off the host at the rates recorded in Table 2 (*a*). 101 nymphs fed fully and dropped off within an average of 7 days (range 4–10 days), when the mean laboratory temperature varied between 77° and 81·1° F. About 50 per cent of the engorged nymphs dropped off within 4 days and the next largest number of engorged nymphs (27) fell off in 5 days. It will be seen that 76 out of 101 of the engorged nymphs were collected in the morning.

Table 2. NYMPHAL STAGE: DURATION OF DEVELOPMENT OF IMMATURE STAGES OF *Boophilus decoloratus* (KOCH, 1844)

(*a*) Feeding on rabbit

No. of mother tick	Date of infesting host	Date engorged nymphs dropped off the host	No. of engorged nymphs dropping off a.m.	p.m.	Laboratory temperature (° F) Range	Mean	Duration of feeding of nymphs (days)
1	21.7	27.7	7	—	78–86	81·1	7
		28.7	6	—	78–86	81	8
		29.7	—	6	76–86	81	9
		1 8	1	—	76–86	80 6	10
5	24.8	27.8	31	19	70–82	77·3	4
		28 8	27	—	70–82	77	5
		29.8	4	—	70–82	77	6
		Total	(101)				(49), Av. 7

(*b*) Moulting off rabbit

No. of mother tick	Date of infesting host	Date engorged nymphs dropped off the host	Date engorged nymphs moulted into adults	No. of nymphs moulting	Laboratory temperature (° F) Range	Mean	Pre-moulting period of nymphs (days)
1	21.7	27.7	13.8	4	72–86	77·9	18
			14.8	11	72–86	77·9	19
			15.8	2	72–86	77 7	25
			16.8	4	72–86	77.3	29
5	24.8	27.8	17.9	30	70–84	77·3	22
			18 9	4	70–84	77·3	23
			19·9	24	70–84	77·4	24
			Total	(79)			(160) Av. 22·9

The average pre-moulting period for 79 engorged nymphs has been recorded in Table 2 (*b*) as 22·9 days (range 18–29 days), within the narrow average laboratory temperature of from 77·4° to 77·9° F. If the rather small number of four engorged nymphs that moulted off the host's body during the longest period of 29 days is ignored, it is clear that the largest quantity of nymphs (34) metamorphosed within 22–23 days at an average laboratory temperature of 77·3° F. Twenty-four moulted in 24 days at an average temperature of 77·4° F, while fifteen changed into adults during 18–19 days at 77·9° F. Only two moulted in 25 days at 77·7° F.

Twenty females from this stock of laboratory-bred adults having hardened in 3–5 days were placed on the ear of the rabbit in order to ascertain the average feeding period of these adult ticks. They attached to the experimental host within 3 h (unlike the larvae and nymphs which attached in less than 2 h) and fed slowly for 5 days. Without becoming even partially engorged, they detached on the fifth day and were collected in a polythene bag. It was thus not possible to obtain any conclusive information on the duration of the feeding period of the female tick.

Table 3. LARVAL TO NYMPHAL STAGE: DURATION OF DEVELOPMENT OF IMMATURE STAGES OF *Boophilus decoloratus* (KOCH, 1844)

(*a*) Feeding on rabbit

No. of mother tick	Date of infesting host	Date engorged nymphs dropped off the host	No. of engorged nymphs dropping off	Laboratory temperature (° F) Range	Mean	Duration of development from larvae to nymphs (days)
1	19 6	9.7	13	72–86	79·3	21
		10.7	19	72–86	79·4	22
		13.7	2	72–86	79·1	25
		Total	(34)			(68); Av. 22·7

(*b*) Moulting of nymphs off rabbit

No. of mother tick	Date engorged nymphs dropped off the host	Date of nymphal moult into adults	No. of nymphs moulting	Laboratory temperature (° F) Range	Mean	Pre-moulting period of nymphs into adults (days)
1	9.7	28.7	10	72–84	77·3	20
	10.7	29.7	18	72–84	77·1	21
	13.7	1.8	2	72–84	77·4	24
		Total	(30)			(65); Av. 21·7

(*Continued on page 77*)

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SCIENCE, MAN AND MORALS

Science, Man and Morals

By Dr. W. H. Thorpe. (Based upon the Freemantle Lectures delivered in Balliol College, Oxford, Trinity Term, 1963.) Pp. xii + 176. (London: Methuen and Co., Ltd., 1965.) 25*s*. net.

THE central themes of *Science, Man and Morals* are defined by the author as "the process of creation" and "the growing understanding of the unitary foundation underlying all experience—scientific, artistic and religious"; but these are dry bones which he brings to life.

Prof. Thorpe assembles an impressive array of evidence, mathematical as well as biological, to prove the fantastic improbability of the idea that the origin of life, or even the simultaneous formation of two or more molecules of any given enzyme, occurred purely by chance. Nor can the emergence and existence of mind be explained in terms of the laws of physics and chemistry. These laws must be obeyed but they do not require the self-consciousness which is the most convincing knowledge that we all have of ourselves. As a world authority on animal behaviour, Prof. Thorpe is eminently able to trace the development of social life and the importance of psychic factors in determining the evolution of human cultures. Moreover, in order to understand the beginnings of abstract ideas and of the language by means of which these may be expressed and communicated, the author has found it helpful to study animal communication, since in many ways the difference between the minds of men and of animals seems to be one of degree.

It is a far cry, however, from a scholarly discussion as to how early ideas came to include primitive ethical concepts, to the pressing modern ethical problems raised by the population explosion, artificial insemination, selective breeding, the welfare state, the uncontrolled exploitation of natural resources, personal problems such as chastity, monogamy and homosexuality and the immediate and imperative problems of aggression and war. Prof. Thorpe does not avoid any of these, but feeling, as he does, so strongly about aggression and war, one could have wished that he had given more than five pages to it.

The discussion of moral, as distinct from ethical, problems brings us into the domain of theology since "there can be no temptation to consider moral concepts as such as derivable from the scientific picture of the origin and development of the living world". Is there any necessary relationship between primitive magic—superstitious behaviour—and religious awareness? It seems not. Religious awareness appears to be a much later development of the search for understanding for its own sake. In spite of the complexities of the modern scientific world picture, the scientist remains obstinately convinced that he is dealing with a real, external world. Religious intuition, the direct knowledge of a world infinitely greater than, but in some sense sympathetic to, ourselves is more nearly akin to the approach of the artist than to that of the scientist, at least in so far as "artistic values are not just the expression of feelings and attitudes . . . but are in fact engendered in the process of establishing a relation with an objective reality transcending ourselves. . . . In theology, the mystical awareness of God as the whole, in some sense the unity of all things, is in effect the basis of all the fundamentals of belief".

The idea of wholeness and the related concept of perfection are shared by a number of religions, but the core of the teaching of Christ is that God is Love and that the greatest achievement of human life is self-effacing love. Moreover, that personality, so far from being a finite attribute of finite beings, is an ideal conception, "the highest category of unity in diversity which we know". "There is a fundamental Christian conviction that somehow there is a realm in which what ought to be is what is; . . . it is impossible to conceive moral judgments being made where there is no moral agent; and a moral agent implies personality."

This is a very inadequate summary of a book that covers a very wide field. It is extremely well documented and the "Notes" which include and amplify the references are well worth reading. The anecdotal illustrations are few but good, and perhaps a non-biological scientist may be excused for wishing that there could have been rather more of them and less quotation from other authorities, even though the quotations are always relevant. The part of the argument that comes through most forcibly is that in which the author expresses his own convictions: and conviction is usually caught, not taught.

KATHLEEN LONSDALE

BRITISH STATESMEN OF SCIENCE

Statesmen of Science

By J. G. Crowther. Pp. xiii + 391 + 10 plates. (London: The Cresset Press, 1965.) 42*s*. net.

THE present place of science and technology in national life has not been easily won. Nor has it been won by those of us now working comfortably in well-equipped laboratories. The victory, in so far as it has been a victory, was won by our predecessors, who showed by their working examples, against the odds, something of what science and technology could do for civilization. They battled with the prejudice of the classical establishment and with the complacency of manufacturers. Most of us to-day are too busy with research, teaching or administration to give much thought to the account of the battle; but this is a pity, for the account is of vital interest. Hitherto, it has had mainly to be sought among contemporary documents, but Mr. Crowther's newest book provides an easy and illuminating access.

Statesmen of Science is a sequence of concise biographies of nine of the principal figures in the battle during the past century and a half: Brougham, Grove, Playfair, Prince Albert, the Duke of Devonshire, Strange, Haldane, Tizard, and Lindemann. Not only are the nine portraits well drawn, but, in addition, around these central figures are ably sketched many others—including Anderson, Babbage, de la Beche, Birkbeck, Faraday, John Herschel, Huxley, Lockyer, Kelvin, Peel, Rayleigh and Roscoe.

Mr. Crowther tempts us to join him in a Baconian analysis of the common factors in the backgrounds of the nine principal figures. Brougham and Haldane, indeed, emulated Francis Bacon as Lord Chancellors who were interested in science and its promotion. Like them, Grove was a lawyer, but was also a practising scientist and the

inventor of the fuel cell. Brougham, Haldane and Playfair were educated in Scotland (Lindemann was at preparatory school there, too) while Strange was of Scottish blood. Playfair, Prince Albert, Haldane, Tizard and Lindemann received part of their education in Germany; and of the nine, Oxford can claim as undergraduates no more than two—Grove and Tizard—and Cambridge but one, the brilliant Duke of Devonshire, second wrangler and Smith's prizeman of 1829 and donor of the Cavendish Laboratory. Prince Albert, Strange, Haldane, Tizard and Lindemann all had direct concern with military affairs and the applications of science to war. Strange and Lindemann contributed to instrument design, and Grove, Playfair and Tizard to fuel technology.

What the analysis does suggest is that these statesmen of science had to draw much of their inspiration from outside England, even though in pure science England was making outstanding contributions. Mr. Crowther suggests that had Britain seen to it that its scientists of the nineteenth century should live and work "within an adequate scientific order", it might not to-day have become a second-class power, and that failure to create this order was "due to the inhibiting effect of principles that are inherent in capitalist society". Certainly we ought to have done much better than we have, especially when to the voices of science were added those of such humane distinction as Jowett, Matthew Arnold, Mark Pattison, the Webbs, and Robert Bridges. But is it clear that the halting response was due to capitalism? Why, if so, did science flower uniquely in a European culture? Is it capitalism that has contributed to the trend noted by Mill: "the general tendency of things throughout the world is to render mediocrity the ascendant power among mankind"? And Mill could say, even in his day: "The greatness of England is now all collective; individually small, we only appear capable of anything great by our habit of combining . . . But it was men of another stamp that made England what it has been; and men of another stamp will be needed to prevent it decline".

Alternative reasons to Mr. Crowther's can be found for the long apathy of Britain: one of much interest has been offered by Sir Peter Medawar in his sensitive Tizard Lecture on "Anglo Saxon Attitudes" (*Encounter*, August, 1965). But whether Mr. Crowther is right or not in his conjecture, he has given excellently fair portraits of even those of his subjects such as Lindemann, whose political views were quite different from his own. He has written with an appreciative eye for obscure but important detail, with an obvious regard for accuracy and balance, and with a sympathy that transcends politics. One of his heroes, Lyon Playfair, wrote in valediction, "I now feel that this work may be left to younger men, but I hope that they will not take their hands from the plough until England has adapted its system of education to the changed conditions of competition". Even now, there is still much to be done, and Mr. Crowther offers the present generation in science much insight into its ancestry and responsibilities.

R. V. JONES

ANCIENT CHINESE TECHNOLOGY

Science and Civilization in China

By Dr. Joseph Needham, with the collaboration of Dr. Wang Ling. Vol. 4: Physics and Physical Technology. Part 2: Mechanical Engineering. Pp. lv+759+plates 124–281. (London: Cambridge University Press, 1965.) 190*s*. net.

VOLUME 4, Part 2, of Needham's monumental *Science and Civilization in China* is concerned with what is called 'mechanical engineering' in the West. It is pre-scientific technology, however, for the theory of mechanics was unknown to the Chinese. They never arrived at an atomism and the idea of a mechanical particle did not arise in Chinese thought. Instead, they developed a kind of continuum conception similar to the *pneuma* of the Stoics; the living human body rather than the machine was the analogy in terms of which the Chinese saw the world. The continuum favours the study of wave motion and, in consequence, optics, acoustics and magnetism were the branches of physics best known in China. Mechanics was little studied and dynamics unknown. Thus, their engineering—although mechanical in the sense that it is concerned with levers, gears and simple machines based on their use—is, like all Chinese technology, pre-scientific. It is not the application of a theory but practical work arising from artisanship and designed for a specific job.

A vast range of implements is surveyed in this book, from levers and pulleys to clocks, power supplies and the kite. A wealth of detail is given and copiously illustrated; and the book is beautifully produced.

It may well be that more inventions than gunpowder, paper and the compass have come to the West from China though it is doubtful, I think, whether there was direct transmission. Similar devices must have arisen spontaneously and independently in many civilizations simply because they represent a practical means of coping with a problem. To assign priorities seems an almost impossible task; so much depends, too, on what item we are willing to accept as a legitimate precursor of a given device. The invention of a mechanical clock is generally credited to Europe and, in the first volume of the present work, Needham himself accepted this view; he feels now, however, that he must reject it. An astronomical clock tower was built by Su Sung in A.D. 1090. The clockwork, driven by a waterwheel, rotated an observational armillary sphere and a celestial globe; its time-announcing function was enhanced by visual and audible signals. Was this machine primarily a time-keeper or was it only a mechanized astronomical instrument? Such machines existed in Europe, too, before the mechanical clock was established.

Science and technology are the products of the mental and social evolution of mankind. This evolution is certainly a continuous and universal phenomenon; but this does not imply that every culture-area must have undergone the same development in the same period of time or reached the same level of achievement. If we want to understand the inter-cultural transmission of ideas, the first thing is to distinguish between science and technology and between the pre-scientific and the post-scientific stages of technical development. Science is not simply the product of practical work, of technology; nor is technology merely the application of science. Science and technology are separate and independent foundations, historically and in principle. The Chinese never passed beyond the pre-scientific stage of technology; until the Renaissance, however, they were well in advance of Europe in this respect. Post-scientific technology got going in Europe only in the eighteenth century and later; traditional practices prevail even to-day in some industries; and scientific methods are never fully exploited everywhere.

Certainly, the stimuli received from Asia and from mediaeval artisans contributed to the European breakthrough in the Renaissance. Certainly, this breakthrough was not merely a matter of re-reading the classical texts. From the point of view of evolution we have to say that a small group of scientists managed to attain a level of 'abstract' thinking that had not been available previously. It became possible only because European society as a whole managed, to some extent, to throw off religious and other dogmas and to arrive at a more progressive social order. The bureaucratic feudalism of China was not conducive to such a development. If we do not see these differences, we are liable to adopt the attitude of the Chinese gentleman who, when seeing an

aeroplane flying overhead, is reported (in this volume) to have remarked, "Ah, a man in a kite!"

E. H. HUTTEN

INVENTION OF THE CALCULUS

The Mathematical Works of Isaac Newton

Vol. 1. Assembled with an Introduction by Dr. Derek T. Whiteside. (The Sources of Science, No. 3.) Pp. xxi+160. (New York and London: Johnson Reprint Corporation, 1965.) n.p.

VOLUME 1 of *The Mathematical Works of Isaac Newton* is the first of two volumes which the Johnson Reprint Corporation have included in their series "The Sources of Science". It reproduces English translations of three Latin texts on which Newton's reputation as a creative mathematician in the calculus was originally founded. These are prefaced by a useful and scholarly introduction by Dr. Whiteside, who emphasizes that it is "the very depth, complexity, and disorganization" of Newton's mathematical papers which have prevented scholars from producing a satisfactory collected edition.

The first work chosen is the short tract entitled "De Analysi per Æquationes Numero Terminorum Infinitas", written in 1669 but not printed until 1729. The English rendering reprinted dates from 1745—John Stewart's "Sir Isaac Newton's Two Treatises of the Quadrature of Curves, and Analysis by Equations of an Infinite Number of Terms". Here is, as Newton says, "rather shortly explained, than accurately demonstrated" a general method for constructing series expansions of given functions, and then using them by simple integration to solve problems in the quadrature and rectification of curves. John Stewart added a "large Commentary, in which the Demonstrations are supplied where wanting, and the Doctrine illustrated, and the whole accommodated to the Capacities of Beginners for whom it is chiefly designed". The original tract had a restricted circulation but was not printed. Newton, later (1671), added it to an earlier tract on fluxions (October 1666), both subjects being amplified and revised. It was usually known by the title "Methodus Fluxionem et Serierum Infinitarum", and is the second of Newton's tracts to be reproduced, in its anonymous English version of 1737, entitled "A Treatise of the Method of Fluxions and Infinite Series, with its Application to the Geometry of Curve Lines". In spite of its obvious importance, Newton, even after several attempts, failed to find a printer. The third reproduction in this volume is an English translation of a treatise published by Newton in 1704 as a (Latin) appendix to his (English) *Opticks*. This is an article, apparently authorized by Newton, printed in the first scientific dictionary—John Harris's *Lexicon Technicum. Or, an Universal Dictionary of Arts and Sciences (1710)* and entitled: "Quadrature of Curves by Sir Isaac Newton".

The first and third tracts in this volume formed the basis for Newton's claim to priority in the dispute with Leibniz over the invention of the calculus.

The successful introduction of ideas of time and motion for the solution of problems in purely static, timeless, classical geometry was an act wholly alien to Greek thought, and only possible to a mathematical genius of another culture. Newton was, as he tells us, a great admirer of the Greeks, and chose to present the *Principia* in Euclidean form when he could have used an analytical approach. It is interesting, therefore, to note a certain disquiet which he expressed, rather obscurely, when he introduced his method of fluxions: "But since we do not consider the time here, any farther than as it is expounded and measured by an equable local motion; and besides whereas things only of the same kind can be compar'd together, and also their velocities of increase and decrease: therefore in what follows I shall have no regard to time formally consider'd, but shall suppose some one of the quantities propos'd, being of the same kind, to be increas'd by an equable Fluxion, to which the rest may be refer'd, as it were to time; and therefore by way of analogy it may not improperly receive the name of Time. Whenever therefore the word Time, occurs in what follows (which for the sake of perspicuity and distinction I have sometimes used), by that word I would not have it understood as if I meant Time in its formal acceptation, but only that other quantity, by the equable increase or fluxion whereof, Time is expounded and measured" (p. 49).

G. BURNISTON BROWN

STRUCTURE OF THE EARTH'S UPPER ATMOSPHERE

Auroral Phenomena

Experiments and Theory. Edited by Martin Walt. (First Lockheed Research Symposium on Space Science.) Pp. vi+170. (Stanford, California: Stanford University Press; London: Oxford University Press, 1965.) 40s. net.

THE ten papers comprising *Auroral Phenomena: Experiments and Theory* encompass theoretical and experimental aspects of aurorae, with emphasis on the latter. The classical techniques of spectrometry and photometry are represented as well as the sophistication attendant on radar, balloon and satellite experiments.

A most useful contribution by A. Omholt summarizes the proceedings of the symposium, setting out the currently available information and suggesting areas of further work. The notion of the aurora as a small part of a cosmic phenomenon taking place between the Sun and the Earth receives considerable attention, along with a discussion on the effects of bombardment of the upper atmosphere by energetic particles on ionospheric disturbances and atmospheric processes. In any event, auroral activity presents an opportunity to investigate atmospheric structure. One important outcome has been the optical determination of temperature as a function of height in the polar atmosphere. Mention is also made of auroral classification according to the excitation mechanism and the type of particles impinging on the atmosphere, rather than one based on geometry and spectral characteristics of the activity. Although satellites offer intensely interesting studies of auroral morphology, ground observations are by no means obsolete for they still are suitable for examinations of auroral details, in particular rapid pulsations and movements. The television film technique described elsewhere in the volume by T. N. Davis is a truly worth-while step in these investigations as the film can be examined at leisure and particular auroral features inspected quantitatively. The technique is extremely sensitive and an important aspect arising from it is that auroral microstructure researches are possible. An obvious extension of the scheme is to aurora and airglow spectroscopy.

So far as optical measurements of aurorae are concerned, A. Vallance Jones directs attention to the suggestion that whereas synoptic observations of the distribution of auroral emissions can be made with satellite-borne photometers, rocket-borne instrumentation is especially valuable for looking at the height distribution of diffuse auroral forms.

The interactions of energetic charged particles with the atmosphere are discussed by A. Dalgarno. Along with a contribution by J. W. Chamberlain on the present status of auroral theory, this comprises the theoretical content of the volume.

Balloon measurements of X-rays in the auroral zone are described by K. A. Anderson with the classification of X-ray bursts according to their association with geo-

magnetic activity, although it must be realized that such a classification might not be fundamentally related to the mechanisms producing the precipitation. The article by B. J. O'Brien on the precipitation of energetic particles into the atmosphere discusses the observational knowledge on the bombardment of the atmosphere by intense fluxes of charged particles, stressing in conclusion that the problem of the origin of the precipitated electrons is still not solved.

The extensive work on radio studies of the aurora is covered in the contribution by R. L. Leadabrand.

The very promising field of co-ordinated measurements on aurorae, for example the distribution of particle fluxes, optical luminosities, and ionospheric electron densities, is discussed by J. E. Evans. A combination of known techniques on a more intense scale than hitherto envisaged would undoubtedly contribute much towards a complete understanding of auroral processes.

The numerous diagrams are extremely well produced. However, it is unfortunate that no index is included.

A. H. JARRETT

RUSSIAN CLIMATOLOGY

Climates of the U.S.S.R.

By Prof. A. A. Borisov. Edited by Cyril A. Halsted. Translated by R. A. Ledward. Pp. xxi+255+2 plates. (Edinburgh and London: Oliver and Boyd, Ltd., 1965.) 75*s*.

A BOOK which can be read from cover to cover in half a day can be no more than a brief introduction to its subject; with that understanding, and with the further limitation that it covers climatology with little physics and no mathematics, we may welcome a useful translation of Prof. A. A. Borisov's 'educational manual' on *Climates of the U.S.S.R.*

There is an introductory chapter on solar and terrestrial radiation, certainly a good feature; on synoptic climate, tracks of depressions, frequency of air masses, the passage of fronts and so forth, very elementary but worth while; and on the so-called moisture cycle, handled in a way which I found confusing. This first chapter forms a 30-page background for the main part of the book, a pleasant enough mixture of the cartographical, statistical and discursive. The climatic elements for the country as a whole are taken in turn—air and soil temperature, humidity, cloudiness and sunshine, precipitation, evaporation, snow cover—followed by regional notes and statistics for some twenty regions.

Among the better things are the maps showing the climatic regions of the U.S.S.R., according to various classifications, including one following the Köppen system, although the explanations in the text are quite inadequate. The narrative contains miscellaneous information of many kinds providing something new for most readers. For example: "Three quarters of the Asiatic part of the U.S.S.R. is occupied by permanently frozen ground or permafrost" (p. 45); "present low level [of the Caspian] has not been exceeded during the last two centuries" (p. 168); "Barnaul lies within foothills that have become especially dry since the felling of the nearby forests" (p. 205).

The book, now it has been translated into English, will, of course, be examined by everyone remotely interested in the subject, and will be enjoyed unless the dialectic is found too irritating. It is a style not unknown among British geographers and explorers, only quasi-scientific, which often tends to obscurity; but it must be partly the added complication of translation which leads to the following maze: "The most essential factor introducing 'intrazonal' differences consists of macro-meteorological processes, which are characterised not only by their 'dynamism' and areal expression, but also by . . ." (p. 91).

Too many blemishes have indeed escaped the editor, but in providing a good bibliography and an alphabetical list of place-names with almost all their latitudes and longitudes, he has done splendid service. A few places are not so located, but one minor mystery can be resolved from the internal evidence. The highest temperature ever recorded in the U.S.S.R., 50° C, assigned to the unlocated Chu-Adzhi on p. 83, identifies that place with Uch-Adzhi which is twice, on pp. 41 and 179, credited with the like achievement. Is it a case of "what I tell you three times is true"?

R. C. SUTCLIFFE

THE CHALLENGE OF BRITAIN'S CHANGING CLIMATE

The Biological Significance of Climatic Changes in Britain

Edited by C. G. Johnson and L. P. Smith. (Proceedings of a Symposium held at the Royal Geographical Society, London, 29 and 30 October, 1964. Symposia of the Institute of Biology, No. 14.) Pp. x+222. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1965. Published for the Institute of Biology.) 42*s*.

AFTER an introductory account of the dimensions of change in the climate of Britain, by H. H. Lamb, *The Biological Significance of Climatic Changes in Britain* is divided into three sections: "The Effects of Climatic Change and their Implications"; "The Manipulation of Materials and Selection of Sites"; and "Perspectives of the Future". Despite variations in quality and aim from paper to paper, the volume is on the whole a stimulating one, though the discussions are often disappointing, sometimes serving simply to underline an unresolved disparity of approach between the contributors from different disciplines.

Two papers on fluctuations in marine communities, one by D. J. Crisp, the other by R. J. H. Beverton and A. J. Lee, are particularly interesting. The first is concerned with inter-tidal molluscs, the second mainly with economically important fish populations. By way of contrast, and lest anyone be tempted to accept simple interpretations of the influence of climatic change on terrestrial productivity, F. L. Milthorpe outlines the many problems posed by any attempt at a quantitative analysis of the relationship between growth or yield and environmental factors. The paper by J. P. Hudson proceeds from a brief practical account of the effect of different types of growing season on different varieties of one and the same crop to a discussion of the potential value of weather forecasts to the farmer or horticulturalist. He emphasizes the value of forecasts 3 to 5 months ahead of the growing season, at the time when crops and particular varieties are being chosen for planting. J. P. Cooper describes what are apparently genecotypic variations in seasonal performance between strains of single forage species, each strain obtained from a different source area. Further appreciation of the physiological and genetic basis for the variations in growth response with source area promises to provide a way of deriving new varieties suitable for special habitats; in the same way, selectively changing biotypes could also help to meet the challenge posed by changes in climate.

After the cautious approach of these and other papers, a return to some of the earlier contributions may come as a mild shock. The interpretation by J. A. Taylor of vegetational changes in parts of Cardiganshire seems very questionable, while his summary of Post-glacial ecological history in upland Wales is also open to serious challenge in the light of recent evidence. The fact is that despite

the elegance with which the experimental biologist demonstrates the subtlety of the relationship between changing environment and plant or animal response, even under the necessarily simplified conditions of his laboratory, most of us are still all too ready both to propose simple climatic explanations for observed biological variations and to rely heavily on evidence from fossil plant and animal remains for quantitative indications of past climatic change. Other lines of evidence, especially for pre-instrumental variations and for very long-term changes, are unfortunately still scarce. However, if it is accepted that we need to know more about long-term climatic variation, and that biological evidence will inevitably contribute to the analysis, then a more critical use of the results in the light of recent work in physiology, experimental taxonomy and genetics is required. In this way, we should perhaps move further away from the dangers of circular argument inherent in so much palaeoclimatology and nearer to providing a factual framework within which the genuinely accurate begins to replace the spuriously precise. Meanwhile, we should take note of the following comment in the paper by Beverton and Lee: "It is deceptively easy to deduce causal connections between two sets of fluctuating data, especially when their reliability and true relationship, if any, are unknown". FRANK OLDFIELD

GEOCHRONOLOGY

Applied Geochronology

By E. I. Hamilton. With a chapter on Comparative Geochemistry by L. H. Ahrens. Pp. xiv+267. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1965.) 59*s*.

THE observation of radiogenic argon-40 in potassium-bearing minerals by Aldrich and Nier in 1948 heralded a new era in geochronology. The seventeen brief years which have elapsed since then have seen the establishment of routine analytical techniques for the dating of rocks and their minerals by the potassium–argon, rubidium–strontium and uranium–thorium–lead methods. Progress has been so rapid, in fact, that a considerable gap has appeared between practising geochronologists and the main body of geologists. It was Hamilton's purpose to help bridge this gap with *Applied Geochronology*.

Each major method has a chapter to itself, with the rubidium–strontium and common-lead techniques receiving the most detailed attention. The book opens with a useful, brief chapter on comparative geochemistry by L. H. Ahrens, followed by a historical sketch of the development of geochronology up to 1945.

Chapter 2 is a review of general methods. The techniques of stable isotope dilution and mass spectrometry form the backbone of geochronology and receive detailed description. However, it should be noticed that the two principal equations of isotope dilution are stated incorrectly and there are several minor errors in the account of vacuum technology.

An extensive summary of the various methods of potassium and argon analysis is given. The section on potassium will be found particularly useful. Since the original measurements of Aldrich and Nier, tremendous strides have been taken, and this method is now being used to date rocks in the age range 30 thousand years to 4·5 billion years. Hamilton rightly emphasizes the contribution of Reynolds with his pioneering applications of the ultra-high-vacuum method. This chapter ends with a short discussion of the problems of interpretation of potassium–argon ages obtained on minerals from igneous, metamorphic and sedimentary rocks.

The principles of the potassium–argon method are immediately apparent to the geologist. The rubidium–strontium, uranium–thorium–lead and common-lead approaches, however, are in general not so readily digested, and each one makes a strong appeal to graphical representation. These three topics are dealt with in Chapters 5, 7 and 9 and occupy almost half the book. Until 1958, it had been usual to examine separated minerals with the rubidium–strontium method. Then, in rapid succession, Schreiner in South Africa and Compston and Jeffery in Australia extended their efforts to include samples of whole rocks. It was clearly demonstrated that it is often a risky business to date a rock solely by examination of its separated constituent minerals. Mild tectonic disturbances readily cause the migration of isotopes in and out of minerals to yield anomalous mineral 'ages'. However, in a very large number of cases the average diffusion length is sufficiently small to allow volumes of the whole rock to remain effectively closed systems. Compston and Jeffery emphasized that not only the age of emplacement, but also the initial strontium-87/strontium-86 isotope ratio may be experimentally determined by whole rock analysis. Hamilton devotes much space to a consideration of their graphical representation of whole rock and mineral data. His treatment of this presentation is, however, not so clear as his account of the graphical plot developed by Nicolaysen. The application of the whole rock method to the dating of sediments is discussed and is illustrated by reference to the work on the Hamilton Shales by Whitney and Hurley. Finally, the petrogenetic significance of strontium isotope ratios is illustrated.

The interpretation of uranium–thorium–lead ages is based on the work of Ahrens, Wetherill and Tilton. Ahrens observed that suites of minerals yielding discordant lead ages produced a straight line when their lead-206/uranium-238 ratios were plotted against the corresponding lead-207/uranium-235 values. It was then shown by Wetherill that such a pattern would result if the minerals were all formed simultaneously and remained completely closed systems apart from undergoing a single period of lead loss during their history. He further showed that the two points of intersection of the straight line with the 'concordia' curve gave the times of mineral formation and metamorphism. Tilton then proved that a similar pattern (over a wide range) ensues if, in fact, lead has been continuously diffusing from the crystals throughout their existence. These various arguments are discussed and illustrated with examples from the literature.

Forty pages are devoted to the intricacies of interpretation of common lead isotope abundances. This is a fascinating topic, intimately bound up with the questions of the age of the Earth, the modes of formation of ore bodies, the growth of continents and the uniformity of the mantle. Hamilton reviews the somewhat contrasting theories of Gerling, Holmes and Houtermans, on the one hand, and Alpher and Herman, and Cumming, Russell and Farquhar on the other. One wonders how many geologists will have the persistence to absorb the whole of this chapter. Their way will be somewhat straitened by the errors in some of the equations, but those who make the effort will be amply rewarded by the challenge to sit back and produce their own interpretations.

Applied Geochronology will certainly acquaint the geologist with the tremendous variety of methods available for determining geological ages. Apart from those mentioned in some detail in the foregoing, Hamilton discusses techniques involving carbon-14, rhenium and osmium, ionium and thorium and radiation damage.

The book also contains a wealth of useful references and deals in a pleasing way with work done in the U.S.S.R.

The geological reader, however, will be disconcerted by the imprecise style and the unusually large number of errors. Equations, numerical values of quantities and details of mass spectrometric technique are particularly ill-treated. The book is well produced and bound and the price is reasonable. DEREK YORK

GEOLOGICAL STOCKTAKING IN SCOTLAND

The Geology of Scotland

Edited by Gordon Y. Craig. Pp. xv+556. (Edinburgh and London: Oliver and Boyd, Ltd., 1965.) 105*s*.

IN his preface to *The Geology of Scotland*, the editor gives a short selective history covering the past 150 years, during which this book may be said to have evolved. After mentioning Hutton he refers to Ami Boué's *Essai Géologique sur l'Ecosse* of 1820 and to Nicol's *Guide to the Geology of Scotland* of 1844, an illustrated work with an up-to-date geological map, a revised version of Macculloch's 1836 map. Nicol's publishers then were Oliver and Boyd, who are the publishers now, 120 years later, of the present imposing volume.

However, just a century after Macculloch's death in 1835, a new geology of Scotland, in all but title, was issued in five parts of *British Regional Geology*. These books from the Geological Survey cover all Scotland and present a well-balanced account of the geology accompanied by some candid remarks on the divergent opinions it aroused. Then, at the International Geological Congress in 1948, came an essay entitled "The Structural History of Scotland", by E. B. Bailey (*Intern. Geol. Congress, Report of the XVIII Session*, 230–255; 1950). This summarized progress and views held up to the end of the Second World War. The present volume continues from there, utilizing the results of research carried out during the past two decades involving the routine use of current bedding technique and the geometry of folds, foliation, concepts in metamorphism and deductions drawn from analyses of radioactive isotopes in rock-forming minerals. Some of these exercises require the acceptance and comprehension of more theory than did any of the publications before this epoch.

Prof. Neville George has written an introductory chapter which covers the long period of some 2,600 m.y. between the early Lewisian and the present time. He uses as check points eight tectonic episodes dated by radioactive work. They are as follows: (1) deformation and metamorphism of Lewisian rocks before the Scourie pegmatites *c*. 2,600–2,250 m.y. ago; (2) deformation and metamorphism of Lewisian rocks after the Scourie basic dykes beginning *c*. 1,600 m.y. ago; (3) early metamorphism of the Moines *c*. 740 m.y. ago; (4) Caledonian folding of the Dalradians followed by metamorphism *c*. 440 m.y. ago; (5) later metamorphism of the Moines *c*. 390 m.y. ago; (6) 'main' Caledonian movements including the Moine thrust; (7) 'main' Hercynian movements *c*. 270 m.y. ago; (8) later alpine movements 25–10 m.y. ago.

Some discussion of the column of strata between the check points is given. Scotland is also divided by convenient faults into five geographical blocks, the Southern Uplands, the Midland Valley, the Caledonian Fold-belt with its orderly metamorphic zones split in two by the Great Glen fault, and the Foreland, west of the Moine thrust. Here the Lewisian, traversed by shatter bands of mylonite, is well exposed and here the unmetamorphosed Torridonian west of the thrust can be compared with the Moines east of the thrust, reputed to be their metamorphosed equivalent, carried here from the east by thrusts. The Caledonian Fold-belt is occupied by the Moines and the Dalradian. Although they occur together the latter, probably unconformably, overlies the former. Complex structures affect both groups, but are more difficult to unravel in the monotonous marker-starved Moines than in the Dalradians with their diversified stratigraphy. The folds and over-folds affected by faults are the result of four phases of folding. The whole Fold-belt is pierced by many granitic intrusions and migmatization affects some areas. South of the Highland Boundary fault the Dalradians are covered by the Old Red Sandstone which, further south, rests unconformably on thick packets of shales and greywackes of Ordovician and Silurian ages. These Lower Palaeozoics have been arched up by compression and wrinkled into small asymmetrical folds or faulted folds. In much of the Midland Valley, Carboniferous and Permian lie in open folds. They are much faulted and fill basement-controlled basins. Round the coasts there is a fringe of Mesozoic formations including relics of Cretaceous which with their cover of Tertiary volcanics are also folded and faulted. They show that restlessness continued until late in, if not all through, Alpine time. Towards the end Prof. George allows himself the luxury of 'imaginative reconstruction' in picturing the development of the scenery since a late Tertiary uplift. By then much of the erupted lava and the dykes cutting it had been eroded and a wave-cut surface formed. It was probably warped as it rose. Its crest may have been in the north-west, anywhere beyond the present watershed; possibly so far west as the Outer Hebrides. As rivers gathered and flowed down-slope they produced a group of roughly parallel valleys discharging eastwards. As time went on and tributaries formed they found north-east–south-west fault planes easy to follow and their valleys were eroded more rapidly than those of the primary rivers. In consequence piracy occurred and some of the primary rivers were beheaded to form a network of streams derived from the intersection of the primary and secondary rivers. When the Ice Age came the ice opened and deepened these north-east–south-west fault zones of which the Great Glen is the type and the Minch possibly another example.

The Geology of Scotland is well illustrated, the diagrams clear and instructive. One figure (6.13) may strike a controversial note, as some readers may prefer a section sketched by Sir James Hall in 1812 (Bailey, E. B., *op. cit.*, p. 230) to that by Dr. Walton in 1963 to illustrate Southern Upland structures. The hallowed ground of the Tertiary Volcanic districts, formerly the precinct of J. E. Richey, is described and augmented with an excellent account of recent surveys in Skye and Rum. The volume is, in every way, a worthy member of a long sequence of distinguished works on Scotland. Fashions will doubtless change in the future as they have done in the past and a time will come when another version will be written, say in A.D. 2015 or even A.D. 2065, when more enigmas will have been discussed and left to the future awaiting a final answer.

J. V. HARRISON

THE PHYSICS OF CRYSTALS

Crystal Physics

By G. S. Zhdanov. Translated and edited by Dr. A. F. Brown. Pp. ix+500. (Edinburgh and London: Oliver and Boyd, Ltd., 1965.) 80*s*. net.

CRYSTAL physics has become a branch of science of interest to workers in many disciplines, and as materials, crystals now assume a special importance in technology since they may possess special mechanical, electrical, magnetic or optical properties. The investigation of crystalline media relates the various physical properties of crystalline solids to chemical composition and atomic structure, in addition to covering methods of crystal production and investigating their behaviour and properties over a wide range of physical conditions. *Crystal Physics*, translated from the Russian by Dr. A. F. Brown, of the University of Edinburgh, attempts to give a unified account of this vast subject which will be of interest to all pure and applied scientists with specialist interests in this field.

A knowledge of crystal physics must be based on an understanding of the atomic and electron structure of matter together with the forces of interatomic and intermolecular interaction, and the author has used this as the underlying theme of his book. In the introductory chapters the results of quantum mechanics, which are of particular application in solid-state physics, are estab-

lished and a concise account is given of the structure of the atom and the consequent build-up of the periodic table. There follows a description of the quantum theory of interatomic and intermolecular interactions, and of the basic types of chemical bond. This introductory information is well presented, although much of it may already be familiar to readers of this type of book.

Crystals are introduced by a consideration of the symmetry of atoms, molecules and crystals, and crystal chemistry; the forces of interaction between particles, which lead to the formation of crystals, and the electron theory of crystals, which is fundamental in determining and explaining crystal properties, also receive very adequate treatment. The book concludes with a detailed consideration of the many properties of crystals which are of particular interest in solid-state physics and materials science.

In a work of this type it is inevitable that certain aspects of the subject tend to be omitted, and in *Crystal Physics* this applies to the optical and thin film behaviour of crystals. The book, which is exceptionally well produced, is based on a series of lectures given by Dr. Zhdanov to postgraduate students some ten years ago, and although written primarily for research workers it is unfortunate that few references to original papers are given and that the subject-matter is apt to be out of date regarding the most recent advances in this field.

Problems are given at the end of most chapters and these are solved by the author in an appendix to the book. In view of a shortage of comprehensive books on this topic it should prove extremely useful as a source of information and an introduction to more advanced reading.

D. T. TURNBULL

PLASTIC FLOW AND FRACTURE —A COMPREHENSIVE APPROACH

The Strength, Fracture and Fatigue of Materials

By Takeo Yokobori. Translated by Shigeru Matsuo and Michiko Inoue. Edited by John D. C. Crisp. Pp. 372. (Groningen: P. Noordhoff, Ltd., 1965.) 48.50 D.fl.

THE Strength, Fracture and Fatigue of Metals is a deceptively slim volume, since it contains in its 372 pages the distilled observations and theories of most of the leading workers in the field of fracture during the past 50 years. References are made to more than four hundred authors and a similar number of papers or books on the subject.

In spite of the lapse of 10 years since the original Japanese edition was published, this translation can still fulfil the aims of the author in providing a comprehensive correlation of the many aspects (atomistic, microstructural, macroscopic and statistical) present in the investigation of the problems of yielding, plastic flow, fracture and fatigue.

The early part of the book, after some general considerations including comments on scatter and rate processes, briefly introduces the dislocation theory. Many readers not familiar with this concept might prefer to have this chapter expanded but references give the reader access to original work.

Yielding, the theory of plastic flow, and fracture are next considered in the sequence of their normal occurrence. Some six types of fracture are described and the following chapters deal with four of them—brittle fracture, ductile fracture, fatigue fracture and creep fracture. The section covering creep fracture is limited in extent but in his preface the author promises a further book devoted to that topic.

Nearly half the book is concerned with fatigue. The various stages of fracture are described and later the theories are examined in the light of these observations. Much of this forms a useful critical review, but since the object of the book is to provide a fundamental approach to a much wider subject it is unfortunate that the earlier topics are not so adequately covered.

In collating work from diverse fields there have obviously been problems of reconciliation of the symbols and terms used. In most cases this appears to have been satisfactorily accomplished though some additional definitions at the end might be helpful. In the fatigue section some confusion appears to remain in the use of the terms 'range of stress' and 'amplitude' and also in the condition of 'understress' when related to coaxing.

Some of the correlation from theory to practice appears to be based on limited evidence as presented, but nevertheless it bears out a point made towards the end of the book that "in order to develop the solid state physics . . . in a proper way, it is necessary to give physical and microstructural meanings to the probabilistic factors".

J. F. ALDER

THE FUEL CELL

An Introduction to Fuel Cells

Edited by Keith R. Williams. Pp. xiv+329. (Amsterdam, London and New York: Elsevier Publishing Company, 1966.) 90*s*.

OF recent years, interest in the production of electrical energy by means of fuel cells has been stimulated both by the recognition that we are approaching the limits of efficiency of heat engines in respect to the temperatures permissible in their working parts, and also by the demands for a generator suitable for space research in which financial considerations play but a minor part. This interest has been reflected in the appearance of a large number of articles and a few monographs on various aspects of the subject.

Under the too modest title of *An Introduction to Fuel Cells*, Dr. Williams has presented us with a volume that is remarkable not only for the completeness of the systematic survey of the various types of fuel cells and the theories as to their mode of operation, but also for the clarity of presentation.

The types of fuel cells available are conveniently grouped into those operating at ambient temperatures and under suitable pressure up to 200° C, fused salt electrolyte cells with working temperatures of 350°–800° C, and finally those with high-temperature solid oxide electrolytes. As might be anticipated, cells with aqueous or fused salt electrolytes have received most attention.

From an operational point of view, fuels and oxidants both soluble in the electrolyte are the simplest, and these include such substances as ammonia, hydrazine and methyl alcohol as fuels and hydrogen peroxide or chlorates as oxidants. It is those cells in which gaseous fuels, especially hydrogen and gaseous hydrocarbons, and air as oxidant are used which are of most interest.

It is clear that the conversion of hydrogen to protons at the electrode surface involves a series of steps, any of which may be rate-determining, dependent on the conditions of operation, and the separate factors such as gaseous diffusion, concentration and activation polarization, chemisorption as well as the magnitude of exchange currents are now reasonably well understood, although the picture is by no means complete. On the other hand, our knowledge of the *modus operandi* of the oxygen electrode is far less satisfactory, for the reaction which involves conversion of oxygen into hydroxyl ions is more complex than is the case for hydrogen. It would appear from experiments on catalytic oxidation that surface oxides are first formed; these can be reduced by water, which in turn is converted into hydroxyl ions. Frequently equations involving two electron transfers are used. It seems preferable to regard the transfer as proceeding by steps of single electron transfer, that is, $O^{2-}{}_2 \rightarrow O^{-1}{}_2 +$

$e \rightarrow O_2 + e$; thus permitting the intermediary alternative step $O^{-1}{}_2 \rightarrow O^{-1} + O + e$.

Investigations of the behaviour of both cathode and anode separately under different conditions of operation of the cell have given us a great deal of information on the catalytic properties of different electrode materials on this all-important electron transfer process.

Since hydrogen is relatively expensive compared with the lighter hydrocarbons or carbon monoxide, the most economical cell in fuel costs would utilize methane or a light hydrocarbon or carbon monoxide. The products of combustion of these fuels include carbon dioxide as well as water, and thus restrictions are placed on the type of electrolyte that can be used.

It seems probable that the reactions of the hydrocarbon at the electrode surface involves a series of catalytic steps, a reforming reaction $CH_4 + H_2O \rightleftarrows CO + 3H_2$ followed by the water gas shift reaction $CO + H_2O \rightleftarrows CO_2 + H_2$, the electrode behaving, in fact, as a hydrogen electrode. Both of the foregoing reactions are susceptible to the influence of appropriate catalysts. Again carbon formation may take place, not only by a cracking reaction, for example, $CH_4 \rightarrow C + 2H_2$, but also by the reaction $2CO \rightleftarrows CO_2 + C$. The thermodynamic data on these reactions which form the basis of fuel technology are well established so the most suitable steam fuel ratio to be used at any given temperature is known.

The most desirable electrode evidently should be an effective catalyst for these basic reactions as well as for electron transfer. I have always had an affection for a hypothetical carbon monoxide air cell in which electron transfer is facilitated by the presence of a *p*-type semiconductor. Self-poisoning of the electrode, a phenomenon common to reactions in which intermediary oxidation products may be formed, has to be avoided.

The practical aspects of cell construction involve consideration of porous electrodes, diaphragms, ionic semipermeable membranes and porous fillings for the interelectrode space.

Dr. Williams has included all this material in his book, together with some interesting information on practical cells which have been constructed in the laboratories of several firms as well as a valuable chapter on the economics of fuel cells in general.

The book is well documented. It does not contain many errors. I cannot commend it too highly for those interested in one of the technological challenges of our time, the fuel cell. ERIC RIDEAL

VACUUM TECHNOLOGY IN GERMANY

Vacuum Technique

(Proceedings of a Meeting of the German Society for Vacuum Technique, Heidelberg, September 18–21, 1962.) Pp. vi+94. (London and New York: Pergamon Press, Ltd., 1965.) 70*s*. net.

EDITED by Prof. G. Günterschulze, *Vacuum Technique* is a handsomely produced book which costs ninepence per page of technical information, most of which has been reported elsewhere and, moreover, is published nearly three years after the conference took place. The book contains seventeen papers, three of which are in English and the remainder in German. The practice of holding a conference concerned with a limited number of papers and yet dealing with vacuum technique and its applications in the broad sense is of doubtful value especially when, as in the present case, there is no central theme at all. Such restricted meetings are probably best confined to a special aspect of this very wide field of science and technology. It is even more doubtful whether it is worth publishing the proceedings in full in an expensive book because most readers will only be interested in the detail of one or two of the contributions. However, to indicate to the potential buyer the value of this costly book a summary follows of its contents.

The papers in English comprise an excellent review by Steckelmacher entitled "Methods of Proving the Gas Tightness of Vacuum Equipment and Components", a paper emphasizing the application of ejector pumps by Savini entitled "Pumping Systems for Freeze-drying Plants" and "Sources of Surface Contamination in Vacuum Evaporation Systems" by Holland.

The papers in German commence with a report of the opening address by Klumb on the present state and tendencies in the development of vacuum technique. The following contributions are on the possibilities of the erection of vacuum apparatus from constructional components (Winkler); constructional details and results obtained with a commercial plant for leak-detection of articles by a radioactive technique using krypton-85 (Uhlemann); mass spectrometer investigations of the gas evolution from plastics (Thieme); the significance of negative ions in residual gas analysis (Ahrens); the production of an electrode-less discharge by the use of concentrated microwaves (Geerk, Kleinwächter and Metze); a universal apparatus for the evaporation of electron microscope preparations and optical glasses (Serwatzky); limitations to the use of vacuum in the preparation of insulating oils for electro-technology (Stoll); the industrial measurement and regulation of vacuum (Schink); the production of microwave tubes by the use of getter-ion pumps (Stolz); metallization in high vacuum of bands of material with special regard to pre- and post-handling (Berisch); a new arrangement for the production of thermal gas streams and the measurement of radiometer forces (Krupp, Robens, Sandstede and Walter); measurement of the sorption, evaporation and decomposition of vacuum materials with an electromagnetic microbalance (Robens, E., Robens, G., and Sandstede); and the application of permanent magnets in vacuum technique (Brinkmann). J. YARWOOD

A LAYMAN'S GUIDE TO THE ATOM

Explaining the Atom

By Prof. Selig Hecht. New edition, with revisions and additional material by Dr. Eugene Rabinowitch. Pp. xviii+237. (London: Victor Gollancz, Ltd., 1965.) 18*s*. net.

"THIS book is for the complete layman", wrote the American author in 1946 as he moulded into book form five lectures for an audience most of whom had had no training in science. Three atomic bombs had been exploded and the Smyth report had been published; Congress had passed a Bill providing the death penalty for giving away the secret of the atomic bomb. Prof. Hecht set out, with very many other scientists, to dissipate ignorance and inform the public of the elementary principles relating to the release of atomic energy and, as Prof. Rabinowitch writes in the foreword to the revised edition of *Explaining the Atom*, he "brought to the campaign his unique gift for clear, simple, and convincing exposition of science".

The result is a most readable book which went into many printings in the United States; it deals briefly with the whole story of the development of atomic theory from the days of Dalton to the end of the nineteenth century when the electron emerged from the 'solid' atom, and then continues with descriptions of the salient experiments from which nuclear theory evolved.

This story has been told, of course, in many British books for the layman and for junior readers, and Hecht's book adds nothing except eminent readability. He seems rather surprised at the excitement created among physi-

cists by the announcement of the discovery by Hahn and Strassman of fission and Miss Meitner's explanation thereof, but the discovery came after four years of searching for the explanation of Fermi's transuranic elements and during that time not one of the great captains of physics, neither Bohr, nor Rutherford, nor others, had even considered the possibility of fission and the great release of energy which must accompany such disintegration. Those who lived through that period will never forget the intense excitement and speculation which occurred almost within days of the news; bombs and power stations at last appeared technically possible. This Hecht explains.

The author died in 1947 and Prof. Rabinowitch revised the book in 1954, recounting again the sad story of the attempts to establish international control of atomic weapons, and also describes the fusion of hydrogen into helium and its application in 'super-bombs'. Although *Explaining the Atom* has had some further revision, the peaceful applications of atomic energy are not adequately dealt with and there is little to indicate the vast strides which have been made in the past decade.

The illustrations do not match up to the excellent presentation of the facts. There are too few, some are drawn with errors, and when reference is made to them in the text, the page reference is not given so time is wasted searching for the appropriate illustration.

T. E. ALLIBONE

REDOX POLYMERS

Oxidation-Reduction Polymers

(Redox Polymers). By Harold G. Cassidy and Kenneth A. Kun. (Polymer Reviews, Vol. 11.) Pp. xv+307. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1965.) 95*s*.

OXIDATION-Reduction Polymers, the most recent addition to the well-known "Polymer Reviews" series of books dealing with individual topics in polymer science, is concerned with a relatively new polymer type containing chemical groupings which participate in electron exchange phenomena.

After a brief general review of the field, the synthesis of these polymers is discussed. The two major approaches are considered separately, namely the polymerization of a suitable monomer which contains appropriate redox groups or the chemical attachment of redox groups on a preformed polymer. To the former approach there are many obstacles, since the redox centres may be expected to interfere with the free radical, ionic or condensation polymerization. Individual examples are discussed in detail and techniques for blocking reactive groupings described so that the polymerizations are unaffected by inhibition or transfer reactions. The second approach of modifying a prepared polymer may be achieved by precipitating or adsorbing the redox material in an inert matrix, or by chemically bonding it to the polymer backbone.

The succeeding chapter gives a concise account of oxidation-reduction theory and the setting up of electrochemical cells for the measurement of electrode potentials. The special problems associated with insoluble redox resins are dealt with and also the use of mediators to overcome them. Typical titration curves for single substances and for mixtures are described and their interpretation discussed in detail.

The largest chapter in the book concerns the measurement of the properties of redox polymers. The principal difficulties of measurement are connected with the high molecular weight and hence relative insolubility of these systems. There is no discussion of the measurement of the usual polymer properties, the main interest lying in the electrical properties and microstructure. The apparatus used for carrying out potentiometric titrations is described in detail, together with that necessary for the control of ambient conditions. In this connexion, it is of the utmost importance that reagents and solvents should be prepared in a standard way, and special difficulties arise since non-aqueous media must on occasion be used. It is also necessary to be able to define the micro properties of these polymers in terms of surface area, pore size, pore size distribution and pore structure, and here a number of conventional techniques with appropriate modifications can be applied. The measurement of redox capacity of a resin is also dealt with.

The properties of individual redox polymers are dealt with in a classified chapter in which polymers are separated according to mode of preparation, solubility and composition of the functional groups. The physical, optical and electrical properties are detailed and discussed in the light of the general theories elaborated earlier in the book.

The final section of the book deals with the applications of oxidation-reduction polymers in ion-exchange; in utilizing the self-buffering action of appropriate copolymers; as catalysts for the formation of hydrogen peroxide; for the de-oxygenation of water and reduction of boiler corrosion; for water purification and waste disposal as well as more specialized uses in biological studies; chemotherapy; photography; electric battery construction and as a dielectric. The authors also speculate on further uses of these substances.

In addition to the references which are provided for each chapter, there is an extensive annual bibliography dealing with not only works and articles but also patent applications. These references are also annotated in such a way as to provide a better indication of the content of individual items. The book is well illustrated where appropriate with diagrams and figures.

This specialized text will appeal to anyone engaged in the study of redox polymers and to those in the general field of polymerization who wish to get a clear, concise account of the special properties of these substances. As a source-book it will find much use by those who wish to extend present understanding and use of oxidation-reduction polymers.

JAMES C. ROBB

AERODYNAMIC AESTHETICS

Theory of Optimum Aerodynamic Shapes

Extremal Problems in the Aerodynamics of Supersonic, Hypersonic, and Free-Molecular Flows. Edited by Prof. Angelo Miele. (Applied Mathematics and Mechanics: An International Series of Monographs, Vol. 9.) Pp. xix+455. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 132*s*.

APPLIED mathematicians approach their work in different ways. One way is to master and develop a particular mathematical technique and then apply it to as many physical problems as possible. Prof. Angelo Miele has followed this path with distinction; he is an authority on variational methods which he has applied to an extremely wide range of optimization problems in the fields of aerodynamics, flight mechanics and astronautics.

In *Theory of Optimum Aerodynamic Shapes* Prof. Miele restricts his attention to the determination of the external shape of bodies, subject to various geometrical constraints, giving minimum drag in certain flight régimes. At supersonic speeds optimum shapes of two-dimensional wings and bodies of revolution are discussed on the basis of linear and second-order aerodynamic theories, taking into account skin friction effects; optimum shapes for rocket nozzles are also described. At hypersonic speeds similar problems are solved assuming first the Newtonian pressure law and then the Newton–Busemann pressure law. A short section is concerned with the optimum-shaped body of revolution in free molecular flow.

The presentation of all this information is somewhat unorthodox. As an introduction, the first four chapters

are devoted to a lucid and rigorous review of the elements of the calculus of variations. The remainder of the book is a collection of twenty-five important papers published in recent years; about half these papers are by Prof. Miele and his associates, the rest are by well-known authors including R. T. Jones, C. Ferrari, G. V. R. Rao, K. G. Guderley, W. D. Hayes, A. J. Eggers and G. G. Chernyi. All the papers, each designated as a separate chapter, have been extensively edited to ensure uniformity of formulation, standardization of notation and consistency of style—a creditable achievement.

Unfortunately groups of these papers are so closely related in content that for each paper to be treated separately leads to a mathematical sameness and an extremely slow development of the main points of interest. For example, six chapters, or papers, are concerned with the determination of the optimum shapes of bodies of revolution in Newtonian hypersonic flow; different initial assumptions are made in each case: slenderness is first assumed without skin friction, then with constant skin friction coefficient, then with variable skin friction coefficient and then the exercise is essentially repeated for non-slender bodies. There is insufficient mathematical difference between these various aspects to warrant separate chapters. From a practical point of view each chapter in this sub-group is made obsolete by the slightly more generality of the following chapter. The ultimate disillusionment comes in the next section in the book where it is established that bodies with non-circular cross-sections can have lower drags than optimum bodies of revolution. Some discussion of the philosophy of the formulation of optimal problems would have been welcome.

This final point leads on to a further comment. Only those problems are discussed which can be formulated within the framework of the calculus of variations. Thus the brilliant concept of the Busemann supersonic biplane is not mentioned. Neither is there any reference to the vast volume of literature on the transonic and supersonic 'area' rule techniques for minimizing drag of wing-body combinations. It might be argued by Prof. Miele that his terms of reference are not intended to cover such topics, but nevertheless the title of the book does suggest considerable generality.

In conclusion, recognizing the book as a text on the calculus of variations with some applications to the determination of optimum aerodynamic shapes, it is a valuable contribution to aeronautical literature. But it is not so comprehensive as the title implies. In my opinion it could have been condensed with, one hopes, a corresponding reduction in price. G. J. HANCOCK

OPERATORS AND TRANSFORMS

The Dynamics of Linear and Non-Linear Systems

By P. Naslin. Translated from the French. Pp. xxvii+586. (London and Glasgow: Blackie and Son, Ltd., 1965.) 105*s*. net.

IT is now many years since Gustav Doetch showed that the Heaviside operational calculus could be given formal justification in terms of the Laplace transform, which is now accepted as the appropriate tool for solving problems concerning linear systems set in motion at $t = 0$, the subsequent history of which is to be determined. As such it now features in the curriculum of almost all engineering courses of degree or comparable standard.

It therefore seems a pity that the author of *The Dynamics of Linear and Non-Linear Systems* relegates it to a brief appendix. At one place he objects that it involves initial conditions, at another that it is too powerful a tool for the job to be done.

When an input and an output function are connected by a linear differential equation with constant coefficients he replaces d/dt by p and expresses the ratio output to input as a rational function of p which he calls the transmittance. It includes both impedances and admittances when the quantities are electrical.

In a note on the symbols s and p he says that s has the specific meaning of the complex variable of Laplace transform theory, whereas p simply stands for the operator d/dt and thus has a purely symbolic value. The author is right that the transmittance is not a Laplace transform, but it seems just as much a misuse of terms to call it, as he does, a 'p-transform'. Equally to regard p as meaning simply d/dt is absurd when the poles and zeros of a transmittance are considered.

The only logical point of view is that the transmittance is the transmission ratio when the input and output depend on time through the exponential factor exp pt. This justifies at once replacing d/dt by p and the differential equation may be recovered by reversing the steps. The steady-state transmission ratio is obtained by setting $p = j\omega$, and, replacing p by s or not, the transmittance function coincides with the Laplace transform of the output when the input is a unit impulse.

Linear systems are represented by block diagrams. Thus if a battery e.m.f., E, is the input to a simple potential divider with resistances R_1 and R_2 in series, the output being the potential v across R_2, this is represented by applying E through an adder to a multiplier $1/R_1$ which produces the current i. This applied to a second multiplier R_2 gives v, while a feed-back path with multiplier -1 runs from v back to the adder so that $E-v$ is actually applied to the first multiplier.

Instead of multipliers there may be integrators, differentiators or more complicated systems. The block diagram may be modified in a variety of ways without altering its overall transmittance. It may be arranged so that only adders, multipliers and integrators are used when it gives the lay-out for an analogue computer simulation of the system. Its use is illustrated in a variety of examples always with application to control systems in mind. The block diagram is easily converted to a signal flow graph for which Mason's formula expresses the transmittance in terms of the transmittances of open paths and closed loops on the graph.

Transients and the sinusoidal steady-state are discussed very thoroughly in close relation to each other and to stability and damping. There is a short chapter on sampled data systems.

The later chapters treat non-linear systems but the emphasis is still on situations where linear theory can be applied: first, the so-called describing function procedure when the effects of harmonics above the first may be neglected, and secondly, phase plane methods applied mainly to problems in which different linear approximations may be used in different ranges of the variables.

In this part of the book some of the figures are not so accurate as they purport to be, possibly through being copied by a draughtsman unfamiliar with the problems represented. The layout of the text and figures is attractive, but for a rather large number of trivial printing errors such as a symbol missing from a formula.

In one respect there is a curiously old-fashioned air about this book. Laborious step-by-step graphical and tabular solutions are described, sometimes with the comment that all the calculations were done on a slide rule. Surely, in these days, every design office concerned with this type of problem has a digital computer available, and some work on numerical analysis features in all engineering courses so that it should scarcely be necessary to devote a final chapter to describing the simplest Euler and modified Euler methods of solving differential equations.

Despite its faults, this book contains a lot of useful and interesting material particularly relevant to the design of control systems. A. W. GILLIES

LECTURE NOTE VOLUMES

Brandeis Summer Institute in Theoretical Physics

Vol. 1: Lectures on Strong and Electromagnetic Interactions. By P. T. Matthews, D. R. Yennie and M. E. Mayer. Pp. iii+343. 24*s*. Vol. 2: Lectures on Astrophysics and Weak Interactions. By S. Hayakawa, H. Y. Chiu, G. Feinberg and M. Dresden. Pp. v+471. 32*s*. 6*d*. (Waltham, Mass.: Brandeis University. Distributed by Gordon and Breach, New York, and Blackie and Son, London, 1964.)

IN the quickly developing areas of science, such as those to which *Lectures on Strong and Electromagnetic Interactions* and *Lectures on Astrophysics and Weak Interactions* offer contributions, the pressure for shorter and shorter publication times for more and more material has extended from the research journals to the text-books; hence the spate of 'lecture note volumes', as they are called. Notes of lectures at the various summer schools are written up either by the lecturer himself, or more commonly by a student attending the school, and published as quickly as possible, usually in paper-back and usually with a minimal amount of editing. Their purpose must be considered primarily didactic. As the editor of the volumes reviewed here claims, "These notes should provide a valuable introduction to areas of contemporary research interest".

A "valuable introduction" for whom? All three articles of Volume 1 would make little sense to a reader without at least some familiarity with the ideas of elementary quantum field theory. Given this background, the notes of the lectures by Matthews provide a readable introduction to the S-matrix and its uses in strong interaction physics. There are also useful sections on applications to low-energy π–N and $\bar{K}$–N scattering, the determination of the quantum numbers of resonances and high-energy scattering. I am not so confident of the usefulness of the notes of the lectures by Meyer, which purport to be an introduction to the SU_3 symmetry scheme of strongly interacting particles. The lectures seem to have been a review very much for the experts; terms such as "Weyl's branching theorem", "Casimir operator", "Okubo's form for the mass matrix" are bandied about without being adequately explained. More readable introductions to the SU_3 scheme now exist in print which cover essentially the same material. Yennie's article is perhaps more specialized and detailed than the other two articles of Volume 1, but certainly no less readable, nor requiring any more background knowledge. It gives an exposition of the techniques associated with the author's name for making the Lamb shift calculations, and an account of the treatment of the infra-red divergence problem and radiative corrections to high-energy scattering.

There are even more variations in the assumed background knowledge of the readers of the various contributions to Volume 2. The title of the lecture notes of Chiu, "Neutrino Astrophysics", is a little misleading. They are divided into two parts and only the second deals at all with neutrino processes, stellar collapse and neutron stars. I can recommend the first part as a very readable brief introduction to the ideas of astrophysicists on stellar sizes, stellar structure, nuclear reactions in stars and stellar evolution. Much of the material requires as background no more than an honours degree course in physics. The other astrophysics article, by Hayakawa on "The Origin of Cosmic Rays", precedes that of Chiu in the volume. However, it would not be intelligible to anyone who has not already a fair familiarity with the ideas presented in Chiu's article. The other two articles in Volume 2 are for specialists in elementary particle physics. That of Feinberg is largely on the intermediate vector boson theory of weak interaction processes, its field theoretic formulation, and possible effects of higher order graphs. The article of Dresden is a very complete and carefully written account of theoretical speculations on the possible existence of particles obeying neither Bose–Einstein nor Fermi–Dirac statistics.

One might well question the wisdom of the continuing publication of these lecture note volumes. They are not books. For, in the interests of speed, there is no attempt on the part of the editor to make uniformity either in lay-out or notation or even in method of referencing. In the second volume even the ordering of the articles seems inappropriate. In all but two cases, the lecturers did not even write their own contributions. In the foreword the editor points out that two of the lecturers "generously devoted time to reviewing the work of their notetakers, but have not undertaken extensive revisions". It seems to me a question not of the generosity of the lecturer but of his responsibility to ensure that whatever is published under his name is as accurately as possible what he wants to say. The editor says that "the notes are intended to be informal presentations, not formal monographs". But the informality of a presentation certainly does not add to its clarity, if it entails, as it seems so often to do in these volumes, undefined notation, carelessly stated principles and badly labelled diagrams. In spite of it all, some things come through and some of the articles will be useful to some people.

J. E. PATON

SPECIAL CERAMICS

Special Ceramics 1964

Edited by P. Popper. (Proceedings of a Symposium held by the British Ceramic Research Association.) Pp. xii+341. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1965. Published for the British Ceramic Research Association.) 90*s*.

THE term ceramics, be they special or not, includes a vast range of solid materials. In the past few years the definition, which originally included only materials based on clay, has been widened progressively so that we now readily accept such statements as "In materials there exist some that are neither metals nor elements nor polymers, these are ceramics". *Special Ceramics 1964* indicates that even this is not wide enough and 'polymers' must be limited to purely organic polymers, since we find included three papers dealing with inorganic polymers. There are twenty-two papers in all and, with the vast range of topics available, it is scarcely surprising that they are as heterogeneous as some of the materials they describe. Although the majority of authors are British, there are also contributions from continental Europe, the United States and Australia. Generally speaking, this is not a book for the theoretician, but rather for the practical scientist who wants to know how to make things, how and where to apply them, what their important properties are, and how to measure them.

Topics are divided roughly into six sections: preparation of non-oxides; pyrolytics and inorganic polymers; oxides; properties; fabrication techniques; and applications. The first section has one paper on the preparation of new high-temperature materials for atomic energy usage, another in which modern theory is applied to an old sintering problem, and the third is a structure analysis of zirconium oxycarbides. The first two of the second group of papers deal with the technologically very interesting formation of hard, impervious, inert layers of silicon carbide on various substrates. The second paper, on inorganic polymers, contains much introductory and background information and, I feel, could well have come first in the section. The other two deal with the preparation of specific polymers. The four papers in the oxide group consist of one on the preparation of high-purity powders, another on the problem of alumina analysis, a third on the improvement of high-temperature structural material by metal infiltration,

and a final paper on preparation and properties of spinel. There are five papers dealing with properties. Three concern electrical properties. Under electrical stress, especially at elevated temperatures, ions from electrodes and from the ceramic material itself migrate, causing rapid degradation of electrical characteristics. This important commercial topic is dealt with at length. The other two papers are concerned with thermal conductivity and friction characteristics of some special compounds. Of the three fabrication papers, two discuss hot-pressing techniques, and the third electron beam melting. In two papers on applications, emphasis is placed on the use of ceramics in high-performance gas-turbine and jet engines.

Most of the papers are short and the inclusion of numerous photographs, figures, graphs and good abstracts makes for easy reading. The British Ceramic Research Association is to be congratulated for producing this useful book, and for playing their full part in leading ceramics out of the empirical wilderness. J. H. MANNING

CHEMISTRY OF NATURAL PRODUCTS

Recent Developments in the Chemistry of Natural Carbon Compounds

Vol. 1. Edited by R. Bognár, V. Bruckner and G. Fodor. Pp. 319. (Budapest: Akadémiai Kiadó, Publishing House of the Hungarian Academy of Sciences, 1965.) 14.70 dollars.

THE Chemical Department of the Hungarian Academy of Sciences has set itself the task of publishing a series of monographs on natural products, dealing mainly with the elucidation of structures. It is claimed as an individual feature of the series that it will collect for the first time relevant work of Hungarian chemists and offer to the English-speaking reader material that may only have been accessible previously with difficulty.

Recent Developments in the Chemistry of Natural Carbon Compounds, the first volume in the series, comprises three monographs by distinguished authors. About half the book (146 pp.) is taken up by a very detailed, lively and critical review by G. Fodor entitled "New Methods and Recent Developments of the Stereochemistry of Ephedrine, Pyrrolizidine, Granatane and Tropane Alkaloids". The major part of this monograph concerns, of course, the tropane alkaloids which have been so thoroughly investigated by Fodor and his collaborators, who have published much of their work in the *Journal of the Chemical Society*. The second monograph (70 pp.), entitled "Relationships between the Structure and Pharmacological Activity of Tropeines", by K. Nádor, continues the alkaloidal theme and is complementary in a sense to that of Fodor. It discusses parasympatholytic, ganglion-blocking, and local anaesthetic activities of tropeines, and the curare activity of *bis*-quaternary tropeines; biological activities relative to those of standard compounds are given in convenient tabular form. The third monograph (85 pp.) entitled "Achievements in the Total Synthesis of Natural Steroids", by the Russian author, I. V. Torgov, is a more general and objective review of the methods that have been developed for the total synthesis of steroids. All the important routes are discussed in logical order, but, as there have been no Hungarian contributions to this field which is already well known to English-speaking readers, the reason for the inclusion of this admirably succinct contribution in the present volume is not clear.

The book is well produced and the standard of the English is quite high, but it suffers from two defects. In the first place, though published as recently as 1965, the three monographs would appear to have been written about 1960 and such references as are given to later work appear as footnotes. Secondly, the analytical contents list at the beginning of the book is scarcely adequate to serve as an index to the very considerable amount of factual information contained between its covers. In spite of these minor shortcomings the book can be recommended for stimulating accounts of the three selected topics. J. WALKER

VALENCE THEORY

Valence Theory

By Prof. J. N. Murrell, S. F. A. Kettle and Prof. J. M. Tedder. Pp. xiii+401. (London and New York: John Wiley and Sons, 1965.) 50*s*.

DURING the past thirty years, many, many books have been written on the subject of valence theory and its quantum mechanical basis. Some set out to explain the basic theoretical methods and to show how wave functions may be determined and used. Others aim to give a qualitative picture of the results of quantum mechanics as applied to molecular structure and to consider the application of these to a wide range of actual molecular examples. Because of the dangers of falling between two stools, it is particularly essential in this field for an author or authors to have a clear idea of who they are writing for. There are so many alternative approaches and needs that to attempt all may easily be to achieve nothing.

One great difficulty is, of course, that there are many chemists who need to have as good a grasp as possible of the ideas and results of quantum mechanics but who, because of the many other things they must learn, have only limited, and usually it must be accepted, rather inadequate, time to devote to it. Different teachers of valence theory would take different views as to how much it is reasonable to expect such experimental chemists to assimilate and how best to avoid the dangers of partial knowledge and limited understanding, but none, I think, would deny the greatness of the problem.

Murrell, Kettle and Tedder have in fact set themselves the very difficult task of trying to bridge the gap between the two types of approach as illustrated by those excellent books *Valence*, by C. A. Coulson, in which the approach is semi-qualitative, and Eyring, Walter and Kimball's more formal *Quantum Chemistry*. They have produced a book which will be valuable to experimental and semi-theoretical chemists, whether they are interested in inorganic or in organic chemistry. There is a short introductory section dealing with such topics as the hydrogen atom and basic principles of valence theory. There follow a few chapters dealing with the mathematical foundation, symmetry and angular momentum. By this stage we are about one-third of the way through the book. Next there come chapters dealing with molecular orbital and valence bond theory, first in diatomic and then in polyatomic molecules. More specialized chapters follow which are devoted to ligand field theory, electron-deficient molecules, π-electron theory, induction, hyperconjugation, reactions in organic chemistry and weak chemical bonds. *Valence Theory* is written to be used by university students, and problems are included throughout; useful hints on their solution are given at the end of the book together with answers.

The material is presented clearly and the printing of formulae, tables and diagrams is of a high standard. The only worry I have is that, in attempting to 'fill in the gap', the authors have at times involved themselves in fairly sudden changes in the difficulty of the material presented. For example, there are many who will find the change from the chapter entitled "Basic Principles" to the one entitled "Mathematical Foundations" quite

difficult to manage. It is to be hoped that they will not be scared into proceeding no further; this would be a pity because such readers will find, if they go on, that later chapters are very useful indeed.

One could not say, with all honesty, that one welcomed all the books that had been written on valence theory, quantum theory for chemists, etc. However, with *Valence Theory*, it is possible to say with sincerity that it is a most welcome book. It provides a reasonable coverage in a reasonable space starting with the elements of the subject and proceeding well on into the more difficult parts. It does this clearly and is well produced. The authors must be congratulated on producing a book which has so much of value in it and which is so well suited to the needs of chemistry students in many British and other universities.

J. W. LINNETT

ASPECTS OF POLYMER CHEMISTRY

Analysis and Fractionation of Polymers

Edited by John Mitchell, jun., and Fred W. Billmeyer, jun. (American Chemical Society Symposia held at Chicago, September 2–3, 1964. *Journal of Polymer Science*, Part C, Polymer Symposia, No. 8.) Pp. vi+314. (New York: Interscience Publishers, a Division of John Wiley and Sons, Inc., 1965.) 96*s*.

ANALYSIS and Fractionation of Polymers contains papers presented at symposia on "Analysis of High Polymers" and "Characterization and Fractionation of Polymers", held at the American Chemical Society meeting in Chicago in September 1964. Some of the papers are written in general form and may be regarded as being in the nature of reviews; other papers are devoted to new information on specific polymer systems. Pyrolysis gives information on polymer composition as well as on kinetics of polymer degradation, and the first group of papers describe pyrolytic techniques, with emphasis on methods of detection of products. This is followed by a description of the use of high-resolution differential thermal analysis and the new technique of dynamic electro-thermal analysis. The next series of papers is devoted to recent applications of infra-red spectroscopy and, in particular, to fluorine-containing polymers and polyacrolein. Other papers describe the use of tritium or carbon-14, and viscosity methods in the study of polyacrolein. The application of high-resolution nuclear magnetic resonance spectroscopy to the investigation of fluorine-containing polymers, polypropylenes and relaxation phenomena are described in detail in succeeding papers.

The second half of the volume is devoted to the topic of the fractionation of polymers and their characterization in terms of molecular weight. The first two papers on molecular weight distribution and methods of fractionation provide valuable and up-to-date reviews of these subjects. The following papers deal with different aspects of solution-gradient electron fractionation and the theory, practice and instruments for gel-permeation chromatography. The remaining papers cover various topics such as the interpretation of molecular weight distribution data, the bulk fractionation of linear polyethylene, the determination of molecular weight distribution by sedimentation velocity, ultracentrifugation and the use of light-scattering techniques.

The papers not only summarize 'the state of the art' regarding the more familiar methods but also describe some of the newer techniques of analysis, characterization and fractionation. The volume will be of considerable interest and usefulness to those interested in these subjects.

C. E. H. BAWN

LIGNIN BIOCHEMISTRY

Lignin Biochemistry

By Walter J. Schubert. Pp. ix+131. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 8 dollars.

NATURE has defied chemists in their attempts to write a single structural formula for the lignin molecule, and present-day ideas on the structure are usually related to a 'statistical' model incorporating a variety of phenyl-propane derived units linked together in different ways to take account of the known analytical data. The pulp and paper industries have been even less successful in their efforts to find profitable outlets for the vast quantities of lignin-derived waste which at present only represents an often embarrassingly large disposal problem. The microbiological utilization of lignin and its derivatives would appear to be a worth-while field of study for both chemists interested in lignin structure and for industries faced with the problem of its disposal. However, to date, such research has provided little other than corroborative information on lignin structure and no practical help to engineers concerned with the disposal of pulping wastes.

Dr. Schubert and his colleagues have been studying the biosynthesis and the fungal degradation of lignin for several years and *Lignin Biochemistry* is based largely on the work of the Fordham University school.

The book is arranged in four chapters. Chapter 1, occupying approximately one-third of the book, deals with the chemistry of lignin, a knowledge of which is essential to an understanding of its biosynthesis and degradation. The field is covered quite comprehensively and some of the more valuable techniques and many of the difficulties facing the lignin chemist are described.

Chapter 2 discusses aromatization in micro-organisms and is a much less full treatment of the subject than that given to the chemistry of lignin. The shikimic acid pathway in bacteria is briefly dealt with and its application to the biosynthesis of tyrosine and phenylalanine described. The biosynthesis of aromatic metabolites by wood-destroying fungi is then discussed in detail. However, the significance of this to the aromatization process in plants and to lignin biosynthesis is not obvious and could have been dealt with much more briefly.

The biosynthesis of lignin in plants is covered in Chapter 3. Tracer studies have established the biosynthetic routes to the lignin 'building stones' but the final stage in lignin synthesis, the polymerization of these precursors within the plant cell wall, is still a matter for conjecture. Freudenberg's hypothesis that soft-wood lignin is synthesized from coniferyl alcohol by dehydrogenation and free radical polymerization is discussed and its limitations pointed out.

The final chapter deals with the microbiological degradation of lignin. The emphasis is very much on the part played in the lignin cycle by wood-destroying fungi, other organisms being almost entirely ignored. Lignin degradation by these fungi is considered to be an oxidative process and the difference between laccase and phenolase, the two commonest oxidative enzymes associated with these fungi, is clearly described. However, while the importance of laccase and phenolase in the classification of wood-destroying fungi is emphasized, their over-riding importance in lignin degradation has yet to be established, a point which is not so clearly brought out.

The book is very well produced, but at 8 dollars for 111 pages of text and eight of references, it is expensive and the cost may well limit its appeal. To the non-specialist it offers an introduction in some depth to a complex subject but the specialist reader may find that as a review it suffers from being too personal an account with a consequent imbalance and incompleteness in places.

N. J. KING

DIAZONIUM AND KINDRED ORGANIC COMPOUNDS

Methoden der Organischen Chemie

Von Houben/Weyl. Vierte völlig neu gestaltete Auflage. Herausgegeben von Eugen Müller. Band 10, Teil 3: Stickstoffverbindungen, 1/3. Herausgegeben von Rudolf Stroh. Pp. xliii+971. (Stuttgart: Georg Thieme Verlag, 1965.) 255 D.M.

IN the new edition of *Methoden der Organischen Chemie*, two volumes, 10 and 11, are devoted to the preparation and reactions of organic nitrogen compounds. The work reviewed here is Part 3 of Volume 10, and its publication has preceded that of Parts 1 and 2 of this volume. Parts 1 and 2 of Volume 11 have already appeared: Part 1 deals solely with amines and Part 2 with alkylinimines, amino-acids, lactams, quaternary ammonium salts, and nitrogen–sulphur compounds.

Part 3 of Volume 10 is a substantial work of 971 pages, of which almost the first half is devoted to the two most important chapters, namely, those on aromatic diazonium salts (212 pp.) and on diarylazo compounds (254 pp.) respectively. The first chapter deals in detail with the preparation of diazonium salts from amines by diazotization under various conditions, according to the nature of the amine used, and by other methods such as oxidation of aromatic hydrazines or reduction of aryl nitramines: a short section deals with the diazotization of heterocyclic amines. The second part of the chapter deals with the reactions of diazonium salts with a large number of reagents under a variety of conditions and thus illustrates fully their synthetic applications, other than those discussed later in the book.

The second chapter, on diarylazo compounds, describes the formation of these compounds by the coupling of diazonium salts again with a wide variety of compounds, with the special conditions which the nature and position of substituents in these compounds may make necessary. Other reactions are surveyed, and a very interesting discussion is given of the types of complexes which diarylazo compounds, with suitable substituents to form chelating groups, may form with metallic salts, particularly with those of chromium, cobalt and nickel.

This very concise summary of these two important chapters cannot do justice to their contents. It must be emphasized that in this volume, as in others in the series, an exhaustive and up-to-date account is given of almost all recorded synthetic methods, both of the main compounds and the products to which they can give rise, and this account includes experimental details for various typical preparations, and tables listing the known examples of the compounds prepared by particular reactions, the whole accompanied by a wealth of references. Each of these two chapters could well be published as a separate book, and each would be of considerable value to the organic chemist.

Other shorter sections deal with aromatic–aliphatic azo- and azoxy-compounds, aryl hydrazones, formazanes, aromatic triazenes and higher azo homologues, aromatic azoxy compounds, organic azido compounds, and nitrile oxides.

It is noteworthy that in this volume, as in earlier ones, the potentially dangerous nature of a compound is emphasized, as in "Das (*cancerogene*) β-Naphthylamine".

This volume will be invaluable to chemists engaged mainly in synthetic work involving aromatic nitrogen compounds, and will be of particular interest to those in the dyestuffs industry.

The excellent presentation and printing of the text are of the same very high standard as that of earlier volumes.

F. G. MANN

PROTEINS AND THE ALIMENTARY TRACT

The Role of the Gastrointestinal Tract in Protein Metabolism

Edited by H. N. Munro. (A Symposium organized by the Council for International Organizations of Medical Sciences, established under the joint auspices of UNESCO and WHO.) Pp. xviii+402. (Oxford: Blackwell Scientific Publications, 1964.) 75*s*. net.

THE Role of the Gastrointestinal Tract in Protein Metabolism presents the twenty-five papers delivered at a three-day international symposium held in Glasgow in August 1963. The objective of the meeting was "to provide a comprehensive picture of the role of the gastrointestinal tract in protein metabolism". To this end the subject was divided into six sections, but all thirty-four participants listened to each paper and this ensured a lively multi-disciplinary discussion which is faithfully reported in the book.

Section 1 deals with the digestion of proteins and has papers on factors affecting transfer rates along the gastrointestinal tract, on the heterogeneity of gastric proteinases and on the effect of soybean trypsin inhibitor on amino-acid availability.

Section 2 has five papers on the origin, magnitude and nutritional significance of endogenous secretion in non-ruminants. There was general agreement that such secretion is equivalent to a significant proportion of the normal dietary protein intake and therefore of great importance under conditions either of dietary inadequacy or of pathological failure to reabsorb the protein secreted into the gut. There was less unanimity on the suggested homeostatic function of endogenous nitrogen secretion in maintaining a uniform concentration and pattern of amino-acids for absorption.

Section 3 extends this discussion to ruminants in which the obligatory loss of nitrogen in faeces is shown to be a major determinant of nitrogen requirements. On the other hand, microbial activity in ruminants may actually enhance the value of dietary protein by amino-acid supplementation. It was suggested in the discussion that because the intestinal flora is quantitatively less important in healthy non-ruminants this does not exclude the flora assuming disproportionate importance under conditions of malnutrition and in the pathogenesis of the malabsorption syndrome.

Section 4 deals with protein digestion in relation to nutrition. It has a paper showing that the gastrointestinal hypertrophy of lactation is related to the increased intake of food. At the other end of the nutritional scale were three papers dealing with the biochemical and morphological changes in the gastrointestinal tract in experimental and clinical protein–calorie deficiency. A fifth paper reviews modern work on the diet–host relationships of the digestive flora, particularly in relationship to the use of antibiotics as growth stimulants.

Section 5 has four papers on the absorption of amino-acids, including fundamental studies on the mechanism of absorption and the practical possibility of using variations in portal amino-acid concentrations to investigate factors in feeds and rations affecting the digestion and absorption of dietary protein.

Section 6 deals with protein metabolism in subjects with enteropathies and other alimentary diseases, and has four papers on the progress being made in differentiating the metabolic lesions in diseases with common symptoms and end results.

Some of the papers represent the polished products of years of research; some are more in the nature of stop-press articles or progress reports. This, together with the discussion, makes for lively reading which can easily be extended because of the adequate treatment of references

and the efficient cross-indexing provided by the editor. The price is modest in relation to the central importance of the subject to anyone interested in the health or disease of animals or man. C. R. C. HEARD

GENES AND HISTONES

The Molecular Biology of Development

By James Bonner. Pp. vi+155. (Oxford: Clarendon Press; London: Oxford University Press, 1965.) 25*s*. net.

THE Molecular Biology of Development, written during the author's tenure of an Eastman visiting professorship at Oxford during 1963–64, is for the most part a résumé of several papers from his own laboratory. Its racy, colloquial style reads like, as it probably is, a transcript of the lectures he gave during this assignment. It certainly conveys his vivid enthusiasm for his thesis, which is that the histones are the natural gene inhibitors so that if the gene is covered with histones it is repressed, while if it is uncovered it is active. This is, of course, an attractive hypothesis and James Bonner makes the most of the limited evidence which can at present be adduced. A more cautious and critical survey of the evidence and possibilities would have been more useful.

Prof. Bonner states that his object is to apply the "framework of molecular biology" to the biological problems which remain to be solved. "It is my thesis", he says, "that we can now profitably go back and re-examine what it is that makes the differences between the different kinds of cells of a higher organism." The book may do good in directing the attention of research students to an enormous field of study which is just becoming accessible to investigation, but in which, although a few clues have been obtained, reliable signposts are still obscure. The fact is that the hypothesis by itself is not enough to explain the activity or inactivity of genes. It would be necessary in addition to explain what puts histones into their proper places and takes them off when required. The author has little to say on this, except for some mention of the possible intervention of hormones and other substances.

Although the enthusiasm is there, the facts are often presented in a slipshod and sometimes misleading fashion, and students would be well advised to supplement their reading by other sources. For example, the account given of the fractionations of histones in our laboratories can only be described as a travesty of the facts; three distinct methods are mixed up and as presented would certainly not work. Prof. Bonner would probably say that he did not aim at giving a precise account of these fractionation methods. But rather than a misleading account of details it would be better to say simply, as he indeed concludes, that "a variety of methods lead to the separation from calf thymus histone of four principal fractions".

Another example of loose reporting is the attribution to "Murray, 1964" of information on the *N*-terminal groups of histones. In fact all that K. Murray states in the paper quoted is that "Determinations of the N-terminal groups of the various histone fractions also indicate that the fractions are not homogeneous". Prof. Bonner's conclusion is "there is an interesting lack of heterogeneity amongst the histones with respect to the N-terminal groups". Conceivably both these statements hold some truth, depending on the point of view, but it is not reasonable to put forward a paper in which the only statement on the subject is that quoted first as a justification of the second. The fact that the information so far obtained is largely due to D. M. P. Phillips is not mentioned.

Readers who expect to learn much about development will be disappointed as the chapter on this subject (Chapter 12) is highly hypothetical and consists of a translation in 'computer language' of a sequence of processes involved in bud growth. The whole mechanism is described in a flight of fancy as "Digital organ generator Model A (DOGMA)". Evidently Prof. Bonner also has some doubts, since he concludes, "we do not yet know if the approach will be a fruitful one". J. A. V. BUTLER

PENICILLINS

The Penicillin Group of Drugs

By Prof. Gordon T. Stewart. Pp. xii+212. (Amsterdam, London and New York: Elsevier Publishing Company, 1965.) 55*s*.

THE discovery of penicillin is one of the few that deserve the hackneyed description 'epoch-making'. Fleming's original work in 1929 and the later development by Chain and Florey literally marked the beginning of a new era in the history of man. This antibiotic era has already profoundly changed the whole practice of medicine, but the special fascination of the penicillin group of drugs lies in their unique combination of medical and scientific interest. Prof. Stewart has tried, as he puts it, "pulling some of the strings together", and *The Penicillin Group of Drugs* is intended to illustrate "a continuing interaction of intellectual and practical endeavour".

The aim of the book then is clearly conceived, and the author has shown imagination and enthusiasm in defining it. But the achievement does not match the conception and the result is rather disappointing.

After a short preface setting out the purpose of the author in writing the book, the first three chapters deal with the history of the penicillins. Then follow chapters on the acid-stable penicillins, a chapter comparing the oral penicillins, and then one on methicillin. Three chapters are devoted to the mode of action of penicillins, and separate single chapters to toxicity (and pharmacology), resistance, allergy and epidemiology. The final chapter is on the cephalosporins.

The historical chapters are perhaps the best in the book. They are interestingly written, though sometimes in a semi-popular and rather dramatic style, and they give a good account of the various stages in the development of the different penicillins. But after these, in the chapters dealing with individual penicillins, the departure from chronological order has no advantages and something nearer the historical order of their discovery would have been in fact better. The isoxazole penicillins come more logically after methicillin than as part of a section on oral penicillins, since their penicillinase stability is a far more important reason for their use than their acid-stability, though this gives them one (sometimes doubtful) advantage over methicillin.

As regards the factual content of the book, there are many examples of facts incorrectly or ambiguously stated. On page 48, the statement that up to 50 per cent of strains of *E. coli* in some situations may form amidase is misleading and probably inaccurate. On p. 72 it is stated that methicillin has a high affinity for staphylococcal β-lactamase, but on page 145 it is stated to have a low affinity. On page 105 it is stated that the lactam-dihydrothiazine ring of 7-aminocephalosporanic acid is intrinsically more stable to most forms of β-lactamase than the lactam–thiazolidine (ring) of 6-aminopenicillanic acid. This is a loose and misleading statement: with some strains the reverse is true.

There are numerous mis-statements due to misprints, mis-spellings or verbal mistakes. For example, on page 149 the part played by *Trichophyton* species in protecting hedgehogs from infection by staphylococci (except by penicillinase-producing strains) is attributed to their ability to produce penicillinase. What is really meant is their ability to produce penicillin or a penicillin-like substance, as is correctly stated on page 161. On pages

92 and 107 'spheroblasts' should be 'spheroplasts'. On pages 38 and 39, in Formulae 3 and 4, for isoxazole penicillins R should be 'C_6H_4Cl', not 'Cl'. In Table 8 on page 100, the formulae of benzyl penicillin, methicillin, oxacillin and penicillin *N* are wrongly given. These and numerous other small misprints should be corrected in any future edition.

The title of the book alone is bound to appeal to many different types of reader, precisely because its subject-matter covers such a wide range of medical and scientific interests. In this sense the author has attempted to meet a real need, but because of its general and rather superficial nature the book steers a somewhat uncertain course between the extremes of an authoritative treatise on the one hand and a semi-popular essay on the other.

R. KNOX

CONTROL OF BACTERIAL DISEASES

The Battle Against Bacteria

A History of the Development of Antibacterial Drugs, for the General Reader. By P. E. Baldry. Pp. ix+102+8 plates. (London: Cambridge University Press, 1965.) 22*s*. 6*d*. net; 4.50 dollars; Paperback 10*s*. 6*d*. net.

THE belief that diseases were the results of supernatural invocations was prevalent until the first century B.C., when Varro postulated invisible organisms as the causative phenomena. Although knowledge was accumulated on the spread and control of diseases such as leprosy and plague, the identity of these organisms remained a mystery until the development of lens systems in the seventeenth century enabled Van Leeuwenhoek to describe bacteria. Later poor sanitation conditions were recognized as potential sources of cholera, typhoid and dysentery and the significance of 'carriers' also established. However, the battle was only fully engaged during the nineteenth century when methods of growing bacteria *in vitro* and evaluating some of their characteristic activities were devised. To-day the essential techniques pioneered by men such as Pasteur, Lister and Koch are taken for granted, but the whole of modern microbiology and therapeutics is based on their painstaking investigations and application to the task of allaying disease. Koch's now famous postulates, prompted earlier by his teacher Henle, were timely in dissuading bacteriologists from making unwarranted conclusions and established a strict protocol for the unequivocal identification of pathogens.

The assault on pathogenic bacteria came from two quarters: on the one hand, defensive measures in the form of vaccination, initiated by the work of Jenner on smallpox and enthusiastically developed by Pasteur and others; on the other hand, offensive measures using chemicals to kill bacteria *in vivo*. Here Ehrlich's concept of antimetabolism provided a basis for the chemotherapeutic attack, and the programme of antimetabolite research pursued by Ehrlich and Domagk led to the fulfilment of their aspirations with the discovery of sulphanilamide. Unfortunately, no mention is made in *The Battle Against Bacteria* of D. D. Wood's elucidation of the mode of action of this drug and the impact that it had on the later course of medical microbiology. The random approach to chemotherapeutics embodied in the search for antibiotics is treated in the final chapters where discussion is centred on a detailed examination of penicillin and streptomycin.

Dr. Baldry concludes by emphasizing the potentiality of many pathogenic bacteria to acquire drug resistance. The countermeasure of prescribing mixtures of drugs, as in the treatment of tuberculosis, was not, and indeed is not, always appreciated; as a result, gonorrhoea and streptococcal infections, for example, are not always susceptible to penicillin—the one-time panacea for all ills. Finally, our thoughts are focused on the increased longevity of man, due, at least in part, to drug therapy. In somewhat Malthusian terms, the author highlights the question of food supply and population control, and the consequences attending an increasing proportion of old people in the community. We are cautioned prudently that " . . . drugs have produced their own problems which, unless wisely handled, can lead to as much misery as the diseases themselves".

Dr. Baldry has produced a concise, accurate account of the development of therapeutic agents and, throughout, the book creates a sense of excitement in the reader, scientific fact being interspersed with profiles of the major figures, anecdotes and social comment. Aimed at the general reader, *The Battle Against Bacteria* will be read by non-specialist and specialist alike with much profit and enjoyment.

ALAN T. BULL

THE CELLULAR BASIS OF RADIATION LETHALITY

Mammalian Radiation Lethality

A Disturbance in Cellular Kinetics. By Victor P. Bond, Theodor M. Fliedner and John O. Archambeau. (American Institute of Biological Sciences and U.S. Atomic Energy Commission. Monograph Series on Radiation Biology.) Pp. xvi+340. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 76*s*.

TO understand the effects of radiation on higher organisms it is necessary to appreciate that the processes of cell division are disrupted by doses which are too small to produce measurable effects on the functions of fully differentiated cells. Thus tissues in which little or no cell division normally occurs may not appear to be affected by radiation, in contrast to those in which the population of functional cells requires continual renewal. In particular, animals will die if either the haemopoietic tissue or the epithelial lining of certain portions of the gastrointestinal tract became too depleted of stem cells and ultimately, therefore, of the functioning cells which arise therefrom.

Mammalian Radiation Lethality reviews most of what is known about the cellular kinetics of these two systems, and deals also with the central nervous system. One of the aims of the authors is to show how the perturbations introduced into the cellular kinetics by irradiation give rise to such radiation syndromes as are observed. Those portions of the book which deal with the kinetics of cell renewal are skilfully and adequately handled, and well documented, reflecting the many contributions in this field made by the authors, in particular Dr. V. P. Bond. Their touch is less sure, however, when they deal, in rather meagre fashion, with the fundamentals of cellular radiobiology, and it follows that the message of the book is not as forcibly delivered as it might have been. One chapter only, entitled "Basic Radiobiological Principles Derived from Tissue Culture Systems", is devoted to this major aspect of their main thesis, and an example of its sketchy nature is the omission of any references whatever to fractionation studies. Yet experiments carried out by tissue-culture techniques have demonstrated patterns which have been most impressively repeated in studies on the whole animal, and indeed, the authors have occasion to quote one such experiment, the very point of which was the evidence it provided that it was justifiable to interpret animal deaths in terms of dose effect curves pertaining to cells.

It is perhaps a part of the comparative inadequacy in the cellular radiobiology section that the documentation

is rather poor: for example, some data have been presented without references, although they were culled from only one or two papers, and there are some lapses in linking textual material with the references quoted at the ends of chapters.

The authors, in their preface, express the hope that "the material will prove useful not only to the advanced student but to the mammalian radiobiologist and the physician as well". This hope will be fulfilled if such readers concomitantly have at their disposal fuller and more authoritative sources of information on basic cellular radiobiology. On the other hand, this book will be invaluable to the cellular radiobiologist who is interested in the impact of his work on radiobiological investigation at a higher level of organization. When, after the Second World War, data on 'acute' radiation death started accumulating, it scarcely seemed possible that all the facts could ever be accommodated within one simple pattern. During the past decade it has become ever clearer that they can be, and much of the evidence, therefore, is here most usefully collected together.

TIKVAH ALPER

THE FUNGAL CELL

The Fungi

An Advanced Treatment. Edited by G. C. Ainsworth and Alfred S. Sussman. Vol. 1 : The Fungal Cell. Pp. xvi+748. (New York: Academic Press, Inc.: London: Academic Press, Inc. (London), Ltd., 1965.) 192*s*.

THE Fungal Cell forms the first volume of a three-volume treatise, of which the later two volumes will deal with fungi as whole organisms and with fungal populations. The purpose of the whole work is, to quote from the editors' preface, "to summarize what is known about fungi as fungi" and to serve as a reference book.

The fungal cell in its various aspects is dealt with in twenty-seven chapters by different authors. The first two chapters are introductory to the whole treatise, the first being a most readable historical introduction by G. C. Ainsworth, and the second, a summary outline for the non-mycologist of the structure and organization of different fungal groups, by C. J. Hickman. There follow five chapters on structural components of fungal cells (including cell walls, flagella, nuclei and ultrastructure of the cytoplasm), two on chemical constituents of fungi, five on cellular metabolism, fourteen on nutrition and growth, and a final chapter on gene action.

In general the coverage is very complete, but there are some gaps, especially in the section on cellular metabolism. D. J. D. Nicholas deals with the utilization of inorganic sources of nitrogen, but there is no chapter on amino-acid metabolism even though this subject offers scope for the kind of comparative treatment which would fit very well in a book such as this. More important, nothing is said about the regulation of cell metabolism by induction or repression of enzyme synthesis or by end-product inhibition of enzyme activity. The question of how far fungi share the regulatory mechanisms which have been demonstrated in so much detail in bacteria is an important one, about which a considerable amount can now be said. Perhaps the book was written a year or so too soon for a chapter on this subject.

Concerning the chapters which have been written, I have few serious complaints. Naturally enough some seem more interesting and readable than others, but all are written from expert knowledge and nearly all have excellent bibliographies. Here and there it seems that a greater integration of the different contributions would have been desirable. For example, C. F. Robinow and A. Bakerspigel deal with nuclear division in vegetative cells, while L. S. Olive covers meiosis and also touches on the post-meiotic divisions in asci. This somewhat artificial division of subject-matter is adhered to rather strictly, and one is left in some doubt as to whether there really is an important difference between mitosis in the ascus and mitosis in the vegetative mycelium, or whether the difference lies more in the different points of view of the respective authors. Each of these chapters is extremely interesting within its own terms of reference.

In one way this volume must have posed problems for the editors which will not arise with the later volumes. At the cellular level, especially in most of their metabolism, fungi are much the same as other organisms. One possible approach, and one which the reference by the editors to "fungi as fungi" might be understood to favour, is to take for granted, or to sketch in briefly, those aspects of cell structure and metabolism which are common to fungi and to other organisms, and to concentrate on those features of fungal cell biology which give the group its special characteristics. R. T. Moore, in his well-illustrated chapter on ultrastructure, takes this approach, dealing with cell-wall structure and septal pores, the behaviour of the nuclear membrane during mitosis, sporogenesis and haustoria. It might, in fact, have been better to have included some reference to, for example, fungal mitochondria, even if it was only to say that they were similar in structure to those of other organisms. One of the most interesting chapters dealing with the specifically fungal nature of fungi is that of N. F. Robertson on hyphal extension and branching, but the brevity of his account serves to emphasize how far we are from being able to account for even simple morphology in biochemical terms. The alternative point of view is represented by the chapters on glycolysis, by H. J. Blumenthal, on the tricarboxylic acid cycle, by D. J. Niederpruem, and on terminal electron transport by A. Lindenmeyer. Much of the space in these chapters is taken up with documenting the unsurprising proposition that fungi have, for the most part, the same enzymatic equipment as other organisms. This will no doubt be valuable for the fungal specialist, even though not very exciting for the general biologist. D. G. Catcheside's chapter on the rapidly moving subject of gene action has a rather different *raison d'être*. Here, although the general conclusions drawn are probably as applicable to other groups as to fungi, much of the best evidence comes from work with fungi, especially with *Neurospora crassa*.

Some diversity of treatment and approach is inevitable in a compendium of this kind, and it cannot be said to detract seriously from the value of the volume. This and the companion volumes seem likely to remain standard works of reference for many years to come, in spite of their extremely high price.

J. R. S. FINCHAM

TEA-GROWING

Tea

By Dr. T. Eden. Second edition. (Tropical Agriculture Series.) Pp. xvi+205+73 photographs. (London: Longmans, Green and Co., Ltd., 1965.) 45*s*. net.

THE first edition of *Tea* appeared in 1958 (see *Nature*, 182, 1577; 1958) with a second impression in 1960. This new edition does not differ fundamentally from the first, although a few sections have been altered or rewritten. The size of the book has increased by only four pages and the price from 35 to 45 shillings.

Tea is one of those tropical crops, like sugar cane, that are voracious consumers of nitrogenous manures, and these are all-important. The section on nitrogenous manuring has been rewritten and the results of recent experimental work incorporated. A feature of tea cultivation is the use of shade trees, usually leguminous. "Shade

and "Greenmanuring" is another chapter which has been redrafted and much additional or recent information provided. Among shade trees several species of *Albizzia* and *Erythrina* are discussed.

In the chapter on insect and other pests there are some alterations, notably in regard to mites. With tea the behaviour of mites is variable and a species economically important in one country or district is unimportant in another. Mite damage to tea is often seasonal and the usual sulphur sprays cannot be used because of taint to the tea, a non-tainting acaricide being necessary. The section on nematodes as pests of tea has also been rewritten, where experimental work with soil fumigants and the recent use of marigolds (*Tagetes*) for control is referred to.

Where tea is cultivated, in the wet tropics, the problem of weeds and weeding is usually especially severe. Weeding ranks second in labour costs on the average tea estate. The section on chemical weedkillers has been rewritten, which is not surprising having regard to the advances in this field in the past few years. Some of the modern herbicides that have shown promise in controlling weeds in tea plantations are referred to. Present indications are that weedkillers will secure adequate control but not eradication.

Another part of the book that has been altered or brought up to date is the final chapter ("Statistical Review") where acreage, production, consumption and distribution are dealt with. As the author points out, in a book of this kind great detail is neither possible nor desirable, and it must suffice to present the main data and point out the trends that seem significant, remembering that statistics can become out of date in a remarkably short time.

F. N. HOWES

BLAST DISEASE OF RICE

The Rice Blast Disease

(Proceedings of a Symposium at the International Rice Research Institute, July, 1963.) Pp. x+507. (Baltimore: Md.: The Johns Hopkins Press; London: Oxford University Press, 1965. Published for the International Rice Research Institute.) 15 dollars; 120*s*. net.

THE ideals behind the cliché that the object of agricultural research is to make two blades of grass grow where one grew before lose their force if those extra blades are diseased. Despite a good deal of research during the past thirty years the basic food of half the world, that is, rice, has been seriously curtailed by blast disease, which continues to spread geographically.

In 1949, the Food and Agriculture Organization set up a commission to place rice research on an international basis and a working party made steady progress towards this end, but it was not until July 1963 that an international symposium on blast disease was organized by the International Rice Research Institute (established in 1962) in the Philippines.

The Rice Blast Disease, which is the report of this symposium, is comprised of twenty-eight invitational papers contributed by participants from Asia and the United States, with proposals for intensifying international co-operation by the use of uniform blast nurseries, uniform procedures for resistance testing, and a joint study for differentiating races of the fungal pathogen. There is also an excellent bibliography of nearly nine hundred titles.

The range of research is comprehensive, covering the causal fungus, *Pyricularia oryzae*, the disease and its development, physiological races, the mechanism and genetics of resistance, breeding for resistance, and other methods of control. The morphology, taxonomy, host range, and life-cycle of *P. oryzae* are described, the author avoiding the pitfalls of early taxonomic literature. Nutritional requirements of the pathogen are dealt with, including the relationship of nitrogenous metabolites to the production of toxins and hence, probably, to greater plant susceptibility. Nutritional and cytological factors are further analysed in investigations of the physiology of the fungus and its variability in artificial culture.

Field studies have not been neglected and there are good accounts of the effects of environment and cultivar on disease incidence and the methods adopted for estimating crop losses and for forecasting disease outbreaks.

The problems of physiological races, the nature of genetics of resistance, the selection of test differentials, in fact, breeding for resistance at large, have a familiar ring, and it is admitted that information on these subjects is as yet meagre. There is need for a good deal more co-operation on an international basis in this sphere with the aim of much stricter standardization of techniques and materials, particularly genetically pure seed supplies.

Concluding papers on disease control recount the successful adoption on a field scale of phenylmercuric fungicides both in seed-beds and the field, and also the promising use of antibiotics. No mention, however, is made of mammalian toxicity of these fungicides except for a casual reference to eye injury by 'Blasticidin-*S*'.

It is a pity that such a report is marred by indifferent illustrations. It is not clear what useful purpose is served by many of the black-and-white text figures, and the coloured plates of leaf lesions are disappointing.

J. C. F. HOPKINS

DEVELOPMENT OF PLANTATIONS

Plantation Agriculture

By Dr. P. P. Courtenay. (Bell's Economic Geographies.) Pp. viii+208. (London: G. Bell and Sons, Ltd., 1965.) 25*s*. net.

THERE is a need for a general text-book on plantation agriculture, setting out the subject for the specialists in the natural and social sciences. Dr. Courtenay is a geographer. His background knowledge is therefore appropriate to the authorship of a book of this type; the stated aim is "to establish and analyse the distribution of this (plantation) distinctive type of crop production". His facts which, from the definition of the subject, are drawn from a wide variety of disciplines are accurate and well chosen; he emphasizes the important point, which has been obscured by political controversy, that many of the less-developed tropical countries owe much of their development to plantation enterprise.

However, as with many geographically biased books, the objective tends to be lost in the attempt to create a narrative. The treatment of the first part of *Plantation Agriculture*, concerned with the establishment and history of such agriculture, is better than the analytical treatment of the second part. Further, there tends to be a repetition of statements; this criticism applies particularly to the third section, in which Dr. Courtenay discusses in detail as case-studies the two plantation crops, rubber and tea. A more satisfactory arrangement would have been to conclude on discussion of the theme "the future of plantation agriculture", a subject of intense interest to students of tropical development.

A weakness is his approach to the crop environment, particularly rainfall variability in the so-called tropical 'regular-rainfall regions', to which the technologists, who are advancing plantation agriculture, are now paying much attention. For example, he states "the shoots of the tea plant grow with a distinct rhythm, unconnected with climate or other environmental conditions, which produces recognised flushing and dormant periods". Yet

he recognizes the 'over-wintering' of rubber and the enforced rest period in the northern areas of tea production.

The most satisfactory treatment is that of the social and economic aspects of plantation development. Siting of the first plantations was based on military security and ease of transport by water. Later sitings showed improved fit to agronomic criteria, but ease of communication remained important; plantations followed development of railways, not vice versa; and these social and economic factors, including those affecting supply of labour, still determine distribution to a large extent.

Dr. Courtenay rightly emphasizes the very large amounts of capital required to establish some types of plantation; the unit cost for a sugar factory, for example, is £2·25 million.

Diversification is considered to be a relatively recent policy, but in fact plantation owners were seeking diversification in the first half of the nineteenth century. To-day, plantation cropping is considered in terms of perennial crops; but this was not always so; formerly both tobacco and cotton were typical plantation crops. Dr. Courtenay's accounts of past economic difficulties help to put those of the present in their true perspective.

In considering the factors operating at the present time, Dr. Courtenay points out that price control, which has always been considered a prerequisite for the salvation of the smallholder, actually favours the plantation system. He also points out that in the total economy of a country, the cost of supporting services and social services must be added to small-holder production costs, whereas the plantation industry is self-supporting. In spite of these advantages he suggests that plantation agriculture may be on the decline.

As a whole, the book is of the right length for an introduction and is an appropriate short text-book for courses in tropical agriculture, agricultural geography and economics, and the specialists in the natural sciences.

W. R. STANTON

RAT GENETICS

Genetics of the Norway Rat

By Roy Robinson. (International Series of Monographs in Pure and Applied Biology. Division: Zoology, Vol. 24.) Pp. ix+804. (London and New York: Pergamon Press, Ltd., 1965.) 200*s*. net.

MOST people concerned with rodent genetics who, at some time or another, have had occasion to consult Grüneberg's excellent *Genetics of the Mouse* will probably have wished that a companion volume dealing with the rat also existed. This 800-page hand-book is a candidate for stopping that particular gap, and it would be a pleasure for me to be able to say that it succeeded in doing so in a manner worthy of its illustrious predecessor dealing with the companion species. Unfortunately, such is not the case, and this is for a variety of reasons.

Mr. Robinson has attempted an exhaustive treatment of the genetics of *Rattus norvegicus* in the form of an account of all the research bearing on such topics as colour variation, reproduction, endocrinology, resistance to disease and cytogenetics. There are seventeen such chapters in all, each of which is essentially self-contained. They can almost be regarded as separate monographs in themselves. This vast labour has been carried out in a most thorough but, it must be admitted, pedestrian fashion. The account of each investigation is usually given in the words of the original author, so that terminology frequently sounds old-fashioned. Moreover, the approach is essentially non-critical. Manifestly inadequate work is often given equal weight with more refined experimentation, even though minor criticisms are offered, albeit diffidently, from time to time.

But for anyone wishing for an introduction to one of the fields which Robinson has covered, the appropriate chapter will prove a veritable open sesame to the literature. Details of experimental procedures, often with diagrams of apparatus, are given, and the closest collation of different papers by the same authors attempted. Tables of results are often given in full, together with diagrams and graphs of the data. Coverage is essentially exhaustive. Thus the section on psychogenetics, which covers 167 pages, appears to lack only one reference which it might reasonably be expected to contain, and presents a wealth of new ones in every branch of this new and rapidly developing field. Indeed, since much of the significant work in psychogenetics has been done on the Norway rat, this chapter constitutes the most up-to-date review available of this modern specialization, and certainly the most comprehensive ever for the older work. Similar aspects of other chapters in this compendium could be singled out for praise, and Robinson's practice of repeating the details of experimentation relevant to the different topics he is dealing with, while it increases the length of the book, dispenses with the need for extensive cross-referencing and means that the chapters can be consulted separately with little fear of missing essential points.

Except in the case of a few diagrams where the word 'intercross' is shown as 'intercourse', an error which, while unfortunate, serves to reveal as much as to obscure, the book is relatively free from misprints. But it does suffer from numerous infelicities of style, which will grate on any reader with a sensitive ear for English, and from a determined confusion over the plural of the word 'datum'. Moreover, Robinson indulges a penchant for the rare word and the near neologism of which 'independency' and 'exercisation' are examples.

But these are relatively minor matters, which, while they detract from the overall impression of the book, must not be allowed to obscure its sterling worth, and can therefore be forgiven. What it is more difficult to forgive is the lack of a more adequate subject index than the present one, which occupies less than 1·3 per cent of the length of the book, and the price of £10 which the publishers have seen fit to attach to it.

For the manifest virtues of the compendium those concerned with any aspect of the genetics of this most important experimental organism will be grateful. The strictures I have felt it necessary to lay on the work as a whole are essentially blemishes, serious blemishes though they may be, but ones which can readily be cured in a later edition, the appearance of which will, no doubt, be eagerly anticipated by the many readers who will certainly find themselves indebted to Robinson before long.

P. L. BROADHURST

BRITISH GRASSHOPPERS AND CRICKETS

Grasshoppers, Crickets and Cockroaches of the British Isles

By Dr. David R. Ragge. (Wayside and Woodland Series.) Pp. xii+299+22 plates. (London and New York: Frederick Warne and Co., Ltd., 1965.) 42*s*. net.

THE group name Orthoptera, as Dr. Ragge admits in the introduction to *Grasshoppers, Crickets and Cockroaches of the British Isles*, applies to "a very diverse assemblage of insects", and its use, as well as a conservative classification, might be justified by a wish not to confuse a general reader. However, there are as yet very few British naturalists interested in these insects, and the new devotees who are certain to appear as a result of this book should have no difficulty in learning the system now adopted by specialists.

Introductory sections of the book deal clearly and competently with the methods of collecting, preserving and keeping the insects alive; the latter is particularly welcome because grasshoppers and crickets are undoubtedly more lively and interesting pets than the sluggish exotic stick insect, which is an old favourite. The chapters on the structure and habits are, inevitably, at the beginning of each group, because there is nothing in common in these respects between cockroaches and grasshoppers. Keys and descriptions of species are simple and clear but it is odd that the author measures the size of specimens as the distance from the front of head to the tip of flexed wings, or the end of abdomen, whichever is the greater; this practice differs from that adopted by all students of these insects and makes it almost impossible to compare the size of British specimens of a species with continental ones, although in some cases the differences suggest subspecific separation; no reason for this idiosyncrasy is offered. Special attention is paid to descriptions of adult coloration and its changes during life; E. J. Clark's system of colour notations is not used, but its exposition is useful, because of its potential value in studies of variation in natural populations; in the book, descriptive names of colour varieties are used—"green", "mottled", "black pale sides", etc. Macropterous forms, which should be called just that, appear under historical Latin names, without mentioning that these have no taxonomic standing. All species are given English names, some not very fortunate; thus while *Myrmeleotettix maculatus* is appropriately called Mottled Grasshopper, the name Lesser Mottled Grasshopper is invented for the unrelated *Stenobothrus stigmaticus*. The distribution of each species is shown on a Webster–Praeger map of counties and vice-counties, but the omission of maps for three localized species (*Decticus*, *Conocephalus concolor* and *Chorthippus vagans*) is regrettable, while the accidental distribution of domestic species is scarcely worth showing on maps.

Notes on habits of crickets, bush-crickets and grasshoppers are most useful; they include diagrams of their songs, and an excellent record of songs of the main species can be purchased with the book. One would wish that other features of behaviour and ecology were given more attention; for example, the eminent suitability of grasshoppers for population studies is not even mentioned.

The twenty-two colour plates, nearly all photographed from fresh specimens by the late H. D. Swain, are the best coloured illustrations of these insects ever published. The superb colour photograph of Great Green Grasshopper is, unfortunately, on the perishable dust-jacket, while it would do well as a frontispiece.

A brief, but pleasant, chapter describes excursions in some more interesting areas, and the book ends with a competent discussion of the distribution and history of the fauna against the background of geological, climatic and vegetational changes. The more recent effects of human activities—forest clearing, agriculture, grazing—are such that at present less than 20 per cent of the area of the British Isles offers suitable habitats for these insects. The rarer species are certainly approaching extinction, but nothing appears to be done for their protection, as is done for some butterflies.

Only few errors and omissions in this excellent book can be noted. It is not correct to say that the stridulatory pegs of female grasshopper are vestigial—they are merely less developed and in some females fully functional. Not all grasshoppers have four nymphal instars, since *Stethophyma grossum* has five. The bibliography omits an important paper by C. and P. Divers (1933) on the ecology and fauna of grasshoppers of South Haven Peninsula and my paper on the composition and origin of the British fauna (1930); interesting observations by K. G. Blair (1944) on *Conocephalus dorsalis* laying eggs in fly galls on reeds are not mentioned either in the text or in the bibliography.

One can confidently say that these long-neglected insects will now, thanks to this book, become as popular with Nature lovers as are birds and butterflies.

BORIS UVAROV

LIFE IS STRIFE

Animal Conflict and Adaptation

By Prof. J. L. Cloudsley-Thompson. Pp. xi+160+29 plates. (London: G. T. Foulis and Co., Ltd., 1965.) 42*s*. net.

PROF. CLOUDSLEY-THOMPSON uses the term 'animal conflict' in a very wide sense and applies it not only to conflict between animals but also to conflict between animals and their ever-varying environment. He regards it as the automatic consequence of change in the environment to which all living organisms must adapt themselves or perish. He points out, however, that the selection pressure of the environment does not directly produce adaptations but merely acts as a sieve that separates the more viable results of gene mutation or recombination from the less viable.

Animal Conflict and Adaptation is divided into four parts; the first gives an account of some of the physiological problems of adaptation such as osmotic regulation, respiration, and the change from aquatic to terrestrial life, and then considers some specific environments. As might be expected the author chooses life in deserts, both the hot ones of the great continents and the cold ones of the polar regions, to illustrate adaptations to particularly exacting environments. His personal experience of these regions and his researches, especially in the hot ones, give these chapters an enhanced value.

Part 2 deals with interspecific conflict, and concentrates attention on food chains and predators, parasites and disease, and how they act on population numbers. Part 3 turns on intraspecific conflict and discusses cannibalism, fighting, displacement activities, and conflict within the individual. Many of the carnivorous invertebrates are adapted to cannibalism, a density-dependent phenomenon especially in some insects that practise egg-cannibalism, but cannibalism is not commonly found among vertebrates, apart from the fishes and amphibians. Intraspecific fighting among the higher vertebrates is rarely fatal, and is generally ritualized so that dominance is established or territory maintained without serious damage to the species.

In the final part the author considers problems of conflict and adaptation in the human species—the menace to health by insects as the vectors of disease, and the human threat to wildlife. The latter is the disgraceful story of the destruction of the natural environment by man in ruthless exploitation, at first through ignorance, by now through greed; "few large animals exist on land to-day except in game parks and nature reserves". Is this destruction an inevitable part of man's evolution? Perhaps his evolution requires the elimination of most of the other land vertebrates to make room for his ever-increasing millions who want to live in an artificial, man-made environment and not in the natural one.

The final chapter brings the author to human population problems, and he suggests that many of them are the result of faulty adaptation and lack of restraint. When biologists consider these problems and think of the ever-surging flood of population growth, they foresee the future by extrapolating from what they know of animal populations. Their visions are not attractive—the human population is increasing at such a rate that within the comparatively near future overcrowding will be so intense that life as we now enjoy it will no longer be possible. It is probably too late now to regulate the size of the population by the

painless method of birth control; numbers will have to be reduced by killing off the excess either by famine, epidemic disease, or nuclear war—probably the latter, for conventional warfare is not effective. This history of civilization has always been discontinuous, and the present phase seems to be bent on its own destruction, after which some totally different phase may, or may not, succeed. It is certain that we cannot continue along our present path for more than a century or two. "Nature leads and science points the way—to the conservation of variety, to the rational exploitation of fisheries and game populations, to the control rather than the elimination of pests and parasites and to the stabilisation of human populations at an economic level. . . . We inhabit a small world and must learn to adapt ourselves to its limitations or perish . . . we are steadily destroying our world and may end up by destroying ourselves."

Prof. Cloudsley-Thompson has written a thoughtful and stimulating book; it is well illustrated, indexed, and provided with references to further reading, but there are too many misprints.

L. HARRISON MATTHEWS

THE BATS OF WEST AFRICA

The Bats of West Africa

By D. R. Rosevear. Pp. xvii+418+2 plates. (London: British Museum (Natural History), 1965.) 155*s*.

THE fascinating mammalian life of tropical West Africa has long been a subject of intense interest to zoologists. Students of the region will be deeply indebted to D. R. Rosevear for this superb monograph on its Chiroptera, the first comprehensive work of the kind yet attempted. *The Bats of West Africa* has been chiefly prepared with a view to the needs of young African scientists, but will prove to be of equal value to specialists in this field elsewhere.

The work covers the whole of tropical West Africa south of the eighteenth parallel of latitude north, to the coast, including Fernando Po Island; the eastern limit is the River Sanaga in Cameroon Republic, and thence north-east to Jebel Marra, following the watersheds dividing the West African drainage area from those draining to the Congo or the Nile.

A systematic check-list gives ready reference to the species occurring in the area and a general introduction to the Chiroptera provides fundamental information on their distribution, habits, reproduction, parasites, sensory perception and other important aspects of bat biology, as well as details of those aspects of structure of systematic importance. Methods of collection and preparation and measurement are also discussed and taxonomic practice and problems briefly treated. The systematic section which follows is well and clearly organized, with keys to the identification of genera and species, and useful synonymies. The information in the text is very full, covering the description, distribution, habits, biology and systematic problems of each species. The text is illustrated with very clear line drawings by Joanna Webb and the author, which greatly enhance the value of the work. A very full list of references, together with a list of journals with their abbreviations, is provided. A useful glossary of technical terms follows, and an illustrated account of taxidermy applied to bats will prove of value for those unfamiliar. A description of the zones of local vegetation is followed by a most valuable geographical gazetteer, supplemented by a folding-map at the back of the volume. *The Bats of West Africa* is a vital need for all serious students of African Chiroptera. It is comprehensive and eminently practical and will be a first source of information on its subject for many years to come.

D. L. HARRISON

FOSSIL MAN

Guide to Fossil Man

A Handbook of Human Palaeontology. By Michael H. Day. Pp. 289. (London: Cassell and Co., Ltd., 1965.) 42*s*. net.

IN the face of the present fashions in physical anthropology, it is a pleasure to review a book on fossil man, a subject seemingly dry as dust, and yet of fundamental importance to anyone interested in the study of man. Whatever we may learn of primate behaviour, it is human palaeontology which forms the basis on which knowledge of our origin and our nature can be built, and which alone can indicate the manner of the evolution of man. This *Guide to Fossil Man* is the first book of its kind ever to be produced in Britain, and we must be grateful to Dr. Day for undertaking such a task.

Apart from two short sections on the environment, culture, dating and anatomy of fossil man (22 pages), the book brings together information relating to forty key sites which have yielded "the bulk of significant hominid fossils". The sites are treated in turn, country by country, in the manner of a catalogue, and the information given for each fossil site is divided under a number of headings; synonyms; found by; geology; associated finds; dating; morphology; dimensions; affinities; original; casts; references. The information given is both accurate and quite extensive, and its value is much enhanced by numerous photographs.

However, this book represents the view of just one man on significant hominid fossils, and subjectivity is apparent both in the selection of the fossils and in their interpretation. It is not difficult to find fault with the selection of fossils which have been included. While on one hand we find an entry devoted to the very insignificant fragments from Ubeideya, of which both date and affinity are uncertain, there is at the same time no reference to any of the important skulls of Middle Pleistocene age from southern Europe, Russia and Persia. In fact the so-called Neandertal remains are very poorly represented, and while Dr. Day may feel that the distribution and character of these people are not of central significance to students of human evolution, they do represent the best recorded phase of this evolutionary process, and as such are wonderful material for students of human palaeontology. I would on no account feel justified in omitting a full consideration of this group in a course in human palaeontology. At the other end of the morphological sequence, it is remarkable that there is a six-page entry for the *Gigantopithecus* remains from China but no reference to the fragments classified as *Ramapithecus*, which have a claim to hominid status at least equal and probably much greater. At the same time, the late age of *Gigantopithecus* makes it clearly an interloper in any study of human evolution, while the early Mio-Pliocene age of *Ramapithecus*, and its remarkable morphology, make it more than merely a probable candidate for a stage in human phylogeny.

In contrast, Dr. Day gives very full treatment to the fossils from the early Pleistocene of Africa, and this part of the book is of real value as a record of what has been discovered in the Transvaal and in Tanzania. But here we must record that a very one-sided account is given of the significance of the Olduvai fossils, and the published opinions of such distinguished palaeontologists as Profs. W. le Gros Clark and J. T. Robinson, which represent an important body of opinion, are quoted neither in the text nor the references. Any book intended for students, let alone for professional reference, should quote widely in its treatment of controversial issues, since one of the most valuable exercises for any student is to make up his own mind on a controversial matter, and for this he must be well informed.

Though I believe these criticisms to be fair and justified there is nevertheless no real question of the value of this

well-produced book. Teachers will use it frequently, and so will students, and it should be on every library shelf. It is more than a pity, however, that every other time one turns to it for information, one draws a blank. The evidence for human evolution is still so slight that we cannot afford to overlook any part of it. The fossils which are missing are not unimportant and it is to be hoped that students will not treat them as such. But for what it contains, this guide fills a unique place as a most valuable text on human palaeontology. BERNARD CAMPBELL

SCIENCE AND SOCIETY

The Rise of the Technocrats

A Social History. By Prof. W. H. G. Armytage. (Studies in Social History.) Pp. vii+448. (London: Routledge and Kegan Paul; Toronto: University of Toronto Press, 1965.) 56*s*.; 9 dollars.

IN title, Prof. Armytage's book recalls Dr. Michael Young's *The Rise of the Meritocracy*, but there the resemblance ends. There is nothing futurist about Prof. Armytage's study of the place of the scientist in society and his influence on public affairs, though, regarded as a social history, the book disappoints. Covering roughly the past four centuries, its span is immense, and it is based on a range of reading that is vast and impressive. Prof. Armytage begins with the botanists and gardeners of the sixteenth century and traces the emergence of the scientific societies in the following two centuries, the beginnings of scientific and of technical education, the founding of the British Association for the Advancement of Science, to the establishment in our own times of the Department of Scientific and Industrial Research and the complex interrelations between Government, industry and the universities. In all this, he scarcely fails to note a significant factor or figure, and it is done, moreover, on an international scale which portrays the interactions of developments in one country or another. Mainly the treatment is pragmatic and practical, but sometimes he ventures into philosophy, as in his final chapter, "An Operational World".

The treatment varies considerably. There are imaginative and sensitive passages, but there are other much more pedestrian, which do not match the originality of the headings, and in which he seems sometimes so overwhelmed by detail that the wood is hidden by the trees. Sometimes, on the other hand, the passage of events is condensed to an extent that gives a picture of the situation which is not entirely accurate, and sometimes again his judgment seems to have been at fault in selecting trivialities, rather than significant factors or events, in order to make his point. Nevertheless, the overwhelming mass of detail which he has examined has not betrayed Prof. Armytage into jargon.

If, as a piece of social history, *The Rise of the Technocrats* disappoints, this is not because too large a canvas has been attempted or because the material is unbalanced. It is rather because Prof. Armytage nowhere defines what he understands by 'technocrat' and shows no clear understanding of the relations between science and technology to-day, particularly of their intellectual value. He describes events without pausing for any searching or satisfying examination of their real causes and leaves the impression at times that he is a little out of his depth. His quotations and references are copious and remarkably up to date, but he has at times a tendency to quote a secondary source when the primary one is even more accessible. Moreover, it is astonishing to find no mention of Tocqueville in an account of Anglo-American relations in the nineteenth century so full as Prof. Armytage gives us. Apart from this, the book is useful for its extensive references and bibliography alone, and the conscientious and painstaking reader should have little difficulty in correcting for himself any passages where either compression or imperfect judgment seems to have distorted the true picture. R. BRIGHTMAN

A HISTORY OF HYSTERIA

Hysteria

The History of a Disease. By Dr. Ilza Veith. Pp. xvi+301+14 plates. (Chicago and London: The University of Chicago Press, 1965.) 7.95 dollars; 59*s*. 6*d*.

DR. ILZA VEITH has essayed a difficult task in choosing hysteria for historical research *Hysteria: The History of a Disease* is timely, for at present hysteria is a controversial subject between neurologists and psychiatrists. The word itself no longer appears in the *Standard Nomenclature of Diseases*, and it was eliminated from the *Mental Disorders Diagnostic Manual* of the American Psychiatric Association in 1952, being replaced by the term 'conversion symptom'.

In spite of the long recognition of hysteria, over the centuries, to be a clinical entity, so eminent a psychiatrist as Dr. Eliot Slater in his Shorvon Lecture[1] holds there is no such disease. Sir Francis Walshe[2] in a recent paper, as a neurologist, defends the clinical unity of hysteria. He writes: "Apart from the mimesis of somatic disease hysteria may present, the dramatizations, the exaggerations and the pathological lying are also behavioural disorders, part of the total expression of the abnormal psychical state which is hysteria. A diversity of symptoms from case to case and, in any single case from time to time, no more destroy the unitary quality of the malady than the same diversities destroy that of disseminated sclerosis or of neurosyphilis". It is to be noted that Walshe also adds: "Nevertheless, Slater's nihilism in regard to hysteria is a challenge to neurologists once again to justify the concept of hysteria as a nosological entity in its own right". Dr. Veith also considers hysteria a disease. The extensive bibliography shows that she has read widely on the subject, and she sets forth much new and interesting information.

From early times in Ancient Egypt, hysteria (derived from ὑστέρα, the Greek word for uterus) was associated with uterine disorders, the symptoms being ascribed to wanderings of that organ. The remedies applied were designed "to cause the womb to go back to its place". The Hippocratic *corpus* described hysterical symptoms, such as the *globus hystericus*, carefully and also associated the disease with uterine displacements. Aretaeus considered the uterus a migratory organ, but noted that the malady also occurred in men. Soranus and Galen pointed out the fallacy of uterine migration.

In Chapter 3, Dr. Veith emphasizes that St. Augustine, Bishop of Hippo, regarded hysteria to be due to demoniac possession, induced by magic or witchcraft. For centuries in the Middle Ages many innocent persons were accused, tortured and executed for witchcraft. William Harvey investigated an accusation of this kind and was more enlightened than Sir Thomas Browne at a later date in that he secured a reprieve for four out of seven of the convicted witches. The sad story of the Salem witches is re-told, whose accusation was supported by so eminent a man as Cotton Mather (1663–1728). Even so late as 1749, a number of nuns were seized with mass hysteria and thought themselves bewitched by a young nun who was condemned and burnt alive in the market-place of Wurzburg.

Magic in the Far East is described and is followed by a chapter on hysteria in England. A striking example, which Dr. Veith does not mention, was the case of Queen Elizabeth I. She was subject to 'a spice of the Mother', as hysteria was termed, and, occasionally, had cataleptic seizures. Her malady was most apparent after the death

of Mary, Queen of Scots, when she held firmly to the belief that she had not authorized her cousin's execution, but had only signed the death-warrant as a precaution in case of Spanish invasion[3].

The medical beliefs about hysteria of later physicians of the past are recounted, including Edward Jorden, Sydenham, Cullen (who returned to the uterine theory), Pinel, Feuchtersleben, Griesingen, R. B. Carter, Jules Fairet, S. Weir Mitchell and Charcot, who studied the malady so brilliantly at the Salpêtrière Hospital in Paris and treated his patients by hypnotism.

Babinski, who was *chef de clinique* to Charcot, was able to show that hysteria was a nosological entity, although certain of its manifestations could be elicited by suggestion and eliminated by persuasion. Walshe[2] refers to this work, and also to the excellent summary of the psychopathology of hysteria by E. Mapother and A. Lewis[4]. The book concludes with an account of the work of Janet and J. Breur. Freud was Breur's pupil and the teaching of his chief led him to the evolution of psychoanalysis and the emergence of psychiatry as a specialized branch of medicine.

"Knowledge grows from more to more", and, as Dr. Veith mentions on p. 164, even Freud stated that the symptoms in some of his patients were not mentally determined or removable by analysis, but must be regarded as direct consequences of disturbed sexual chemical processes[5]. Dr. Veith suggests substitution of the term endocrinological for chemical.

We know to-day that certain manifestations of mental disease, such as general paralysis of the insane, and cerebral syphilis, are due to infection by the *Treponema pallida* and that forms of encephalitis have a virus causation. Again, powerful psychopathic drugs like those of the phenothiazine group produce effects very similar to those seen as sequelae of encephalitis lethargica. Walshe[2] observes that mass hysteria epidemics were attributed to witchcraft or demoniacal possession in past centuries, but "it is likely to-day that we should declare them to be due to some unknown and undiscoverable virus and call the condition encephalitis".

Research workers, including biochemists, may yet discover an organic basis for hysteria. In the meantime, Dr. Veith's admirable study will help reflexion and, possibly, suggest further methods of approach to the problem.

ARTHUR S. MACNALTY

[1] Slater, Eliot, *Brit. Med. J.*, 1, 1395 (1965).
[2] Walshe, F., *Brit. Med. J.*, ii, 1451 (1965).
[3] MacNalty, A. S., *Elizabeth Tudor: The Lonely Queen*, 166 (C. Johnson, London, 1954).
[4] Lewis, A., and Mapother, E., in *Price's Textbook of the Practice of Medicine*, 5th ed., 1870 (1937).
[5] Freud, S., *An Autobiographical Study*, trans. by Strachey, J., 46 (Hogarth Press, London, 1946)

THALASSAEMIA

The Thalassaemia Syndromes

By D. J. Weatherall. Pp. viii+272. (Oxford: Blackwell Scientific Publications, Ltd., 1965.) 45*s*. net.

HAEMOGLOBINOPATHIES arise basically from an absence of normal adult haemoglobin. One mechanism is the replacement of the structural gene for normal adult haemoglobin (haemoglobin A) by one for that of an abnormal haemoglobin. Notably in the homozygote for the abnormal gene a red cell is produced which is too flat and has a shortened life span. Another type of haemoglobinopathy is called thalassaemia and is a consequence of an under-production of the normal adult haemoglobin, without a replacement by an abnormal variant.

The term thalassaemia is meant to denote an anaemia occurring in the Mediterranean, θαλαττα or θαλασσα being the Greek for Mediterranean Sea. The word has come to stay though it is an etymological monstrosity and conveys to a Greek the meaning of 'sea in blood'. Also, while thalassaemia was at first discovered in people of Mediterranean origin, it is now known that this 'Mediterranean' anaemia is widespread and that there are probably more Asiatics affected by this condition than there are Mediterranean people altogether.

The first step in clarifying the diagnosis of thalassaemia was the recognition that this under-production of adult haemoglobin could either be due to a failure in producing the α-chains of haemoglobin A or to one of providing the β-chains. As the α-chains of haemoglobin A ($\alpha_2\beta_2$) are shared with the other physiological haemoglobins F ($\alpha_2\gamma_2$) and A_2 ($\alpha_2\delta_2$), a completely different situation arises in α-thalassaemia from that of β-thalassaemia where only the normal adult haemoglobin is affected. There are numerous byways along which the normal haemoglobin A production can be diverted. Some of them have found their biochemical and genetical explanation as, for example, the formation of haemoglobin Lepore which consists of normal α-chains but has as its non-α-chain one which is composed of parts of the δ-chain and of the β-chain. It seems that thalassaemia may eventually be explained as a disturbance at one stage or another along a sequence of steps. This begins with the regulator gene giving permission to the operator gene to switch on the structural gene in the nucleus of the red cell precursor, and it ends with the assembly of amino-acids into polypeptide chains at the ribosome of the protoplasm under the guidance of the messenger RNA. There still remains a bewildering collection of thalassaemia syndromes which have not been tidied up and have not yet been explained on the basis of their genetics and their molecular biology. Nevertheless there is no field in human biology where insight into the synthesis of a macromolecule and its underlying genetical mechanism has been carried so far as in the investigation of the thalassaemias.

In *The Thalassaemia Syndromes* Dr. Weatherall has not made any concessions to simplification but he has succeeded in describing with great clarity the complexity of thalassaemia from every point of view, including those of genetics, clinical disease and therapy. His volume will be a standard work of help to geneticists and biochemists as well as to physicians and pathologists for many years to come.

H. LEHMANN

MEDICINE AND THE COMPUTER

Mathematics and Computer Science in Biology and Medicine

(Proceedings of Conference held by Medical Research Council in association with the Health Departments, Oxford, July 1964.) Pp. ix+317+12 plates. (London: H.M.S.O., 1965.) 60*s*. net.

THOSE who attended the Medical Research Council conference in July 1964 seem all to have agreed that it was a remarkable success in respect of the quality of papers presented and the opportunities for exchanges of ideas. Was insistence on publication of the *Proceedings* wise? The topics are not sufficiently closely related to provide a comprehensive summary of the present state of research in a field of science, and many papers are too short to stand alone either as broad reviews or as original contributions. This is not a criticism of the authors, whose papers may have been excellent for conference presentation and discussion, but of the belief that no conference is complete without full publication of proceedings.

The papers may be grouped as concerned with medical records, with classification problems, with epidemiology and therapeutics, and with physiological and cytological studies. Much of the first group consists of plans in outline or preliminary reports on record linkage. The range of ideas may have stimulated valuable discussion; in print

the impression is of eight authors making excellent proposals for different problems without the critical synthesis that could help in other situations. Newcombe's paper is outstanding for its account of large-scale record linkage in progress, though much of the content has been published before.

Sneath and Healy have illustrated very different multivariate statistical procedures for generating hypotheses relevant to classificatory problems, probably too briefly to be useful without reference to other papers that they summarize. Bailey's otherwise admirable statement of the logical problems of the logic of medical diagnosis is unfortunately tied to an assumption that circumstances are appropriate to assessment and use of prior probabilities. None of these papers makes much reference to computers except as a means of handling more variates than is otherwise practicable.

Among the papers concerned with epidemiology and drug therapy, Kendall displays his customary lucidity in explaining the mathematics of the spread of infection in a manner intelligible to non-mathematicians: Armitage raises important criticisms of sequential techniques in clinical trials.

About the time of the conference, several British universities were formulating plans for augmenting totally inadequate computing facilities. Projects regarded in the United States as straightforward applications of computers were totally outside the range of British installations, either directly for reasons of size and speed or because appropriate programming expertise could not flourish in the face of these inadequacies, and the gap was steadily widening. It is symptomatic of the attitude of British governments that only after eighteen months (and shortly after publication of this book) has preliminary approval been given to some of these plans. The type of loss we suffer is exemplified by the Lusted and Ruddle paper on analysis of chromosome karyograms, a description of a programming system that relates to a superb research technique and reads as dramatically as a 'thriller'. This paper puts others in its group in the shade, though Rosenblith gives a masterly survey of problems in the quantification of nervous activity. A series of papers on the mathematics of biological function includes interesting contributions on cell dynamics, tissue excitation, and brain topology (all speculative, especially this latter) and Whitteridge's excellent account of his work on the geometry of the visual cortex.

Undoubtedly this book will interest and benefit many readers, but publication of the more important papers in various journals might have made them more widely accessible at smaller cost. D. J. FINNEY

GENETICS AND PSYCHOLOGY

Methods and Goals in Human Behavior Genetics

Edited by Steven G. Vandenberg. Pp. xi+351. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 70*s*. 9*d*.

PSYCHOLOGICAL or behaviour genetics is the concern of an increasingly active group of psychologists and other scientists in the United States. *Methods and Goals in Human Behavior Genetics* is based on a symposium on the subject held in Louisville in 1963 and includes an edited version of the discussion which followed the papers read there. The sixteen contributors include six from University Departments of Psychology and representatives from biochemical genetics, agricultural biometrics, the science of animal behaviour, anthropology, child development and psychoanalysis.

There is a healthy diversity of opinion as to which aspects of human behaviour can most profitably be studied within a genetical frame of reference. Some would regard social criteria as the most important. On this view the contribution of genetic variation to academic achievement, or to the ability to patent scientific inventions, or to socially defined deviant behaviour, would be appropriate fields of investigation, even though simple genetic mechanisms will not be found. Others would prefer to start with traits such as hand clasping or tongue rolling in the hope that they may turn out to be inherited simply, but which may appear psychologically or socially trivial. In either case what is analysed is close to observed data. However, as psychologists, the majority of contributors prefer to analyse variables which they consider to have greater meaning psychologically, even though they may be more remote from direct observation. They may, for example, study scores on factors such as 'surgency', abstracted from responses to paper and pencil questionnaires. The form the genetic analysis typically takes is the calculation of 'heritability ratios' on data obtained from normal monozygotic and dizygotic twin schoolchildren.

It is when the contributors go beyond the calculation of h^2 from twin data that they are of greatest interest. Cattell, Vandenberg and others suggest theoretical extensions and refinements. There is clearly no lack of formulae. Yet others have been put forward in the United Kingdom. The difficulty is that findings are likely to vary widely, not only according to the population studied and the test used but also according to the method of analysis.

Among those who have attempted to replicate studies is Gottesman, who administered the same personality inventory to two different populations of twins. He is also one of the few to test parents as well as twins. After briefly reporting his findings, he goes on to speculate on natural selection and personality. Personality test score and fertility might be a fruitful area of research.

A perennial difficulty about the interpretation of twin data is the weight to be given to any greater similarity in the environments of identical than fraternal twins. R. T. Smith reports the opinions of 13 to 16-year-old twins on such points as whether they have different friends or different eating habits. It is interesting to have data of a more or less factual kind on points such as these, even though genetic factors may contribute to the environment to which a person is exposed. However, we are unable to assess the significance of such differences unless it is shown whether identical twins who differ in their taste in friends—or in vegetables—are in consequence less alike in health or personality.

A twin study which was not based on questionnaires is that of Freedman, using film, on the development of smiling and fear responses in infants. An interesting sidelight here is that the greater resemblance found in identical twins in these basic social reactions was unlikely to be accounted for by the mothers' handling. At that age (up to 8 months) most mothers of identicals thought their twins were fraternal.

It is perhaps typical of the psychologist's rather than the psychiatrist's approach that only one of the studies reported should be based on the relatives of psychiatric patients. This is the chapter by Mednick and Schulsinger, an American psychologist and Danish psychiatrist, who are investigating two hundred and seven children of schizophrenic mothers. The subjects were chosen because they constituted a high risk group. A recent Danish survey confirmed that about 12 per cent may be expected to develop the condition in time. The psychological, physiological and social data being collected will be used to test a particular psychological theory according to which schizophrenia is a learned disorder of thinking. The theory supposes the origins of schizophrenia to lie in the interaction of predisposing factors, possibly though not necessarily genetic, with 'unkind environments'. Already the children of schizophrenics have been found to differ from the matched control subjects in some of the expected directions. Although the hypothesis being

tested is not specifically related to genetics, the chapter fully deserves its place in the book since it illustrates one of the uses—the study of differences in identical twins is another—to which genetically derived material can be put. As most contributors realize, the calculation of numerical estimates of heritability is only one of the goals of human behaviour genetics.

One hopes the book will encourage a growing interest in genetics among psychologists and a reciprocal interest in psychology among geneticists. JAMES SHIELDS

HISTORY OF PSYCHOLOGY

A Source Book in the History of Psychology

Edited by Prof. Richard J. Herrnstein and Prof. Edwin G. Boring. Pp. xvii+636. (Cambridge, Mass.: Harvard University Press; London: Oxford University Press, 1965.) 100*s*. net.

THERE are very few books dealing with the history of psychology, and most of these have been written by Boring. The present *Source Book in the History of Psychology* is no exception, except that Herrnstein, also from Harvard University, has been made co-author. The book contains 116 excerpts from short to middling in length, and ranging in time from Epicurus (*c*. 300 B.C.) to the turn of the present century—and sometimes a little beyond. The arrangement is by topic; there are fifteen of these, covering such aspects as psychophysics, visual perception, cerebral localization, the reflex, association, and learning; within each topic the arrangement is in date order. The sections are preceded by a general statement in which the authors provide a setting for the reader, and individual excerpts are preceded or followed by quite brief notes which again are invariably helpful to the reader in directing his attention to points of interest, premonitions of later experiments or connexions with other authors.

It is very difficult to review a book of this kind; all that a reviewer could say after all would be whether he liked or disliked the particular choice made by the two authors. There can be few psychologists with sufficient temerity to argue the case with Prof. Boring, and in someone who does not have one-tenth of his historical knowledge or learning it may even seem presumptuous to say that on the whole the choice is excellent. However, taking courage in both hands, a few criticisms may perhaps be voiced, if only *sotto voce*.. In the first place, while we must welcome the inclusion of a section dealing with individual differences, these all deal with intelligence. Surely something could have been provided on personality ? Excerpts that come to mind are Kant's chapter on temperament in his *Anthropologie*, which was extremely influential and has found modern support in many factor analytic studies; Wundt's adumbration of the dimensional approach in his *Physiologische Psychologie*, and Binet's very important description of the personality differences between his two daughters.

These omissions are perhaps symptomatic of the tendency of the authors to restrict the term 'psychology' somewhat unduly; we have Fechner on Fechner's Law (predictably enough !), but why not Fechner on experimental aesthetics ? His discussion of aesthetics (*Von Oben Und Unten*) is a classic which should not be omitted in a book like this. In a similar way we are given Hartley on association but we are not given his extremely interesting and forward-looking discussion of the application of associationist principles to criminology and other important human activities. It might be objected that where something new comes in, something else has to go out, but in answer one might perhaps feel that some of the selections are more relevant to physiology than psychology.

It will be seen that even these criticisms are more a reflexion of my own preferences than firm indications of the dereliction of the authors; they certainly do not for a minute diminish the importance of this book, which may serve to lead many psychologists into an appreciation of historical writings which at the moment is so sadly lacking. We already owe so much to Boring that it is a pleasure to welcome this latest Harvard effort, and to wish it the success it so richly deserves. H. J. EYSENCK

INVESTIGATION OF SOCIAL STATUS

Roles

An Introduction to the Study of Social Relations. By Michael Banton. Pp. ix+224. (London: Tavistock Publications, Ltd., 1965.) 21*s*. net.

WRITERS who concern themselves with social class are legion; few approach their chosen causes in any spirit of scientific enquiry. Yet better regard for this complex field of social relations is essential for human and social development and, in *Roles*, Prof. Michael Banton has not only made a timely and conspicuous contribution to existing knowledge but has also provided a great stimulus to further investigation.

Using material obtained from studies of primitive, peasant and industrial societies, he analyses different kinds of social structure in terms of roles. Banton's approach is different from that of other sociologists, however, in that he is less concerned with the behaviour of people who occupy various roles (their 'context') than in the implications of the inter-relations between roles. Various definitions of concepts like status, class, prestige and esteem are examined and from these Banton builds up a theory of roles involving a rough, threefold classification into basic, general and independent. His basic roles are concerned with distinctions of sex and age, race and rank, and "are those which predetermine most of the positions open to an individual and which have implications for the way the parties behave towards one another in a correspondingly large proportion of social situations". General roles are usually allocated to individuals in accordance with their qualifications and are frequently associated with activities of a religious or political character. Independent roles are associated with individual merit, examples being most leisure roles and many occupational roles in industrial societies. From these definitions, Banton expands to examine, in greater depth, the connexion between economic development and role strains; role signs; role-changing; roles and the self; the compatibility of roles; the prestige of roles; and the relation of social density to order and progress.

Such a thoughtful and original work could scarcely be free from error or issues where different observers would place different emphasis or interpretations. Banton, for example, has misjudged the pace at which men are taking over home roles of women (for example, matrons in hospitals) and the reason that doctors prefer not to treat members of their own families. He labours the psychological ramifications of incest and neglects genetic drawbacks. He also needs to delineate more clearly the difference between job status and personal prestige and their significance for all kinds of workers in industrial communities. In a book which is based on wide survey and scholarship in many disciplines, however, Banton expostulates so persuasively that most readers should welcome *Roles* as a worthy descendant of T. H. Marshall's study of social class in 1934. The transparent simplicity of the writing adds value to the text and altogether makes this one of the most outstanding contributions to sociology during the past three decades. It could profitably be used in the education of all who are concerned with people, whether primitive or from industrial societies. This

volume, and others which should stem from it, should lead in constructive manner to greater understanding and skill in social control. T. H. HAWKINS

THE BEST USE OF MATHEMATICAL INNOVATION

The Best Use of Economic Resources

By L. V. Kantorovich. English edition edited by G. Morton. Translated from the Russian by P. F. Knightsfield. Pp. xxxiii+349. (London and New York: Pergamon Press, Ltd., 1965.) 100*s*. net.

THE Best Use of Economic Resources is an important book for the historian of economic thought, a valuable one for the economist anxious to improve his technical capacity, and a fascinating one for those interested in the development of the economy of the U.S.S.R.

Linear programming is a mathematical technique with many important applications in economics. Kantorovich was probably the first to use it (in 1938) and to publish on it (from Leningrad University in 1939). The first Russian edition of this book was published by the Academy of Sciences in 1959. This edition in English now enables a comparison to be made between Kantorovich's approach and the techniques independently developed since, mainly in the United States.

The book confirms the view, or prejudice, that to find close similarities in independent discoveries of mathematical techniques is less surprising than, for example, in theoretical model building. Even where Kantorovich considers that his methods differ significantly, for example, from Dantzig's simplex-method, the similarities seem greater than he imagines. The references Kantorovich cites to the American work are very early ones and perhaps he was unaware of the extent to which their refinements paralleled his own.

Kantorovich's exposition of his techniques is admirably lucid, well stocked with helpful numerical examples and requires only a minimum of elementary algebra from the reader. One problem dealt with is how best to allocate a desired output of two (later three) products between a number of factories. Another is how to determine the best combination of factors of production with which to produce a given commodity. Kantorovich's concept of 'objectively determined valuations' plays a key part in his techniques for taking optimum decisions. These 'shadow prices' are used to determine the least-cost combination of factors of production and the allocation of output between enterprises. These methods of picking out least-cost solutions from a wide range of complicated possibilities are surely among the most important contributions made by mathematicians to laying foundations for human happiness.

Where Kantorovich touches on the relationship between the process of pricing and allocating factors of production on the one hand and the pricing of commodities on the other, his exposition is not so clear. When he wrote this book Kantorovich was trying to convert Russian planners to the use of more cost-conscious criteria when taking decisions about production. This ran into ideological resistance, because of the view that any identification of costs with scarcity was a challenge to Marx's labour theory of value. Several critical qualifications of this sort are made by the late V. S. Nemchinov in an editorial preface. To argue that scarcity should determine commodity prices was open to additional criticisms, of subjectivity and of suggesting that market forces should supplement or supplant decisions on priorities taken by the planners. Planning methods in the U.S.S.R. have made progress in the seven years since this book was first published, with enterprises enjoying greater autonomy to enable them to respond to the market forces which now exert considerably greater influence on the pattern of production. Much that was either obscure to Kantorovich (or obscured by him) would probably now be treated with greater clarity and directness.

Even now, of course, Russian economists would not claim that this partial equilibrium analysis could be integrated into a general equilibrium system. Their situation in this respect is little different in practice from that of Western economists, who recognize that because of the structure of industry in advanced capitalist economies the integration of their partial and general equilibrium analyses can only take place in theory. But it is useful to have a general equilibrium theory, if only to provide a contrast to the way the economy works in practice. Kantorovich's book is not an attempt at a general equilibrium theory of a planned socialist economy, and so far as I am aware, Russian economists have not yet produced such a general idealized model of their planning system. The changes in economic thinking and practice in the U.S.S.R. bring nearer the time when somebody could. K. J. W. ALEXANDER

RULE-OF-THUMB STATISTICS

Statistical Methods

By Prof. Stefan Szulc. Translated from the Polish by J. Stadler. Translation edited by H. Infeld and C. D. I. Forrester. Pp. viii+666. (London and New York: Pergamon Press, Ltd.; Warszawa: Państwowe Wydawnictwo Ekonomiczne, 1965.) 105*s*. net.

THE title *Statistical Methods* is a slight misnomer. The book is almost exclusively concerned with the collection and presentation of statistical data, mostly of a demographic or economic nature. It discusses, though not very thoroughly, data processing, but punched cards and the machines associated with them are described as if they were the most modern thing in a statistician's equipment; electronic computers are not mentioned at all. This probably explains why the calculation of multiple correlation coefficients is regarded as "very arduous" (p. 489).

The concept of probability is discussed only in a chapter of sixteen pages towards the end of the book. An idea of the vagueness of the treatment can be derived from the following 'definition', set out in italics: "*The probability of an event is the ratio of the number of cases favouring the event to the number of all possible cases (those favouring the event and those not) assuming that all these cases are equally possible*" (p. 505). The late author admits, it is true, that "this definition is not correct from the formal point of view", but no correct definition is given later. Thereafter one is not surprised to find no mention of the concept of a probabilistic model, without which no statistical interpretation of raw data is possible. This applies, in particular, to the problem of smoothing statistical data, eliminating random disturbances, and discovering trends; but then this question is treated in advance of the little chapter on probability. The vagueness of the approach to theoretical considerations is further illustrated by the following sentence: "The property of the averages is that by substituting an appropriate average for the particular values of the variable we obtain for the whole population the same result as we would get by taking into account individual values corresponding to particular observations" (p. 170).

This quotation gives also an idea of the quality of the translation. One cannot expect high standards when publishers use translators whose work has to be 'edited' by somebody else. In any event, the editors failed, for example, to provide references to existing English translations of books quoted in Polish translations; however,

they seem to have been careful about the syntax of the first thirty pages.

It is a pity that the Polish statistical literature, rich as it is in excellent publications, should now be represented by a book which was obsolescent when the original appeared twelve years ago. However, a practical statistician can still find in it a great deal of shrewd advice on gathering and presenting statistical data, and even curious examples of deliberately misleading graphs. The wealth of examples is certainly instructive in many ways, and notes on the early history of statistics, going as far back as Biblical times, are very interesting. A few statistical tables are always welcome. The book is well produced.

S. K. ZAREMBA

ESSAYS OF TEILHARD DE CHARDIN

The Appearance of Man

By Pierre Teilhard de Chardin. Translated by J. M. Cohen. Pp. 286. (London: William Collins, Sons and Co., Ltd., 1965.) 25*s*. net.

THE Jesuit Teilhard de Chardin was a really great man. He was born in Central France, the son of what in England would have been called a 'gentleman farmer'. In his maturity, he was in the front rank as a palaeontologist, he was a first-rate geologist, a philosopher of note, and a profound Christian. Attention has to be paid to the opinions of such an individual. His books are widely read and have been translated into English. Indeed, at the moment, quite a cult of Teilhard de Chardin has grown up.

In spite of the foregoing facts, what, one is driven to ask, is the object of publishing or republishing old essays, some of which date back to 1913 and 1921? The subjects which Teilhard professed are rapidly expanding and changing as new information comes to hand. In 1921 Boule's book was the last word on the subject of fossil man and Teilhard's opinion of it was at that time well worth knowing; but nowadays much has changed—including, naturally, Teilhard's own attitude to many problems. Indeed, the later essays in *The Appearance of Man* show this to have been the case. Of course, where he is writing of factual finds, of excavations in which he took part, the articles are dateless and invaluable. Such is the case for the two essays on Pekin Man. Again the illustrations in the essay entitled "The Question of Fossil Man" are particularly well chosen and useful: if one were asked to produce an illustration of Baron Blanc's Mousterian find of the Monte Circeo skull one would be hard put to it to recall where this was published, but it appears in this essay. Naturally the later articles are of much more contemporary interest, especially those written after de Chardin's visit to South Africa towards the end of his life.

As an old friend of Teilhard I am glad to possess this book because, as well as being pregnant with new ideas, it also shows the evolution of the author's own thought, and perhaps it is in some sense as a historical document that it has been published. But anyone reading it for instructional purposes should note carefully the date when any particular essay was written. There is a preface by Desmond Collins which indeed briefly attempts to bring the reader up to date, and this, of course, is very helpful. Nevertheless this volume should not be classed with such works as *The Phenomenon of Man*, *Le Milieu Divin*, or *The Future of Man*. It is obviously not fair to compare a collection of purely scientific essays with those great works of philosophic thought, but I can only feel that Teilhard himself would have gently but gravely protested against the republication of some of his writings which he would have been the first to describe as *vieux jeux*.

M. C. BURKITT

JOHN HUNTER'S MUSEUM

History of the Trustees of the Hunterian Collection

By Sir Victor Negus. Pp. viii+132+7 plates. (Edinburgh and London: E. and S. Livingstone, Ltd., 1966.) 30*s*. net.

AN inestimable debt of gratitude is owed to two medical men who have enriched knowledge by their collections, now held by trustees for the nation. One was the physician, Sir Hans Sloane, founder of the British Museum, the other the surgeon, John Hunter, whose memory is perpetuated by the Hunterian Collection.

Hunter in his Will desired that his museum should be kept intact and if no other purchaser appeared that it should be offered to the Government. He died in 1793. Soon afterwards England was at war with France. Dr. Matthew Baillie, Hunter's nephew, and Everard Home, his brother-in-law, approached the Prime Minister, William Pitt, who said: "What! Buy bottles? We have not enough money to buy gunpowder".

Nevertheless, in 1796, a committee was appointed to report; they advised purchase of the collection. Three years elapsed, and then in 1799 the Company of Surgeons, reconstituted by Royal Charter, received a parliamentary grant of £15,000 for the purchase and undertook to maintain the museum. Further grants were given later. In 1800 the Company became the Royal College of Surgeons of London. Trustees, pre-eminent in science and public life, were appointed and William Clift, Hunter's devoted assistant, was appointed curator. The Government also made certain terms and conditions of the Trust including a course of lectures on comparative anatomy. By 1818 the museum found a permanent home in the Royal College of Surgeons in Lincoln's Inn Fields.

The original collection comprised some 13,682 specimens. The physiological section was in two divisions; the first illustrated the structure and parts of a plant, animal or man, and the second concerned the reproduction of the living individual, thereby continuing its species. Another division included pathological specimens and illustrated the effects of disease or injury on normal structures. Other, smaller sections contained specimens of human and comparative osteology, geology, monsters and malformations, undissected animals, vertebrates and invertebrates and stuffed animals and birds.

History of the Trustees of the Hunterian Collection recounts the later history of the Trustees and the museum. Sir Victor Negus gives concise notes of the eminent scientists who have cared for and increased the value of the collection; for example, Sir Richard Owen, Sir William Flower, Sir Arthur Keith and Dr. Wood Jones, the first Sir William Collins professor of anatomy and conservator of the Anatomy Museum.

During the First World War, storage in a sub-basement sufficed for protection of the collection. In the Second World War, although extra precautions including strengthening of the sub-basement were adopted in 1940, they proved inadequate in view of the increasingly destructive power of high explosives. On the night of May 10–11, 1941, a high-explosive bomb destroyed two-fifths of the original Hunterian specimens and three-fifths of the 60,000 specimens including the valuable collection made in the First World War. By careful salvage, about 26,000 out of 60,827 specimens were saved. The president of the College, Sir Hugh Lett, and the acting chairman of the Museum Committee, Sir Gordon Gordon-Taylor, procured accommodation for the salvaged collection at various institutions and private centres in the country.

At the conclusion of the War the collection was gradually brought back to Lincoln's Inn Fields. The specimens, with further additions, have now been arranged by Miss Jessie Dobson, the anatomy curator, in the rebuilt portion of the College. Generous contributions have been made by the Wolfson Trustees, by the MacRae Webb-Johnson Fund and by Fellows of the College.

Sir Victor Negus has dedicated his excellent *History* of the Hunterian Trustees to the memory of John Hunter. It shows how the Trustees have well maintained the purpose of the collection to teach scientific surgery to undergraduate and postgraduate students. Hunter brought an observant scientific mind into the study and practice of surgery and in the Hunterian Museum this abides to-day.

ANTARCTICA

Antarctica

Edited by Trevor Hatherton. Pp. xvi+511. (London: Methuen and Co., Ltd., 1965.) 90*s*. net.

EVEN a cursory acquaintance with conditions in Antarctica is sufficient to enable one to understand why it has stimulated the interests of explorers and, more recently, of earth scientists even if, to date, its economic importance is represented only by a declining whaling industry. For those who approach the Antarctic via the South Atlantic, the first view of a typical landscape is South Georgia, a mountainous island with extensive glaciers and a fringe of vegetation consisting of lichens and coarse grasses. The fact that it is, nevertheless, at the same latitude as Sheffield emphasizes the contrast between the southern and northern hemispheres: that civilized life can exist in northern Europe at latitudes which involve considerable survival problems in the southern hemisphere.

Less than one-tenth of 1 per cent of the fourteen million square kilometres of continental Antarctica is free from the ice which averages 2 km in depth over the continent as a whole. The removal of this ice would raise the level of the oceans by 60 m, while the land surface of the continent would rise by some 600 m through isostatic compensation. Nevertheless, Antarctica is an arid region with a precipitation of only 12–15 cm equiv. of water per year. This, however, is sufficient to account for the vast tabular icebergs which break away from the perimeter of the continental ice shelf. Antarctica also has its oases, like Lake Vanda with a permanent ice cover 4 m thick and yet with a temperature of 26° C at its bottom. Is there net accumulation of ice over the continent? What is the explanation for the thermal balance in the 'oases'? Almost every statistic about Antarctica raises questions to which answers are still being sought.

Antarctic research is carried on by some twelve nations and, although the primary scientific reason is the extension of man's knowledge of his global environment, there are respects in which it may contribute to his more immediate well-being. The extension of meteorological observations is relevant to weather prediction throughout a vast area of the hemisphere while ionospheric data are important in connexion with radio communications, which are especially difficult in polar regions (due to auroral and associated phenomena).

The heroic era of Antarctic exploration which commenced with Captain James Cook's circumnavigation in 1772–75 finally gave way to intensive scientific investigation, with the final great journey in 1957–58 of Fuchs and Hilary coinciding with the International Geophysical Year which brought into being a vast international scientific programme. This has largely been maintained and, indeed, has been extended during the International Years of the Quiet Sun of 1964–65. Intensive land-based scientific work on any scale is very recent indeed and may be said to date from the creation, in 1943, of the Falkland Islands Dependencies Survey with the establishment of a number of stations in the Antarctic Peninsula (formerly Graham Land) and the initiation of a detailed survey, regular weather observations, etc.

The present volume, entitled *Antarctica*, is edited by Trevor Hatherton on behalf of the New Zealand Antarctic Society. Hatherton was New Zealand's chief scientist for the Antarctic during the International Geophysical Year and his twenty-two contributors are drawn from the United States, New Zealand and the United Kingdom. The subject-matter is divided into four main parts: the nations in Antarctica; the southern ocean; the antarctic continent; the south polar atmosphere. Each author is a specialist in his field and to some extent this is a book for specialists. On the other hand, it is characteristic of Antarctic research that few who embark on it can avoid developing wider interests outside their own speciality. The present volume will therefore help the individual to acquaint himself with what his colleagues in other scientific disciplines are doing and enable him to set his own work in a wider context.

With so many contributions it is unfortunately possible only to make a selective review.

The first chapter offers a historical survey of national interests and activity in Antarctica from its discovery until the Antarctic Treaty. Some may regard this latter as the most important outcome of the International Geophysical Year, representing a political reconciliation of competing territorial claims in which the U.S.S.R. and the United States joined in taking a leading part. The Treaty is quoted in full in an appendix and it is, perhaps, worth recalling here the following extracts:

". . . Recognising that it is in the interest of all mankind that Antarctica shall continue forever to be used exclusively for peaceful purposes and shall not become the scene or object of international discord; . . . There shall be prohibited, *inter alia*, any measures of a military nature. . . Freedom of scientific investigation in Antarctica and cooperation toward that end, as applied during the International Geophysical Year, shall continue, . . .".

Chapters 2 and 3 deal with "Techniques of Living, Transport and Communication" and with "The Mapping of Antarctica" respectively. While the advent of the aeroplane together with the tellurometer has revolutionized mapping, it is nevertheless true that dogs are in many cases still the safest and often the only suitable means of transport. Living has its own problems, especially when men must live underground like rabbits while maintaining apparatus for scientific observations above the surface.

Chapters 4–7 deal with oceanic Antarctica, the movements of both water and ice pack, the ocean floor, the fauna and the abundant higher species of birds and mammals. The section on the continent, Chapters 8–14, discusses the physical characteristics and dynamics of production and wastage of the Antarctic ice, the distribution of land below the ice and its geology, as well as the flora and fauna.

The final section (Chapters 15–18) is concerned with the south polar atmosphere, its meteorology, the upper atmosphere and ionosphere, and the polar geomagnetic field and its fluctuations. The Aurora Australis, like its associated Boreal phenomenon, occurs most frequently in a zone within the polar region and is allocated a special chapter (Chapter 16). The aurora is closely associated with geomagnetic disturbance produced by electric currents flowing in the auroral regions, and it is interesting to note that it was this connexion that led C. F. Gauss to speculate so long ago as 1839 on the existence of an electrically conducting upper atmosphere. We now know that a primary mechanism in auroral production is the precipitation, down magnetic lines of force, of energetic electrons and protons. Thus one important aspect of Antarctic auroral research is concerned with studies conducted simultaneously in both hemispheres from stations which are connected by the same geomagnetic field line. Gauss spoke of the "enigmatic phenomena of the Polar Aurora"; after a lapse of more than a century it still has its mysteries to be resolved.

Antarctica is, technically and scientifically, a volume of considerable merit and is provided with an up-to-date map (scale 1 : 16,000,000) on which are indicated the locations of the various scientific bases. T. R. KAISER

SHORT REVIEWS

Satellites of the Solar System

By W. Sandner. Pp. 151. (London: Faber and Faber, Ltd., 1965.) 36*s*.

THIS is an interesting book in that it confines its attention almost entirely to the natural satellites of the planetary system; reference to the primaries is made only when necessary to clarify some point of the description. After a brief introduction to the history of observational work and the laws of Kepler and Newton, the author deals first with the satellites of the planets beyond Mars and returns to the inner worlds near the end of the book.

The work suffers little in the translation, and will appeal to the interested layman as well as to the amateur astronomer; indeed, some of the references to early observational work are rarely seen in more advanced works, yet they can, when separable from defects of optics and technique, be quite useful.

The few faults in mathematical notation will not distract the casual reader, and those who do detect them will also realize what is actually intended. This book is a useful contribution to the literature for those at the beginning of an interest in our closer neighbours in space.

L. WILSON

Elements of Geochemistry

By Prof. Yasuo Miyake. Pp. 475. (Tokyo: Maruzen Company, Ltd., 1965.) 12 dollars.

THIS text-book is said to be a modernized revision and translation of a work in Japanese first published in 1954. Although it bears a 1965 date-line, reference to investigations published later than 1958 is restricted to the mention of a few papers from Japan; and as a consequence of this lack of observations drawn from the recent vigorous growth of geochemical science elsewhere, some of the information which the book presents is obsolete. For example, a section on 'the oldest rocks' is based on a paper of 1952, giving maximum ages of no more than 2,200–2,400 m.y. instead of around 3,500 m.y. Not one of the great range of contributions published since 1956 in the Russian periodical *Geokhimiya* is referred to, although this journal is available in English translation. In his preface, the author rightly observes that he has paid more attention to the boundary problems between astronomy, geophysics and geochemistry than to a general review of geochemical science; and he claims that more space is devoted to cosmochemistry and to the geochemistry of the atmosphere and hydrosphere than in other texts of this kind. Since, however, his treatment of the chemistry of the Earth's crust compares somewhat unfavourably with that of geologically oriented works available at a lower price, it is difficult to recommend the book as a teaching text, particularly as it has received insufficient editing to ensure uniform clarity of presentation and eradication of the very many misprints.

C. F. DAVIDSON

The Collected Papers of Lord Rutherford of Nelson, O.M., F.R.S.

Vol. 3: Cambridge. Published under the scientific direction of Sir James Chadwick, F.R.S. Pp. 428+10 plates. (London: George Allen and Unwin, Ltd., 1965.) 90*s*. net.

THE third volume of *The Collected Papers of Lord Rutherford of Nelson* contains his work while Cavendish professor at Cambridge from 1919 to 1937. This was his mature period when, having laid the foundations of nuclear physics, he directed an already famous laboratory with very great distinction. Naturally the earlier papers in the volume are the most important; presumably later in the period he devoted more of his time to the general welfare of the laboratory and to advising and encouraging the many able physicists who visited the Mecca of nuclear physics.

The most important papers deal with the very occasional production of long-range protons when light nuclei are bombarded by α-particles. Rutherford correctly interpreted these observations as evidence for the absorption of the α-particle by a nucleus with the subsequent emission of the fast proton. For the first time the disintegration of light nuclei had been observed.

Rutherford's Guthrie Lecture of 1927 on "Atomic Nuclei and their Transformation" is a very clear summary of all the work of himself and his colleagues on the interactions of α-particles, including the rare disintegrations. He speculated freely about nuclear structure and nuclear forces and produced some fascinating nuclear models.

Together with some young colleagues, Rutherford performed several beautiful experiments on the very sure long-range α-particles emitted from radium *C* and correctly interpreted the complex phenomenon in terms of excited states in nuclei.

Later on we find Rutherford using both protons and deuterons to probe the structure of nuclei although he frequently reverts to the use of his beloved α-particles. At the end of his life he was searching for the mass-3 isotopes of hydrogen and helium.

Many of the papers reproduced in the volume are classic examples of scientific exposition. All students of physics would benefit greatly from reading them. It is not usually possible to find time to search out the originals so we are greatly indebted to the late Paul Rosbaud, who inspired Sir James Chadwick to collect together all of Rutherford's written work into this invaluable series of volumes, which are a truly worthy monument to his work as a physicist.

C. C. BUTLER

Weltkarten zur Klimakunde

Von H. E. Landsberg, H. Lippmann, KH. Paffen und C. Troll. Zweite auflage. Herausgegeben im Auftrage der Heidelberger Akademie der Wissenschaften von E. Rodenwaldt und H. J. Jusatz. Pp. viii+28+5 Weltkarten. (Berlin: Springer-Verlag, 1965.) 30 DM.

THE title page, contents list, foreword and text of this publication are presented in both German and English; a pocket at the back encloses five world maps (scale 1 : 45,000,000, equal area projection) of January sunshine, July sunshine, annual sunshine (hours), annual total of global (that is, direct plus diffuse solar) radiation (cal cm^{-2}) and 'seasonal climates of the earth'. There is not, therefore, a lot of new information for 30 DM, but it must be conceded that the printing and binding are of the highest quality.

The maps are intended, in conjunction with an atlas of epidemic diseases already published, as a contribution to medical meteorology, a subject which has traditionally excited more interest in German- than in English-speaking circles, both medical and meteorological. It is hard to see how such relatively small-scale maps, necessarily displaying only very generalized isolines, can advance the subject much, though it no doubt ill becomes a non-medical meteorologist to say so. The maps are, of course, authoritative. Dr. Landsberg, who is director of climatology in the United States Weather Bureau, is responsible for those of solar radiation and contributes a terse note on radiation climatology. Prof. Troll, of the University of Bonn, is meticulous, though verbose, in explaining his

classification which is the basis of the climatic map. The whole of the information can be found elsewhere without too much difficulty; the book is a well-made luxury.

G. D. ROBINSON

Plasticization and Plasticizer Processes

A Symposium sponsored by the Division of Industrial and Engineering Chemistry at the 147th Meeting of the American Chemical Society, Philadelphia, Pa., April 6–7, 1965.) (Advances in Chemistry Series, No. 48.) Pp. ix+200. (Washington, D.C.: American Chemical Society, 1965.) 7 dollars.

PLANS for considerable increases in production capacity for polyvinyl chloride announced by manufacturers in Britain will be accompanied by similar increases in plasticizer output and interest in both plasticization and the production of plasticizers, and makes the publication of this book timely. It includes specialist papers of both a review and an experimental nature on a number of different aspects of the subject, which were presented at a symposium in the United States in 1964. Its title is, therefore, perhaps misleading since it is not a reference book. Unlike a number of other books published in recent years it is international in character, papers being presented from five different countries. These cover the theory of action of plasticizers; although it is clear that the user will still have to make his choice of plasticizer largely by means of empirical tests. Esters still predominate as plasticizers, and there are some interesting papers discussing recent developments of interest to the manufacturer of plasticizers, in particular further work on the production of ester plasticizers directly from olefins, and also the use of amphoteric type catalysts in esterification. Other papers include a review of polymeric plasticizers, analytical aspects and some highly specialized papers of rather limited interest. This book is probably the most authoritative to appear since the publication of Doolittle's book in 1954 and will be of considerable value to research workers who are interested in either the production of plasticizers or in obtaining a greater understanding of the action of plasticizers. It is reasonably priced.

R. J. WICKER

Heat Transfer in Structures

By H. Schuh. (International Series of Monographs in Aeronautics and Astronautics, Vol. 7.) Pp. xviii+338. (Oxford, London and New York: Pergamon Press, Ltd., 1965.) 70*s*.

THE author has set out to strike a balance between the interests of all workers in the heat-transfer field and the special interests of those in the field of aeronautics. Although his examples are taken wholly from aeronautics, *Heat Transfer in Structures* is certainly of considerable interest to a wider public.

After dealing with external heat-transfer to structures in high-speed flight, and the fundamental laws for heat-transfer within structures, the author deals separately with (*a*) analytical, and (*b*) numerical methods of calculation for (1) steady, and (2) transient heat flow. He finishes the work with a discussion of heat flow in particular structural elements, the use of analogues, the similarity laws and model testing for heat flow in structures.

Complex shapes, found in other fields such as turbine rotors and casings, and steam header pipes, are not mentioned. It is as well to remember while reading the book that the applications discussed are, on the whole, applications to simple shapes. This must be particularly borne in mind when assessing the justification for the author's happy conclusions regarding the small errors in his calculations.

Nevertheless, the author's approach is a fundamental one and everyone has much to learn. Many problems common to aeronautical and other fields are discussed, and particularly interesting are the effects of the flight plan on heat-transfer and his comparison of the effects of the modes of heat-transfer.

RUSSELL HOYLE

Impeller Pumps

By Stephen Lazarkiewicz and Prof. Adam T. Troskolański. Pp. xiv+648. (London and New York: Pergamon Press, Ltd.; Warsaw: Wydawnictwa Naukowo-Techniczne, 1965.) 120*s*. net.

IMPELLER Pumps is a good book, well written and well translated. The authors, one an eminent consulting engineer, the other a distinguished teacher, have revised and enlarged their first book, which was in Polish. Prof. S. P. Hutton has written the preface. The volume is an excellent treatise and book of reference for student, designer, draughtsman, consultant and contractor. Comprised of twenty-three chapters with 506 figures, the book deals with theory and design practice, installation and maintenance. Worked examples demonstrate methods where necessary and each chapter has a list of references which show that the authors have used books from ten different countries.

Diagrams, figures and printing are excellent. Proof reading has been good—there are few printers' errors—and a useful list of symbols is provided. Acknowledgment is made to thirty-nine pump-makers who have contributed to the book in some fashion; nineteen of these are British. Name index and subject index are adequate, although I feel that the latter might have had broader coverage. Centrifugal, mixed-flow, diagonal and propeller pumps are discussed in detail. Attention is given to hydraulic side-thrusts, volutes, recuperators and diffusers of all types, and reference is made to peripheral and water-ring pumps.

Quite properly, the authors have a large chapter on fundamental theory of impeller flow. The longest chapter of all is that dealing with pumps for special duties, such as pumped storage, boiler-feed, corrosive liquids, solids in suspension, etc. Constructional and design details are given for castings, balancing, sealing-rings, stuffing-boxes, shaft sizes, bearings, sudden temperature changes, etc. Much space is allotted to discussing impeller flow, number of blades and choice of blade angles. Dynamical similarity and scale-model tests are fully discussed, as well as full-scale and air testing. The sections on self-priming, pump driving, pump–pipeline systems and discharge regulation are useful, but little or no space is given to water-hammer or surge problems in connexion with complete impeller head–discharge characteristics.

Modern theories of cavitation phenomena are clearly expounded and in a long chapter on cavitation the authors give a full and lucid explanation of net positive suction head or, as they prefer to call it, anti-cavitation pressure margin. The book ends with a short section listing common faults in pump operation and how to correct them.

Undoubtedly, coming as it does from the authors' own experience, this volume is a welcome addition to the literature on the growing science of pump design.

A. S. THOM

Science of Ceramics

Proceedings of the 2nd Conference held under the auspices of the British Ceramic Society and the Nederlands Keramische Vereniging, at Noordwijk aan Zee, May 1963. Edited by G. H. Stewart. Vol. 2. Pp. 431. (London and New York: Academic Press, Inc., 1965.) 84*s*.

SCIENCE of Ceramics records the papers presented at a joint meeting of the British and Dutch Ceramic Societies held in Holland in 1963, which was the second of a series planned by the two societies to provide opportunities for the exchange of views and information in the rapidly growing and peculiarly challenging field of ceramics. The

book contains twenty-eight papers, of which twenty-one are in English, four in German and three in French. Eleven of the papers are by British authors.

The first group of papers includes an important account of the characteristics of fine oxide powders (down to below 100 Å) and a 'new look' at kaolinitic clays which challenges X-ray and differential thermal analyses and proposes an ingenious, simple alternative method of estimating the nature and proportion of clay mineral in a kaolinitic clay. The second group covers process problems: one paper deals with the principles of comminution and another with compaction of powders. A study of torsional hysteresis in plastic clay throws new light on 'plasticity'.

The third group, covering behaviour during the firing process, presents a number of papers on sintering, ranging from the dynamic properties of grain boundaries to the effect of atmosphere. The final group covers structures and properties, and ends with a short survey of glass-ceramics. Another paper deals with solid solutions of certain oxides which can be used as solid-state electrolytes.

While the general reader must not expect to get from this book a coherent picture of ceramic science and technology, he will be provided with a wide sample of some of the problems, and at the same time the specialist will find a good deal of useful material here. The book is well produced and well documented, and one looks forward to the steady progress of this new series. N. F. ASTBURY

Annual Survey of Organometallic Chemistry

Vol. 1. Covering the Year 1964. By Dr. Dietmar Seyferth and Dr. R. Bruce King. Pp. viii+330. (Amsterdam, London and New York: Elsevier Publishing Company, Ltd., 1965.) 110*s*.

THE annual review type of literature is one of the most useful forms of secondary research source: it suffers from a disadvantage in that the work reported is disjointed and undigested, but has the great advantage that this is red-hot from the journals. Even the lack of digestion is sometimes an advantage; it does not impose a point of view and chance scraps of information or chance juxtapositions can be very evocative of new ideas.

The primary literature in the rapidly growing field of organometallic chemistry can be very confusing, and an annual survey such as this performs a real service. In order to systematize the subject, it is divided according to the periodic table, but with a separation of main group metals from transition metals. The form of presentation is similar to the staccato one familiar to readers of the *Annual Reports* of the Chemical Society, but coverage is far more extensive and there is a welcome abundance of formulae. The aim has been to pick significant contributions, and although this is clearly a subjective matter, the authors appear to have performed their herculean task with skill and industry.

Whether for browsing by inorganic or organic chemists who are just interested in a general way, or as an entry into the literature for research students, this book, and no doubt its successors, will be worth having on the bookshelf. A. J. BIRCH

Introducing the Insect

By Prof. F. A. Urquhart. Pp. x+258. (London: Frederick Warne and Co., Ltd., 1965.) 30*s*. net.

IT is difficult to know what to say in fair comment on *Introducing the Insect*. It opens with the words: "This book is designed for the beginner . . . ", and on this basis it should be judged. It certainly qualifies in simplicity, clarity and directness. The style is conversational, without being jocular, slangy or journalistic. The text is clearly arranged, broken into short sections with headings in bold type, and no time is wasted by either the writer or the reader in getting to the point. There are keys to orders and to families, and plenty of simple, clear drawings.

The author is a professional entomologist of international standing, known for his detailed studies of the Monarch butterfly. Entomologically, therefore, the book is sound, and much of the information comes from the author's own experience. In fact what the book says is admirable; it is what it omits that causes misgiving.

The keys provide an example of this. Pages 34–37 discuss how to use a key, and as an example a simple key is constructed to four types of screws and nails. "You can see how easy it is to use identification keys, and how helpful they are." This works satisfactorily so long as you know that there are only four items, and that your choice must be one of these, but how far would anyone get who took such a little key into an ironmonger's shop? All the keys in the book suffer in like manner. An incomplete key to orders, omitting those that are 'obscure', is probably justified, and is helped out by the generally excellent little drawings. But is the reader really helped by: "17. Antennae usually thread-like or feebly clubbed; usually found feeding on leaves of plants. *Leaf-eating Beetles*" (p. 155)?

The function of any book of instruction is not only to give the reader information, but also, and equally important, to leave him clearly aware of the limits of his knowledge. The most serious criticism of the present book is that it does not do so; that, indeed, the formal arrangements of keys and text gives a misleading impression of near-completeness. This book does not compare in quality with the books of the "Wayside and Woodland" series by the same publisher, which are listed on the back of its book-jacket. HAROLD OLDROYD

The Coccidian Parasites (Protozoa, Sporozoa) of Rodents

By Norman D. Levine and Virginia Ivens. (Illinois Biological Monographs, No. 33.) Pp. 365 (48 plates). (Urbana, Illinois: University of Illinois Press, 1965.) 8.50 dollars.

THIS book should certainly be in the library of every parasitological department. Its authors are well known throughout the world for their research on, and wide experience of, the coccidian parasites, not only of rodents, but also of birds and other hosts as well. Well and clearly written, and illustrated by copious figures assembled in forty-eight plates which are either photographs or very clear line drawings and two text-figures, all of which together show clearly the diagnostic features of the species of coccidia described, the book considers and defines 176 species of the genus *Eimeria*, nine species of the genus *Isospora*, one species each of the genera *Caryospora*, *Dorisiella*, *Wenyonella klossia* and *Tyzzeria*, two species of the genus *Cryptosporidium* and three of the genus *Klossiella*. The characteristics of the species of these genera found in rodents are summarized in Tables 1–29. Table 31 gives the characteristics of the species of the genus *Eimeria*, and Table 32 summarizes the results of cross-infection experiments carried out with species of *Eimeria* found in rodents. The extensive list of references to the literature adds greatly to the value of the book, which is beautifully produced and is printed on glossy paper throughout, the whole production reaching the high standard we have learned to expect from these publishers. Every parasitologist will agree that the authors have performed a great service by writing this book. G. LAPAGE

Brave New Victuals

An Inquiry Into Modern Food Production. By Elspeth Huxley. Pp. 168. (London: Chatto and Windus, Ltd., 1965.) 21*s*.

MRS. HUXLEY'S new book deals in the usual old victuals. Although it is sub-titled *An Inquiry Into Modern Food Production*, it covers everything from factory farming to fall-out, and takes in hormones,

insecticides and chemical weed-killers *en route*. In short, it examines all the 'unnatural' procedures, except artificial insemination, which have recently been deliberately or accidentally introduced into modern farming. While the book says nothing new, it does summarize most of the arguments for and against intensive farming and the uses of chemicals in food production, in fifteen concise and reasonably up-to-date chapters. There are some minor errors of fact, and some of the references in the chapter-by-chapter bibliography are too incomplete to be of use to the lay reader.

The book betrays its origins as a series of articles in *Punch*, not only because each topic gets nearly equal treatment, but also because clarity of presentation is often sacrificed to journalism. ("Were *they* happy? Who knows? The crated calves I saw did not *look* unhappy . . .", may involve the reader emotionally, one way or the other, but it scarcely helps understanding.) Intensive animal husbandry has been more rationally, and more effectively, considered in the recent report of the Brambell Committee[1].

The journalistic approach also oscillates between recognizing the need to be modern and 'with it', and the appeal of supporting the 'natural' order. So, while the need to meet food shortages is recognized, a bias towards old-fashioned techniques tends to show through and discussion of pros and cons becomes clouded. There are no suggestions about acceptable alternatives, and no detailed analyses of the reasons why accountants are taking over from farmers: the book is descriptive rather than constructive. Not surprisingly, then, it concludes with a mystical discussion ("Is what is wrong, sheer numbers?") and ends with the suggestion that the missing factor in our approach to food production is charity: the computer, calculating profitability, cannot fit love into the farming equation.

J. H. SANG

[1] *Report of the Technical Committee to Enquire into the Welfare of Animals kept under Intensive Husbandry Systems.* (Cmnd. 2836.) (London: H.M.S.O., 1965.)

Differential and Riemannian Geometry

By Prof. Detlef Laugwitz. Translated by Fritz Steinhardt. Pp. xii+238. (New York: Academic Press, Inc., ; London: Academic Press, Inc. (London), Ltd., 1965.) 68*s*.

DIFFERENTIAL and Riemannian Geometry is a translation of the 1960 Teubner book, *Differentialgeometrie*. Its main feature is that it combines an account of classical differential geometry (of curves and surfaces) with an introduction to the tensor calculus and Riemannian geometry. It is a refreshing text, among books in English on the subject, because it contains various results not usually found in English texts. Among these special mention might be made of the results on curves and surfaces in the large, given in the final chapter.

A short first chapter on the local geometry of space curves is followed by one on surfaces. The tensor calculus and Riemannian geometry are then introduced. The next chapter commences with an account of spaces of constant curvature and non-Euclidean geometry. After a section on mappings there is an interesting and important discussion on Riemannian spaces and analytical dynamics. Finally there is a section on the characterization of Riemannian geometry.

In the final chapter, on differential geometry in the large, considerable use is made of Herglotz's method of integral formulae. The four vertex theorem is proved, and also Fenchel's theorem that for every closed space curve, the total curvature is at least 2π (and is 2π only for plane convex curves). These results are followed by theorems on surfaces in the large.

For a fairly extended honours course in differential geometry this book would be most stimulating. It contains more than one hundred and fifty exercises and many references to original papers. There is a short appendix on the history of differential geometry and another appendix on some relevant theorems of analysis.

L. S. GODDARD

The Measurement of Efficiency of Scientific Research

By Dr. Ben-Ami Lipetz. Pp. xv+262. (Carlisle, Mass.: Intermedia, Inc., 1965.) 7 dollars.

THE main purpose of this book is to clarify the meaning of efficiency in scientific research and to indicate specific approaches by which the efficiency of such research could, in practice, be measured with the view of increasing productivity. The book is refreshingly free from jargon, for the most part it is well written and its lucid argument supports its emphasis on the importance of clear writing in publications on research. It is, moreover, thoroughly pragmatic and the author shows himself well aware of the essential factors in creativity research and the problems that confront the administrator. He recognizes, for example, that the effective utilization of manpower is one of the most acute problems, and that while there has been little or no improvement in the efficiency of scientific research, improvement must come essentially from the administrator's own hard-won experience. Dr. Lipetz's criticism of methods proposed for evaluating or comparing some contributory methods is sound but, nevertheless, rejecting the idea that any research project is unique in its objectives and achievements and therefore beyond comparison, he proceeds to outline possible procedures for comparison.

This is done in successive chapters on the validity and limitations of the efficiency concept, the recurrent products of scientific research, the objective measurements of scientific achievement and measurements of the scientific content of communications and the problems presented. For his purpose, he relies essentially on a comparison of the reports or scientific publication resulting from the research, in spite of the admitted difficulties to which he frankly directs attention. For all the clarity of the exposition of the need or desirability for some comparison of scientific research, the author is not convincing in his account of how in practice this is to be done, and the latter part of the book disappoints the expectations aroused by his initial aproach. There are ample references which cover the limited literature reasonably, and there is a good bibliography, but the book deserves an index.

R. BRIGHTMAN

The World of Learning 1965–1966

Sixteenth edition. Pp. xiv+1442. (London: Europa Publications, Ltd., 1965.) 140*s*. net.

THE sixteenth edition of *The World of Learning* contains classified information on the universities, colleges, learned societies, libraries, museums, art galleries and research institutes of 158 countries. The new edition is not greatly increased in bulk but has been considerably extended by the use of three columns to a page for the greater part of the university and college sections. This has enabled the editors to include more than 1,500 institutions not previously listed, 625 of which are in the United States. Other features include an up-to-date account of the United Nations Educational, Scientific and Cultural Organization and a section dealing with the International Council of Scientific Unions. The sections on universities and colleges give details of the professorial staffs, rectors, registrars, librarians and deans of faculties, and include list of publications and details of the numbers of students. The sheer bulk of this volume bears witness to the spread of cultural and especially scientific activity at a high level throughout the world. This book now has an honoured place as a guidebook to world scholarship, and the editors and publishers are to be congratulated on the careful presentation and arrangement of their material.

(*Continued from page 44*)

In the case where the pattern of behaviour as a two-host tick was displayed, some of the larvae that had been experimentally fed did not drop off from the rabbit at all. They moulted into nymphs at their feeding sites. The nymphs also fed but when fully replete they dropped off on the dates shown in Table 3 (*a*).

Table 4. LARVAL TO NYMPHAL STAGE FEEDING SITES OF *Boophilus decoloratus* (KOCH, 1844)

No of mother tick	Date of infesting host	Sites of the host at which engorged nymphs were observed										
		Head	Ear		Eyelid		Back	Neck		Nose	Flanks	
			Left	Right	Left	Right		Left	Right		Left	Right
268	25.5	—	—	—	—	—	—	7	9	—	—	—
1	19.6	—	5	1	4	2	—	—	—	5	—	—
5	24.8	—	11	10	—	—	35	—	—	—	10	15
	Total	—	(27)		(6)		(35)	(16)		(5)	(25)	

The adults bred from the above stock of nymphs could not attach to the rabbit for more than 2 days when attempts were made to feed them on the ear of this animal.

When the host's body was gently stroked in order to locate the small engorged larvae several such immature individuals were dislodged at the same time. It was therefore not easy to make specific quantitative observations on the predilection sites for the engorged larvae. However, most of these seemed to drop from the trunk region of the host. Table 4 (*b*) shows the distribution of the engorged nymphs on the body of the rabbit. It will be seen that the head region, including the ears, the area around the eyes, and the nose appeared to be most heavily infected by the nymphs.

The water dropping from the feeding bottle in the ventilated cage and the urine of the confined rabbit maintained a relative humidity inside the cage as high as 90–95 per cent. In spite of this condition and the apparently favourable laboratory temperature range, it was remarkable that throughout the breeding experiments no larvae were able to develop continuously through the nymphal stage into engorged adults on the body of the rabbit; this is especially remarkable because tick species of the genus *Boophilus* are noted for their one-host type of life-cycle[1–5].

The earlier record from Ghana of atypical behaviour of *B. decoloratus* as a two-host tick species was with reference to its principal domestic host, the ox[1]. This contribution would seem to record for the first time two patterns of atypical behaviour in this tick species on the rabbit, namely, a three-host type and a two-host type of behaviour.

As *B. decoloratus* is a one-host tick, the hosts of its larvae are the same as those of the adults[2]. Hoogstraal[2], Tromsdoff[6] and Bedford[7] have listed hares (*Lepus* sp.) among the wild hosts of *B. decoloratus*. Both the hare and the rabbit belong to the same order—Lagomorpha[8]. It is possible that the atypical patterns of behaviour displayed by this tick species on the rabbit could occur unnoticed in Nature, in the case of the hare.

I thank the Ghana Academy of Sciences for enabling me to undertake this work at the Animal Research Institute.

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ROLE OF PLANT GROWTH IN REGULATING PHOTOSYNTHESIS

By G. B. SWEET and PROF. P. F. WAREING
Department of Botany, University College of Wales, Aberystwyth

IT has long been known that the rate of photosynthesis is one of the more important factors affecting plant growth and that any external conditions affecting the photosynthetic process will, as a consequence, affect growth. It is only comparatively recently, however, that evidence has been put forward to suggest that growth itself can affect the photosynthetic rate, and that instead of there being a one-way relationship, photosynthesis → growth, there may, in fact, be an interrelationship, photosynthesis ⇌ growth.

Went[1] postulated that in some circumstances the rate of photosynthesis might be limited by growth rate, and suggested that a slow rate of translocation of photosynthates out of leaf cells could limit the rate of carbon dioxide reduction. This hypothesis has since been supported by the work of Hartt[2], who found a reduction in the rate of photosynthesis of detached sugar cane leaves following exposure to light of saturating intensity, the decrease in photosynthetic rate being associated with a build-up in sucrose-levels. Similarly, Humphries and Thorne[3], working with detached leaves of dwarf beans growing in nutrient solution, found an increase in the rate of photosynthesis associated with increased translocation following development of roots from the petiole. Such experiments, although not carried out on whole plants, do support the possibility that in whole plants the rate of growth may itself directly affect photosynthesis, by affecting the rate of removal of assimilates from the production areas.

An experiment using whole plants gives further evidence in this direction. Thorne and Evans[4] inter-grafted in all possible stock–scion combinations sugar beet which has a large storage root and a high net assimilation rate with spinach beet having a smaller storage root and a lower net assimilation rate. Grafts with sugar beet roots had a greater net assimilation rate than grafts with spinach beet roots, irrespective of the type of scion used. The results were interpreted to suggest that sugar beet roots increased the net assimilation rate (and thus net photosynthesis) of scions grafted on to them by providing a more efficient 'sink' for assimilates than spinach beet roots, thus again implying a direct effect of growth on photosynthesis.

An experiment with apple trees by Maggs[5], involving part removal of leaves, roots, or leaves and roots in combination, showed that growth rate and net assimilation rate were affected by these treatments. A change in net assimilation rate implies a change in rate of net photosynthesis, and if, as seems likely, the treatments did not affect the rate of photosynthesis directly, the results again imply an effect of growth on photosynthesis.

So far as we are aware, the experiments described here are the first in which direct measurements of photosynthesis have been made on whole plants in order to investigate an alteration in photosynthetic rate caused by a change in internal growth conditions and to examine the mechanics of this change.

Experiment 1. 16-month-old seedlings of *Pinus radiata* grown in compost in 4-in. pots were used for the experiment, which was carried out in December 1964. At this time, while the plants had not formed winter buds they were making slow growth only. Three treatments were applied. In each of these, the plant apex (including all primary needles which had not fully elongated) was removed. The treatments differed only in the time interval between removal of the apex and measurement of photosynthesis, these intervals being as follows:

Treatment 1	8 days
Treatment 2	16 days
Treatment 3	No time interval. This treatment constituted the control.

The plants in series 1 made no obvious new growth between treatment and measurement of photosynthesis, but those in series 2 produced new needles from the primary leaf axils during that period.

Rates of uptake of carbon dioxide by the plants were measured at four different light intensities by infra-red gas analysis using an open-circuit system. Each replicate of each treatment consisted of two plants the roots of which were washed clean of soil and placed in plastic containers of water in the assimilation chamber. The experiment was run with air from outside the laboratory, brought up to a temperature of 20° C and a relative humidity of 80 per cent. The light source was a 6,000 W high-pressure xenon lamp.

Results of net carbon dioxide uptake are presented for the treatments in Fig. 1. Because of the difficulty of measuring leaf area in pines, the data have been expressed as units of carbon dioxide uptake per unit of leaf dry weight per unit of time. Differences were significant at the 1 per cent level.

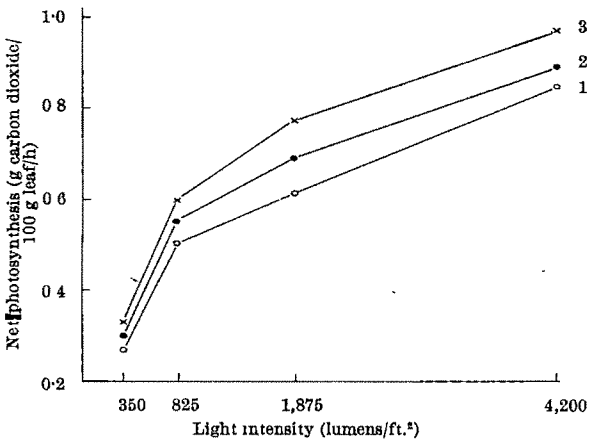


Fig. 1. Rates of net photosynthesis for 16-month-old seedlings of *Pinus radiata* with the plant apex removed The time interval between removal of the apex and measurement of photosynthesis was as follows: series 1, 8 days; series 2, 16 days, series 3, no time interval. Treatment differences are significant at the 1 per cent level

Fig. 1 shows that treatments 1 and 2 gave a decrease in rate of net photosynthesis compared with that of the controls (treatment 3). This decrease was greatest at high light intensities and least at low light intensities. Treatment 1 depressed the net rate of carbon dioxide uptake more than did treatment 2.

Each of the three treatments resulted in removal of the same proportion of leaves from the plants, the only difference between treatments being in the time interval between removal of the apex and measurement of photosynthesis. It is clear, therefore, that the treatment itself could have had no direct effect on the rate of net photosynthesis. Rather, the rate of photosynthesis must have been affected by the changes in internal growth conditions, which probably included the following: (1) A reduced overall 'sink' for assimilates as compared with the controls; (2) a reduced auxin-level in the plants compared with that of the controls.

If either of these changes did, in fact, cause a limitation in the rate of photosynthesis, the effect should have been reduced when new growth commenced after treatment. The plants of series 2 did show new growth prior to measurement of photosynthesis and, in fact, their rate of net carbon dioxide uptake at the time of measurement showed a reversion to a position mid-way between that of series 1 and of the controls. Thus the postulated result was observed.

Experiment 2. Actively growing 11-week-old seedlings of *Pinus radiata* grown in compost in polythene bags were used for this experiment, and four different treatments were applied to them.

Treatment 1	All fully expanded leaves removed 6 days before measurement of photosynthesis.
Treatment 2	One-third of the fully expanded leaves removed 6 days before measurement of photosynthesis.
Treatment 3	All fully expanded leaves removed immediately prior to measurement of photosynthesis.
Treatment 4	Controls. No leaf removal.

Rates of carbon dioxide uptake of the plants were measured as in Experiment 1. However, this time each replicate in each treatment consisted of five plants, and measurement was made at six different light intensities. In addition, respiration rates in the dark were determined. The results are presented in Fig. 2.

It is apparent that treatments 1 and 2 caused a significant increase in the rate of net photosynthesis over that of the controls (treatment 4). This increase was not very great at low light intensities, but at saturating light intensity it was very pronounced, the increase in net photosynthetic rate of treatment 1 over that of the controls being 27·4 per cent. The plants of treatment 3, from which fully expanded leaves were removed immediately before measurement of photosynthesis, showed essentially the same photosynthetic rate as those of the controls, and

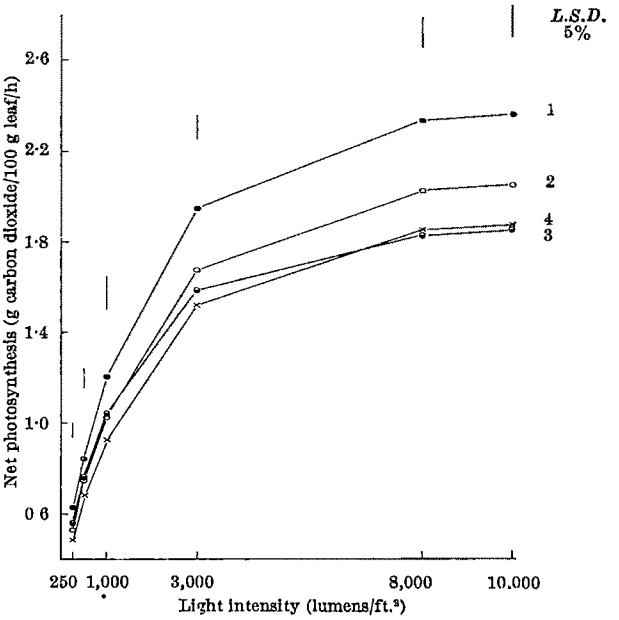


Fig. 2. Rates of net photosynthesis for 11-week-old seedlings of *Pinus radiata* with four treatments applied as follows: series 1, all fully expanded leaves removed 6 days before measurement of photosynthesis; series 2, one-third of the fully expanded leaves removed 6 days before measurement of photosynthesis; series 3, all fully expanded leaves removed immediately prior to measurement of photosynthesis; series 4, no leaf removal. Treatment differences are significant at the 0·1 per cent level

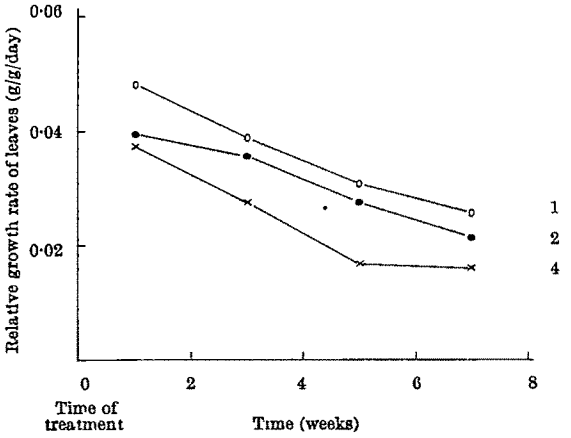


Fig. 3. Changes in leaf relative growth rate with time for three series of *Pinus radiata* seedlings. At time 0, series 1 had all fully expanded leaves removed and series 2 had one-third of the fully expanded leaves removed. Series 4 with no leaf removal formed the control. Treatment differences are significant at the 0·1 per cent level

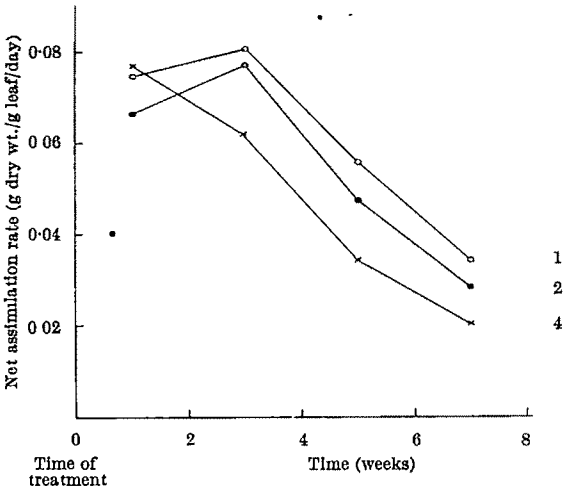


Fig. 4. Changes in net assimilation rate with time for three series of *Pinus radiata* seedlings. At time 0, series 1 had all fully expanded leaves removed and series 2 had one-third of the fully expanded leaves removed. Series 4 with no leaf removal formed the control. Treatment differences are significant at the 0·1 per cent level.

treatments 1 and 3, which differed only in the time of leaf removal, caused significantly different photosynthetic rates at every light intensity.

There was no significant difference between the plants of the four treatments in dark respiration rate expressed on a whole plant basis. As the treatments differed in the ratio, weight of photosynthesizing tissue : weight of total respiring tissue, it is not valid to compare respiration rates calculated on a leaf basis, or gross photosynthesis expressed per unit of leaf tissue. However, the lack of significant difference between treatments in respiration rate of the whole plants makes it clear that the pattern of gross photosynthesis for the treatments was essentially the same as that shown for net photosynthesis.

The significant differences between series 1 and 3 indicate that, as in experiment 1, the observed effect must have been an indirect one, with the treatments affecting the internal growing conditions of the plants, and thus, in turn, the photosynthetic mechanism.

That the treatments also affected the growth of the plants was shown by a growth analysis experiment carried out on plants treated in the same way as those for which photosynthetic rates were measured. Results of this are shown in Figs. 3 and 4. The treated plants had both a higher relative growth rate of the leaves (Fig. 3) and a higher net assimilation rate (Fig. 4) than the controls, both differences being significant at the 0·1 per cent level. Eight weeks after treatment, the rates of relative leaf growth and net assimilation rate showed little sign of reverting to the level of the controls.

The treatments, removal or partial removal of fully expanded leaves, have with time had two obvious effects on growth of the plants, namely: (1) While leaving the actively growing 'sink' for photosynthates comparable with that of the controls, they reduced the photosynthesizing area of leaf. If, as shown by Maggs[5], leaves normally operate at levels well below the maxima of which they are capable, such an effect might be expected to increase the photosynthetic activity of the remaining leaves. (2) They have increased the relative growth rate of the leaves compared with that of the controls. This could be expected to increase further the 'sink' for photosynthates, and might also be expected to increase the auxin production of the treated plants, since young leaves appear to produce higher levels of auxin than mature leaves.

Thus, as in experiment 1, the reason for the change in the rate of photosynthesis may lie either in a 'metabolic sink' effect, or in an effect of auxin-level on the rate of photosynthesis. In support of this latter possibility, Turner and Bidwell[6] have demonstrated an increase in the photosynthetic rate of bean leaves following spraying with indolyl-3-acetic acid (IAA) solution. This increase was smallest at low light intensities, but was markedly greater towards saturation light intensity, a pattern which parallels that shown in the two experiments reported here. They interpret their results as an effect by IAA on some mechanism which normally limits the rate of the dark reaction in photosynthesis.

At this stage it is not possible to state with certainty whether the auxin or the 'metabolic sink' interpretation is correct, or whether the two may be interrelated in their effect. Davies and Wareing[7] have shown that in *Pisum sativum* IAA applied to a decapitated stem stimulated the movement of phosphorus-32 from a lower part of the stem to the locus of application where it accumulated, and that this effect was apparently independent of growth. It seems possible, therefore, that the movement of metabolites out of leaves may also be to some extent under auxin control. Humphries[8] stressed that where rates of photosynthesis were limited by a reduced 'sink' for metabolites, the factor limiting photosynthesis was not the build-up of photosynthates *per se*, but rather the reduction in the rate of translocation from the leaves. It is possible, therefore, that although the reported changes in photosynthetic rate have been caused by a change in the rate of movement of metabolites out of the leaves, this movement has in turn been affected, at least partially, by the auxin-level in the plants.

Certainly much more work is necessary before the mechanism involved can be understood with any certainty. What is clear, however, is that the rate of photosynthesis in *Pinus radiata* seedlings is in some part subject to control by the growth of the plants, and that photosynthetic behaviour can be altered by manipulation of internal growth conditions. Rates of photosynthesis can either be increased or decreased by such means. The fact that rates of photosynthesis can be increased to well above normal levels should be of interest to plant breeders, and it is likely that with further knowledge of the processes involved, it may be possible to tap this unused potential for the production of faster growing plants.

This work was carried out while G. B. Sweet was on study leave from the New Zealand Forest Service, Forest Research Institute, and in tenure of a David Henry scholarship.

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LETTERS TO THE EDITOR

RADIO ASTRONOMY

Low-frequency Spectrum from Radio Source 3C84

RECENTLY, Roger *et al.*[1] reported some measurements of the flux densities from the discrete source 3*C*84 at two frequencies—10·03 Mc/s and 22·2 Mc/s. These and measurements reported by other research workers[3,4] were used to plot the radio spectrum of 3*C*84 in the frequency range 10–10^4 Mc/s.

Roger *et al.* reached the conclusion that the source 3*C*84 (which has been identified as the Seyfert Galaxy NGC 1275 (ref. 2)) has an unusual spectrum both at high and low frequencies. They commented that the spectrum of the source 3*C*84 has a slope of about -2 at the frequencies $-f > 20$ Mc/s, instead of $-0{\cdot}7$ at the frequencies 10^2 Mc/s $\leqslant f \leqslant 3 \times 10^3$ Mc/s; moreover, they found an anomaly in the radio spectrum range 10–20 Mc/s.

In earlier publications[5,6] we described some measurements of flux densities of the source 3*C*84 on frequencies 20, 25, 31 and 38·5 Mc/s. Flux densities were measured relative to Cassiopeia-*A*(3*C*461), and the absolute calibration of the radio telescope system was made at seven frequencies for Cassiopeia $-A$ in the frequency range 12·5–40 Mc/s (ref. 7). The slope of the spectrum of 3*C*84 for frequencies from 20 Mc/s to 150 Mc/s was found to be $-1{\cdot}15$ (Fig. 1).

For $f \geqslant 150$ Mc/s, flux densities were taken from ref. 8.

It can be seen from Fig. 1 that the flux density for 3*C*84 at 22·2 Mc/s as given in ref. 1 fits the spectrum with a slope of $-1{\cdot}15$.

It should be pointed out, however, that the flux density given by Roger *et al.* for 3*C*84 at $f = 10{\cdot}03$ Mc/s is incorrect. As an absolute calibration of the system was not available at 10·03 Mc/s, they used the flux densities measured relative only to 3*C*274 (Virgo-*A*), and the flux density of 3*C*274 was obtained by linear extrapolation of the measured values from very high frequencies. But it is well known[7] that for Virgo-*A* the flux density, S_D, is almost constant in the region from $f = 35$ Mc/s to $f = 12{\cdot}5$ Mc/s; moreover, $S_D \approx 5 \times 10^{-23}$ Wm^{-2} (c/s)$^{-1}$ (ref. 7) and not $S_D \approx 10^{-22}$ Wm^{-2} (c/s)$^{-1}$ as given by Roger *et al.* Thus the flux density of 3*C*84 at $f = 10{\cdot}03$ Mc/s must equal $(490 \pm 170) \times 10^{-26}$ Wm^{-2} (c/s)$^{-1}$, that is, half the value given by them.

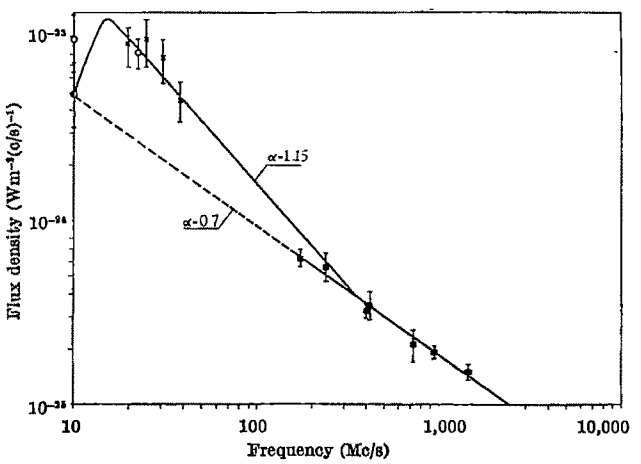


Fig. 1. Radio spectrum of source 3*C*84. ×, Flux densities from refs. 5 and 6; O, flux densities from ref. 1

All the experimental points fitted a smooth solid curve and the shape of the spectrum of 3*C*84 can be seen in Fig. 1. Between 10 and 20 Mc/s there is a sharp change in slope and the curve becomes very steep.

It is obvious that the exact shape of the spectrum of 3*C*84 in the frequency ranges 10–20 Mc/s and 50–150 Mc/s is unknown: it will be necessary to make many further measurements before the spectrum of 3*C*84 is established.

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GEOPHYSICS

A Helium Whistler observed in the Canadian Satellite *Alouette* II

LIKE her sister *Alouette* I, the *Alouette* II satellite carries a broad band, very-low-frequency receiver, but the pass band has been extended to (0·05–30 kc/s) from (0·4–10 kc/s). The *Alouette* II orbit has almost the same inclination as that of *Alouette* I ($\sim 80°$), but is elliptical with a perigee of 501 km, and an apogee of 2,983 km, rather than the almost circular 1,000 km orbit of *Alouette* I. One of the significant discoveries made by analysis of very-low-frequency data recorded by the *Alouette* I satellite[1] was that an atmospheric (an electromagnetic impulse originating from a lightning flash) that had propagated upwards to the satellite was often immediately followed by a novel type of very-low-frequency signal. Initially, the frequency of this signal rose rapidly; the rate of increase then gradually diminished until the frequency approached a nearly constant value. These signals have been termed proton whistlers, and analysis[2] has shown that they are due to coupling of wave energy from the (electron) whistler mode into ion cyclotron waves. Such coupling is possible only at frequencies below the proton gyro-frequency. The coupling occurs over a range of heights below the satellite, with a given frequency component coupling most strongly at a height determined solely by the positive ion composition of the ionosphere. The resulting ion cyclotron waves propagate upwards to the satellite and suffer increasing time delay as their frequency approaches the local proton gyro-frequency. This interpretation explains the form of dispersion exhibited by proton whistler signals, and why such signals are observed only after the occurrence of a normal short fractional hop whistler as well as the manner in which the wave energy enters and propagates through the lower ionosphere.

At the time that this interpretation of the proton whistler was developed it was realized that a similar phenomenon might occur in the vicinity of the helium gyro-frequency. However, none of the very-low-frequency

receivers that were in orbit at that time had sufficient sensitivity at low frequencies to detect signals at or below the helium gyro-frequency. In order to determine whether helium whistlers could be detected, the very-low-frequency receiver of the *Alouette* II spacecraft was designed so that it would be sensitive down to frequencies of about 50 c/s. On one of the first *Alouette* II very-low-frequency records from the southern hemisphere, proton and helium whistlers were both observed (see Fig. 1). Analysis of this signal has shown that it is not a particularly good example of a short fractional hop whistler nor of a proton whistler, but it is the best example of the several helium whistlers obtained during this pass. The temporal variation of the helium whistler frequency is of the same form as that of the proton whistler and the values of gyro-frequency derived from the helium and proton signals are in the ratio 4 : 1 to an accuracy of about 0·2 per cent.

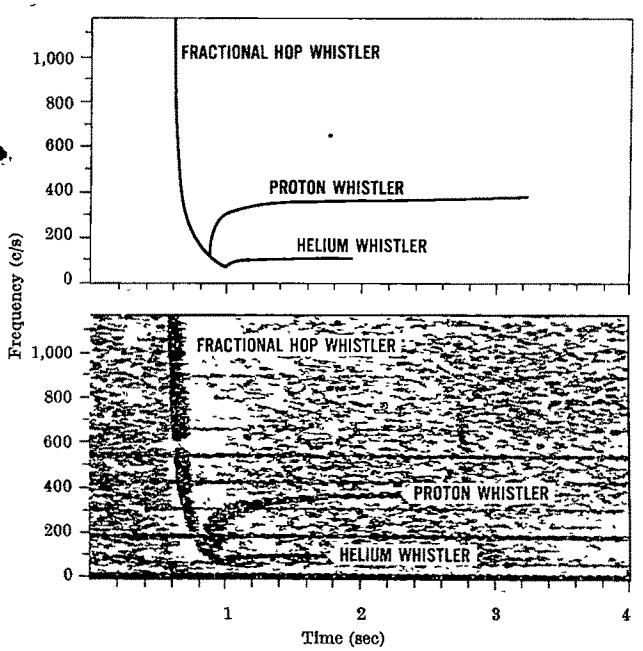


Fig 1. A proton and helium whistler observed in the *Alouette* II satellite. The lower part of the figure shows the spectrum of this signal as produced by a 'Sona-Graph' spectrum analyser The upper portion of this figure is an idealized representation of this event Ororal, Australia, December 3, 1965, 1221.15 U.T., 132° E. long , 32° S lat., 2,007 km height

It has been shown[3] that the frequency variation of a proton whistler can be used to determine the number density of protons in the vicinity of the satellite. In a similar manner the number density of helium ions can be derived from the frequency variation of a helium whistler. The helium and proton whistlers of Fig. 1 have been analysed in considerable detail to yield the number density of protons and helium ions at the satellite. The frequencies at which the proton and helium whistlers separate from the (electron) whistler signal are termed 'cross-over' frequencies and depend only on the fractional abundances of the positive ions comprising the medium[2]. The two cross-over frequencies of the whistler signals shown in Fig. 1 were measured, and used to derive the relative abundances of O^+, He^+ and H^+ ions. In determining the abundances of these ions, it was assumed that they are the major constituents of the ionosphere at the satellite height. The results of this analysis are summarized in the following:

	H^+	He^+	O^+
Number density	6.5×10^3/c.c.	270/c.c.	—
Fractional abundance	0·89	0·038	0·07

From the number density and relative abundance of H^+ the electron number density was found to be $7{\cdot}3 \times 10^3$/c.c. Since independent determinations of the number densities and fractional abundances of H^+ and He^+ are made, two measures of the ratio $n(He^+)/n(H^+)$ can be calculated. The ratio of the number densities of these ions is 0·042 and the ratio of their fractional abundances is 0·043, indicating quite good agreement between the different methods of measuring this ratio. It is highly significant that, in the atmospheric region where this event was observed, O^+ was more abundant than He^+ in spite of the fact that H^+ was by far the dominant constituent. These facts suggest that if an approximation to diffusive equilibrium exists during this measurement, then He^+ must be a relatively minor constituent of the ionosphere at all heights at this time and place.

In summary it can be stated that the simultaneous observation of proton and helium whistlers provides a direct and accurate means of measuring the composition and electron density of the ionosphere at great heights above the Earth's surface. Since these parameters are determined from a radio propagation phenomenon of large wave-length they are representative of a relatively large volume of the medium at the height in question and are not subject to the difficulties of techniques that directly sense the medium in the vicinity of the spacecraft. The greatest drawback to the very-low-frequency technique is that it depends on a sporadic natural phenomenon, that is, a lightning discharge, which usually produces a short fractional hop whistler, but only occasionally generates ion cyclotron whistlers. In spite of these limitations, the newly discovered helium whistler in conjunction with the proton whistler is a valuable addition to the methods available for studying the high ionosphere.

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GEOLOGY

An Estimate of the Rate of Continental Drift in the Permian Period

Continental drift in the Wegenerian sense is now an established theory. Also, it is believed that the supercontinent Gondwanaland itself was drifting around before it was split up. This was suggested by Ahmad[1], on the basis of his study of the glacial deposits in Gondwanaland, although Du Toit[2] had earlier envisaged 'drift *en masse*', without elaborating the point further. The respectability the hypothesis has gained in recent years is, however, due entirely to palaeomagnetic studies[3–6].

The difference between the results obtained by the two approaches is that the path chalked out by Ahmad[1,7], and altered slightly by Ahmad and Ahmad[8], is rather tortuous and complicated. Fig. 1 is based on these. The paths deduced by the palaeomagnetic data, Fig. 2 (ref. 5), are more or less straight, and hence considerably shorter. This is mainly because the latter presumed paths are based upon data for successive geological ages and sometimes longer. What happened in between any two horizons found suitable for palaeomagnetic determinations could not be known.

Also, estimates of the rate of drift have occasionally been attempted on the basis of the palaeomagnetic data[9]. It now appears that a rough idea may be deduced from evidence based on the vicissitudes in the climate of the Salt Range area in the Permian period.

Permian beds, in the area, unconformably overlie the Cambrian System rocks, and are followed by the Triassic beds, without any visible break in sedimentation. The

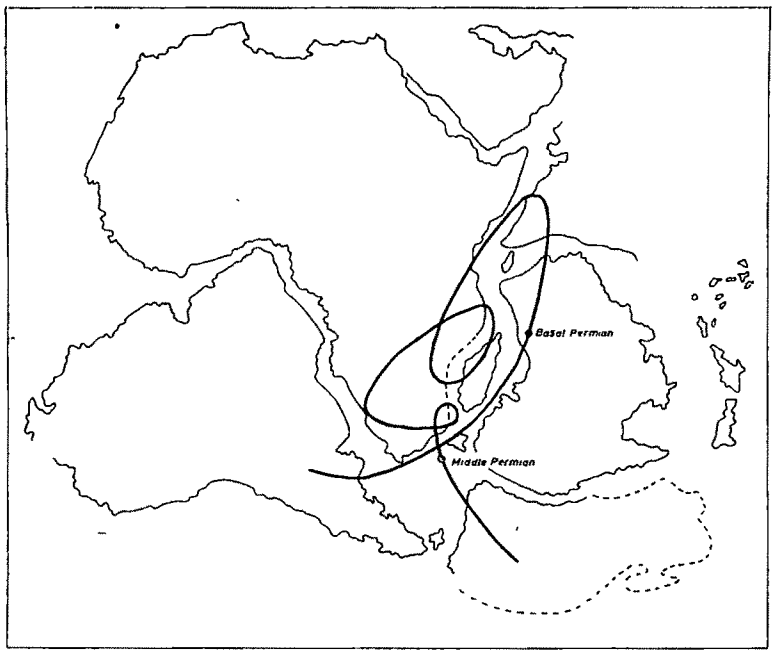


Fig. 1. Probable polar positions in the Lower Permian Period and the route of drift

Boulder Bed at the base of the Permian system is, in places, typically tillitic. Overlying this is the *Eurydesma–Conularia* Bed, 100–300 ft. thick. The lower part of this formation is of continental origin and carries the *Gangamopteris* flora. The *Eurydesma* fauna was adapted to cold climatic conditions and is elsewhere known to occur within the glacial deposits. It entered the area with the first sign of transgression. Considerable time might, therefore, have elapsed between the deposition of the glacial bed and that of the *Eurydesma–Conularia* bed, the two being separated by the fresh-water sediments.

Overlying this bed are 50–100 ft. of barren Lavender Clays and above these come the *Productus* Limestones, carrying a rich and varied fauna. Dunbar studied the fusulines from this horizon and Schuchert[10] concluded that the Upper and Middle *Productus* Limestone fauna indicated a warm, tropical climate. This is also suggested by the richness of the fauna, the deposition of thick, clean, limestone beds, and the presence of reef-building corals. The Lower *Productus* Limestone, however, does not conform to this environmental set-up. The fauna is comparatively sparse and several of the characteristic genera are absent. Schuchert[11] suspected that this sparse fauna, without corals and cephalopods, may indicate that cold waters were holding on. Thus, although it was obviously getting warmer, it was yet not warm enough for the tropical fauna to appear. It came in the Middle *Productus* times. Thomas and Dickins[12] opined that 'A distinct difference also exists between the fauna of the Lower and of the Middle and Upper *Productus* Limestone'. In Western Australia, too, the Lower and Upper Permian faunas have few species in common, and Dickins[13] thought that this indicated 'an important change in the palaeogeographical conditions'.

It therefore seems to follow that, by the end of the Lower *Productus* times, the climate changed from cold and glacial to tropical. If this change was due entirely to a change in the geographical location of the area, it may be placed in about 25° latitude, maybe less. Tillites from polar ice caps are rarely deposited in lower than 60°–65° latitudes, so that the Salt Range area had, in the meantime, moved through about 35° of latitude. It would scarcely be justifiable to assign more than half the Permian period to the deposition of these 700–800 ft. (225–275 m.) of sediments, that is, it might have taken about 25 × 10^6 years. Indeed, even this may be excessive.

A shift of 35° in 25 × 10^6 years means a rate of 0·49 ft. (15 cm) in a year. The figure would be higher if the time taken for the deposition of these beds was less, or if the loops shown on Fig. 1 correctly represent the path of the pole. On the other hand, if it be presumed that the entire Permian succession in the Salt Range was deposited without any significant breaks, and the rate of sedimentation of limestones, shales and sandstones be taken as 1 : 5 : 10, the deposition of *Eurydesma* Bed–Lower *Productus* Limestone would have taken about 17 × 10^6 years. This would mean a rate of drift of about 0·71 ft. (21·3 cm) per year. In contrast Athavale *et al.*[9] estimate a drift of 1·7 cm (0·056 ft.) per year during the last 750 million years. However, accepting even the lower of the two estimated rates of drift given above (15 cm per year), the continents must, then, have been 'sprinting', not just 'drifting'.

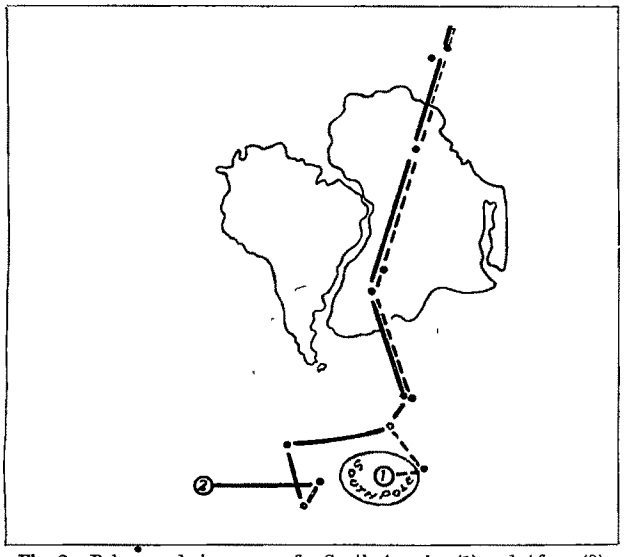


Fig. 2. Polar wandering curves for South America (1) and Africa (2) for Upper Palaeozoic time. (After K. M. Creer (ref. 5))

It, then, seems possible that the widespread occurrence of the Permian glacial deposits was due to the fact that for some reason the rate of continental drift during the Lower Permian period was considerably higher, and consequently widespread areas in different continents came successively under the polar ice cap. If this is accepted, it follows that, whatever might have been the then shape of Gondwanaland, when the Salt Range lay in the tropical belt, South and South-Western Africa must have been in the frigid zone and consequently glaciated, and vice versa. It follows as a corollary that the tillites in different parts of Gondwanaland were not coeval and also that the central part of Gondwanaland must have seen several vicissitudes of climate during the Lower Permian times. Not only is it the case in eastern Australia but the South African Dwyka Tillites may also carry deposits of more than one glacial age[14].

What caused this fantastic rate of drift in the Lower Permian period is not known at present. Stronger convection currents might have been responsible for this, and these, in turn, might have been due to an accumulation of heat in the interior. It is possible that the Hercynian movement, too, formed a link in this chain of events. Escape of heat through volcanic eruptions and

granite emplacements in the mobile belts following the Hercynian orogeny could cause the currents to subside, and the drift to decline.

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Loss of Argon from Biotite in a Thermal Metamorphism

ANALYTICALLY, the simplest example of isotope migration in a geological environment is afforded by the loss of radiogenic argon-40 (one of the daughter products of the potassium-40 decay scheme) from minerals in a thermal metamorphism. By suitably selecting the thermal source, and assuming that the argon is lost by a diffusion process having only a single activation energy (E) of argon release, a relatively simple mathematical model of the system can be constructed. The most probable value of E can be deduced by finding for what value of the parameters in the diffusion equation the theoretical argon losses approach closest to those observed. The significance of E is that it affords a convenient numerical criterion for the argon-retentivity of a mineral; the higher the value of E, the more firmly is the argon retained.

The present communication outlines a recent investigation undertaken at the Department of Geodesy and Geophysics, University of Cambridge, into the diffusion of argon from the biotite of a mica schist due to the intrusion of a dyke. The value of E inferred from the diffusion process using the foregoing approach is compared with that obtained in complementary laboratory experiments, with the same biotite, on the kinetics of argon release in a vacuum.

For the geological investigations, a 20-ft. Tertiary dyke was selected in the pelitic Moine Schist area in the East of the Ardnamurchan peninsula, Argyllshire, Scotland. Samples of the schist were taken at the contact and at various distances along in a line perpendicular to the strike of the dyke. The apparent potassium/argon ages of the biotite separated from these samples within 10 ft. from the dyke were found to decrease regularly from the accepted value of 420 m.y. for the Moine Schist to about 100 m.y. at the contact.

For the mathematical treatment, several idealized models of a cooling dyke were considered[1]. In each case, the dyke was visualized as an infinite flat sheet, and the schist as having uniform composition on either side of the dyke. Computations of the temperature distributions resulting from these models showed that the incorporations of a latent heat raised contact temperatures by about 100° C relative to models without it, but that the way in which the latent heat was liberated was unimportant. Consequently, the mathematically simplest case of a dyke evolving its latent heat of solidification (80 cal g^{-1}) entirely at its temperature of intrusion (1,150° C) was adopted for calculations of the theoretical distributions. The thermal conductivities, densities and specific heats of the schist and solidified basalt were taken to be identical (0·005 cal cm^{-2} sec^{-1}; 2·7 g cm^{-3}; 0·22 cal g^{-1}, respectively)[2,3].

The biotite flakes were assumed to have lost argon by radial diffusion, involving a simple exponential dependence of the diffusion constant on E and temperature[1], namely, $D = D_0 \exp(-E/RT)$. For computational purposes it is unnecessary to know either D_0 or the average biotite grain radius, 'a', since they occur only as the unit D_0/a^2. However, to guard against the possibility of several combinations of the variable parameters E and D_0/a^2 giving equally good agreement with the observed argon distribution, the range of D_0/a^2 was restricted to the neighbourhood of the value $D_0/a^2 = 1{\cdot}52 \times 10^3$ sec^{-1}; this number was arrived at by taking D_0 to be[4] 0·342 and 'a' to be the average measured biotite grain radius of 0·015 cm.

Fig. 1 (*a*) shows the computed argon retention versus distance from the dyke for various values of D_0/a^2 and E. Distances are in multiples of the half-width of the dyke. Fig. 1 (*b*) compares the observed argon distribution with the theoretical distribution for $E = 40$ kcal/g atom and $D_0/a^2 = 1{\cdot}52 \times 10^3$ sec^{-1}.

Out of interest, the foregoing model was modified to correspond to the geological conditions met in a similar, but independent, investigation by S. R. Hart in Colorado[5,6]. Fig. 2 shows that for $E = 40$ kcal/g atom, $D_0/a^2 = 12{\cdot}0$ sec^{-1}, reasonable agreement is obtained between theory and observation (compare $E = 16$ kcal/g atom inferred by Tilton and Hart[5]). The departure of theory from observation in both cases may reflect some over-simplification in either the thermal or the diffusion model; or in both. Laboratory experiments on the kinetics of the release of argon from biotite when heated *in vacuo*[7] do, in fact, show that the loss of the bulk of argon is associated not with one, but two activation energies. Below 800° C, the release appears to be described by a cylindrical volume diffusion model having $E \simeq 35$ kcal/g atom, and above 800°

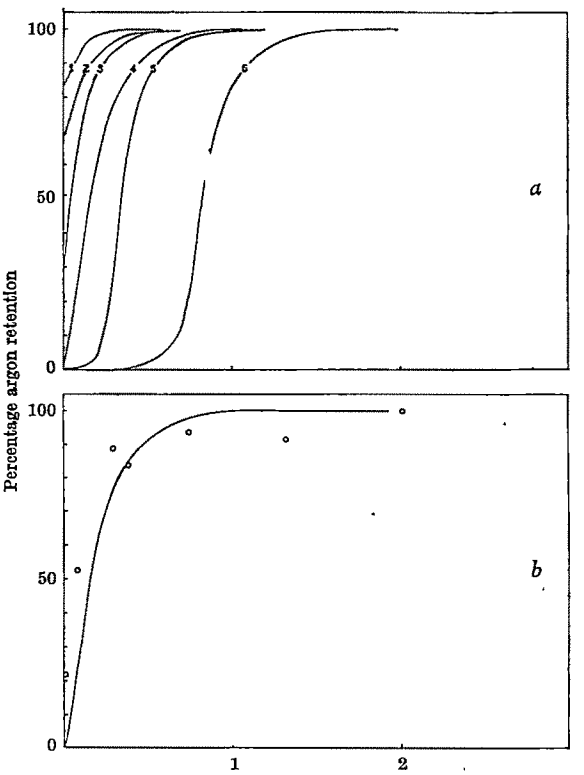


Fig. 1. *a*, Argon retention (theoretical). (1) $E = 48$, $D/a^2 = 1{\cdot}52 \times 10^3$; (2) $E = 48$, $D/a^2 = 3{\cdot}8 \times 10^2$ (3) $E = 48$, $D/a^2 = 0{\cdot}95 \times 10^2$; (4) $E = 40$, $D/a^2 = 1{\cdot}52 \times 10^3$; (5) $E = 40$, $D/a^2 = 0{\cdot}95 \times 10^2$; (6) $E = 30$, $D/a^2 = 1{\cdot}52 \times 10^3$. *b*, Comparison of observed distribution with theory ($E = 40 \times 10^3$ cal/g atom, $D_0/a^2 = 1{\cdot}52 \times 10^3$ sec^{-1})

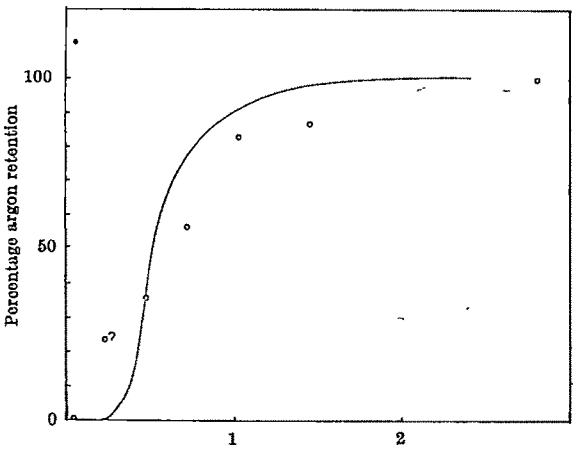


Fig. 2. Comparison of Hart's (1963) results with theory ($E = 40 \times 10^3$ cal/g atom, $D_0/a^2 = 12{\cdot}0$ sec^{-1})

C by such a diffusion mechanism with $E = 49$ kcal/g atom.

These results are of further interest in that they show a close agreement between a laboratory-determined E for a mica, and one inferred from the effects of a thermal metamorphism on isotopic ages. From the available values of E, it would appear that a laboratory value is usually higher than one determined geologically (Table 1).

Table 1. ACTIVATION ENERGIES OF ARGON LOSS FROM MICAS

Mineral	E(kcal/g atom)	Method	Reference
Muscovite	85	Laboratory	Gerling and Morozova[8]
Phlogopite	67	Laboratory	
Biotite	57	Laboratory	
Margarite	54	Laboratory	Fechtig *et al.*[9]
Phlogopite	48	Laboratory	Amirkhanoff *et al.*[4]
Muscovite	90	Laboratory	Sadarov[10]
Biotite	35;49	Laboratory	Present work
Biotite	27	Geological	Hurley *et al.*[11]
Biotite	16	Geological	Tilton and Hart[5]
Biotite	<28	Geological	Hart[6]
Biotite	40	Geological	Present work

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PHYSICS

Laminar Plasma Jets in Nitrogen

VARIOUS authors[1-3] have reported that under certain conditions the high-temperature jet issuing from the throat of a direct-current plasma torch can be given a laminar form and extended from its normal length of a few centimetres to about one metre. This communication describes some results of a series of experiments in which the development and structure of laminar jets in nitrogen have been studied with the aid of schlieren and spectroscopic techniques.

The structure of the jet produced at the outlet of the throat (diameter 3 mm, length 1 cm) of an experimental plasma torch[4] at a current of 100 amp and a gas flow rate of 20 l./min is shown in Fig. 1. Region I extends as a cone 5 mm in length, over which distance it produces spectral lines, in varying intensity, of tungsten (W) and of the ionized molecular and excited atomic levels of nitrogen (N^+_2 and N). The presence of tungsten shows that some erosion of the cathode is occurring, while the excitation of the nitrogen levels indicates temperatures in the range from 7,000° K to 11,000° K. Enveloping I is a cone 4 cm long which has a highly turbulent boundary; the spectral lines of tungsten and ionized molecular nitrogen which were observed from the central regions of this cone indicated temperatures from 4,000° K to 7,000° K. Schlieren photographs showed that on slightly reducing the gas flow rate from 20 l./min the turbulent boundary near the throat outlet became laminar although the flow pattern at the jet extremity did not change. On further reduction of the gas flow rate, the length of the laminar boundary increased so that the turbulent regions at the jet extremity moved increasing distances from the throat outlet; at 8 l./min they were at a maximum distance, for the present experimental arrangement, of 45 cm. The structure of the jet produced at this gas flow rate is shown in Fig. 2. The length of region I is about 1 mm, one-fifth of that in the normal form of the jet. Region II is 12 cm long and produces from its central regions spectral emission of tungsten and ionized molecular nitrogen; only spectral lines of tungsten are produced from the central regions of region III. The expansion of the jet from 3 mm (diameter) at the throat outlet to 1·5 cm in the laminar flow at the onset of the turbulent termination region was not constant, the diameter increasing to 8 mm over the first 12 cm corresponding to the length of region II.

Other experiments[4] have shown that in the normal form of the jet (Fig. 1), the gas flowing through the throat of the plasma torch extends the arc column into a 'hairpin' form. This extension continues until the voltage drop along the column becomes greater than the breakdown potential between the column and the throat

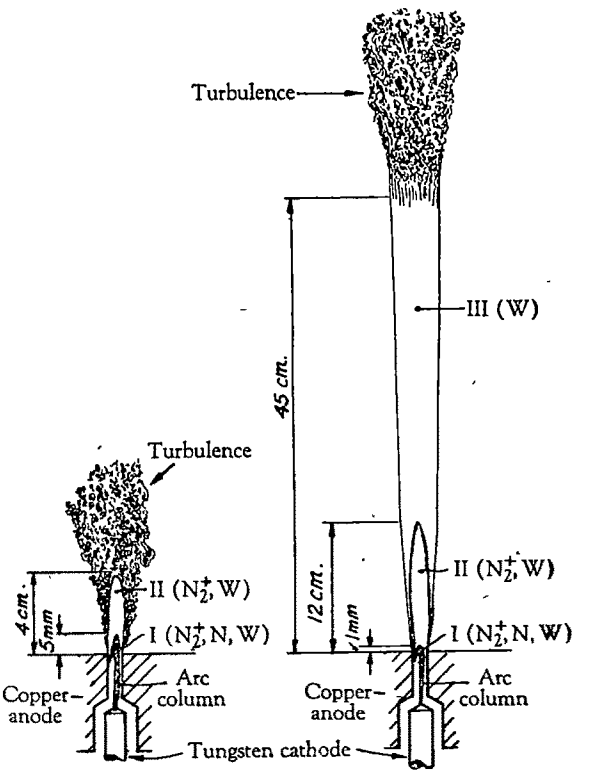


Fig. 1. Normal jet structure (20 l./min)

Fig. 2. Laminar jet structure (8 l./min)

(anode), so that the column short-circuits to the throat. This process of lengthening and short-circuiting repeats itself continually at an amplitude and frequency dependent upon the current and gas flow rate and is revealed in the arc voltage developed in the torch which exhibits a saw-tooth waveform.

In the laminar jets investigated in these experiments, the arc voltage fluctuated in this manner, suggesting that the arc column process of continual lengthening and short-circuiting is also present in this form of jet. The amplitude and frequency of the saw-tooth voltage fluctuations in the laminar jets always tended to be lower than those measured, at the same current, in the normal jets. For example, in a 100 amp jet in its normal form (Fig. 1) the voltage fluctuation had an amplitude of 22 V and a frequency of 23 kc/s, while the fluctuation produced at the same current when the jet was in its laminar form (Fig. 2) had an amplitude of 7 V and a frequency of 12 kc/s. This reduction in amplitude and frequency with gas flow rate is similar to that reported previously[4]. The decrease in the length of region I with the reduction in gas flow rate, and the observations from this region of spectral lines of atomic nitrogen, suggest that it contains the maximum extension of the main conduction path of the arc column.

To determine whether the development of the laminar jet is dependent upon the Reynolds number of the emergent gas, the gas enthalpy was derived from the measurement of the temperature rise and flow rate of the cooling water. The known enthalpy-temperature relation for nitrogen[5] was then used to determine the mean gas temperature and thus the density, viscosity[6] and, from the gas flow rate, the mean velocity. In turbulent form (100 amp, 29 l./min) the emergent gas had a mean temperature of 6,600° K and thus the density (ρ) of $5{\cdot}2 \times 10^{-5}$ gm/c.c., viscosity (η) $1{\cdot}6 \times 10^{-3}$ poise, velocity (U) $1{\cdot}1 \times 10^{5}$ cm/sec, and using the expression

$$\text{Reynolds number} = \frac{Ud\rho}{\eta}$$

where d is the throat diameter (3 mm), a Reynolds number of 1,040. In laminar form (100 amp, 8 l./min), the gas temperature of 7,100° K gave density, viscosity and velocity of $4{\cdot}8 \times 10^{-4}$ gm/c.c., $1{\cdot}8 \times 10^{-3}$ poise and $4{\cdot}6 \times 10^{4}$ cm/sec, respectively, and a lower Reynolds number of 368. This dependence of the jet form on the Reynolds number is similar to that reported for diffusion flames[7] in which the turbulent region terminating the laminar flame was observed to move increasing distances from the burner outlet with decreasing Reynolds number. The turbulent termination region was thought to be due to friction in the boundary layer between fuel and air, causing changes in the viscosity and density of the flame. Similar boundary layer phenomena may be occurring in laminar jets. Further investigations will be concerned with this aspect and will involve measurements of the velocity and temperature changes produced in these jets in the region of the laminar flow below the turbulent termination region of the jet.

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Mechanism of Viscous Flow in Water

BY treating viscous flow, self-diffusion and dielectric relaxation as rate processes, it has been shown that in water the energies of activation associated with the foregoing processes not only exhibit the same temperature dependence but have identical values[1,2]. The dielectric relaxation time τ of a polar liquid has been expressed in terms of the viscosity η by the Debye equation:

$$\tau = \frac{4\pi a^3 \eta}{kT} \qquad (1)$$

where a is the molecular radius. Equation (1) does not hold for hydrogen-bond-associated liquids (such as 2-methyl heptan-3-ol and octan-1-ol)[3], yet it holds for water, and the value of $a = 1{\cdot}4$ Å obtained from a plot of τ^{-1} against η/T agrees well with recently published data[4]. The foregoing findings can be explained with the aid of the 'flickering cluster' model for water, described by Frank and Wen[5], on the basis that the time taken for a translational jump or a molecular re-orientation is less than the mean life-time of the clusters, which may be of the order of 10^{-11} sec. Thus, in a discussion of viscous flow, the nature and concentration of non-hydrogen-bonded water must be considered a major factor. This is made possible by using the cluster model of Nemethy and Scheraga[6]. For ease in computation the clusters are taken to be spherical and, from a consideration of their total area and volume, the x_i parameters (mole fractions of i-bonded water) are calculated as functions of temperature. The molar volume of water, V_{H_2O}, is the sum of the volume of clusters, V_b, and the volume of dipolar water, V_u, and it is given by:

$$V_{H_2O} = (x_0 + x_1)\, V_u + (x_4 + x_3 + x_2)\, V_b$$

(In this representation of the molar volume of water lies one of the main differences between this model and that proposed by Frank and Quist, in whose gas hydrate type structures unbonded water molecules can only fill vacant sites in the clusters and therefore cannot contribute to V_{H_2O} (ref. 10).)

where V_b can be taken to be the extrapolated molar volume of ice, and V_u can be calculated at each temperature from a knowledge of x_i, V_{H_2O}, and V_b.

Thus on a time scale of 10^{-11} sec liquid water can be treated as a suspension of incompressible spheres in a dipolar fluid of viscosity η_0, and as a first approximation the viscosity of such a suspension can be written as:

$$\eta = \eta_0 (1 + 2{\cdot}5\, \varphi_b) \qquad (2)$$

where φ_b is the volume fraction of clusters. φ_b was obtained as a function of temperature, by using the calculated values for x_i, V_u and V_b, and hence η_0 was obtained at each temperature. An Arrhenius plot of log η_0 against $1/T$ was linear above $\sim 40°$ C but showed a curvature, convex to the $1/T$ axis, below that temperature. Since equation (2) is a limiting equation, it was concluded that at lower temperatures φ_b became too large for this equation to hold. Accordingly, two other equations were used to calculate η_0:

$$\eta = \eta_0 (1 + 2{\cdot}5\, \varphi_b + 7{\cdot}35\, \varphi_b^2) \qquad (3)$$

and

$$\eta = \eta_0 (1 + 2{\cdot}5\, \varphi_b + 7{\cdot}17\, \varphi_b^2 + 16{\cdot}2\, \varphi_b^3) \qquad (4)$$

Equation (3) has been applied to concentrated suspensions[7], and equation (4) also allows for the effect of binary encounters of suspended spheres on the viscosity of the suspension[8]. When plotting log η_0, as obtained from equations (3) and (4), against $1/T$, linear relationships were again obtained above $\sim 40°$ C, although with somewhat smaller slopes than that given by equation (2). The results obtained from equation (4) are shown in Fig. 1. The values of η_0 are of the same order of magnitude as are the viscosities of other dipolar, non-associated liquids. The energy ΔH_η, and entropy, ΔS_η, of activation are 1,400 cal. mole^{-1} and 1·0 e.u., and these values approx-

imate closely to those obtained for liquid ammonia and sulphur dioxide. By inserting the value for η_0 in equation (1), the relaxation time τ_0 of unbonded water can be estimated; at 40° C this is found to be 1×10^{-12} sec. This value is consistent with the assumption that the rate-determining process is the decay of the hydrogen-bonded clusters, and that compared to this the time required for the secondary process is small.

In order to confirm that the nature of the results shown in Fig. 1 was not fortuitous, the viscosity data were also tested against the water model of Wada[9]. This postulates a simple equilibrium between ice-like and monomeric species. According to Wada's calculations, x_b decreases from 0·42 at 0° C to 0·20 at 100° C. From a knowledge of ΔH^0, the enthalpy change associated with the formation of the ice-like species from the monomeric species, the equilibrium constant, and hence x_b, could be obtained over the temperature range 0°–100° C. The derived η_0 values, obtained with equation (4), are included in Fig. 1 and it is seen that here again the unbonded water appears to behave as a normal fluid above ~ 40° C. Since the structural model of Wada is based on very different premises from that of Nemethy and Scheraga, we may conclude that the results are not restricted to a particular formalism adopted in the development of the structural model and in the calculation of x_b (T), but that they do apparently reflect a property of liquid water.

In order to calculate the effect of pressure on the transport properties of unbonded water, its compressibility, β_u, must be known, as well as $(\partial x_u/\partial P)_T$, where $x_u = (x_0 + x_1)$. Assuming that ideal mixing occurs between clusters and unbonded water:

$$-\frac{\Delta V^0}{RT} = [x_u\,(1 - x_u)]^{-1}\left(\frac{\partial x_u}{\partial P}\right)_T \qquad (5)$$

where $\Delta V^0 = V_u^0 - V_b^0$. Following Nemethy and Scheraga, the compressibility of liquid water can be written as:

$$\beta_{H_2O} = -(V_{H_2O})^{-1}\,[x_u(\partial V_u/\partial P)_T + x_b(\partial V_b/\partial P)_T + \Delta V^0(\partial x_u/\partial P)_T] \qquad (6)$$

For β_{H_2O} mean values between 1 and 1,000 atm. were used, and for $(\partial V_b/\partial P)_T$ the compressibility of ice at 0° was used. Values for the term $(\partial x_u/\partial P)_T$, included in the structural contribution to β_{H_2O}, were obtained using equation (5). Knowing $(\partial V_u/\partial P)_T$ and $(\partial x_u/\partial P)_T$, φ_b was calculated as a function of temperature, and hence η_0 could be obtained for water under a pressure of 1,000 atm. It was found that the particular model gave reasonable results up to 60° C; above this temperature $(\partial V_u/\partial P)_T$ increased with rising temperature.

The calculated viscosities of unbonded water at a pressure of 1,000 atm. are included in Fig. 1. It can be seen that a plot of linear log η_0 against $1/T$ is again obtained above 30° C, but that at lower temperatures the deviations from the linear relationship are not as pronounced at 1,000 atm. as they are at 1 atm. As is the case for normal fluids, a rise in pressure leads to an increase in the viscosity of unbonded water, and also to an increase in ΔH_η (1·5 kcal. mole^{-1}). From the pressure coefficient of ΔH_η, the volume change of activation, ΔV_η, is found to be + 5 c.c. mole^{-1}, which again is of the right order of magnitude for a non-associated liquid with a molar volume of 16 c.c. mole^{-1}.

All curves in Fig. 1 show that at low temperatures $\Delta C_{p\eta}$ is negative and it approaches zero between 30° C and 40° C, that is, at the temperature at which C_p for liquid water passes through a minimum.

On the basis of the formalism adopted in the interpretation of the viscosity of water, above ~ 35° C the results are consistent with the cluster model which allows for an unbonded species acting as a normal dipolar fluid. Below 35° C a temperature-dependent ΔH_η indicates that this model is not valid.

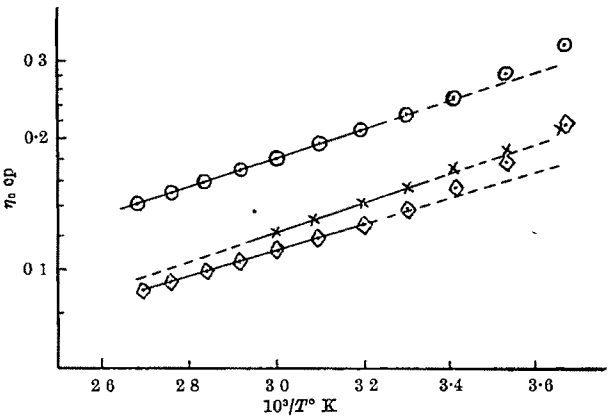


Fig. 1. Viscosity of unbonded water, as given by equation (4), and based on the structural models of Wada (ref. 9) (○) and Nemethy and Scheraga (ref. 6) (◇, at 1 atm., ×, at 1,000 atm)

It is therefore reasonable to conclude that at low temperatures the interstitial model may provide a more realistic representation of the state of liquid water, that is, unbonded molecules can exist only as guests inside a quasi-crystalline framework[10]. With rising temperature, however, x_b decreases, and a physical limit of this model is set by the condition $x_b < \nu/(\nu + 1)$, where ν is the number of molecules in the structural species which are required for the provision of each interstitial site. Frank and Quist have pointed out that, as x_b approaches this limiting value, another species (State III) must provide the predominant contribution towards the observed properties, and they state that the temperature at which this occurs may well be in the region of 35° C.

The results show that this "State III" may well consist of the same two species but that a transition from a quasi-crystalline structure to a suspension of clusters in a dipolar fluid takes place, so that the thermodynamic state of the unbonded water undergoes a change.

Such a transition in the region of 35° C may be responsible for the reported discontinuities shown by many physical properties in aqueous systems, for which there is an increasing amount of evidence[11,12].

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Determination of Radial Distribution Functions by Elastic Electron Diffraction

THE ordering of matter in amorphous materials can be characterized by a radial distribution function of density $4\pi r^2 \rho(r)$, and a difference radial distribution function $4\pi r^2(\rho(r) - \rho_0)$. The latter is obtained[1] from experimental scattering data $I(s)$ by the Fourier inversion:

$$4\pi r^2\,(\rho(r) - \rho_0) = 8\pi r \int_0^\infty s\left(\frac{I(s) - f^2}{f^2}\right)\sin\,[2\pi r\, s]\,ds$$

where $s = 2\sin\tfrac{1}{2}\beta/\lambda$, β is the scattering angle, and f is the

atomic scattering factor. f is a smoothly decreasing function of s and the quantity to be inverted is the ratio of the ripple of $I(s)$ about $f^2(s)$, to the smooth curve $f^2(s)$.

Attempts have been made to obtain the radial distribution function using electron scattering. These attempts have always used the total scattered electron intensity $I_t(s)$, from which electrons scattered inelastically were not removed. We have recently measured both the filtered intensity $I_f(s)$ and the total intensity $I_t(s)$ scattered from a number of materials using the scanning electron diffraction system developed in this laboratory[2–4].

Fig. 1 shows the scattering curve from a thin evaporated carbon film (55 Å thick, 20 kV electrons) with and without removal of loss electrons. The filtered trace contains no electrons which have lost more energy than eV_m, 5 eV. The important differences between the elastic and total intensity curves are: an additional ring at about 0·2 Å^{-1}, three outer rings at s values > 1 Å^{-1}, and true maxima for some of the rings; the unfiltered data (V_m = 20 kV) show no true maxima. The relative ripple values measured in the two cases are therefore very different; when multiplied by the factor s the differences are enhanced. A pronounced difference is obtained between the two radial distribution curves (Fig. 2). The elastic data lead to density functions with greater fluctuation and more order at longer range. Fig. 3 shows marked differences between filtered and unfiltered data from germanium (150 Å film, 20 kV electrons), a fairly heavy element. Grigson and Dove (unpublished) found similar differences in amorphous silicon.

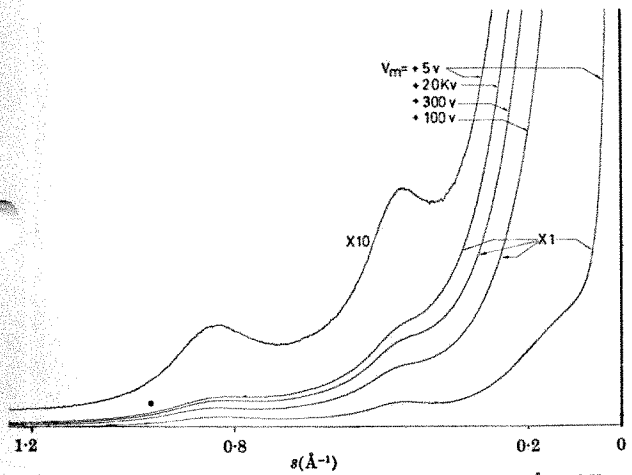


Fig. 1. Filtered and unfiltered scattering curves for carbon, 55 Å, 20 kV

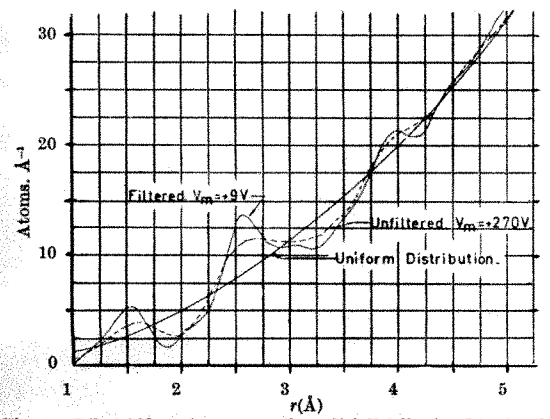


Fig. 2. Effect of loss electrons on the radial distribution function for evaporated carbon, 55 Å, 20 kV, M = 0·1

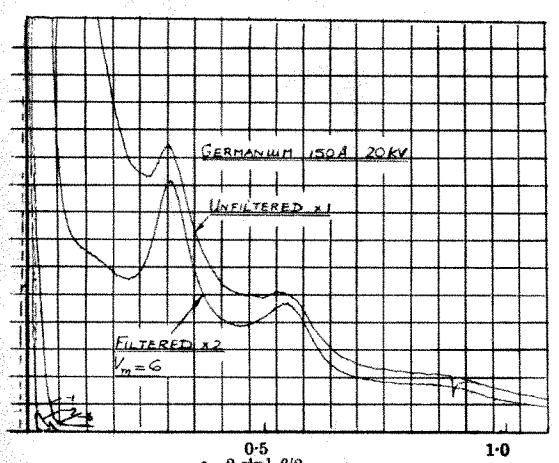


Fig. 3. Germanium, 150 Å, 20 kV

We conclude that unfiltered electron diffraction patterns must not be used for computing radial distribution functions and that when the elastically scattered data are used they are likely to reveal more order at longer range.

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CHEMISTRY

Solute–Water Interactions and the Solubility Behaviour of Long-chain Paraffin Hydrocarbons

THE effect of non-polar groups on the structural properties of liquid water is of interest in several fields of enquiry. The most direct method of studying such systems is by the determination of hydrocarbon solubilities, but, until recently, reliable experimental data have been confined to the four lowest members of the paraffin series[1,2]. McAuliffe has provided a valuable extension to the existing data by his study of the solubilities of twenty-three aliphatic and aromatic hydrocarbons[3]. His results show good agreement with earlier data for the C_1–C_4 paraffins[1,2], but his values for the C_5–C_8 members of the series are lower by more than one order of magnitude than the only other recorded solubilities of these hydrocarbons[4].

McAuliffe's results, obtained by a gas chromatographic technique, have recently been confirmed by Baker, who used tritiated and carbon-14-labelled hydrocarbons, in conjunction with a liquid scintillation spectrometer[5]. Baker also extended the scope of his investigation to include the normal C_{10}, C_{18}, and C_{36} paraffins. He found that, although the solubility decreases with increasing chain length, the solubility decrease for each additional CH_2 group is considerably smaller for the long-chain members of the series ($> C_{10}$) than it is for the shorter-chain paraffins.

In order to investigate further this dependence of solubility on chain length, the solubilities of the C_{10}, C_{12}, C_{14}, and C_{16} paraffins were determined at 25° C by gas-liquid partition chromatography[6]. Purum grade (Fluka) hydrocarbons were used; chromatographic analysis indicated levels of impurities (homologues and branch chain isomers) of less than 3 per cent. After equilibrating the hydrocarbon/water mixtures in a thermostat at 25° C [7], up to 0·5 ml. of the aqueous phase was injected into the fractionator fitted to the chromatographic column, and the water was removed by 'Drierite'. The hydrocarbon concentrations were obtained from the peak areas, after initial calibrations, in the usual manner. Replicate determinations indicated an average experimental error of ±12 per cent in the solubilities.

The mean solubilities of the four hydrocarbons, expressed as mole fractions, x, were obtained as follows:

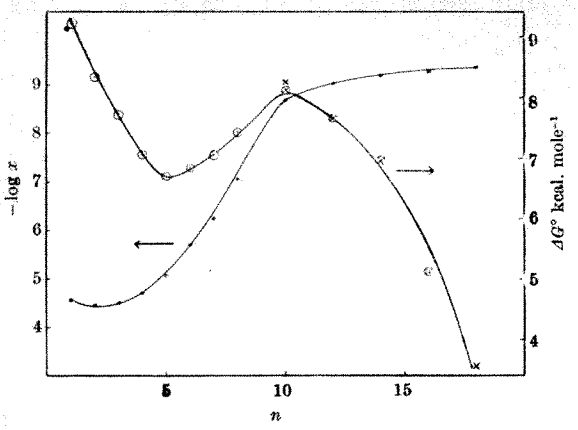


Fig. 1. (Left) Mole fraction solubility and (right) $\Delta G°$ of the norma paraffins as a function of the carbon number n. ○, from ref. 3; ×, from ref. 5; ⊗, this investigation

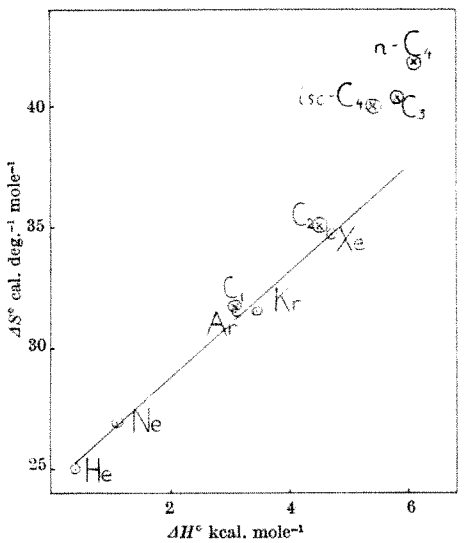


Fig. 2. Barclay–Butler plot for non-polar gases in water. The points for the hydrocarbons are mean values derived from refs. 1 and 2

n-decane $2{\cdot}5 \times 10^{-9}$, n-dodecane $8{\cdot}9 \times 10^{-10}$, n-tetradecane $6{\cdot}3 \times 10^{-10}$, and n-hexadecane $5{\cdot}0 \times 10^{-10}$.

There have in the past been several attempts to express x as a linear function of some property of the hydrocarbons, such as molar volume[8]. McAuliffe points out that a plot of log x against molar volume is linear only for the liquid hydrocarbons. Since the molar volume is a linear function of the carbon number n, it is simpler to plot log x against n, and Fig. 1 shows such a plot for the normal paraffins. It is evident that the linear relationship, if indeed it has any meaning at all, only holds for the C_5–C_{10} members, and the higher members of the series are increasingly more soluble than would be predicted by such a relationship.

A more realistic method of comparing the solubilities of members of a homologous series is to consider the transfer of the solutes from some common standard state to the aqueous solution. Fig. 1 includes a plot of $\Delta G°$, the standard free energy change associated with the transfer of 1 mole of gaseous hydrocarbon at a pressure of 1 atm. to an aqueous solution of a hypothetical unit mole fraction, that is, $\Delta G° = RT \ln (p/x)$, where p is the vapour pressure of the hydrocarbon at a temperature $T°$ K. For the lower members of the series, hypothetical values of p at 298° K were obtained by extrapolation. It is apparent that $\Delta G°$ decreases with n and reaches a minimum for pentane, corresponding to a maximum solubility. The trend is then reversed, and a minimum solubility is observed for decane. For the higher members $\Delta G°$ decreases rapidly with n; this effect is so marked that it would still be apparent, even if the error in the determination of x for, say, hexadecane exceeded two orders of magnitude. The results indicate that the long-chain hydrocarbons are vastly more soluble than would be expected from an extrapolation of $\Delta G°$ values of the lower members of the series. It would be interesting to speculate on the significance of this observation to the properties of lipids in an aqueous environment or to the property of micelle formation which is common to the derivatives of these higher homologues.

Clearly, then, the solubility behaviour of the members of the paraffin series is not as simple as might have been expected; the extrema in the $\Delta G°(x)$ plot probably have their origin in complex enthalpy and entropy effects which are related to the modifications in the structure of liquid water produced by the presence of the solutes. Although, at the present time, $\Delta G°$ data for the higher members ($> C_4$) are available at only one temperature, their nature indicates that the linearity between $\Delta H°$ and $\Delta S°$, which has been observed for different solutes in a given solvent, and which has become known as the Barclay–Butler rule[9], cannot apply to aqueous solutions of hydrocarbons. This already becomes apparent from a consideration of the experimental $\Delta H°$ and $\Delta S°$ values for the C_1–C_4 paraffins[1,2]; Fig. 2 shows that, whereas this rule is valid for a number of simple gases[10], the paraffins increasingly deviate from this linear trend. A fuller discussion of the hydrocarbon solubilities, in terms of water structure, will be published elsewhere.

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Preparing Dispersions of Zinc Oxide Particles for Electronmicroscopic Investigation

PARTICLES of a zinc oxide powder cluster together due to the electrostatic forces acting between the individual particles. These clusters can be reduced to a minimum by the technique described here, and a homogeneous distribution of the particles is obtained throughout the sample. This homogeneous distribution is almost a necessity for the investigation of the grain-size distribution of the zinc oxide powder, utilizing microscopic techniques, as it reduces the time involved in the counting procedure.

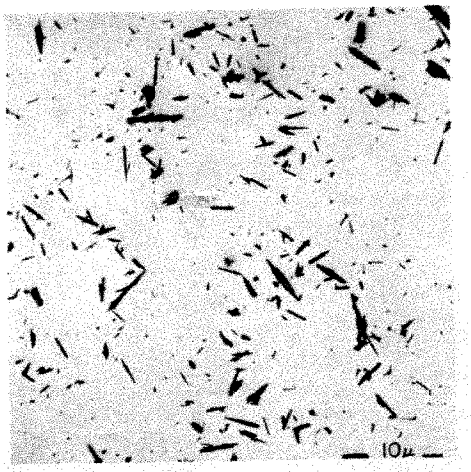


Fig. 1. Needle-shaped particles

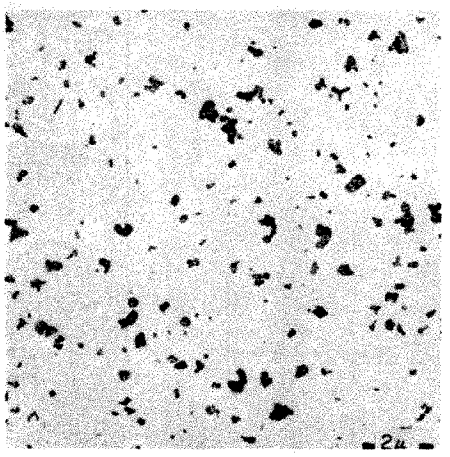


Fig. 2. Particles of different shape (their cross-section is essentially hexagonal)

Optimum results were gained when a paste technique was used. The procedure was very simple and the various steps involved were: (1) Zinc oxide powder was placed in a flat agate mortar. The quantity of powder used depended on the shape and the size of the individual particles, but 2·5 mg proved to be satisfactory in most of our experiments. (2) Ten drops of a 0·1 per cent (by weight) solution of 'Formvar' in chloroform were added. The mass was then thoroughly mixed and mulled to the dry state. (3) Step 2 was repeated and in order to extend the time of mixture some pure chloroform was added until the paste was just becoming weak. (4) Twenty drops of the same 'Formvar' solution were added to the paste. After mixing, a drop of the fluid paste was spread out on a glass plate. (5) After drying, the 'Formvar' film in which the zinc oxide particles were incorporated was floated off on a water surface. The film was collected on copper grids and dried. The film was then ready for examination with the electronmicroscope.

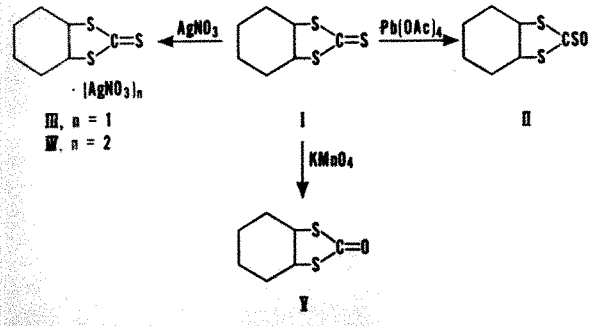
The dispersion obtained is not a function of the shape of the particles, as can be seen from Figs. 1 and 2.

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Reactions of *Cyclo*hexene Trithiocarbonate with Oxidants

LEAD tetraacetate oxidation of sugar derivatives containing a thiocarbonyl group was shown previously to give the corresponding carbonates, disulphides, or oxythiocarbonyl derivatives, depending on the structure of the parent compound[1]. To determine more fully the effect of oxidants on the thiocarbonyl group, the investigation has been extended to the reactions of *cyclo*hexene trithiocarbonate (I) with lead tetraacetate, silver nitrate, and potassium permanganate.



When I (prepared according to Culvenor *et al.*[2] and dissolved in glacial acetic acid) was treated with lead tetraacetate at 25° C, a rapid consumption of one molecular equivalent of oxidant was observed. The oxidized product (II) was isolated as needles (m.p. 96°–97° C); an analysis of this indicated the addition of one oxygen atom to I (calculated for $C_7H_{10}OS_3$: C, 40·8; H, 4·9; S, 46·2, found: C, 40·8; H, 5·0; S, 45·9).

Compound II gave a negative test with sodium azide[3] for the C=S group. Its ultra-violet spectrum in methanol showed absorption maxima at 347 mμ (ε11,700) and 284 mμ (ε4,900), whereas the spectrum of I had a maximum at 318 mμ (ε14,000). On treatment of a methanol–water solution of II with two molecular equivalents of L-cysteine, the *cyclo*hexene derivative was reduced, and I was recovered in 91 per cent yield and L-cystine was recovered in 92 per cent yield. The reduction of sulphoxides under these conditions has been reported[4,5]. These results are in agreement with the proposed structure of II as being an oxythiocarbonyl derivative of *cyclo*hexene trithiocarbonate. This structure is analogous to that formed on lead tetraacetate oxidation of 1,2-*O*-isopropylidene-5,6-dithio-β-L-idofuranose 5,6-trithiocarbonate[1].

When an acetone solution of I was treated at 25° C with an aqueous solution containing one molecular equivalent of silver nitrate, none of the corresponding carbonate or oxythiocarbonyl was formed. Instead, a crystalline product (III) (m.p. 108°–109° C) was isolated which on elemental analysis showed the addition of one molecular equivalent of silver nitrate to I (calculated for $C_7H_{10}S_3 \cdot AgNO_3$: C, 23·2; H, 3·6; S, 26·6; Ash, 30; found: C, 23·4; H, 2·8; S, 26·0; Ash, 33). The infra-red spectrum of III showed a band characteristic for an ionic nitrate group at 1,390 cm^{-1}, whereas no absorption was found in this region with the parent compound I. The grey adduct product was stable when kept at −5° C but darkened on standing at 25° C for a few hours. This product was insoluble in water, acetone, alcohol and chloroform, but was readily soluble in a mixture of water and acetone or alcohol. Such a solution of III gave no precipitate on addition of diluted hydrochloric acid. However, on treatment of III with 1,2-*O*-isopropylidene-α-D-glucofuranose 5,6-thionocarbonate, silver sulphide rapidly formed along with I and 1,2-*O*-isopropylidene-α-D-glucofuranose 5,6-carbonate.

That the C=S group is not involved in the adduct formation, as has been shown for 1,2-dithiole-3-thione compounds[6], is suggested by a positive sodium azide test for III and by its ultra-violet spectrum which showed an absorption maximum at 318 mμ (ε16,000 in methanol : water 9 : 1 v/v). The spectrum of I had a maximum at 318 mμ (ε14,000 in methanol).

When an acetone solution of I was treated with an excess of silver nitrate, a yellow compound (IV) was obtained as needles (m.p. 144°–146° C) which contained silver nitrate and I in a molar ratio of 2 : 1, respectively. (Calculated for $C_7H_{10}S_3 \cdot 2AgNO_3$: C, 15·8; H, 1·9; S, 18·1; Ash, 40·5; found: C, 15·2; H, 2·1; S, 17·9; Ash, 40·7.) The infra-red spectrum of IV was almost the same as III. The adduct product containing two molecular equivalents of silver nitrate was insoluble in most organic solvents and rapidly decomposed on addition of water. On standing at 25° C, IV was readily decomposed with evolution of acidic vapours.

Presumably the addition of two molecular equivalents of silver nitrate to I is due to the presence of two alkyl sulphide groups since it has been shown[7] that alkyl sulphides combine with 1 mole of silver nitrate to give unstable adduct products.

On reflux of an acetone solution of I with an acetone solution of potassium permanganate the corresponding *cyclo*hexene dithiocarbonate (V) was formed, m.p. 106° C. The course of oxidation was followed spectrometrically, and by the end of the reaction practically no absorption was found at 318 mμ, whereas a strong carbonyl peak

showed in the infra-red region. (Calculated for $C_7H_{10}OS_2$: C, 48·3; H, 5·7; S, 36·8; found: C, 48·2; H, 5·8; S, 36·0.) Razuvaev *et al.*[8] reported the preparation of V by oxidation of I with 60 per cent nitric acid.

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Helical Conformation of Poly-γ-benzyl-L-glutamate

RECENTLY, Luzzati *et al.*[1] and Spach *et al.*[2] have suggested that the helical form of poly-γ-benzyl-L-glutamate (PBLG) in dilute solution is that of a $3{\cdot}0_{10}$ helix rather than an α-helix. They reached this conclusion after performing both X-ray diffraction and hydrodynamic experiments on dilute solutions of the polymer in appropriate solvents. They further reviewed the present light-scattering data and concluded that this supported the $3{\cdot}0_{10}$ helical conformation and confirmed the slight flexibility of the helices. They showed that the parameter h, which is the distance between two successive residues projected on to the helix axis or the length of the equivalent rod divided by the degree of polymerization, varied with the molecular weight M and, furthermore, that h approached a value of 2·0 Å as the value of M approached zero. For an α-helix $h_{M\to 0}$ should be about 1·5 Å. More recently, Parry and Elliott[3] have repeated some of the X-ray diffraction experiments of Luzzati *et al.*, using *m*-cresol as solvent, and suggest that the conclusions reached by the latter are erroneous, not because of faulty measurements but rather because of incorrect assumptions regarding the packing of the molecule. Parry and Elliott demonstrated that the α-helix is the probable conformation of the molecule of the PBLG sample investigated.

Prior to the study of PBLG in dichlorethane by light scattering when the solution was subjected to electric fields[4], we made conventional light scattering measurements. We evaluated our data after the method of Zimm[5], and in order to obtain information about the polydispersity of the sample we followed the treatment devised by Reichmann[6]. Using this method, a value for the weight-average length L_w is obtained from the slope of the high-angle asymptote of a graph of Kc/R_θ^1 against $\sin\theta/2$. The factor K is the usual constant prevalent in light scattering theory, c is the concentration, θ the angle between the forward direction of the incident beam and the scattered beam, and R_θ^1 is the Rayleigh ratio corrected by the term $\sin\theta/(1+\cos^2\theta)$. The following results were obtained: weight average molecular weight $2{\cdot}5 \times 10^5$, weight average degree of polymerization, $\overline{N}_w$, $= 1{\cdot}14 \times 10^3$, $\overline{L}_w = 980 \pm 50$ Å, $\overline{L}_z = 1550 \pm 80$ Å, $h = 1{\cdot}3$ Å and $h^1 = 0{\cdot}86$ Å. Here $\overline{L}_z$ is the $[Z(Z+1)]^{\frac{1}{2}}$ average length obtained from the Zimm plot data alone and $h = \overline{L}_z/\overline{N}_w$, whereas $h^1 = \overline{L}_w/\overline{N}_w$.

In their appraisal of the light scattering data taken from the literature, Luzzati *et al.* have given a curve of h against M (Fig. 6 of ref. 1) and have not considered polydispersity to be important. Our value of h fits their curve very well but the seemingly more correct value of h^1 falls considerably below the curve and indicates that if polydispersity were taken into account it would lead to a value of $h_{M\to 0}$ more nearly equal to 1·5 Å than to 2·0 Å. We suggest that the effect of polydispersity is much more important than Luzzati *et al.* have assumed. Our suggestion is further supported by the work of Spach *et al.*[2], from which, by reference to Fig. 4 of their paper, a value for $h_{M\to 0}$ of 1·5 Å is indicated.

We conclude, therefore, that if due attention is given to, and allowance made for, the polydispersity of the samples studied, then both the hydrodynamic and light scattering data will give a value of $h_{M\to 0}$ for the glutamate molecule indicative of the α-helix rather than the $3{\cdot}0_{10}$ helix.

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A Means of Increasing the Oxidation Resistance of Diamond

IN the search for techniques by which to determine small amounts (0·01–1 per cent) of light-atom impurities in laboratory-prepared diamond[1], we have attempted to use strongly oxidizing melts and combustion in oxygen to destroy the host. Useful results for the carbon-13 distribution in undoped diamonds[2] have already been obtained by the direct combustion technique. However, straightforward combustion in oxygen did not produce complete oxidation of boron-doped diamonds. Microscopic examination of the incompletely burned diamonds and their behaviour toward alkali suggested that the residue was covered with a layer of boric oxide, which, being continuous and of low vapour pressure, inhibited further attack of oxygen on the diamond. Complete oxidation of the sample could be achieved only by alternately heating the sample at 800° C in oxygen and washing the residue in the platinum boat with successive portions of 1 N sodium hydroxide and water. The boric oxide that was removed from the diamond surface by these washing steps was determined by potentiometric alkalimetric titration of the borate in the combined wash solutions.

The oxidative stability of boron-doped diamonds, as disclosed by our attempts to analyse them, is illustrated by the following comparison with the oxidative stability of undoped man-made diamonds (RVG grade, commercially available from Metallurgical Products Department, General Electric Co., Detroit, Michigan) and with natural diamonds (from South West Africa). The boron-doped diamonds were prepared according to the class of conditions outlined in previous papers[1,3].

(1) Complete oxidation (800° C, pure oxygen) of all samples of undoped man-made diamonds and of natural diamonds was achieved within 24 h; in most cases oxidation was complete after 1 h.

(2) With one boron-doped sample a residue equal to 60 per cent of the initial sample weight still remained after an 8-h period of combustion; with another sample, 38·5 per cent of the initial sample weight remained after a 24-h period of combustion. The rate of weight loss was about 1/70 that of the RVG and less than 1/100 that of the natural diamonds.

(3) The residues from the boron-doped diamonds suffered no further weight change during a second 4-h period of combustion.

(4) Further oxidation of the residues from the boron-doped diamonds was achieved only after they had been washed with alkali.

(5) A marked increase in the oxidative stability of the RVG diamonds was observed when they were mixed with some powdered boron before combustion.

In addition to their potential technological importance, these results have an important scientific implication. The thermal oxidative etching of diamond is an investigatory technique used in the study of the origin of natural diamond, and inferences have been drawn from the results concerning the microstructural characteristics and thermal history of the diamonds. However, as the work reported here shows, small amounts of impurity can markedly change the oxidative behaviour of diamonds.

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BIOCHEMISTRY

Glyoxylate Reductase Activity of Lactate Dehydrogenase

LACTATE dehydrogenase (L-lactate: NAD oxidoreductase, *EC* 1.1.1.27) occurs in most animal tissues in five different forms[1,2], which can easily be separated by electrophoresis, and these dehydrogenases are enzymes of substrate specificity. Meister[3], however, has found that a series of α,γ-diketo acids are reduced at an essentially uniform rate which is about one-tenth that for pyruvate. Markert and Møller[1] observed that a series of α-hydroxy acids are oxidized at different rates by the isozymes of bovine heart extract, and Kun[4] has shown that β-mercaptopyruvate is reduced by crystalline heart lactate dehydrogenase. It has been shown (unpublished) that β-hydroxybutyrate is a slowly reacting substrate. The present report presents evidence for the glyoxylate reductase activity (*EC* 1.1.1.26) of lactate dehydrogenase based on quantitative and qualitative studies of purified enzyme (Boehringer rabbit muscle lactate dehydrogenase) or rat liver extract. Quantitative determination of dehydrogenase activities was carried out by measurement of the diminution or production of reduced nicotinamide dinucleotide ($NADH_2$) (at 23° C and at a wave-length of 340 mμ).

A qualitative study of cellulose acetate electrophoresis[5] was performed at 4° C, and the enzyme activity was observed by staining the $NADH_2$ produced by the enzyme reaction with phenazine methosulphate and nitro-blue tetrazolium. The results are shown in Table 1 and Fig. 1.

Table 1. ACTIVITY OF THE PURIFIED LACTATE DEHYDROGENASE WITH TWO SUBSTRATES

(Specific activities are expressed as μmoles of $NADH_2$ reduced or produced/100 μg of protein/h)

Substrate		Specific activity
Pyruvate	0·0125 M	111·6
Glyoxylate	0·0125 M	105·6
Lactate	0·0125 M	1·2
Glycolate	0·0125 M	0·96

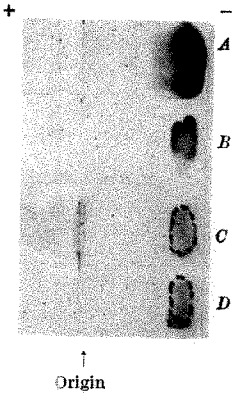


Fig. 1. Results of cellulose acetate, electrophoresis. *A*, Purified Boehringer rabbit muscle lactate dehydrogenase; *B*, rat liver extract; *C*, purified Boehringer rabbit muscle lactate dehydrogenase; *D*, rat liver extract. *A* and *B* developed with lactate; *C* and *D* developed with glycolate

Our results show that the lactate dehydrogenase catalyses glyoxylate reductase.

Except for the intensity of the bands, the patterns obtained on the cellulose acetate preparations, from the two different substrates, were identical. When glycolate was used instead, the pattern was much fainter. In view of the similarity in structure between the molecules of pyruvate and glyoxylate, and lactate and glycolate, the finding that these substrates are metabolized by the same system is not surprising.

Kun[6] has indicated that glycolate is oxidized to glyoxylate by a soluble liver enzyme, but its exact properties have not yet been established. It is well known that some single enzymatic proteins are capable of catalysing reactions with more than one substrate. These results would seem to provide further evidence for this concept, but more extensive investigations will be required to rule out the possibility of enzymatic contamination.

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Peroxidation of Lipid from Normal and Paroxysmal Nocturnal Haemoglobinuria Erythrocytes

PREVIOUS investigations in this laboratory demonstrated that *in vivo* red cell damage in animals and humans during exposure to oxygen under high pressure was associated with peroxidation of erythrocyte unsaturated fatty acids[1,2].

In paroxysmal nocturnal haemoglobinuria (PNH), a rare disease characterized by intravascular haemolysis accentuated during sleep, the primary defect is in the stroma of the erythrocyte. The underlying mechanism of red cell destruction has not been clarified in spite of intensive investigation. Since some investigators reported that PNH erythrocytes contained increased quantities of unsaturated fatty acid, we examined the possibility that the lipid of PNH erythrocytes would form more lipid peroxides than that of normal erythrocytes under conditions known to cause lipid peroxidation.

Erythrocytes were obtained from three patients with documented PNH and compared to those of six normal control subjects. Lipid extractions were performed simultaneously on PNH and normal erythrocytes in each investigation and exposed simultaneously to ultra-violet radiation (known to cause lipid peroxidation)[3]. Lipids were extracted from 4 ml. of washed packed erythrocytes by a modification of the chloroform–methanol method described by Bligh and Dyer[4]. The final chloroform layer was evaporated to dryness under vacuum at 37° C and the lipid evaporate resuspended in 16 ml. of physiological saline and used immediately or frozen under nitrogen for storage.

Portions of extract were placed in 200-ml. quartz Erlenmeyer flasks and shaken at 100 c/m, 40 cm below an ultra-violet source (Westinghouse Sterilamp *G*15 *T*8). Lipid peroxides were determined before and after varying times of ultra-violet exposure (30 min, 1 h, 2 h, 3 h and 4 h) by the reaction of 2-thiobarbituric acid with malonylaldehyde. This reaction measures the products of lipid peroxidation rather than lipid peroxides themselves, and is generally accepted as a measure of lipid peroxidation[5]. A standard curve was prepared using 1,1,3,3-tetraethoxypropane which hydrolyses to yield malonylaldehyde[6]. Values for lipid peroxides were thus expressed as moles of malonylaldehyde.

Table 1. LIPID PEROXIDE FORMATION IN ERYTHROCYTE LIPID AFTER 30 MIN EXPOSURE TO ULTRA-VIOLET RADIATION

	Lipid peroxides mμmoles malonylaldehyde per ml. lipid extract
Normal subject	9
PNH No. 1	20

Values are the average of duplicate determinations in a representative experiment.

Table 2. LIPID PEROXIDE FORMATION IN ERYTHROCYTE LIPID AFTER 1 H EXPOSURE TO ULTRA-VIOLET RADIATION

	Lipid peroxides mμmoles malonylaldehyde per ml. lipid extract
Normal subjects	8–12
PNH No. 1	25
PNH No. 2	18
PNH No. 3	23

Values are the average of duplicate determinations in a representative experiment. The range of normal mean values is given.

Lipid from PNH erythrocytes consistently formed more lipid peroxides than that of normal erythrocytes (Tables 1 and 2). This difference was even apparent after only 7·5 min of ultra-violet exposure. Quantities of lipid peroxides determined by the 2-thiobarbituric acid reaction paralleled peroxide activity measured iodometrically.

There is general agreement that the basic lesion in PNH is intracorpuscular rather than extracorpuscular. Although Munn and Crosby in 1957 reported elevated unsaturated fatty acids in PNH red cells[7], other investigations failed to confirm these findings[8]. In the investigations described herein lipid extracts of PNH erythrocytes exposed to ultra-violet radiation yielded consistently higher lipid peroxide levels than the lipid extract of normal red cells. Since peroxidation involves unsaturated fatty acids, the increased lipid peroxide levels in PNH lipid extract most likely reflect elevated levels of stromal unsaturated fatty acid in PNH erythrocytes.

Recent work in this laboratory has shown that PNH erythrocytes were more susceptible to lysis by agents capable of peroxidizing lipid than normal erythrocytes. It is therefore possible that peroxidation of erythrocyte lipid may bear some relationship to the haemolytic mechanism in PNH. Cell damage could occur as a direct result of membrane damage by rupture of fatty acid double bonds, or as a result of the known toxic effects of lipid peroxides on protein and enzymes[9].

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Search for a Naturally Occurring Amylase Inhibitor in Human Serum

IT has been postulated that an amylase inhibitor exists in normal human serum, but not in the serum of patients with acute pancreatitis[1]. The principal evidence for the presence of this inhibitor was based on observations that the average amylase activity of a group of sera was nearly tripled after subjecting the specimens to filter paper electrophoresis. It was found that when eighteen normal sera having a mean amylase value of 106 Somogyi units were separated electrophoretically, the total amylase recovered by extracting the various protein fractions with saline averaged 311 Somogyi units[1]. However, a similar electrophoretically-induced enhancement in amylase activity could not be demonstrated in sera from patients with acute pancreatitis. Dreiling *et al.*[2] substantiated these observations and suggested that although increased quantities of pancreatic amylase enter the blood in acute pancreatitis, the circulating level of the amylase inhibitor is decreased in this disorder. These workers further concluded that, in cases of pancreatic cancer, hyperamylasaemia was not accompanied by any diminution in the enzyme inhibitor.

Proof of the existence of an inhibitor of amylase has been derived exclusively from iodometric measurements of amyloclastic activity. Several independent investigations have recently indicated that certain protein components of serum are capable of interfering with the formation of starch-iodine colour and thereby altering amylase measurements based on this chromogenic response[3–5]. The dinitrosalicylic acid colour reaction introduced in 1921 by Sumner[6] for measuring reducing sugars is not adversely affected by serum proteins[7] and has been found applicable for the determination of amylase in serum[8]. It seemed desirable, therefore, to verify the existence of the postulated amylase inhibitor, by using the dinitrosalicylic acid method to investigate the distribution of amylase activity in normal and abnormal sera before and after paper electrophoresis.

Aliquots of 0·85 ml. of serum were applied to large (28·5 × 35 cm, Whatman 3*MM*) filter paper rectangles moistened with veronal buffer (*p*H 8·6, ionic strength 0·075). Electrophoresis was performed in a horizontal cell at a constant potential 80 V for 16 h. Following the separation, a 1·5-cm strip was cut longitudinally from the filter paper and stained for protein. The remainder of the rectangle was cut into 0·5-cm segments that were individually extracted with 0·85 per cent sodium chloride solution. The amylase activity in the saline eluates was

measured by the modified dinitrosalicylic acid procedure[8] and protein content was estimated at 280 mμ in a Beckman '*DU*' spectrophotometer[9].

The electrophoretic distribution of amylase was examined according to the described procedure in the sera of normal rabbits[10], normal and pancreatectomized dogs[11], healthy individuals and patients with various disorders[12]. Amylase recoveries from the electrophoretic strips ranged between 75–110 per cent of the total amylase of unfractionated serum, except in specimens from patients with acute pancreatitis. Amylase recoveries in the latter cases were always much lower than those obtained with normal human serum. In no instance did subjection to electrophoresis greatly increase amylase activity in any of the normal or abnormal specimens investigated.

In an attempt to demonstrate an amylase inhibitor, aliquots of pooled normal human serum were subjected to electrophoresis in duplicate. The segments from one strip were eluted with saline, whereas those of the other strip were treated with a mixture of saline and serum (250:1). After correction for the amylase activity of the serum in the saline–serum mixture, the electrophoretic distributions of saccharogenic activity were quite similar in both types of eluates (Fig. 1). The recovery of serum amylase activity in both cases was approximately 80 per cent. Of special interest is the fact that the presence of normal human serum in the saline eluting fluid did not depress amylase activity as might have been expected if the extractant mixture had contained an inhibitor.

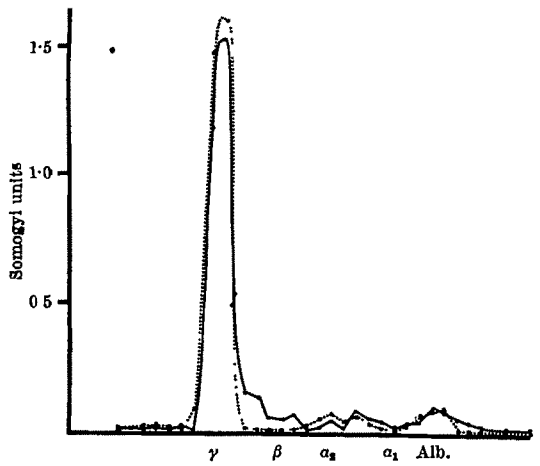


Fig. 1. Electrophoretic distribution of saccharogenic activity on strips prepared from pooled normal human serum and extracted with saline (——) or a saline–serum mixture (- - - -)

The major part of the saccharogenic activity in normal human serum and in the sera of patients with various clinical disorders is consistently found in the gamma-globulin zone[8]. Only trace amounts of activity of questionable significance are isolated from the other electrophoretic areas. These observations are in sharp contrast to the patterns obtained when an amyloclastic method is used to determine amylase activity. With an iodometric procedure, amylase activity is evident in both the albumin and gamma-globulin regions, but particularly in the former.

Exposure of electrophoretic eluates to boiling water for 30 min completely abolishes amyloclastic activity in the gamma-globulin zone[13]. Such treatment, however, enhances the amylase activity in the albumin-area extracts. It is unlikely that this increase in apparent amyloclastic activity in the heated albumin-containing eluates is enzymatic in nature, since incubation in boiling water would be expected to destroy most enzymes. Protein, particularly serum albumin, has been shown to interfere with the starch-iodine reaction[14]. The strikingly increased recoveries of amylase in the albumin zone of heated serum eluates as determined by the iodometric method could therefore be accounted for by protein interference with the starch-iodine colour reaction.

The low saccharogenic recovery of amylase after electrophoresis of serum from acute pancreatitis patients is worthy of special note. The magnitude of the amylase activity isolated from the electropherograms prepared from these sera was found to be dependent on the time of exposure to the electric current. For example, lesser amounts of saccharogenic activity were recovered from the gamma-globulin zone following 16 h of electrophoresis as compared to an 8-h separation. It is possible that heat generated during electrophoresis may be a factor contributing to the loss of enzymatic activity. It is interesting to note the nearly complete recoveries of amylase obtained following fractionation of serum from acute pancreatitis patients by gel filtration. Heat is not evolved during this chromatographic procedure.

In order to investigate the sensitivity of the enzyme to heat, amylase activity was measured saccharogenically in serum pooled from normal subjects and serum pooled from patients with acute pancreatitis. After the samples of each pool had been incubated at 45° C for 16 h, the amylase determinations were then repeated (Fig. 2). This storage temperature had little effect on the saccharogenic activity of the normal pool. However, exposure of the abnormal specimen to 45° C caused a marked reduction in amylase activity. This behaviour suggests a possible physicochemical difference between amylase which circulates normally and that present in the serum of patients with acute pancreatitis.

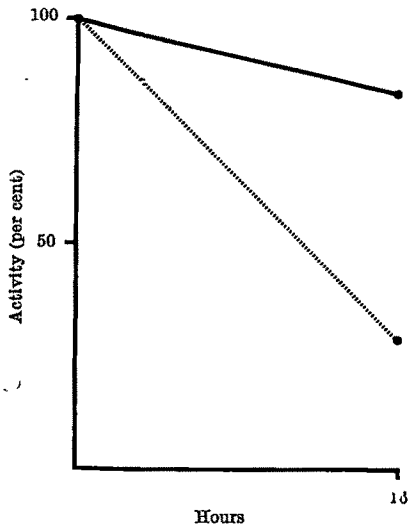


Fig. 2. Effect of a 16-h incubation at 45° C on the saccharogenic activity of pooled normal human serum (——) and serum pooled from patients with acute pancreatitis (- - - -)

The apparent increase in normal serum amylase activity measured iodometrically following electrophoresis is probably due to protein interference with the starch-iodine colour reaction rather than inactivation of a hypothetical amylase inhibitor. Use of a saccharogenic method to measure amylase failed to provide evidence suggesting that normal human serum contains an electrophoretic-labile inhibitor of this enzyme. Reduced electrophoretic recoveries of amylase in specimens from acute pancreatitis patients may result from the presence of a molecular form of amylase that is highly susceptible to heat. This heat-labile amylase fraction may be derived from the inflamed pancreas and could conceivably be an isoenzyme.

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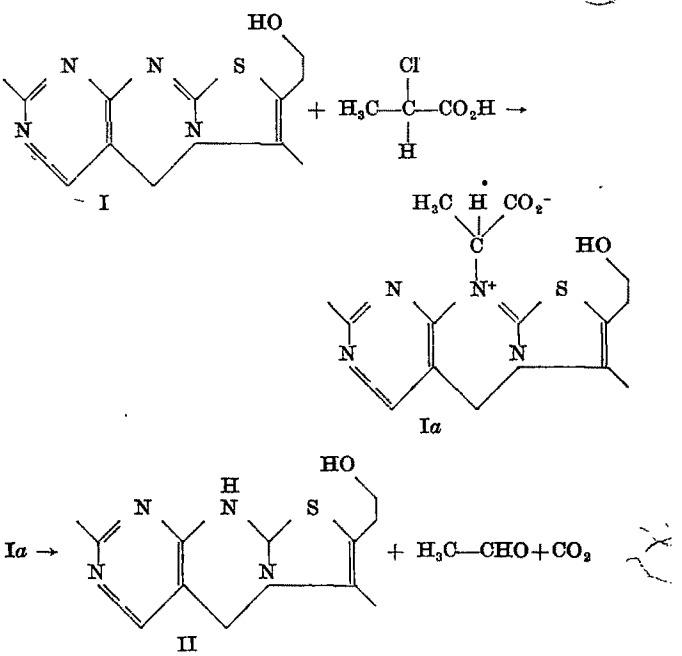
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Reductive Conversion of Thiochrome to Thiamine

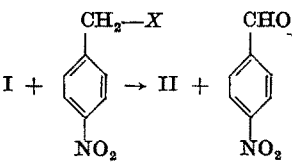
ALTHOUGH the chemistry of thiochrome was thoroughly developed during the period of thiamine exploration in the 'thirties[1,2], the biochemical aspects of this pigment were neglected, mainly because of the early reports on its apparent inactivity in the carboxylase reactions[3]. Recent investigations by Titaev[4], however, have suggested a major role for thiochrome; these reports have alleged that an enzyme, thiamine dehydrogenase, catalyses the oxidation of thiamine to thiochrome with the subsequent reduction of oxidized adrenaline. The existence of this enzyme system, however, has been both refuted[5] and affirmed[6] in recent years, and at the present time it is difficult to assess the biological significance of the reaction.

The mechanism of the model thiamine-catalysed reactions has been of interest to us for several years and recently we have investigated the chemistry of both thiochrome and dihydrothiochrome in connexion with these reactions[7]. The oxidative conversion of the vitamin to thiochrome, to our surprise, was found to be rapid and occurred under mild conditions. When one suspends the vitamin in methyl alcohol and then adds three equivalents of potassium carbonate it is found that a brilliant blue fluorescence is produced after a few minutes. Analysis of the alcoholic solution by paper chromatography indicates that thiochrome is the fluorescent derivative. Under these conditions, the oxidation is incomplete; however, the oxidized products are thiochrome and thiamine disulphide. This result has led us to investigate a variety of oxidizing agents, and at the present time we have found that potassium permanganate, manganese dioxide, nitrobenzene, nitrosobenzene, iodine, cupric acetate, alloxan, ninhydrin, amyl nitrite and quinone are effective in this conversion (thiochrome, 4 per cent; thiamine disulphide, 85–90 per cent isolated). The conditions for the reactions are as follows. The appropriate oxidizing agent (10^{-3} moles) was dissolved in absolute methanol (50 c.c.) and the mixture was stirred to facilitate solution (additional alcohol was added if solution was not complete). Thiamine (10^{-3} moles) was then suspended in the alcohol. After stirring for a few minutes, sodium methoxide or potassium carbonate (3×10^{-3} moles) was added and this reaction mixture was stirred for 1 h. At the end of this time, the reaction was neutralized with glacial acetic acid and filtered. The concentration of thiochrome was determined by measuring an aliquot of this solution at 375 mμ on the Beckmann '*DB*' Spectrophotometer. The remaining solution was evaporated to dryness on a rotary evaporator and the residue was then suspended in a water–acetone mixture overnight. Recrystallization of the semi-solid from acetone gave thiamine disulphide (85–90 per cent).

This rapid non-enzymatic conversion is the basis for the dispute over the existence of thiamine dehydrogenase, since it is difficult to ascertain whether or not the enzymatic oxidation is significant. Since the oxidation is disputed and the reduction of thiochrome has not been investigated, it was concluded that a reduction study might afford information on the ease of such a conversion, if not direct evidence for the enzymatic reaction. If one were to make an *a priori* judgment on the biochemical mechanism of such a conversion, it would be concluded that three reaction pathways would be favoured: (1) a reductive decarboxylation; (2) a reductive isomerization; (3) a direct reduction. Conditions for (1) and (2) have already been described[8,9]. When thiochrome is reacted with α-chloropropionic acid in the presence of alkali, thiochrome (I) is converted to dihydrothiochrome (II):



Similarly, the reductive isomerization was also accomplished by reacting thiochrome with *p*-nitrobenzyl halides under alkaline conditions. This reaction gives dihydrothiochrome, and *p*-nitrobenzaldehyde:



Although these reactions are of interest to the model thiamine-catalysed reactions it is unlikely that they are of significance to the biochemical reactions. Instead, it would be expected that the third mechanism, a direct reduction with DPNH or other suitable reducing agent, would be the most likely reaction pathway. In order to investigate this pathway, an investigation was undertaken of both model and enzymatic reductions of thiochrome.

It was concluded that the simplest experimental approach to this problem would be to assay for carbon

dioxide produced from the thiamine-catalysed decarboxylation of pyruvic acid. Since thiamine is relatively unstable under the alkaline conditions of the model reactions, a standard (corresponding to 100 per cent reduction of thiochrome) was compared with the various runs containing thiochrome and the appropriate reducing agent. The results of this experiment are summarized in Table 1.

Table 1. MODEL THIOCHROME REDUCTIONS

Reducing agent	μl. Carbon dioxide/h
(1) Standard	34
(2) Benzaldehyde	20
(3) DPNH	6
(4) Lactic acid	5
(5) Acetaldehyde	4
(6) Ascorbic acid	0

Standard double-arm Warburg flasks were used. In the side-arms were placed the appropriate reducing agent and concentrated sulphuric acid. Thiochrome (1×10^{-5}) and sodium phosphate buffer (0·2 moles, *p*H 8·3) were placed in the main compartment. The flasks were equilibrated under a nitrogen atmosphere at 30° C for 15 min. The reducing agent was then poured into the main compartment and the reaction was allowed to run for 1 h. At the end of this time, acid was added to stop the reaction, and final readings were taken after re-equilibrating for 15 min. Under these conditions, it was found that most of the reducing systems did not yield sufficient thiamine (at least not at a sufficient rate to overcome the base catalysed decomposition of the vitamin) to show significant reduction of thiochrome. The notable exception, however, was benzaldehyde which apparently reduced the pigment by way of a Cannizzaro reaction to give approximately 57 per cent thiamine. Based on the results of the model system, an attempt was also made to reduce thiochrome enzymatically. This investigation, however, was not successful because it was found that thiochrome inhibited the decarboxylation of pyruvic acid to a considerable extent. When the method developed by Schuster and Lohman was used[10] it was found that a reaction mixture containing benzaldehyde, thiochrome and yeast afforded 50 μl. of carbon dioxide; a similar mixture without thiochrome afforded 85 μl. Similar results were found with the other reducing agents.

In conclusion, it can be stated that the chemical conversion of thiamine to thiochrome occurs under mild conditions, and this reaction may be of biochemical significance. The transformation of thiochrome to the vitamin by biochemical agents does not, however, occur at a sufficient rate to allow a firm decision to be made on its possible physiological importance.

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Serum ATP : Creatine Phosphotransferase Activity in Guinea-pigs with Experimental Muscular Dystrophy

HOSENFELD *et al.*[1] have recently reported investigations of plasma creatine kinase (ATP : creatine phosphotransferase) activity in mice with hereditary muscular dystrophy indicating that the disease in mice is more closely related to the limb girdle type of human muscular dystrophy than the Duchenne type of muscular dystrophy.

Experimental muscular dystrophy in guinea-pigs, induced by placing young guinea-pigs on a vitamin E-deficient diet, has also provided evidence of a similarity to human dystrophy when studying the tissues of guinea-pigs in the 'pre-clinical' and early clinical stages of the disease. Schottelius *et al.* have demonstrated altered mechanical properties[2], significant changes in myoglobin concentration[3–5], and oxygen consumption in the resting and stimulated muscle[6]. Bird and Szabo have recently correlated these changes with ubiquinone and tocopherol concentrations and lipid peroxidation of the tissues[7]. The object of the present investigation was to compare the serum ATP : creatine phosphotransferase activity in developing experimental muscular dystrophy of the guinea-pig with results reported for hereditary muscular dystrophy of the mouse and the human myopathies.

Thirty-day-old male English strain guinea-pigs, average weight 325 g, were maintained on one of the following dietary regimens: 'Purina Lab Chow' pellets (normal), synthetic vitamin E-deficient diet[2] or the synthetic diet supplemented with *dl*-α-tocopherol. The *dl*-α-tocopherol was suspended in olive oil (15 mg/0·5 c.c.) and 0·5 c.c. administered orally every third day. Food was withheld 4 h prior to supplementation and replaced 4 h after supplementation. On days of tocopherol supplementation, animals on normal and vitamin E-deficient regimens received 0·5 ml. of olive oil without tocopherol. All animals received 0·5 ml. of cod liver oil and 25 mg of sodium ascorbate on days tocopherol supplement was not given. Fresh diet was prepared twice a week.

Blood was drawn by heart puncture following light ether anaesthesia and allowed to clot at room temperature for 1–2 h. The clotted blood was centrifuged at 2,500 r.p.m. for 15 min; the serum was separated and again centrifuged for an additional 15 min to remove any remaining cells. Duplicate determinations were made 2 h after killing the animals.

ATP : creatine phosphotransferase activity was determined by the method of Tanzer and Gilvarg[8] in which the ADP, formed in time with creatine as substrate, is phosphorylated by phosphoenolpyruvate in an auxiliary reaction catalysed by pyruvate kinase: the pyruvate formed is determined with the indicator reaction catalysed by lactic dehydrogenase; the rate of oxidation of DPNH is a measure of the ATP : creatine phosphotransferase activity. Results of enzyme activity are expressed in International Units (I.U.).

Student's small sample '*t*' test was used for the statistical analysis with a probability of $P \leqslant 0{\cdot}01$ being accepted as revealing a significant difference.

Table 1 shows the mean serum enzyme activity of the guinea-pigs at feeding durations of 15, 21 and 30 days. The data indicate that the enzyme activity is not significantly different in the serum from the animals from the three dietary regimens at 15 days of diet. At 21 and 30 days of diet, serum from vitamin E-deficient animals is significantly greater in activity than either the normal or vitamin E-supplemented animals, with no significant difference between the normal and vitamin E-supplemented animals. It is also noted that there is no significant difference between the normal animals for the three feeding durations. Vitamin E-deficient enzyme activity is significantly greater in the 21-day animal serum than the 15-day vitamin E-deficient serum, and continues to be high in the 30-day animals.

A point of emphasis in this communication is the investigation of guinea-pigs in the beginning stages of experimental muscular dystrophy. Secondly, we have used a young herbivore in which the muscle fibres are still in the process of biochemical and structural maturation[9], and are more sensitive to the presence of highly unsaturated fatty acids such as are provided by supplementation of cod liver oil. Older animals acquire greater tissue concentrations of vitamin E through dietary sources[10], and relatively long periods of time are required to induce the manifestations of vitamin E-deficiency.

Table 1. GUINEA-PIG SERUM ATP: CREATININE PHOSPHOTRANSFERASE ACTIVITY

(I.U. units)

Diet	Feeding duration 15 Days	21 Days	30 Days
Normal	(6) 1·44 ± 0·30*	(12) 1·76 ± 0·32	(13) 1·94 ± 0 28
Vitamin E-suppl.	(8) 1·17 ± 0·36	(6) 1 40 ± 0·39	(8) 2·91 ± 0·33
Vitamin E-defic.	(9) 2·95 ± 0·62	(8) 7·49 ± 1·12†	(6) 4·76 ± 0 38†

* Mean ± standard error; parentheses indicate number of animals studied in each group.
† Significantly different from normal and vitamin E-supplemented animals ($P \leq 0$ 01).

Growth of the guinea-pigs on the synthetic diet was not optimal even when supplemented with reasonably large amounts of vitamin E. For the first 5 days, animals fed the synthetic diet supplemented with vitamin E had a slower growth-rate than those animals fed the 'Purina Lab Chow' diet, but thereafter had the same growth-rate for the duration of the experiment[7]. The increase in enzyme activity of the vitamin E-supplemented animals at 30 days of diet is a further indication of the sub-optimal conditions of the synthetic diet.

Previous investigations in our laboratory and others, using guinea-pigs and the same dietary regimen, have shown metabolic evidence of dystrophy at 15 days of diet. We have termed this stage as 'pre-clinical', in that there was no creatinuria, significant histopathological lesions, or typical external gross symptoms. Serum ATP : creatine phosphotransferase activity, which is considered as a clinical diagnostic test, was shown in this study to be not significantly different in the three groups of animals at 15 days. Since serum ATP : creatine phosphotransferase is of muscular origin[11], and increases in serum activity are more likely a function of membrane alteration[12], it appears that the permeability of the muscle cell membranes to this enzyme has not been altered in this early stage of vitamin E-deficiency. Several investigators[13–15] have postulated that vitamin E exerts its effect by neutralizing free radicals which arise in the initial stages of an antioxidative chain-reaction, and lack of the vitamin results in peroxidation damage to cellular structures. Previous investigations in our laboratory have shown no significant change in muscle lipid peroxidation, even though there was a significant decrease in α-tocopherol concentration at 15 days of vitamin E-deficiency[7].

After 21 days of vitamin E-deficient diet, the animals have creatinuria, typical histological changes in the muscles, significant decreases in muscle myoglobin[3–5], and oxygen consumption[6], and significant increases in lipid peroxidation[7]. The significant increase in serum ATP : creatine phosphotransferase at this time would conform with the idea that the serum enzyme is of muscular origin, and is released into the blood when metabolic events are such as to alter the permeability of the muscle membrane. The initial biochemical lesion remains to be demonstrated.

Comparing our results with those of others working with different species we find: the mean serum enzyme activity of normal and vitamin E-supplemented animals to be in the same range as healthy humans[12], and much lower than the activity reported for normal Swiss albino mice and heterozygote litter mates of dystrophic mice[1]. The evidence indicates that the enzyme activity increases in the initial stages of vitamin E deficiency, and then has a tendency to decrease as the disease progresses ($P = 0{\cdot}08$). We hesitate to conclude that these conditions have an identity with human types of dystrophy since it is likely that a variety of metabolic and membrane defects in muscle cells can alter the escape of the soluble muscle proteins, including enzymes[16].

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Response to Vitamin D in Adrenalectomized Animals

IN recent years there have been repeated attempts to establish that the action of vitamin D is in some way mediated through the adrenal glands. Perhaps the first such suggestion came from Raoul and Gounelle[1], who described an increase in an ultra-violet absorbing material in the adrenal glands of rats given vitamin D. The same authors also reported that the adrenals accumulate a large fraction of the administered vitamin shortly after supplementation. Neither Kodicek *et al.*[2], Blumberg *et al.*[3] nor Norman and DeLuca[4], using either ^{14}C- or tritium-labelled vitamin D, were able to confirm the latter observation. In fact, in this laboratory no more than 0·3 per cent of the labelled vitamin administered has ever been found in the adrenals.

Recently, Quarterman[5] has been able to repeat Raoul's experiments demonstrating an increase in a 262, 275 mμ absorbing material in the adrenals of rats following vitamin D administration, again raising the question of the participation of the adrenal glands in vitamin D action. Additional evidence for this idea has been produced by the experiments of Sallis and Holdsworth[6] in which an inhibitor of adrenal function, *o-p'*-D,D,D[1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2-dichloroethane], appeared to block the vitamin D stimulation of calcium absorption. These experiments therefore made it desirable that the question should be examined by a direct approach: can adrenalectomized rats respond to vitamin D?

Weanling male albino rats (Holtzman Co., Madison, Wisconsin) were fed a purified diet containing adequate calcium and phosphate for at least 3 weeks as described previously[7]. At the end of this period severe vitamin D deficiency was evidenced by a low serum calcium concentration (Table 1) and by reduced growth[8]. At this time one group of animals was adrenalectomized and sham operations were carried out on the second group. Adrenalectomized rats were given a 1 per cent sodium chloride solution in place of their usual water supply. All animals were allowed to recover for 5–7 days before vitamin D

treatment. At this time half the rats from each group were given 2,000 I.U. of vitamin D_3 orally in 0·1 ml. of cottonseed oil, while the remainder received the same amount of cottonseed oil without the vitamin. All animals were killed 15 h later by decapitation, their blood was collected and everted intestinal sacs were prepared as described previously[9]. The sacs were filled with Krebs–Ringer bicarbonate containing 10^{-4} M $^{40}Ca - ^{45}Ca$ and 0·01 M glucose. They were incubated in the same solution at 37° C under an atmosphere of 95 per cent oxygen, 5 per cent carbon dioxide for 1·5 h. On completion of the incubations, portions were taken from the sacs and the incubation medium, plated as infinitely thin samples and the calcium-45 present was counted in a thin end window Geiger–Müller counter. The ratio of the calcium-45 concentration inside the sac to that in the incubation medium was taken as a measure of calcium transport against the concentration gradient[10]. The collected blood was allowed to clot and then centrifuged to collect the serum. Calcium of the serum was determined by the method of Webster[11].

The results (Table 1) clearly demonstrate that adrenalectomy does not prevent the well-established responses to vitamin D. In fact, the serum calcium-levels both in the presence and absence of vitamin D are not in any way affected by the presence or absence of the adrenal glands. However, it is of interest that the adrenalectomy did increase the ability of the gut sac from vitamin D deficient animals to transport calcium. This is in harmony with the observation of Harrison and Harrison[12] that the adrenal steroid cortisol inhibits calcium transport. Similar results were obtained by Sallis and Holdsworth[6], although they demonstrated that a single dose of cortisol stimulates calcium absorption while chronic treatment inhibits it. It would therefore appear that the adrenals themselves must influence calcium transport in the intestine quite apart from the action of vitamin D. Why adrenalectomy did not increase calcium transport in rats given vitamin D cannot be determined at the present time, although it is possible that a maximal rate of calcium transport has already been attained in these animals.

The present experiments cast doubt on the physiological significance of the data of Quarterman[5] and Raoul and Gounelle[1] with regard to the action of vitamin D. There seems little doubt that production of an ultra-violet absorbing material by the adrenals is stimulated by vitamin D administration. However, this material certainly does not appear to be involved in the physiological action of the vitamin, but may reflect an influence of vitamin D in adrenal metabolism.

We have also found that 90 mg of *o-p'*-D,D,D given to each rat failed to block the vitamin D induced rise in serum calcium. Either the *o-p'*-D,D,D does not function in the rat as in the chicken, or insufficient dosages were used. The fact that animals will respond to vitamin D in the absence of their adrenals may suggest that the *o-p'*-D,D,D inhibits the action of vitamin D directly. This can only be answered by a more detailed investigation. The present data are, however, sufficient to rule out the possibility that adrenal glands are required for the characteristic actions of vitamin D.

Table 1. FAILURE OF ADRENALECTOMY TO BLOCK VITAMIN D ACTION

	Serum calcium mg per cent	Intestinal ^{45}Ca transport 'ratio'*
Vitamin D deficient (sham operated)	5·1 ± 0·2†	2·09 ± 0·15†
Vitamin D deficient + adrenalectomy	5 1 ± 0 6	2·96 ± 0 51
Vitamin D deficient + vitamin D	7 7 ± 0·7	3·66 ± 0·44
Vitamin D deficient + adrenalectomy + vitamin D	7·9 ± 0·7	3·87 ± 0·53

Vitamin D deficient rats were either sham operated or adrenalectomized and 7 days later were either given 2,000 I U. of vitamin D_3 in 'Wesson' oil or 'Wesson' oil alone. They were killed 15 h later and their serum calcium and gut sac transport of calcium-45 determined.

* 'Ratio', calcium-45 serosol side: calcium-45 mucosal side.

† Standard deviation = $\sqrt{\frac{\Sigma d^2}{(n-1)}}$. There were at least nine rats in each group

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Mechanism of Action of Bacterial Endotoxin

THE amount of endotoxin required to produce fatal traumatic shock by the intracerebral route is roughly one-twentieth of that required by the intravenous route[1]. The smaller dose is harmless when given intravenously. Shock following the injection of endotoxin into the brain is therefore the result of an action on the peripheral vessels from a distance—through the sympathetic nervous system. Since the site of the critical lesion produced by sympathetic nerve hyperactivity is in the splanchnic viscera, blockade of the coeliac plexus was used in an effort to prevent the critical lesion. In a previous communication we reported that coeliac blockade by ganglionectomy or by a long-lasting regional anaesthetic prevents death in dogs and rabbits from shock produced by endotoxin given intravenously or intracerebrally[1]. Some 65 per cent of dogs and rabbits survived a degree of shock that would have produced a mortality exceeding 85 per cent in animals without blockade. Therefore, while endotoxin appears to be capable of acting directly on certain tissues (for example, granulocytes, platelets), the lethal effects of endotoxin appear to be mediated by the catecholamines released in the splanchnic tissues.

We have recently confirmed this observation by the following means. A sublethal dose of endotoxin given intravenously to 24 rabbits was followed within one hour by a much smaller dose. The mortality was 100 per cent, although the mortality from the sum of both doses given as a single dose was zero.

This experiment was repeated in twenty-four additional rabbits, but the coeliac plexus was blocked by percutaneous one to two per cent xylocaine some 15–30 min before the second dose. Twenty-two rabbits (91 per cent) survived. The two rabbits that died were killed by a needle tear of the aorta.

Since the first dose of endotoxin makes the rabbit extremely hypersensitive to the second smaller dose[2], the vasoconstrictor damage done by the first dose is to the system involved in detoxification of the second dose—the reticulo-endothelial system (RES) in liver and spleen. The additional ischemia produced by the second dose results in fatal collapse of the peripheral circulation. By abolishing vasoconstrictor activity in the liver and spleen, coeliac blockade prevents damage from the second dose and allows the RES system to recover. We shall present data elsewhere to show that blockade prevents the loss of the detoxifying function of spleen and the

severe intracellular structural damage that is seen by electron microscopy.

The defenceless state of the animal the RES of which is damaged by a sublethal dose of endotoxin is transient—for if the second fractional dose is given 4 h rather than 1 h after the first, the mortality is very much less (less than 50 per cent), and still less (about 25 per cent) if the interval is 12 h (ref. 2). These data are a rough measure of the rapidity with which a severely damaged RES recovers normal function.

From these data we confirm the conclusion reached previously that (*a*) endotoxin kills by virtue of an effect in the splanchnic viscera that it cannot produce when the splanchnic nerves are blocked, and (*b*) the shock produced by endotoxin is the result of excessive catecholamine activity in the splanchnic circulation, and not a consequence of whatever effect endotoxin may be capable of inflicting elsewhere. Thus we conclude that death from endotoxin is caused by the loss of integrity of the blood vessels in the splanchnic area as a result of excessive and unrelenting activity of the catecholamines produced by the splanchnic sympathetic nerves.

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Separation of Two Different Substrates for Plasma Kinin-forming Enzymes

THE formation and possible function of plasma kinins have attracted considerable interest during recent years. The kinins have been shown to be formed from a fraction within the α-2 globulins of the plasma by the action of several enzymes. Kinin-forming enzymes are present in several glandular secretions, such as saliva and sweat. Another type of enzyme, called plasma kallikrein, is present in an inactive form in plasma. The first two plasma kinins identified were bradykinin[1] and kallidin[2,3]. Recently a third, methionyl-lysyl-bradykinin, has been prepared from bovine plasma[4]. It is not known, however, whether the various kinins are formed from one and the same substrate or if they originate from different substrate fractions within the α-2 globulins of the plasma. Nor is it known whether the various enzymes attack one and the same substrate molecule, or if they act on different ones.

Margolis and Bishop[5] showed that the rapid kinin formation with plasma kallikrein involves only about one-third of the total plasma substrate (kininogen), whereas trypsin or glandular kallikreins release kinins from the whole of the substrate. Their experiments could not tell whether two different substrate entities or only different parts of the same substrate molecules were involved in these reactions.

During experiments on lymph, evidence was obtained on the existence of two separate plasma substrates for plasma kinin-forming enzymes[6,7]. It has now been possible to separate two substrates from plasma by gel filtration through a 'Sephadex *G*-200' column. The substrates can also be separated by using a DEAE-'Sephadex *A*-50' anion exchange column, and the two separation procedures can be combined.

Two sources of plasma kallikrein were used; either a pseudoglobulin preparation from human plasma[8] or freshly glass-activated human plasma[9]. The pseudoglobulin preparation, which had been dialysed for more than 48 h, did not develop any kinin on incubation with saliva or with trypsin, and was thus itself without any substrate for kinin-forming enzymes.

The sources of glandular kallikrein used were cell-free saliva[10] or 'Padutin' (Bayer, Leverkusen, Germany).

The plasma or plasma fraction to be tested for substrate content was incubated at 37° C with one of the previously mentioned sources of enzyme. Aliquots of the mixture were removed at intervals and tested for kinin activity on the isolated test organ, which was either the virgin rat uterus or the guinea-pig ileum preparation. Kinin activity developed was evaluated by comparison to the effect of known doses of synthetic bradykinin (Sandoz, Ltd., Basle).

The separation procedures were as follows: citrated plasma was prepared from blood (one volume of 3·1 per cent sodium citrate dihydrate solution to 9 volumes of blood) taken with silicone technique. 50 ml. of this plasma was applied to a column (diameter 2·5 cm, height 120 cm) of 'Sephadex *G*-200' equilibrated with a 0·1 M *tris*-HCl buffer, *p*H 8·0, containing 0·2 M sodium chloride. Elution was carried out with the same buffer, and the flow rate was 20 ml./h. Fractions of 5 ml. were collected using an 'LKB' fraction collector. Substrate 1 was found in some of the earliest fractions containing protein. Substrate 2 occurred in later fractions (Fig. 1). Substrate 1 could be shown to develop kinin rapidly on incubation with glandular as well as with plasma kallikrein (Fig. 2).

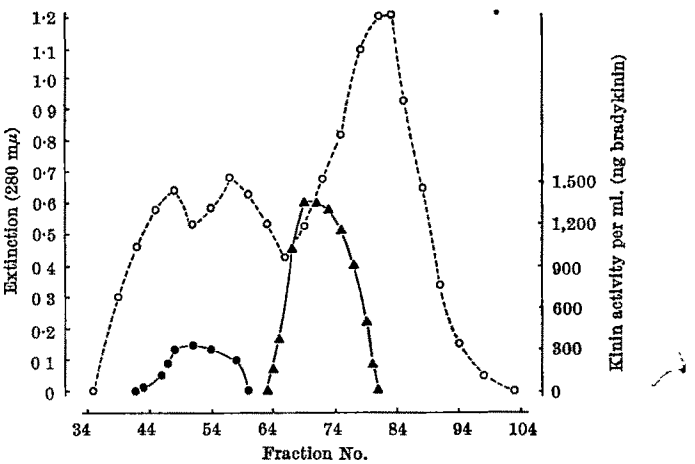


Fig. 1. Content of protein and of two substrates for kinin-forming enzymes in consecutive fractions (5 ml.) of human plasma from a 'Sephadex *G* 200' column (see text). O---- O, content of protein; ●——●, content of substrate 1; ▲——▲, content of substrate 2. Substrates are expressed in terms of kinin that could be developed per ml., synthetic bradykinin being used as reference. Protein content is expressed as extinction at 280 mμ, using a Zeiss spectrophotometer (Model '*PMQ* II') and a 1 : 16 dilution of the fractions

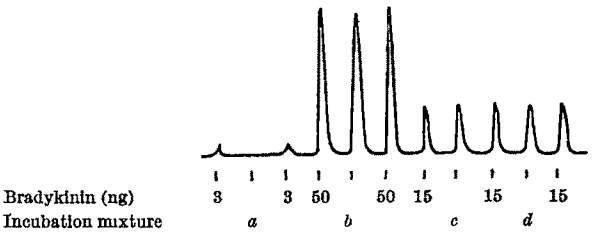


Fig. 2. Maximal kinin activity found in various incubation mixtures at 37° C. 0·1 ml. of the various mixtures added to the isolated rat uterus preparation in a 5 ml. organ bath (de Jalon solution, 29° C). Incubation mixtures: *a*, 0·2 ml. of a substrate 2-containing fraction and 0 1 ml pseudoglobulin preparation (plasma kallikrein); *b*, 0·2 ml. of a substrate 2-containing fraction and 0 1 ml. saliva; *c*, 0·2 ml. of a substrate 1-containing fraction and 0·1 ml. pseudoglobulin preparation; *d*, 0·2 ml. of a substrate 1-containing fraction and 0·1 ml. saliva. The fractions containing substrate 1 and 2 respectively were obtained by gel filtration through a 'Sephadex *G* 200' column. Standard doses of synthetic bradykinin added in between as indicated

Equal amounts of kinin activity could be obtained from this substrate with either of the two enzyme types. After maximal kinin formation by one of the enzymes, addition of the other enzyme did not cause any further kinin formation. The maximal activity from this substrate was obtained within 1 min on incubation with our plasma kallikrein preparations and within 4 min when saliva was used.

With substrate 2, only glandular kallikrein or trypsin caused a rapid kinin formation. With the plasma kallikrein preparation added to this substrate no kinin activity could be detected after 15 min of incubation (Fig. 2). On very prolonged incubation (36–48 h), however, substrate 2 could also be depleted by plasma kallikrein. Trypsin seemed to give somewhat more kinin activity with this substrate than did glandular kallikrein.

A DEAE-'Sephadex *A*-50' anion exchange column (diameter 1·1 cm, height 10 cm) equilibrated with 0·05 M *tris*-HCl buffer, *p*H 8·0, was also used for separation of the two substrates from citrated plasma or for further purification of the substrate fractions obtained by the gel filtration. Five ml. of plasma or 15–20 ml. of the fractions were first dialysed against 0·05 M *tris*-HCl buffer, *p*H 8·0, and then applied to the column. Elution of the column was carried out with the same buffer, its sodium chloride concentration being increased stepwise. Substrate 2 was eluted at a concentration of 0·2 M sodium chloride in the buffer, substrate 1 at a concentration of 0·3 M sodium chloride.

Fractions with substrate 1, which had been obtained by the gel filtration and by subsequent purification by the ion-exchange procedure, contained no plasma kallikrein and no kininase activity, whereas the fractions with substrate 2 contained a little kininase activity and some inactive plasma kallikrein. This plasma kallikrein could be removed by two successive exposures of the substrate 2 fractions from gel filtrations to large amounts of a silicate powder ('Speed plus', Great Lakes Carbon Corp., Los Angeles). The fractions were shaken with 100 mg of powder per ml. for 10 min at 37° C. After centrifugation at 2,200*g* for 5 min, the supernatant was again shaken with 50 mg silicate powder per ml. for 60 min at 37° C, followed by further centrifugation for 10 min at 2,200*g*. Finally, the fraction was filtered through a Whatman No. 1 paper before being applied to the ion exchange column.

The two substrates could be mixed with each other and re-separated again by gel filtration, each of them again occurring in the same fractions. No tendency towards transformation of one of the substrates to the other was seen on repeated gel filtration.

These findings of two separate substrates for kinin-forming enzymes may be of considerable physiological interest. It would be of importance to know which of the substrates is being utilized in the various tissues and situations where kinin formation is believed to play a part. It would also be interesting to know if the two substrates give rise to the same or to different kinins. Work is now in progress in our laboratory to obtain information on the type of kinins that are being formed from the two substrates with the various enzymes.

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Formation of Esters from Acids by Brewer's Yeast: Formation from Unsaturated Acids

ESTERS are supposed to be of importance as flavour determinants in many products. A considerable number of esters have been detected in fruits[1–8] and in fermented beverages[9–11]. Jennings[4] has shown that the desirable pear aroma is largely due to volatile esters. Esters are also used in essences (primarily the esters with 6–8 carbon atoms in acid + alcohol)[1,7] and are generally accepted to be important contributors to the flavour and aroma of beer and wine. The threshold concentration for flavour perception is very low for some esters: it is 0·01 p.p.m. for ethyl isobutyrate for example, as has been reported by Harrison[12]. Sihto and Arkima[13] have reported that isopentyl acetate is deleterious to the flavour of beer at rather low concentrations.

Esters are formed as by-products of alcoholic fermentation[14]. The acid moiety must be activated to the corresponding acyl ~ SCo*A* derivative. In previous papers, I have reported that the amount of an ester formed depends on the supply of the corresponding alcohols[15] and acyl ~ SCo*A*-compounds[14,16,17]; these can be supplied by the metabolism of the yeast or via substances added to the medium[14]. For example, if valeric acid is added to the medium, considerable quantities of ethyl valerate are formed[16]. The correlation between ester formation and acid concentration can be expressed by means of a modification of the Briggs–Haldane equation for monomolecular enzyme reactions[18]. Some acids (propionate, isobutyrate and isovalerate) were not esterified at all[16]. At low concentrations of the acids a constant fraction was esterified. This fraction increased with increasing molecular weight of the acids; it was 2·6 per cent for butyrate and 12 per cent for caproate–caprylate[16].

The experimental approach of adding an acid (and in other experiments alcohols) to the medium increased the amounts formed considerably which facilitated the analyses. It also made the results independent of the formation of the acid during fermentation; the concentration of the acid was essentially constant during the experiment[16].

Unsaturated esters have been shown to be important to the flavour of fruits[5]. It was then thought to be of value to study the formation of esters from unsaturated esters.

A synthetic medium[19] (80 g/l. of glucose, 6·7 g/l. of Difco yeast nitrogen base and 36 mM of citrate, *p*H 5·3) containing different amounts of vinylacetic acid or hexen-3-oic acid was fermented with a strain of *Saccharomyces cerevisiae* (brewer's top yeast) at 20° C subjected to continuous stirring in flasks closed by means of one-way valves[19,20]. The media were analysed by means of gas chromatography for volatile esters[16] when the glucose was completely exhausted (as has been described elsewhere).

The results of the experiments are reported in Fig. 1. For comparison, experiments with butyric acid and caproic acid added to the medium are included[16]. Considerable amounts of ethyl esters were formed from vinylacetic acid and hexenoic acid; however, the curves for these two esters were significantly below the curves of the saturated isomers. The calculated value of the fraction esterified at low concentration of the acid has been tabulated in Table 1.

Table 1. KINETIC CONSTANTS OF ESTER FORMATION FROM SOME ACIDS IN FERMENTATION OF A SYNTHETIC MEDIUM WITH A STRAIN OF BREWER'S TOP YEAST

Calculated from Figs. 1 and 2

Acid	Michaelis constant (mM)	Maximum velocity (μM)	Esterified fraction at low conc. of the acid (μM)
Butyric	8	220	2·6
Vinylacetic	20	60	0·6
Caproic	1·4	130	12
Hexen-3-oic	5	70	2·5

Straight lines were obtained in double reciprocal graphs (Fig. 2) (Lineweaver–Burk plots). From the intercepts, the results of Table 1 were obtained. The Michaelis constants were higher and the maximum velocities were lower for the unsaturated acids than for the saturated acids (Table 1).

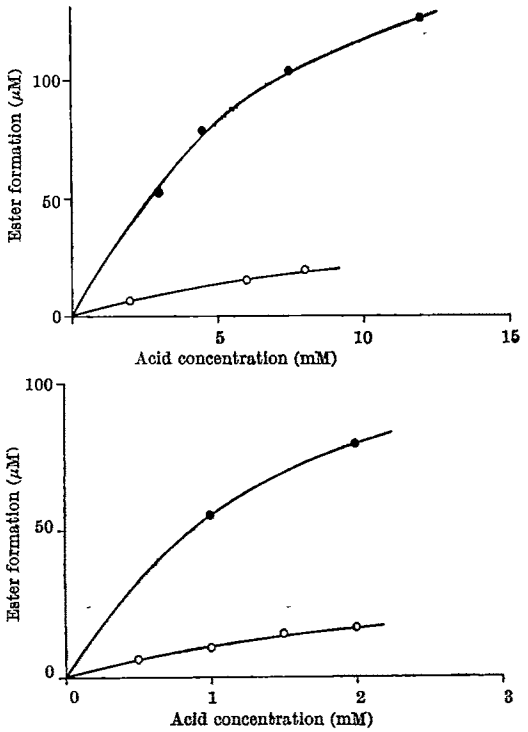


Fig. 1. Ester formation from some acids in fermentation of a synthetic medium with a strain of brewer's top yeast. Upper diagram, butyrate (●) and vinylacetate (○). Lower diagram, caproate (●) and hexen-3-oate (○)

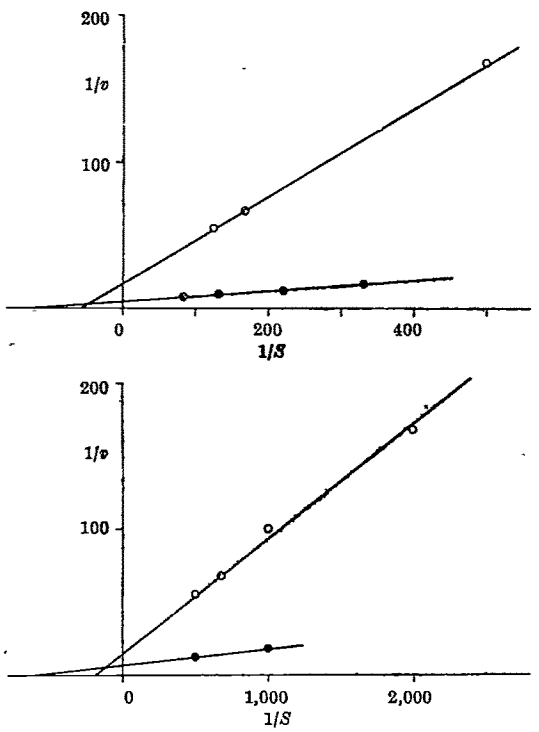


Fig. 2. Double reciprocal graph $1/v$ against $1/S$, where v is the formation of various ethyl esters (mM), and S the concentration of the corresponding acids (M). Upper diagram, ethyl butyrate (●) and ethyl vinylacetate (○). Lower diagram, ethyl caproate (●) and ethyl hexen-3-oate (○)

Thus, unsaturated acids were esterified, but to a lower extent than saturated acids. Whether this finding is of importance in brewing, for example, is uncertain since few quantitative results have been reported concerning the acids found in beer. Åyräpää *et al.*[21] have found about 1 p.p.m. of hexenoic acid in Swedish beers. About 0·03 p.p.m. of ethyl hexenoate is to be expected from the results of Table 1 and Fig. 1. This value is presumably of the same order of magnitude as the threshold value for flavour perception[12].

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A Simple Medium for the Propagation and Maintenance of Human Diploid Cell Strains

THE experiences of many workers propagating human diploid cell strains (HDCS) suggest that these cells are uncommonly exacting in their medium requirements[1,4]. Eagle's basal medium (BME)[2] and minimum essential medium (MEM)[3] have given most satisfactory growth, but careful adherence to these formulae in preparing the media has not always guaranteed their suitability[4]. Differences in the purity of the constituents may account for the variability of different batches of media[4]. The use of standardized Eagle's media[4] has largely overcome these difficulties, but it is of interest to report that a simplified Eagle's medium has also been used successfully for the growth and maintenance of cells in our laboratory.

For the purpose of this study it was decided to investigate the amino-acid content of BME, and to determine whether the quantities used in Eagle's medium were critical for the propagation and maintenance of HDCS. It was also decided to investigate whether, in the absence of defined concentrations of the amino-acids used in BME, these amino-acids contained in the serum added to BME might be present in quantities adequate to support cell growth. Preliminary investigations were concerned with screening tests to preclude any unnecessary amino-acids. In subsequent studies only salts, vitamins and two amino-acids were incorporated in BME. The efficacy of experimental media was assessed by comparing results with those obtained using complete BME as a control medium.

BME contains thirteen amino-acids, nine vitamins, salts and glucose. Several modifications of this medium were made as follows: (i) Twelve combinations based on BME, but each deficient in one of the amino-acids. (ii)

A medium (S-BME) which was simplified BME containing only two of its amino-acids. In addition, galactose was substituted for glucose, and no sodium bicarbonate was present. The formula is as follows:

Salts and galactose (modified Hanks's balanced salt solution)	(g/l.)
NaCl	8·0
KCl	0·40
$MgSO_4 7H_2O$	0 20
Na_2HPO_4	0 05
$CaCl_2$	0·14
Kh_2PO_4	0·06
Galactose	2·0
Amino-acids (quantities as in BME)	**mM**
L-cystine	0 05
L-glutamine	2·0

Vitamins (Based on BME)	(mg/l.)
Biotin	1·0
Choline chloride	1·0
Folic acid	1·0
i-Inositol	0·2
Nicotinamide mononucleotide	1·0
Calcium pantothenate	1·0
Pyridoxal hydrochloride	1·0
Riboflavin	0·1
Thiamine hydrochloride	1·0
Phenol red	10·0

The *p*H of the medium was adjusted to 7·1–7·2 using N/10 sodium hydroxide.

(iii) Complete BME, but with the sodium bicarbonate concentration reduced to 0·1 per cent.

In all these media the vitamins and amino-acids were obtained from Nutritional Biochemical Corporation, Cleveland, U.S.A., and the salts from Hopkin and Williams, Ltd., Chadwell Heath, England.

For the maintenance of cells, no serum was added to these media but for serial propagation unheated calf serum was added to a final concentration of 10 per cent. None of the media contained antibiotics.

Cultures of two normal human foetal lung cells were used: (i) *WI*-38, developed at the Wistar Institute, Philadelphia, by Dr. L. Hayflick[5]; (ii) *MRC*-1, developed at the Medical Research Council Laboratories, Hampstead.

Quantitative analysis of serum for the thirteen amino-acids used in BME was carried out chromatographically, using the method of Stein and Moore[6]. The same batch of calf serum was used in all media.

Cells were propagated by the method described by Hayflick and Moorehead[7] using 4-oz. medical-flat bottles. For maintenance studies they were incubated in stationary 4-oz. bottles, but rolled when in test-tubes. Cultures used for maintenance studies did not have the medium replaced; all cultures were incubated in rubber-stoppered containers at a temperature of 36°–37° C. Cell counts were made in a haemocytometer on both unstained and trypan blue-treated cells.

Initial studies on the propagation of cells indicated that the omission of L-cystine and L-glutamine gave medium unsatisfactory for cell growth, whereas the exclusion of any of the other amino-acids did not seem to impair growth. Satisfactory results for cell propagation were obtained using S-BME with added serum, although cells maintained readily on this medium in the absence of serum. Substituting galactose for glucose reduced fluctuations in the *p*H of the medium during cell propagation.

The normal life span of these cells *in vitro* is 50 ± 10 passages. Cell senescence in this study began at the 44th to 47th passage as indicated in Table 1. Since the limited life of both cell strains is shown to be similar in both control and experimental media, it is clear that the use of S-BME has not altered the life span of the cells.

Table 1. PROPAGATION OF CELLS IN BME AND S-BME

Cell	Passage No.	Medium	No. of sub-cultures
WI-38	24–44	BME	22
WI-38	24–45	S-BME	23
MRC-1	26–47	BME	22
MRC-1	26–47	S-BME	22

Table 2. MAINTENANCE OF CELLS IN BME AND S-BME

Cell	Passage No. Bottles*	Tubes†	Medium	No. of days during which cells remained satisfactory‡ Bottles	Tubes
WI-38	24	21	BME	10	10
WI-38	24	21	S-BME	12	11
MRC-1	26	21	BME	9	12
MRC-1	26	23	S-BME	8	12

* Four bottles per medium.
† Ten tubes per medium.
‡ Earliest sign of cell degeneration.

Cell counts made on four occasions from confluent monolayers of cells after the same time interval for both control and experimental cultures showed that the rate of growth of cells in S-BME was slightly reduced, the average ratio of cells grown in experimental medium to those grown in control medium being 87 : 100.

Not only do the cells propagate satisfactorily in S-BME but the data in Table 2 show that they maintained equally well in both S-BME and BME.

The effect on the amino-acid content of adding serum to the medium is shown in Table 3, from which it is clear that in no case has the addition of serum resulted in a substantial increase in the concentration of any of the amino-acids; indeed, most are increased by no more than 20 per cent.

Table 3. CONCENTRATIONS OF AMINO-ACIDS IN BME AND IN CALF-SERUM SUPPLEMENT

Amino-acid	Concentration in BME (mM)	Concentration in serum (mM/100 ml.)*	Percentage of increase in amino-acid concentration after serum added†
L-Arginine	0·1	0 020	20
L-Cystine	0·05	0·023	46
L-Histidine	0 05	0·028	56
L-Isoleucine	0·2	0 012	6
L-Leucine	0·2	0·027	14
L-Lysine	0·2	0 051	26
L-Methionine	0·05	0 002	4
L-Phenylalanine	0·1	0·009	9
L-Threonine	0·2	0·017	9
L-Tryptophan	0·02	0·003	15
L-Tyrosine	0 1	0·006	6
L-Valine	0·2	0·039	20
L-Glutamine	2·0	0 022	1

* Final concentration of serum in growth medium is 10 per cent.
† Maximum value (common 'peak' for serine and glutamine).

These findings show that a defined medium of simple composition, with added serum, is suitable for the propagation of human diploid foetal lung cell strains. They also show that confluent sheets of non-replicating cells can survive in this medium in the absence of serum. Although the rate of growth of cells in the medium is slightly reduced compared with that in the more complex BME, this is not considered to be a practical disadvantage. By substituting galactose for glucose no difference in the rate of cell propagation occurs but it results in diminished glycolysis, so that the *p*H range is more uniform during cell growth.

The quantities of amino-acids provided by the added serum are insufficient, in themselves, to account for the satisfactory growth and survival of cells. It would seem that, on a quantitative basis, apart from cystine and glutamine the other eleven amino-acids used in BME need to be added in appreciably smaller quantities, or in some cases may not need to be added, to produce satisfactory propagation of cells, and that for the survival of cells these eleven amino-acids can be omitted. Optimal ranges or concentrations of amino-acids have not been determined, but smaller quantities of these components than present in BME are shown to be adequate.

Further investigations of a complementary nature on the vitamin requirement of the cells and an extension of these studies to a quantitative determination of the minimal and optimal nutritional requirements are contemplated.

I thank Dr. D. G. Smyth for determining the concentration of amino-acids in serum.

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PHYSIOLOGY

Action of Aldosterone on the Lachrymal Gland

THE lachrymal gland in mammals has acinar tissue leading to ducts lined with columnar epithelium showing no regional differentiation. The Na/K ratio of an established secretion shows no alteration in relation to variation in secretory rates, nor is there other evidence of sodium absorption from the acinar secretion associated with exchange for other ions[1]. There are no records of experiments on the effect of mineralocorticoids on the sodium–potassium relation of lachrymal secretion. In view of the fact that sheep were available with graded sodium depletion, and therefore proportional aldosterone secretion rate[2], saliva and tears were collected for sodium and potassium assay.

The sheep were Merino cross-bred. Four (two ewes and two wethers) had a parotid fistula from the start; a third ewe (Gretel) was used for two collections of tears over a fortnight and then a parotid fistula was made. Up to 450 m.equiv. sodium were lost daily from the fistulae and the degrees of sodium depletion were regulated by varying the amount of sodium bicarbonate solution given to drink[2]. Tears were collected by placing a 100-μl. capillary pipette at the lateral canthus of the eye and lachrymation was stimulated by blowing ammonia fumes at the conjunctiva. Precautions were not taken against evaporation because our interest was in the Na/K ratio. Parotid salivary samples were taken just before the tear collections. The sodium and potassium concentrations in each were estimated using a Beckman flame spectrophotometer.

Four animals were otherwise normal. The exception was Maxine, which had had bilateral Goldblatt clamps applied 12 months previously and had, at the time of the experiment, a systolic blood pressure of 150–180 mm mercury.

The results are shown in Table 1. This shows that the range of the Na/K ratio in tears is from 8·8 to 4·5, while that for saliva, which is known to reflect the degree of sodium depletion and aldosterone secretion[2], was from 41 to 0·58; in terms of concentration of potassium, there was variation by a factor of × 1·6 for tears and × 26 for saliva. The variation of lachrymal and salivary Na/K was not regularly concomitant, and it is likely that the variation of the potassium concentration of tears was due to the 'start-up' discharge of potassium at the onset of increased secretory rate which has been described for tears[3], for sweat[4,5] and for salivary glands[6–8].

As an extension of these results, it was decided to test the effect of aldosterone infusion in a sheep when sodium-replete and when sodium-deficient, in which latter instance the sensitivity of the parotid gland to aldosterone is increased[9]. Flora, a bilaterally adrenalectomized ewe, was normally maintained on 25 mg cortisone acetate and 5 mg DOCA daily. These hormones were withdrawn 36 h before experiments were performed. Tears and saliva were collected before and 150 min after the start of intravenous infusions of *d*-aldosterone at 20 μg/h.

Table 1 LACHRYMAL SODIUM AND POTASSIUM CONCENTRATIONS AND THE PAROTID SALIVARY Na/K OF FIVE SHEEP WITH SODIUM STATUS VARYING FROM REPLETE TO MODERATE TO SEVERE DEFICIENCY

Sheep	Salivary Na/K	Tears Sodium (m.equiv./l.)	Tears Potassium (m.equiv./l.)	Na/K
Gretel	(Before fistula)	162	19	8·5
		153	19	8·0
	41	140	17	8·2
	28	137	24	5·7
	6·1	141	27	5·2
	5 4	140	28	5·0
	1·06	142	20	7·1
Cecil	6·7	133	20	6·7
	4·0	144	23	6·3
	0 87	142	26	5·5
	0·58	127	28	4 5
Arianne	8·1	153	24	6·4
	3·3	168	19	8·8
	1·3	138	28	5·0
Finochio	13·2	146	24	6·1
	1·8	142	27	5·2
Maxine	8·5	138	20	6·9
	3·6	137	28	4·9
	0·85	152	27	5 6

Table 2. FLORA—THE LACHRYMAL SODIUM AND POTASSIUM CONCENTRATIONS AND THE PAROTID SALIVARY Na/K BEFORE INTRAVENOUS INFUSION OF *d*-ALDOSTERONE, AT 20 μg/H AND AFTER 150 MIN OF INFUSION, WHEN THE SHEEP WAS (1) SODIUM-REPLETE AND (2) SODIUM-DEFICIENT

Experiment	Salivary Na/K	Tears Sodium (m.equiv/l.)	Tears Potassium (m.equiv/l.)	Na/K
(1) Sodium-replete				
Before aldosterone	23·6	142	20	7·1
During aldosterone	5·9	132	27	4·9
(2) Sodium-deficient				
Before aldosterone	13·8	138	30	4·6
During aldosterone	1·6	130	23	5·6

The results in Table 2 are similar to the findings in sodium depletion (Table 1).

The lachrymal gland, in common with the pancreas and sublingual and palatine glands, does not show regional differentiation of ducts, and presumably does not have a sodium reabsorption and exchange mechanisms[1]. (Yoshimura and Hosokawa[10] state that lachrymal potassium increases proportionally to secretory rate and conclude that the gland actively secretes potassium without exchange for sodium or hydrogen. This behaviour would be different from that of the parotid, submaxillary and sweat glands in which, over a limited range, potassium decreases with increased secretory rate and sodium concentration. The conclusions of these authors should be accepted with caution because the high secretory rates occurred at the onset of increased secretion rates; constant secretory rates at several levels were not produced.) Because it shows trifling change in Na/K as compared with parotid saliva in sodium depletion and aldosterone infusion, and because such variation as does occur is not concomitant with the salivary variation and is explicable on grounds other than mineralocorticoid effect, it is reasonable to regard the gland as insensitive to aldosterone. A similar conclusion about the pancreas can be drawn from the observations that the potassium concentration of pancreatic juice did not rise in humans depleted of sodium by duodenal fistula[11]. In sheep the salivary Na/K of the palatine gland did not change in sodium depletion as did the Na/K of all the other salivary glands tested[12]. The insensitivity of these several glands to a level of aldosterone which has a large effect on the parotid and kidney[13] is consistent with the general proposition of Thaysen[6] that aldosterone may act at the sodium reabsorption and exchange mechanisms, but not at the acinar sodium secretory mechanisms.

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Subcomponents of *PIII* in Cold-blooded Vertebrate Retinae

IT is generally accepted that the *PII* component of the vertebrate electroretinogram (ERG) arises in the bipolar cell layer. The origin of the *PIII* component, however, is still the subject of controversy. From experiments on the mammalian retina *in situ*, Brown and Wiesel[1,2] and Brown and Watanabe[3] conclude that the receptor cell layer is the origin of *PIII*, while Tomita[4], working on cold-blooded retinae, emphasizes the bipolar cell layer as the main origin of *PIII*. He observed more recently that, in the excised opened eye of the frog, a small fraction of *PIII* persists distal to the bipolar cell layer after the abolition of *PII* and the greater part of *PIII* by sodium azide. This suggests that *PIII* is made up of more than one component from different retinal layers[4]. This suggestion is strongly supported by our depth-recording experiment on isolated inverted retinae of cold-blooded vertebrates. The carp, frog and turtle were used with substantially the same result.

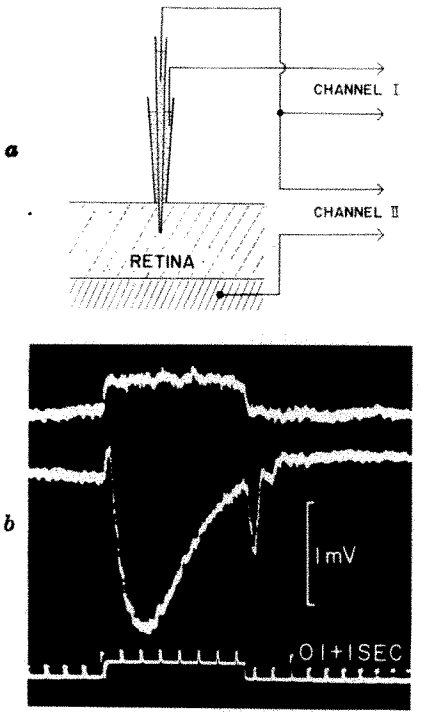


Fig. 1. (*a*) Experimental arrangement for depth recording of ERG with pencil-type coaxial microelectrode. (*b*) ERG of frog recorded with coaxial microelectrode. The inner pipette penetrated 120μ from the outer margin of the receptor layer, while the outer one was located on the receptor surface. The upper tracing shows the potential between the two pipettes (channel I in *a*) and the lower tracing shows the potential between the inner pipette and the indifferent electrode (channel II). Elevation of the time scale indicates the duration of illumination

After dark-adaptation, the retina was detached from the pigment epithelium and mounted with the receptor side up on a small piece of Ringer-soaked black paper, which thus constituted the indifferent electrode together with a chlorided silver plate underneath. The experimental arrangement is shown schematically in Fig. 1*a*: channel I records the potential between the inner and the outer pipette of a pencil-type coaxial micro-electrode, and channel II the potential between the inner pipette and the indifferent electrode. The outer pipette was located on the receptor surface throughout. These two potentials were displayed simultaneously on the screen of a dual-beam oscilloscope. All experiments were conducted at room temperatures of 18°–20° C.

Fig. 1*b* is an example of simultaneous recording in the frog through both channels with the inner pipette 120μ beneath the receptor surface. At this location of the inner pipette a sustained positive potential (which appears to correspond to the small fraction of *PIII* observed by Tomita[4]) was isolated after application of sodium azide on channel I at the largest amplitude and was little contaminated by *PII*. This will be called 'distal *PIII*', since it is located more distally than the remaining *PIII* fraction. It is clear from a large *a*-wave in the lower tracing (channel II) that the ERG thus recorded between the inner pipette and the indifferent electrode is made up not only of *PII* but also of a large fraction of *PIII*. This fraction of *PIII* has recently been localized in the bipolar cell layer[4,5] and will be called 'proximal *PIII*'. The distal *PIII* was maximal in amplitude at about 120μ in the carp and 80μ in the turtle. According to our histological measurement, the thickness from the outer margin of receptors to the outer plexiform layer is nearly 105μ in the frog, 120μ in the carp and 70μ in the turtle. The difference in depth for the maximal distal *PIII* was consistent with the difference in thickness of the receptor layer in the different animals. The following experiments provide evidence that these two *PIII* fractions are different in nature.

The distal *PIII* could be isolated from the proximal *PIII* by application for a few seconds of ammonia vapour from a fine nozzle placed near the retinal surface. As observed earlier, *PII* was most sensitive to this agent[6], and was the first component to be suppressed. The proximal *PIII* on channel II at this stage was larger than the *a*-wave in Fig. 1*b*, due apparently to decay of the *PII* component. By further application of ammonia, the proximal *PIII* was next abolished, while the distal *PIII* persisted, though slightly decreased thereafter in amplitude. The same result was obtained on all the animals used. A lower sensitivity to ammonia than any other ERG components of the distal *PIII* was one of the characteristics of the distal *PIII* sub-component. It is also suggested from the foregoing observation that the proximal *PIII* may be larger in amplitude than the distal *PIII*.

Another approach for discrimination of the distal *PIII* from the proximal one was to compare their latency using a data-processing digital computer ('*ATAC* 401' of Nihon Kohden Kogyo Co., Ltd.), which improved the S/N ratio. The inner pipette was located at the depth where the maximal distal *PIII* was obtained through channel I. This and the rest of the ERG through channel II were put into two separate channels of the computer which added 30–70 successive responses. The base-lines of both tracings were brought to the same level and their slopes were made equal by adjustment of amplifier gain in order to facilitate comparison of the latencies. The result on the frog is illustrated in Fig. 2, in which the tracing '*d*' indicates the start of the distal *PIII* and the tracing '*p*' that of the proximal *PIII* in the *a*-wave. The latency of the distal *PIII* is about 15 msec, being 7 msec shorter than that of the proximal *PIII*. Similar results were obtained on the carp and turtle; the distal *PIII* appeared 3–5 msec earlier than the proximal *PIII*. It is suggested that the distal *PIII* is a manifestation of a visual process that takes place earlier than other components, and probably reflects the process in the receptors themselves.

Intracellular recording in single invertebrate photoreceptors has been reported by many workers, but no work has been done on the vertebrate apart from a recent report on the single receptor potential in the carp by Tomita[7]. It seems reasonable to take this single receptor potential as the constituent of the distal *PIII*, since both of them are localized in the receptor layer. It is difficult to determine the exact ratio of the distal *PIII* to the proximal *PIII* either from the results of depth recording or by means of chemical agents, because of the difficulty of completely separating the two components.

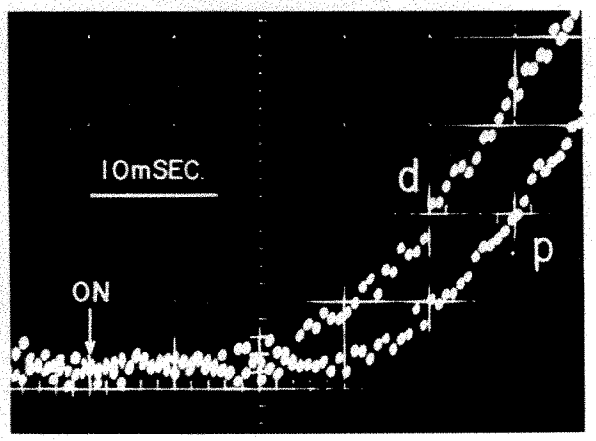


Fig. 2. Commencement of the distal *P*III (indicated as *d*) and the proximal *P*III (indicated as *p*), showing the difference in latency. Each of them was summed 70 times in a digital computer. Arrow indicates the beginning of illumination

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PHARMACOLOGY

Drosophila-tests in Pharmacology

IN a communication under this heading, Lüning[1] has suggested a routine procedure by which potentially mutagenic drugs can be screened in tests on *Drosophila*. He uses developmental delay as the type of somatic damage that is likely to be correlated with whatever genetical damage has occurred. I wish to point out that in the only instance in which, to my knowledge, the effects of the same mutagen on developmental delay and mutation frequency were investigated, the correlation between them was clearly and strikingly negative. When larvae are raised on food to which formaldehyde at a low concentration has been added, high mutation frequencies are obtained so long as development proceeds at the normal speed. Any condition that delays development of the treated larvae—be this shortage of food, poor quality of food, supplementary harmful treatments, or even a more than optimal concentration of formaldehyde—concomitantly decreases mutation frequency[2]. For this particular mutagen, therefore, developmental delay would be a highly misleading guide to mutagenicity. It is, of course, possible that formaldehyde is exceptional in this respect, and that Lüning's test might be more reliable for other chemicals. Before this can be assumed, however, it will be necessary to establish experimentally for at least one member of the group of chemicals to be screened that its mutagenic action is positively correlated with its delaying action on development.

As an additional criterion for potential genetic damage, Lüning uses survival to adulthood, which for some of the drugs used by him went down as developmental time increased. This was also the case in most of the experiments with formaldehyde already mentioned. Since in these experiments genetical damage was negatively correlated with developmental delay, it was also negatively correlated with survival, more mutations being obtained at high than at low survival. In a number of cases it was, however, possible to destroy the parallelism between survival and speed of development, and in these it could be shown that the decisive factor in determining mutation frequency was developmental speed rather than survival. This was most clearly shown in one experiment where mutation frequency among the same batch of treated larvae was about twice as high in those flies that had developed with normal speed as in those that had reached adulthood after a developmental delay lasting 5–6 days. Conversely, in one experiment in which developmental delay had been prevented by the presence of dinitrophenol in the culture bottle, mutation frequency was not affected by a highly significant decrease in survival. It would seem, therefore, that decreased survival not correlated with delayed development might be a guide to genetical damage. This would agree with the findings by Müller's group[3] that X-ray-induced life-shortening in *Drosophila* is mainly caused by somatic chromosome loss. One way of demonstrating this consisted of the finding that, for a given exposure of larvae, survival decreased from females to rod-*X* males to ring-*X* males. Parallel results with a given drug would form strong presumptive evidence of genetic damage.

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Synergistic Interaction of Kethoxal *bis*(Thiosemicarbazone) and Cupric Ions in Sarcoma 180

2-KETO-3-ethoxybutyraldehyde *bis*(thiosemicarbazone) [kethoxal *bis*(thiosemicarbazone); KTS], an agent that chelates metals of the first, second and third transition series[1], has been reported to be an inhibitor of the growth of several transplanted rodent neoplasms[2–5]. The antineoplastic potency of KTS was enhanced by the presence of either cupric or zinc ions[6]; however, the preformed cupric chelate was found to be the most potent of several different metal chelates of KTS[1,5,7]. To investigate the relationship of copper ions to the antineoplastic activity of KTS, the effects of combinations of cupric ions (as cupric chloride) and KTS, both on growth and on the synthesis of nucleic acids and proteins, were measured in sarcoma 180 ascites cells.

The tumour-inhibitory effects of such combinations on neoplastic growth were measured by determining the survival time of adult *Ha/ICR* Swiss mice inoculated with approximately 4×10^6 sarcoma 180 ascites tumour cells by intraperitoneal injection as previously described[8]. Therapy was initiated 24 h after implantation of the neoplastic cells and was continued for six consecutive days. The results obtained are shown in Table 1. Neither KTS in daily doses up to 60 mg/kg, nor cupric chloride in daily doses up to 10 mg/kg, increased the average survival time of tumour-bearing mice. In combination, however, significant prolongation of life was produced at low levels of each drug. No concomitant toxicity to the host was evident; thus, no weight loss from onset to termination of therapy was observed.

To probe for metabolic alterations associated with the growth-inhibitory potency of combinations of cupric chloride and KTS, the effect of a single dose of these agents on the synthesis of DNA, RNA and proteins was

monitored using thymidine-methyl-^{3}H, uridine-^{3}H and DL-leucine-1-^{14}C, respectively (Table 2). Little or no inhibition of these biosynthetic pathways was produced by either cupric chloride or KTS alone; however, combination of these agents caused about 82 per cent inhibition of the formation of DNA, 54 per cent inhibition of the synthesis of RNA, and a 62 per cent depression in the rate of formation of proteins. Although the magnitude of the effects of the combination on DNA were comparable with those produced by administration of the preformed cupric chelate of KTS, [Cu(II)KTS][9], the degree of inhibition of the synthesis of proteins by the mixture of copper chloride and KTS could not be explained by the formation of Cu(II)KTS *in vivo*. Furthermore, the magnitude of inhibition achieved by the mixture of cupric chloride and KTS could not be duplicated by appropriate quantities of Cu(II)KTS in combination with either KTS or cupric chloride[10]. Thus it would appear that cupric chloride, KTS and possibly some resultant Cu(II)KTS produce distinct alterations that result in synergistic inhibition of the synthesis of proteins.

Table 1. EFFECT OF COMBINATIONS OF CUPRIC CHLORIDE AND KTS ON THE SURVIVAL TIME OF MICE BEARING SARCOMA 180 ASCITES CELLS

Daily dosage* (mg/kg) Cupric chloride	KTS	Av. Δ wt.† (%)	Av. survival‡ (days)
0	0	+22·3	12·8 ± 0·5
0·5	0	+20·0	13·8 ± 0·8
1·0	0	+20·8	12·1 ± 0·8
0	2·5	+22·4	13·4 ± 0·6
0	5·0	+26·8	11·2 ± 0·9
0·5	2·5	+5·0	21·6 ± 1·3
1·0	5·0	+0·8	23·3 ± 1·7

* Drugs were administered intraperitoneally once daily for 6 consecutive days, beginning 24 h after implantation of tumour cells, with combinations given simultaneously.
† Average weight change from onset to termination of drug treatment.
‡ Each value represents the average survival time (± standard error) obtained with 9–20 mice.

Table 2. EFFECT OF CUPRIC CHLORIDE, KTS OR THEIR COMBINATION ON THE SYNTHESIS OF DNA, RNA AND PROTEINS IN SARCOMA 180 ASCITES CELLS

Treatment	Specific activity DNA*	RNA†	Protein
None	136·8 ± 10·6	13·05 ± 1·10	3,720 ± 147
Cupric chloride	109·9 ± 13·5	10·45 ± 1·35	3,380 ± 274
KTS	132·9 ± 11·8	8·55 ± 0·90	3,700 ± 147
Cupric chloride + KTS	25·2 ± 6·4	6·00 ± 1·05	1,420 ± 503

Six days after implantation of tumour cells, each mouse received a single intraperitoneal injection of either 2·5 mg of cupric chloride/kg body-weight, 50 mg of KTS/kg, or a combination of the two drugs administered simultaneously. Twelve h later an isotopic tracer was administered intraperitoneally to each mouse, and 1 h was allowed for incorporation. Isolations and analyses were performed as previously described (ref. 13).
* Determined as c.p.m./μmole of thymine × 10^{-3} after administration of 200 μg of thymidine-methyl-^{3}H ($5·7 \times 10^{3}$ c.p.m./μg) to each mouse. Each number represents the mean value (± standard error) obtained with 8–12 mice.
† Determined as c.p.m./mμmole of ribose after administration of 200 μg of uridine-^{3}H ($7·1 \times 10^{3}$ c p.m /μg) to each mouse. Each number represents the mean value (± standard error) obtained with four mice.
‡ Determined as c p.m /mg of residual protein after administration of 125 μg of DL-leucine-1-^{14}C ($1·45 \times 10^{4}$ c p.m./μg) to each mouse. Each number represents the mean value (± standard error) obtained with eight mice.

Table 3. COPPER CONTENT OF SARCOMA 180 ASCITES CELLS AFTER EXPOSURE TO CUPRIC CHLORIDE, KTS OR THEIR COMBINATION

Treatment	mμmoles Copper/g packed cells
None	9·2 ± 3·9
Cupric chloride	38·0 ± 4·4
KTS	10·2 ± 3·3
Cupric chloride + KTS	144·2 ± 29·1

Six days after implantation of tumour cells each mouse received a single intraperitoneal injection of either 2·5 mg of cupric chloride/kg body-weight, 50 mg of KTS/kg, or a combination of the two drugs administered simultaneously. Twelve h later ascites cells were wet-ashed and analysed for copper content as described by Stoner and Dasler (ref. 14). Each point represents the mean value (± standard error) obtained with four mice.

Since Cu(II)KTS, formed from the mixture of cupric chloride and KTS, is a relatively lipid-soluble form of copper and, therefore, would penetrate cellular membranes with comparatively greater ease than would copper ions derived from cupric chloride, the quantity of copper associated with the tumour cells after a single exposure to the drugs alone or in combination was determined (Table 3). The amount of copper/g of wet weight of cells was four times greater in sarcoma 180 cells removed from mice treated with cupric chloride than in untreated controls, whereas KTS did not significantly alter the copper content. Exposure of the neoplastic cells to the drug combination, however, resulted in a copper content 15·5 times that of untreated control cells. Although the large copper content of cells may be responsible for some of the observed biochemical effects, no direct correlation can be made between the quantity of copper found in the neoplastic cells and the degree of metabolic blockade[10].

The enhanced inhibitory effects observed in the present study with copper chloride and KTS are reminiscent of the synergistic chromosomal aberrations produced by combinations of ethyl methane sulphonate and copper ions in *Vicia faba*[11,12]. Investigations are now in progress to define precisely the enzymatic sites involved in the metabolic lesions produced by cupric chloride, KTS and Cu(II)KTS.

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Effect of Phytohaemagglutinin on Guinea-pig Skin *in vivo*

PHYTOHAEMAGGLUTININ (PHA) can stimulate the transformation of lymphocytes into lymphoblasts *in vitro*[1] and a therapeutic stimulant effect on haematopoietic tissue in aplastic anaemia has been claimed for it[2]. We have recently shown that PHA stimulates mitosis in the epithelial cells of the basal layer of adult human skin *in vitro*[3] using a system developed by us for short-term organ culture[4]. We now report the mitogenic effect of PHA on guinea-pig skin *in vivo*.

Three guinea-pigs (each weighing 500 g) were given a series of intradermal injections of PHA at 96, 72, 48, 24 and 4 h before they were killed. One ampoule of PHA-*M* (Difco Co.) was dissolved in 5 ml. Hanks's solution. The first guinea-pig was injected with 0·1-ml. volumes of this PHA solution, of a 1/10 dilution of this solution and of Hanks's solution. The second animal was given a similar series of injections, and 4 h before death received 0·75 mg desacetylmethylcolchicine ('Colcemid', Ciba, Ltd.) subcutaneously at a remote site. The third animal was given a series of injections of 0·1 ml. of (*a*) a mixture of PHA and 'Colcemid' containing an amount of PHA equivalent to that used in the other two animals and 125 μg of 'Colcemid', (*b*) 'Colcemid' solution (125 μg/0·1 ml.) and (*c*) Hanks's solution. Four hours before death 0·75 mg 'Colcemid' was injected subcutaneously at a remote site. Specimens from the injected sites and from normal control sites were fixed in 10 per cent formal saline immediately

after death and histological preparations were stained with haematoxylin and eosin.

Macroscopically, erythema and slight induration occurred at the sites where PHA had been injected at approximately 4 h, became maximal at 24 h and disappeared at 4 days. No visible change occurred at the sites of injection of Hanks's solution or of 'Colcemid'.

Microscopically, there was a marked thickening of the epidermis at the sites of injection of PHA after 4, 3 and 2 days with a lesser degree of acanthosis after 1 day. No acanthosis was seen at the 4-h injection site. Accompanying the acanthosis was a moderate cellular infiltration of the dermis by lymphocytes and polymorphs. A small fibrinous crust was seen overlying the horny layer at the PHA injection site. At the control injection sites a slight inflammatory infiltrate and crust were seen but there was no acanthosis. The acanthotic epidermis at the sites injected with PHA consisted of between eight and twelve cell layers compared with the two to four cell layers of the normal guinea-pig skin. The degree of acanthosis was proportional to the amount of PHA injected, the 1/10 dilution of PHA producing an acanthosis of three to six cell layers. At the sites injected with PHA there was also a thickening of the granular cell layer. This, as well as the acanthosis, diminished progressively away from the injection sites.

In the first guinea-pig there was a threefold increase in the proportion of the basal cells of the epidermis undergoing mitosis at the sites injected with PHA as compared to control sites. In the second animal 'Colcemid'-arrested metaphase mitotic figures occurred in 8 per cent of the basal cells at the sites injected with PHA as compared with 1 per cent at the control sites. In the third animal the range of 'Colcemid'-arrested mitoses at the sites injected with PHA was 6–16 per cent of basal cells compared with a range of 1–4 per cent at the control sites and at the sites injected with 'Colcemid' alone, on corresponding days.

These results show that phytohaemagglutinin has a mitogenic effect on epithelial cells of guinea-pig skin *in vivo*. They do not allow of any conclusions as to its mode of action. Recent work suggests that PHA stimulates the transformation of lymphocytes to lymphoblasts in culture by a direct action on their lysosome membranes[5,6]. This might also be the mechanism of the mitogenic effect of PHA on epithelial cells of guinea-pig skin *in vivo*.

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HAEMATOLOGY

Magnesium, Adenosine Diphosphate and Blood Platelets

RECENTLY we reported[1] a relative plasma magnesium deficiency in a group of forty male patients with acute myocardial infarction (mean of 2·3 mg per 100 ml.) compared with thirty-six normal healthy males (mean of 2·6 mg per 100 ml.) in the same age group. In addition it was shown that magnesium added to fresh whole blood delays the clumping of platelets *in vitro*.

The ability of blood platelets to adhere to one another and form platelet clumps is well known, and work[2–4] on experimental animals has emphasized the part played by the intravascular clumping of platelets in the production of lung and heart lesions. The circumstances that lead to intravascular platelet clumping are still unresolved, but much interest was aroused by Hellem's[5] observation that a heat-stable extract of red cells, factor *R* (later identified as adenosine diphosphate[6]), could induce platelet clumping *in vitro*. More recently, Hovig[7] has shown the release of a platelet clumping substance, identified as adenosine diphosphate (ADP), from damaged platelets. Since Hellem's original observation, Born and Cross[8] and Silver, Stehbens and Silver[9] have induced intravascular platelet clumping in the cat and the rabbit by intravenous or intra-arterial injection of ADP, while Honour and Mitchell[10] report similar results in rabbits when ADP is applied to the outer surface of injured cerebral cortical arteries. In man, Davey and Lander[11] observed a temporary reduction in the number of circulating platelets after intravenous ADP, presumably due to the trapping of platelet clumps in small vessels.

This communication reports on the effect of magnesium on ADP-induced platelet clumping *in vitro* in human blood.

Blood was withdrawn by clean venepuncture into chemically clean siliconed needles and glassware and added immediately to one-fiftieth its volume of (*a*) 0·9 per cent saline alone; (*b*) varying concentrations of disodium salt of ADP (Boehringer Corporation, London, Ltd., or Sigma London Chemical Co., Ltd.) in isotonic saline or (*c*) varying concentrations of a mixture of magnesium chloride and ADP in isotonic saline. The blood was then centrifuged without delay for 1 min at 450*g* and 20° C and the supernatant examined microscopically every 5 min for the presence of platelet clumps, the size of which was determined by the use of a graticule. No anticoagulants were added to these blood samples at any stage. Poor withdrawals of blood were discarded.

Fig. 1 shows the platelet clumping activity of ADP *in vitro*. In general, ADP added to whole blood in a final concentration of 10^{-9} M (about 0·05 μg/100 ml.) does not have a noticeable clumping effect. Five times that amount is needed to cause clumping in whole blood. This clumping activity is a quantitative one, with larger platelet clumps occurring earlier and persisting longer with increasing concentrations of ADP. As platelet clumps occur in the supernatant of each sample, the number of discrete platelets diminishes. This feature is reversed in the biphasic curve produced with 5×10^{-8} M ADP in Fig. 1, when a decided increase in the number of discrete platelets is seen in the supernatant as the size of the platelet clumps become smaller between 15–30 min, with a dispersal of individual platelets from the large clumps. This transient platelet clumping activity with certain concentrations of ADP was also observed by Born[12] and O'Brien[13] using quite different experimental conditions. However, in their experiments this event was completed in 2–8 min using citrated plasma whereas here it takes 30 min. In the present series, when ADP is added in a final concentration of 10^{-7} M (about 5 μg per 100 ml.), massive platelet clumps of 1,000μ or more occur within 5 min and persist without lysis throughout the 35 min of the test period. Scarcely any discrete platelets are present in the supernatant.

It is of interest to note that the platelet clumping potency of commercially available ADP can vary from batch to batch so that quantitative comparisons are best carried out on pooled batches stored in a desiccator at 0° C. Solutions of ADP should be used within 2 h of preparation at room temperature.

Since concentrations of ADP in excess of 10^{-7} M cause the early lysis of platelets, 10^{-7} M was chosen as a suitably large final concentration of ADP with which to examine the influence of magnesium on platelet clumping. Fig. 2 shows the substantial reduction in the clumping activity of ADP in the presence of added magnesium. In addition to reducing the size of the platelet clumps it also reduces

their number and discrete platelets become more plentiful with increasing concentrations of magnesium. Magnesium at $0{\cdot}4 \times 10^{-3}$ M completely abolishes the effect of ADP and delays the clumping of platelets beyond that of the control blood.

It is important to realize the differences between our method and those of Hellem[14], Born[12], O'Brien[13,15] and others who used platelet-rich plasma obtained from anticoagulated blood of variable age. In doing so they exclude the influence on platelet behaviour of continuing metabolism in withdrawn blood, of the presence of red cells and of the effects of anticoagulants (such as citrate, ethylene diamine tetraacetic acid and heparin) on calcium and magnesium ions, enzyme systems and substrates. In fresh whole blood where electrolytes and enzyme systems are kept as near normal as possible, the platelets are at least twenty times more sensitive to ADP and its effect is much more prolonged.

In the present series of experiments the ADP is mixed with the magnesium before the addition of the whole blood, and it is possible that ADP is inactivated by magnesium before the blood is added. However, if the magnesium is first mixed with whole blood and then added to the adenosine diphosphate such a procedure might be expected to reduce the effectiveness of the magnesium, but it does not. Adenosine diphosphate is said to require the presence of ionized calcium before it will cause platelet aggregation[16,17] and Hellem and Owren[18] have proposed that ADP is bound to the platelet surface and to a second molecule of ADP by calcium, thus linking the platelets into an aggregate or clump. If the effect of magnesium is to reduce or prevent this activity at the surface of the platelet it is possible that its action might result from cation antagonism between magnesium and calcium[19].

We have already described[1] how the deficiency of plasma magnesium in patients with acute myocardial infarction might be easily replaced by taking orally an absorbable form of magnesium, for example, in the beverage 'Oxo'.

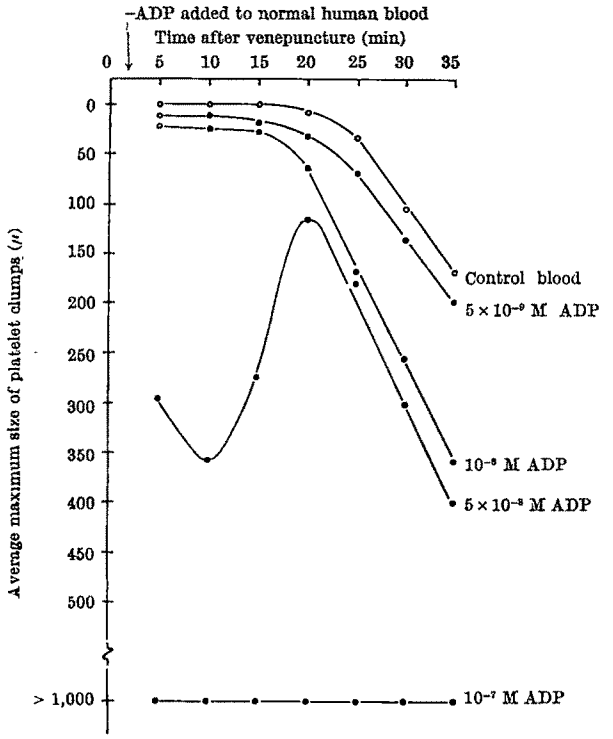


Fig. 1. Platelet clumping activity of adenosine diphosphate added to normal human blood *in vitro* in the absence of anticoagulants. Each point is the mean of eight to eleven results. The ordinate represents the average maximum size of platelet clumps (in μ) present in the supernatant of whole blood added to varying concentrations of ADP in isotonic saline (●——●) and in blood added to 0·9 per cent saline alone (○——○). The supernatant is examined microscopically every 5 min

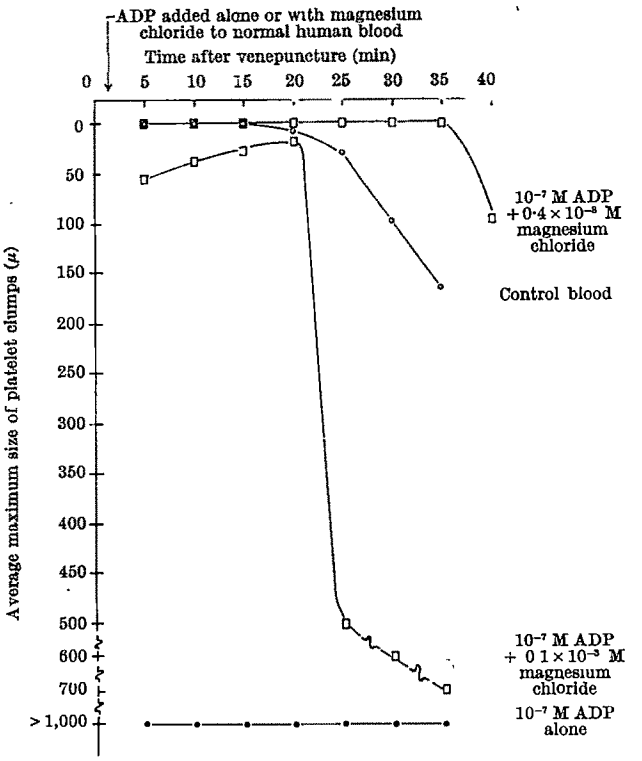


Fig. 2. The antagonism of the platelet clumping activity of adenosine diphosphate by magnesium in normal human blood *in vitro*. Each point is the mean of seven to eleven results. The ordinate represents the average maximum size of platelet clumps (in μ) present in the supernatant of whole blood added to mixtures of magnesium and ADP in isotonic saline (□——□), in blood added to ADP alone (●——●) and in blood added to 0·9 per cent saline (○——○). The supernatant is examined microscopically every 5 min

Final concentrations of added magnesium of 0·1 to $0{\cdot}4 \times 10^{-3}$ M (0·24 to 0·96 mg per 100 ml.) are within the range of supplements that would be needed to adjust to normal the low levels of plasma magnesium in patients with myocardial infarction.

Thus, if local increases of adenosine diphosphate play a part in intravascular platelet clumping these results suggest that magnesium might have a place in its antagonism.

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IMMUNOLOGY

Graft-versus-host Specificity of Lymphoid Cells transformed from Bone Marrow Allograft in Supralethally X-irradiated Dogs

It has been shown that bone marrow allografts in supralethally X-irradiated dogs transform into lymphoid cells[1]. Soon after transplantation, so-called 'hyperbasophilic' cells may be observed in bone marrow punctures, and within 6–7 days they and transitional to small lymphocyte forms appear in great numbers in the peripheral blood. We have supposed that these lymphoid cells transformed from bone marrow stem cells are immunologically competent cells which are able to induce the 'graft-versus-host' reaction. This communication presents some evidence for this hypothesis. The participation of these cells in transplantation immunity was tested by their ability to induce a hypersensitivity reaction of the delayed type, called a 'transfer reaction' by Brent *et al.*[2].

Within 7 days of allogeneic bone marrow transplantation in dogs X-irradiated with a dose of 1,000 r., their own blood lymphocytes were injected intradermally into each dog. Leucocytes were isolated from 100–200 ml. of heparinized arterial blood, washed twice with medium 199 and resuspended in this medium to make up a count of nearly 5×10^7 per ml. 0·1 ml. volumes of cell suspension were injected into the lateral skin of the chest; two injections were normally made. In evaluating the reaction erythema, induration and swelling were taken into consideration, but the diameter of the skin lesion was considered the most important sign when scoring. The approximate scores given for the various diameters of the skin lesions were: +, (weak, 6–10 mm); 2+, (moderate, 11–15 mm); 3+, (strong, 16–20 mm); 4+, (21–25 mm); 5+, (very strong, 26–30 mm); and > 5+, (strongest, more than 30 mm). Reactions less than 2+ were considered as unreliable.

Five out of six animals demonstrated strong to strongest skin lesions within 18 h (see the upper part of Table 1). Surprisingly, in one dog the reaction was unreliable; other workers[2,3] have also noticed that not all experimental animals respond to immune lymphocytes. In one animal, the skin lesion presented a huge haemorrhage in the form of a circle with diameter 30 mm.

The immunological specificity of the reaction described is high. Lymphocytes tested in other dogs, intact or irradiated, either provoked moderate reactions (in about half the animals) or did not provoke any reliable reaction (see the lower part of Table 1). These strong skin lesions are therefore the result of a specific interaction between transplantation antigens and the immune lymphocytes committed against them. Some positive non-specific reactions may perhaps be explained by the presence of common transplantation antigens in pairs of animals selected at random. Nor can the possibility be excluded that immune lymphocytes quickly degenerate in the sites of injection, releasing some active substances which damage tissues non-specifically. It should be mentioned that, according to our unpublished data, these cells have a sharply decreased viability in tissue culture.

Table 1. Skin Reaction to Transformed Lymphoid Cells

Lymphocyte donors No.	Lymphocyte recipients No.	Leucocyte No. in injection ($\times 10^6$)	Percentage of lymphoid cells in injection	Reaction in 18 h
116	116	5·0	100	3+
118	118	4·8	97·5	+
139	139	5·0	100	>5+, 2+
143	143	4·1	94	>5+, 3+
154	154	3·5	100	4+, 3+
159	159	4·0	74	3+, 3+
143	142*	4·1	94	2+, 2+
156	154	6·4	81	2+, +
139	Intact 1	5·0	100	±
154	,, 3	3·5	100	+, +
154	,, 4	3·5	100	2+, +
154	,, 5	3·5	100	2+, 2+
156	,, 3	6·4	81	+, ±
159	,, 6	4·0	74	±, ±
159	,, 7†	2·4; 3·2	74	±, ±

* Thymectomized dog, 1,000 r. + bone marrow allograft.
† Dog survived 1·5 years after 1,000 r. + two bone marrow allografts.

Non-immune lymphocytes in amounts up to 4×10^6 provoked no reliable reaction either in autologous or in allogeneic injections both in intact and irradiated dogs. The latter fact again confirms the belief that the 'graft-versus-host' reaction is the only immunological component of the skin reaction provoked by tested lymphocytes in X-irradiated allografted dogs.

These experimental results support the hypothesis that the stem cells of bone marrow allografts in supralethally X-irradiated dogs transform into immunologically competent lymphoid cells which are directed against the transplantation antigens of the recipient. Such a transformation leads to interruption of the normal haemopoiesis of bone marrow allograft and removes its protective effect; the aggressive trend of the transformed cells against the recipient can only aggravate the recipient's radiation disease. The picture that we have observed in concentrated form in skin lesions can be expected to hold throughout the body, and it is a matter for speculation whether this reaction has a total diffuse character or is directed against certain 'targets'.

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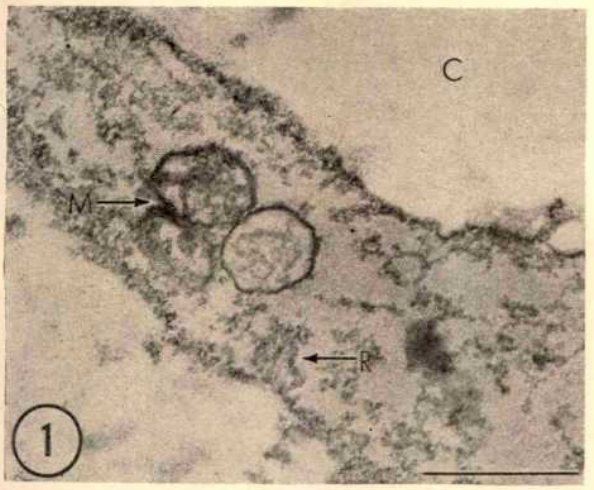
HISTOLOGY

Mitochondria in Odontoblastic Processes

The odontoblastic processes of human dentin are projections of the odontoblast cytoplasm into spaces in the dense collagenous, highly mineralized dentin matrix[1]. These projections can be up to several mm in length resulting in the cytoplasm being a long distance (2,000–3,000μ) from the odontoblast nucleus. The contents of odontoblastic processes in published electron micrographs have shown a varied appearance. In general they display a granular texture but do not contain recognizable cytoplasmic organelles[2–4]. Most of these specimens were prepared with standard osmium fixatives which have a very poor penetrating ability. We have investigated the ultrastructure of dentin fixed with glutaraldehyde which is particularly favourable for the preservation of membrane systems and has good penetrating ability. A freshly extracted tooth (severed from its blood supply no longer than 8 min) was broken into small fragments with at least one dimension 0·5–1 mm thick. These were immersed in cold fixative containing 1·25 per cent glutaraldehyde in *s*-collidine buffered to *p*H 7·4–7·55 and adjusted to a final osmolality of 360 milliosmols. Samples were post-fixed in 4 per cent osmium tetroxide for 48 h and then decalcified in a nitric acid–formalin mixture[5] for 12 h. They were then dehydrated in an alcohol series and embedded in 'Epon' *B* (ref. 6). Sections were cut with a diamond knife on an *LKB* 'Ultratome' and examined in an RCA *EMU*-3-*G* electron microscope.

Figs. 1 and 2 show sections of odontoblast processes from the root of an impacted lower left 3rd molar of a 26-year-old woman. The sections of processes were taken from a point midway between the cementum–dentin and the dentin–predentin junctions. The odontoblast cytoplasm contains (Figs. 1 and 2) mitochondria, membranous elements of endoplasmic reticulum and free and associated ribosome-like granules.

The mitochondria are spheroid, with a very distinct double membrane, and contain a variable number of inter-



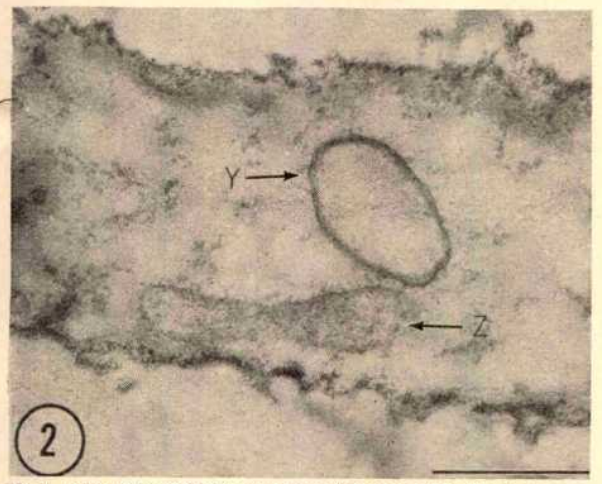


Fig. 1. Oblique to longitudinal section of human odontoblastic process. *C*, Dentin matrix; *M*, mitochondria; *R*, ribosome-like granules. Scale is 0·5μ

Fig. 2. Oblique to longitudinal section of human odontoblastic process. *Y* shows a vesicle surrounded by double membranes; *Z* shows a dumb-bell shaped structure surrounded by double membranes. Scale is 0·5μ

nal cristae. They range in size from 0·3 to 0·5μ in diameter. Occasionally one sees vesicles surrounded by double membranes but containing no cristae. These may be mitochondria either in the process of formation or degeneration. Fig. 2 contains a dumb-bell shaped mitochondrion suggesting the process of division.

Membranous elements of the endoplasmic reticulum are mostly in the form of cisternae. Ribosome-like granules are mostly associated into groups of strings with only a few free forms.

The presence of mitochondria and endoplasmic reticulum in the odontoblast processes of mature dentin suggests that there is considerable metabolic activity in the odontoblastic process cytoplasm even in those portions far removed from the odontoblast nucleus and even when they are encased in fully formed dentin matrix.

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PATHOLOGY

Preservation of Trachoma Agent in HeLa Cells

UNLIKE most other members of the psittacosis-lymphogranuloma trachoma group, trachoma and inclusion blennorrhoea (TRIC) strains cannot usually be passed serially in cell cultures although they grow well in chick-embryo yolk-sac. A few TRIC strains have, however, been adapted to tissue culture, notably the T'ang strain, now designated TRIC/China/Peking-2/OT. Such strains may be useful when preparations free from contaminating yolk are needed. A limitation of the use of tissue culture strains is their lability on storage at low temperatures; survival of infective particles when frozen in tissue culture fluid is generally very poor.

The finding that cells can be successfully preserved at low temperatures in media containing dimethyl sulphoxide (DMS)[1,2] has been widely applied. It seemed possible that the infectivity of tissue-culture grown trachoma agents might be retained on freezing if the infectious particles could be preserved by this technique in their intracellular environment, that is, in inclusions contained in intact cells.

T'ang trachoma agent which had undergone at least six serial passages in HeLa cells was used. The suspending medium for freezing was Hanks's balanced salt solution without bicarbonate, with 0·5 per cent (w/v) lactalbumin hydrolysate and 25 per cent horse serum. Various concentrations of DMS were incorporated in this medium. Specimens were cooled to −50° C at the rate of approximately 1° C/min, using the device described by Nagington and Greaves[3]. Having attained −50° C ampoules were stored either with solid carbon dioxide or in liquid nitrogen. Infectivity of suspensions was titrated in HeLa cells[4] before freezing and after storage, and the percentage of the original infectivity surviving was calculated. Specimens were thawed at 36° C. Infectivity titres before freezing were generally between 10^6 and 10^7 inclusion-forming units per ml.

In several experiments, preservation of infectivity was found to be improved by freezing suspensions as intact inclusion-bearing cells rather than as particles liberated from the cells by ultrasonic treatment (Table 1).

To determine the optimal concentration of DMS for preservation of infectivity, replicate suspensions containing various concentrations of DMS were slow-frozen; infectivity remaining after storage in a solid carbon dioxide container was compared with that of the original suspension without DMS (Table 2). On these results 15 per cent DMS was selected as the optimal concentration in further experiments.

In these and in other experiments, infectivity was found to decline over a period of several weeks' storage in the solid carbon dioxide container. Infectivity surviving during carbon dioxide storage was therefore compared with survival on storage in liquid nitrogen (Table 3). After titration of an aliquot of infected cell suspension, replicate specimens were slow-frozen to −50° C. Some ampoules were then stored in carbon dioxide and some by

Table 1. PERCENTAGE INFECTIVITY OF TRACHOMA SUSPENSIONS SURVIVING AFTER 6 DAYS' STORAGE IN SOLID CARBON DIOXIDE

T'ang preparation	Basic medium plus	Rate of freezing	Percentage survival
Intact inclusions	No DMS	Rapid	8
Ultrasonic treated	15% DMS	1° C/min	29
Intact inclusions	15% DMS	1° C/min	67

Table 2. EFFECT OF DMS CONCENTRATION ON SURVIVAL OF INFECTIVITY

DMS percentage in medium	Percentage of infectivity surviving after carbon dioxide storage for 1 week	24 weeks
0	15	1
5	39	3
10	49	6
15	61	17
20	41	13

Table 3. COMPARISON OF STORAGE IN SOLID CARBON DIOXIDE AND LIQUID NITROGEN

Weeks' storage	Percentage infectivity after storage in Solid carbon dioxide	Liquid nitrogen
2	36	Not done
12	12	50
26	3	64
32	5	56
42	2	61

immersion in liquid nitrogen. Liquid nitrogen storage appeared definitely superior to storage in a solid carbon dioxide container, though lower levels of infectivity persisted for long periods on carbon dioxide storage. Other experiments confirmed the value of liquid nitrogen in preserving high levels of infectivity for the maximum 1 year testing period.

Freezing of intact inclusions at 1° C/min in medium containing DMS evidently much improves the survival rate, but slow loss of infectivity occurs on carbon dioxide storage. Temperatures in solid carbon dioxide containers undoubtedly fluctuate, and it is possible that infectivity would be better preserved if a temperature of −79° C were steadily maintained. However, thermodynamic changes are known to occur in biological materials at temperatures down to −130° C (ref. 5). Storage at −196° C proved more satisfactory for preservation of infective trachoma agent, though even at this temperature some enzyme systems are not completely stable[6]. In these experiments cell cultures were frozen at the end of one growth cycle, 40–42 h after inoculation. The inclusions then contain a high proportion of mature elementary bodies, but also many less mature forms. Of this varied population of particles presumably only the more resistant survive storage with solid carbon dioxide; a further proportion of particles, perhaps more labile, appears to be preserved in liquid nitrogen.

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Small Virus-like Particles associated with Type *B* Virus Particles in a Transplantable Mammary Tumour of *ICR/Ha* Mice

THIS communication describes the electron microscopic appearance of small (20–55 mμ) virus-like particles which exist in a transplantable mammary tumour of *ICR/Ha* mice, and discusses the possible role of these particles.

A review of previous work on mouse mammary tumour virus, and a description of the appearance and development of *B* particles have already been given[1,2].

Mammary tumours of *ICR/Ha* mice were taken from the first recipients of a transplantable mammary tumour described previously[1]. Tumours were fixed in osmium tetroxide, embedded in 'Epon', stained with uranyl acetate and lead hydroxide, sectioned, and examined with an *EMU*-3 electron microscope.

A deliberate search was made for viral particles in the intermediate stage of development between maturing *B* particles[1] and the *A* particle. All the viral forms described herein were found in the cytoplasm of acinar cells. Many sacs were found which contained type *B* particles with eccentric nucleoids. The sacs ranged in diameter from small (about 380 mμ, Fig. 1), medium (about 1μ, Fig. 2) to large (about 3μ, Fig. 3).

In most sacs, small virus-like particles were present ranging in diameter from 20–55 mμ (Figs. 1, 2 and 3). A few of the small particles seemed to be attached to *B* particles (Fig. 3). Most of the small particles did not appear to be attached to the *B* particles, but were found singly or in groups throughout the sac. In a few cases, the particles were found outside of sacs in the cytoplasm (Fig. 1).

The small particles varied in appearance from that of a completely granular mass circular in shape at one extreme to a hollow ring with radiating spicules at the other extreme.

The significance of the existence of the small virus-like particle suggests the following five alternative possibilities. (1) It may be one form of the mammary tumour virus, possibly an infectious form. Particles of this size

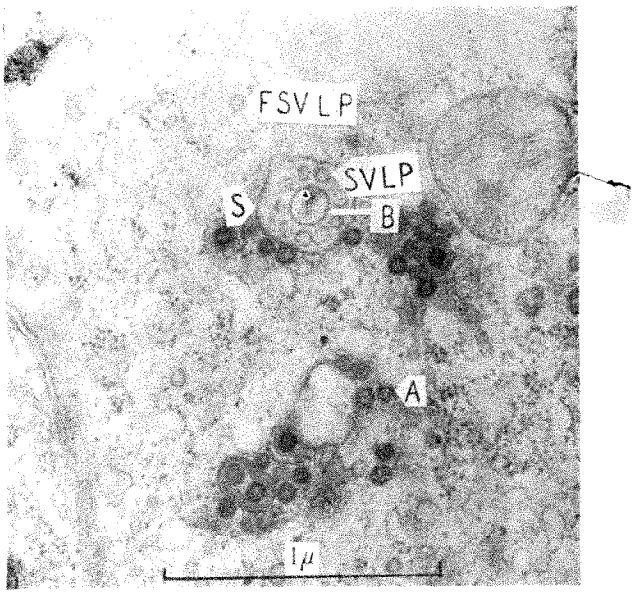


Fig. 1. Electron micrograph of cytoplasm of a transplantable tumour of *ICR/Ha* mouse. A single *B* particle (*B*) is located within a small sac (*S*). Small virus-like particles (*SVLP*) are seen between the *B* particle and the sac wall. A free small virus-like particle (*FSVLP*) is seen in the cytoplasm outside the sac. Numerous *A* particles are seen (*A*)

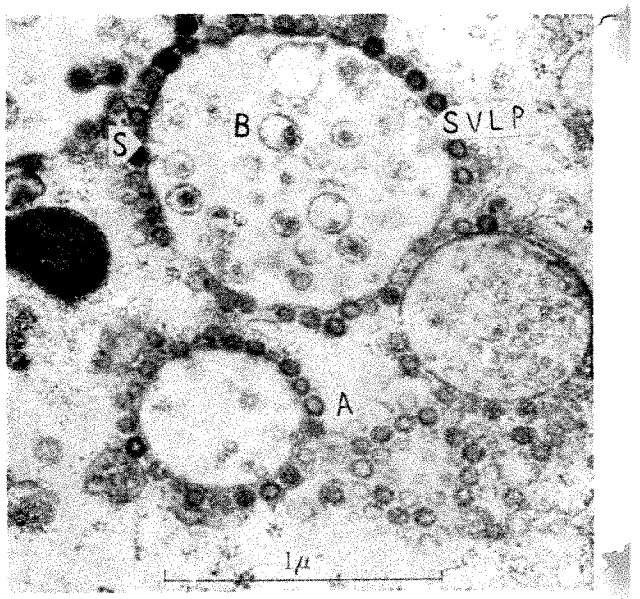


Fig. 2. Electron micrograph of cytoplasm of a transplantable tumour of *ICR/Ha* mouse. Numerous *B* particles (*B*) are present withir medium-sized sacs (*S*). Small virus-like particles (*SVLP*) can be seen within the sacs. Numerous *A* particles (*A*) can also be seen

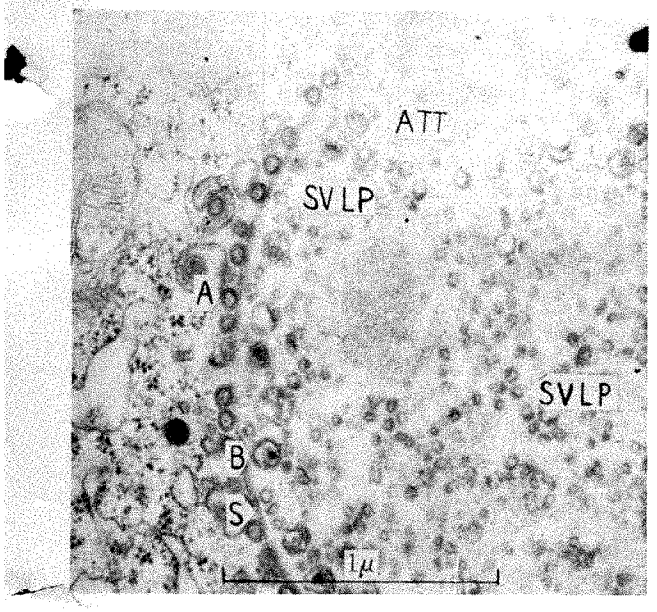


Fig. 3. Electron micrograph of cytoplasm of a transplantable tumour of *ICR/Ha* mouse. *B* particles (*B*) are present within the sac (*S*). Numerous small virus-like particles (*SVLP*) can be seen throughout the sac, and one appears to be attached to a *B* particle (*ATT*). Numerous *A* particles are also visible (*A*)

range were reported to be tumorigenic when separated from milk of mammary tumour strain mice by chemical extraction by Passey *et al.*[3], by filtration by Moore[4], and by irradiation by Moore[4]. Because of its size and location, it might be the precursor of the *A* particle. The presence of many *A* particles outside the sac in which many small particles are present is suggestive that this possibility should be investigated further. (2) It may be another mammary tumour virus not related to the *B* particle. (3) It may be a virus, but not one that will induce mammary tumours. (4) It may not be a virus, but a cell component arising at the site of viral infection. (5) It may be a normal cell component which is more obvious in vacuoles containing virus.

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RADIOBIOLOGY

Radiation Dose Fractionation and DNA Replication

IT has been shown that the magnitude of depression of DNA synthesis following radiation can be altered by altering the dose-rate[1]. These preliminary results suggest that the total dose alone may be inadequate in the assessment of the effects of radiation on DNA synthesis. Dose-rate and the duration of radiation are additional factors which might be needed for an adequate assessment of the effects of radiation on this key biological process. This investigation was carried out on a synchronous population of hepatocytes, near the mid-point of the 8-h replication period following partial hepatectomy. A subsequent investigation was made into the dependence of DNA depression on dose-rate both in synchronous liver cells and in asynchronous cells of the first part of the small intestine[2]. These two series of experiments have permitted comparison of the effects of localized radiation on DNA synthesis both in synchronous liver cells and in asynchronous intestinal cells in the same animal. The curves describing the depression in the two organs were similar in character; however, the magnitude of the depression in the intestines, initially, was greater than in the liver.

Divided doses of radiation, comparable with those used by Elkind *et al.*[3], were used in an attempt to investigate the optimum time of delivery and the minimum dose of radiation needed to produce a maximum depression of DNA synthesis. Male, Lewis strain rats (200 g) were used and experimental conditions were similar to those used in previous investigations[4]. Radiation was given locally to the liver and adjacent intestines through a cone (3 cm in diameter) at a rate of 300 r./min, using 200 kVp. X-rays half value layer 1 cm copper; all radiation deliveries ended 21 h after partial hepatectomy. 50 μc. tritiated thymidine, 3 c./mmole in 1 c.c. normal saline, were given at the end of radiation, and the animals were killed 1 h later. Monolayers of liver cells were made by squashing random samples of finely minced liver. Fixation was carried out by freeze substitution, and all squashes were stained by the Feulgen technique, prior to the application of Kodak '*AR*-10' stripping film.

Two series of experiments were carried out on the effects of radiation on DNA synthesis after fractionated doses of radiation. The first series evaluated the effects of the fractionated dose (375 r. × 2) on cells of the liver, and the second series evaluated the effects of the fractionated dose (750 r. × 2) on cells of both the liver and the intestine of the same animal. The results are given in Table 1 and shown in Fig. 1.

The major depression of DNA synthesis in the (375 r. × 2) series occurred during the first 4 min. When the first dose was given 4–128 min before the second dose, the trend of DNA depression was slightly downward compared with fractionated doses within the first 4 min. The increase in the 256-min value, compared with the 128-min value, suggests that recovery may be beginning to take place between 128 and 256 min.

The major depression of synthesis occurred during the first 2 min in the 750 r. × 2 series. The liver cells showed almost no further change between 2 and 256 min. There is a much greater initial depression of DNA synthesis in the cells of the intestines compared with those of the liver.

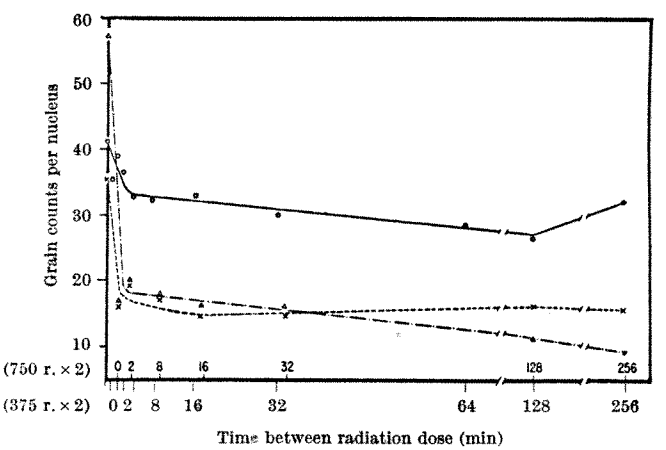


Fig. 1. Relation of radiation dose fractionation to the depression of DNA synthesis (liver and intestines, 21 h after partial hepatectomy). Zero time has been arbitrarily set as the time needed for the delivery of the single dose of 750 r. in the (375 r. × 2) series and for 1,500 r. in the (750 r. × 2) series. This is to separate the two experiments so that each can be graphically shown in the same figure. ○——○, 375 r. × 2 (liver); × - - - ×, 750 r. × 2 (liver); *Δ*— · — · —*Δ*, 750 r. × 2 (intestines)

Table 1. RELATION OF RADIATION DOSE FRACTIONATION TO DNA SYNTHESIS
(Autoradiographic results)

Time between radiation doses (min)	Control	0	1	2	4	8	16	32	64	128	256	
No. of animals	11	8	6	4	6	5	5	5	6	5	5	375 r. × 2 (liver)
Grain counts per nucleus	41·1	35·3*	39·3	36·4	32·9	32·4	32·9	30·0	28·5	27·0	32·1	
± S.E.	3·2	2·4	3·5	2·3	4·1	1·6	3·1	2·6	2·2	2·3	1·7	
Percentage of labelled cells	13·1	13·9	12·1	18·9	11·1	13·2	14·1	12·0	10·1	11·1	5·6	
± S.E.	2·1	1·8	3·0	2·4	3·1	4·0	3·6	1·4	1·7	2·9	2·5	
No. of animals	6	5		6		6	5	6		6	6	750 r. × 2 (liver)
Grain counts per nucleus	35·5	17·0†		20·4		19·6	14·6	14·5		16·2	15·3	
± S.E.	3·4	1·6		2·1		4·1	1·9	1·9		2·2	2·7	
Percentage of labelled cells	19·9	13·7		15·2		15·1	9·5	12·3		8·1	5·6	
± S.E.	4·2	4·3		2·9		3·9	2·4	2·6		2·4	1·3	
No. of animals	6	5		5		6	5	6		6	6	750 r. × 2 (intestines)
Grain counts per nucleus	57·0	17·0†		19·6		17·9	14·5	14·4		13·1	11·4	
± S.E.	3·4	1·9		0·9		4·7	2·2	1·5		1·0	1·1	

* 750 r. was given as a single dose. † 1,500 r. was given as a single dose.

The rate of synthesis is reduced to approximately half the control values in the liver and one-third of the control values in the intestines. The slight downward trend of DNA depression continues up to 256 min in both liver and intestinal cells.

It appears that the first few minutes are critical, since the major depression occurs within 4 min in the (375 r. × 2) series (liver), and within the first 2 min in the (750 r. × 2) series (both intestines and liver). The preliminary data suggest, therefore, that the optimum time of radiation delivery for the immediate depression of DNA synthesis is of the order of a few minutes.

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BIOLOGY

Kiln-firing as a Technique for studying Nest-building Behaviour of Mud-dauber Wasps

IN a study of the utilization of mud by mud-dauber wasps, kiln-firing has been investigated as a technique for quick identification of the mud sources used by the wasps. *Sceliphron cementarium* (Drury) has been found to construct nests using radioactive mud from the waste disposal pits on the Atomic Energy Reservation at the Oak Ridge National Laboratory. *Trypoxylon politum* (Say) seldom used radioactive mud, although it nested in the same sites as *Sceliphron*[1]. Information about the kinds of mud used, and the sources of such muds, was necessary for interpreting the behaviour of the wasps in this unique situation.

Bartram[2] first reported the nests of these wasps as ". . . some curious wasp nests made of clay in Pensilvania", and later authors have stated that the nests are made of clay. According to the *Soil Textural Classification* used by the U.S. Department of Agriculture[3], none of the nests in the Oak Ridge area is made of clay. A textural classification of the nests is to be published elsewhere, but there is from 3 to 30 per cent clay in the mud used by the wasps. Mud-daubers, particularly *Sceliphron* females, tended to use mud from various sources in the construction of a single nest. Organic materials in the muds gave a nearly uniform colour to the nests, but kiln-firing revealed sharp differences in colour contributed by different clays.

Nests were removed from the field, placed in cold kilns at room temperature, and raised to 2,200° F either in 4 h or 12 h. The kiln-fired nests revealed strikingly the heterogeneous composition of the *Sceliphron* nests as the various clays came out in bright contrast (Fig. 1). Firing



Fig. 1. A live female *Sceliphron cementarium* (Drury) posed on a kiln-fired clump nest which shows the patchwork effect from the use of different muds by an individual of the species

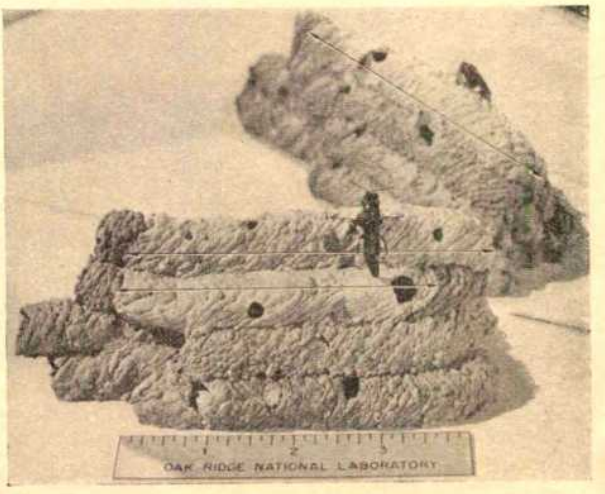


Fig. 2. A live female *Trypoxylon politum* (Say) posed on a kiln-fired pipe-organ nest which shows two tubes made of one mud (mirror image shows rear of one of these) with the remaining tubes composed of another mud

of the *Trypoxylon* nests showed clearly that this wasp is much more constant in its habits when bringing mud for nest construction. From Fig. 2 it can be seen that whole tubes of a *Trypoxylon* nest will be made of mud from a single source before there is a change to another source of mud. Moreover, *Sceliphron* often thickens the outside of the walls of its nests by plastering on balls of mud from a variety of sources (Fig. 1), whereas *Trypoxylon* does any thickening there may be on the inside. Fired *Trypoxylon* nests also reveal that the prepupal cocoon is composed primarily of grains of sand and clay chewed from the inner wall of the cell. The fired cocoon is white with localized spots of mineral colours derived from the clay it contains.

Some of the fired nests gave distinctive colours which were connected with a specific source, so that an estimate could be made of the flight range of the wasps. In a systematic study, kiln-firing would probably indicate the comparative usages by different species, seasonal changes in nest-building activity, and perhaps information about the rate of nest construction as well.

I thank Mr. Douglas Ferguson, of the Pigeon Forge Pottery, Pigeon Forge, Tennessee, for suggesting that kiln-firing might discriminate between different sources of mud.

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Isolation of 2-Aminoethylphosphonic Acid from Phospholipids of the Abalone (*Haliotis midae*)

IN the course of an investigation of the phospholipids of the abalone shellfish (*Haliotis midae*), a ninhydrin-reactive substance was detected which showed properties similar to phosphoryl ethanolamine. For example, no separation between phosphoryl ethanolamine and the unknown compound was obtained by column chromatography on the cation exchange resin 'Amberlite *C.G.* 120' type 2 with an eluting buffer of *p*H 3·25. Hydrolysis with 2 N hydrochloric acid at 120° C for 100 h failed, however, to liberate ethanolamine and phosphoric acid. This substance was identified as 2-aminoethylphosphonic acid.

Horiguchi and Kandatsu[1,2] were the first investigators to describe the isolation of 2-aminoethylphosphonic acid from rumen protozoa and *Tetrahymena pyriformis W*. Recently, this compound has also been isolated from the sea anemone (*Anthopleura elegantissima*) in which it occurs free and esterified with glycerol and sphingosine[3–5]. It appears that the abalone is the highest organism in which the presence of this phosphonic acid has so far been demonstrated.

Lipids of the abalone (shell-free 3,800 g) were extracted with chloroform–methanol (2 : 1 by volume). The water-soluble impurities were removed by two washings according to Folch *et al.*[6]. After taking the material to dryness *in vacuo* it was taken up in petroleum ether (40–60° C) and filtered. The lipids (23·7 g; phosphorus 2·1 per cent), which were obtained after removal of the petroleum ether, were refluxed for 48 h with 600 ml. 6 N hydrochloric acid. The reaction mixture was filtered, the filtrate evaporated to dryness *in vacuo* and the residue taken up in about 20 ml. of water. The phosphonic acid was separated from other compounds by chromatography over a column of 'Zeokarb 225' (20 × 2 cm; 52–100 mesh, hydrogen form, 4·5 per cent divinyl benzene). The eluting agent was 0·1 N hydrochloric acid, 5 ml. fractions were collected and each fraction was tested for the presence of 2-aminoethylphosphonic acid by paper chromatography in 80 per cent *n*-propanol (R_F 0·10). One fractionation was insufficient, due to the presence of large quantities of choline, ethanolamine, serine, etc., and the separation was therefore repeated on an identical column. Fractions containing the compound (tube numbers 8–30 for the first separation and 50–100 for the second) were combined and evaporated to dryness *in vacuo*.

The resulting oil, which could not be induced to crystallize, was taken up in 2 ml. of water and transferred to a small column of 'Dowex 1–X8' (4 × 2 cm; acetate form). Elution was carried out with 0·5 N acetic acid; the first 20 ml. contained all the desired material. After evaporation of the acetic acid the material became crystalline. Recrystallization from 50 per cent ethanol gave 58 mg of 2-aminoethyl phosphonic acid, which melted at 284°–286° C and decomposed at 295° C. Horiguchi and Kandatsu[1] give 295°–297° C (decomposition); Kittredge, Roberts and Simonsen[3] give 280°–281° C (decomposition).

Authentic 2-aminoethylphosphonic acid was supplied by Dr. Horiguchi of the University of Tokyo. No depression in melting point was observed when mixing this substance and the isolated material; moreover, the infra-red spectra were identical. Horiguchi and Kandatsu[7] have recently shown dimorphism in 2-aminoethylphosphonic acid, resulting in two infra-red spectra. The substance described in this report corresponded to the metastable 'α-form' of these authors.

It was shown by column chromatography of the lipids on silicic acid according to Hanahan, Dittmer and Warashina[8] that the phosphonic acid was present in the phospholipids of the abalone. The phosphonic acid-containing lipid, which was eluted from the silicic acid column with chloroform–methanol 3 : 2 (by volume), emerged just before phosphatidyl inositol and phosphatidyl choline. This phospholipid fraction was very rich in sphingosine and it seems, therefore, that 2-aminoethylphosphonic acid is part of a sphingolipid, the structure of which is still to be established.

A complete report of the composition of the abalone phospholipids will be published elsewhere.

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MICROBIOLOGY

Inability of Decenylsuccinic Acid to protect *Escherichia coli* against Damage by Freezing

CHEMICAL protection against injury due to damage by freezing has been extensively studied by various workers[1–6]. Recently Kuiper[9] reported that decenylsuccinic acid induced resistance to desiccation, cold and frost in young bean plants. When decenylsuccinic acid penetrates into the lipid layer of the membrane of bean root cells it increases water permeability. This permeability was only slightly temperature dependent[10]. Our laboratory has been interested in the protection against damage by freezing in bacteria[11]. We obtained samples of

decenylsuccinic acid from B. J. Humphrey, Humphrey–Wilkinson, Inc., North Haven, Conn., U.S.A., and investigated the activity of the compound in *Escherichia coli* cell suspensions exposed to sub-zero temperatures.

E. coli cultures were maintained in nutrient broth and in a chemically defined medium. 24-h cultures were used in the freezing experiments. Aliquots of decenylsuccinic acid were added to suspensions of *E. coli* containing approximately 1×10^7 cells/ml. and the preparations were frozen slowly at $-20°$ C. At intervals of 2, 4, 6, 8 and 10 days duplicate samples were removed from the freezer, quickly thawed in running tap water, and plate-counted. The thawed suspensions were decimally diluted, streaked in duplicate on nutrient agar plates with a bent glass rod, incubated at 37° C for 24–48 h and examined with the aid of a colony counter. The decenylsuccinic acid was dissolved in a few drops of hot ethanol, added to distilled water, and the slightly cloudy liquid was used. The experiments were repeated three times. I also performed experiments to rule out the toxicity of the chemical to *E. coli*.

E. coli frozen and thawed in the absence of decenylsuccinic acid resulted in a survival rate of 1·3 to 1·6 per cent. Cells frozen and thawed in various concentrations (ranging from 2·0 to 7·4 per cent) of decenylsuccinic acid resulted in a survival rate of 0·83 to 1·5 per cent. On the other hand, cells frozen and thawed in glycerol resulted in a survival rate of 11–15 per cent at the lower concentrations and up to 70 per cent at the higher concentrations (7·5 per cent) of glycerol.

This work indicated that decenylsuccinic acid was unable to protect *E. coli* against damage due to freezing measured in terms of colony-forming survivors.

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Effect of Inositol on the Lipids of *Candida albicans*

PREVIOUSLY I have described[1] changes in the morphology and physiology of the cells of *Candida albicans* during early stages of growth. These changes are in part associated with the cell wall in that the cells are flocculant during the lag phase of growth. An analysis of the basic constituents in the cell wall and the variations in the constituents during growth have also been reported[2], and they indicate that the ability of the cells to flocculate might be due to a weakened cell wall during this stage.

Challinor *et al.*[3] reported the effect of inositol on the flocculation of *Saccharomyces cerevisiae*. These investigations indicated that inositol was a primary cell wall constituent, and in its absence the wall was weakened and the cells clumped together and formed aggregates (flocculation). Associated with these characteristics was an increase in the lipid content of the cells (but not the cell walls) and a possible reduction of the phosphorus in the cell lipids. Smith[4] similarly reported that *S. carlsbergensis*, when grown in a medium deficient in inositol, fails to properly divide and forms aggregates up to 50 cells. This communication describes the effects of added inositol to the growth, morphology, and lipid content of *C. albicans* during the flocculant stage of its growth.

The yeast was grown at 24° C in a medium consisting of glucose as the carbon source and ammonium tartrate and ammonium nitrate as the nitrogen sources. The only vitamin added was biotin. This medium is referred to as the defined medium. The supplemented medium consisted of the defined medium plus 10 mg inositol per 100 ml. medium. The cells were grown in flasks on a reciprocal shaker at a speed for maximum aeration for 12–21 h, the time of maximum flocculation, and then gathered by centrifugation, washed three times and freeze-dried.

The amount of growth obtained by the yeast in each of these two media was about the same. The yield (dry weight) of cells from 600 ml. of the defined medium was 3·72 g and that from 600 ml. of the supplemented medium was 3·94 g. Manometric measurements were made to compare the rates of aerobic respiration. The inositol-grown cells were found to possess the same respiratory rate as the cells grown on the defined medium. Microscopic examination indicated further similarities in the cells. The number of cells remaining attached following cell division appeared to be the same and the cells flocculated as well with the inositol as without.

The freeze-dried cells were treated with acetone to help the extraction of lipids by breaking lipid–protein bonds. The acetone was evaporated under nitrogen and the cells extracted with three treatments of a chloroform : methanol mixture (2 : 1 v/v) at room temperature. The three extracts from each cell type were combined, washed, and dried *in vacuo*.

Table 1. LIPIDS EXTRACTED BY CHLOROFORM : METHANOL MIXTURE (2 : 1 V/V) FROM CELLS OF *C. albicans* GROWN ON A DEFINED MEDIUM AND THE DEFINED MEDIUM SUPPLEMENTED WITH INOSITOL
(10 mg/100 ml.)

		Cells grown on defined medium	Cells grown on supplemented medium
Sterol esters	Weight (g)	0·3760	0·3284
	% Lipid	86·9	84·6
	% Cell	10·1	8·3
Triglycerides	Weight (g)	0·0048	0·0040
	% Lipid	1·1	1·0
	% Cell	0·1	0·1
Sterols	Weight (g)	0·0132	0·0104
	% Lipid	3·1	2·7
	% Cell	0·4	0·3
Fatty acids	Weight (g)	0·0140	0·0100
	% Lipid	3·2	2·6
	% Cell	0·4	0·3
Phospholipids	Weight (g)	0·0246	0·0355
	% Lipid	5·7	9·1
	% Cell	0·7	0·9
Weight recovered lipids (g)		0·4326	0·3883
Dry weight of cells (g)		3·72	3·94

The lipid extracts were then fractionated by the technique of Fillerup and Mead[5] using a $2·2 \times 8·0$ cm column of silicic acid, and the sterol ester, triglyceride, sterol, fatty acid and phospholipid fractions separated by successive washes of 1 per cent diethyl ether in petroleum ether, 4 per cent diethyl ether in petroleum ether, 10 per cent diethyl ether in petroleum ether, 50 per cent diethyl ether in petroleum ether, and 25 per cent methanol in diethyl ether respectively. The results of these separations are shown in Table 1. Clearly, the greatest part of the lipid fraction of both types of cells is the sterol esters. The amount of triglycerides, sterols, and fatty acids is effectively the same in the two types of cells. However, the phospholipids are significantly increased in the cells grown in the presence of inositol. This increase in the phospholipids supports the findings of Challinor *et al.*[3]; however, the absence of inositol from the medium did not increase the total lipid content as he found but appears to have decreased it : 11·7 per cent lipid in the absence of inositol and 9·9 per cent with inositol.

The fatty acid fraction, the phospholipid fraction, and the combined fractions of the sterol esters, triglycerides, and sterols were analysed by gas chromatography for individual fatty acids. The fatty acids detected and their retention times are provided in Table 2. Differences in the fatty acid composition of the cells grown in the two types of media are found only in the combined fraction of sterol esters, triglycerides and sterols. Supplementing the growth medium with inositol did not alter the types of free fatty acids formed or the fatty acids in the phospholipid fraction. Differences in the fatty acids from the various silicic acid separated fractions are notable. The sterol ester–triglyceride–sterol combined fraction was transesterified by the anhydrous methanol–hydrochloric acid method of Stoffel *et al.*[6] to form the methyl esters of the fatty acids. Direct methylation of the free fatty acids and phospholipids was accomplished using the diazomethane method of Schlenk and Gellerman[7]. For the identification of the fatty acid methyl esters an *F* and *M* chromatograph (Avondale, Penn.) with a thermal conductivity detector was used. Conditions of the instrument were: injector temperature, 300° C; column temperature, 196° C; detector temperature, 360° C; the column, 6 ft. × ⅜ in., packed with 10 diethylene glycol succinate polymer in 'Celite' (60–80 mesh); and helium flow was 45 ml./min.

Table 2. FATTY ACID CONTENT OF SILICIC ACID-SEPARATED FRACTIONS OF LIPIDS OF *C. albicans* GROWN ON DEFINED (*D*) AND INOSITOL-SUPPLEMENTED (*S*) MEDIA

Presence of fatty acids determined by gas chromatography on a diethylene glycol succinate column at 190° C

Fatty acid carbon chain*	Retention value†	Free fatty acid fraction		Phospholipid fraction		Sterol–sterol ester–triglyceride fraction	
		D	*S*	*D*	*S*	*D*	*S*
8 : 0	0·128	+	?	+	+	+	
10 : 0	0·168			+	+	+	
12 : 0	0·240	+	+	+	+	+	+
?	0·352	+	+	+	+		
?	0·416					+	+
13 : 0	0·480	+	+	+	+	+	+
14 : 0	0·528	+	+	+	+	+	
14 : 1	0·640	+	+				
15 : 0	0·680	+	+	+	+	+	+
?	0·832	+	+			+	+
16 : 0	1·000	+	+	+	+	+	+
16 : 1	1·176	+	+	+	+	+	+
18 : 0	1·472	+	+	+	+	+	+

* Values are listed as number of carbon atoms per number of double bonds.

† Relative to methyl myristate.

? Unidentified component or unclear peak.

The conditions affecting flocculation of *C. albicans* appear to be different from the effects on *Saccharomyces*. Where the lack of inositol in the medium weakens the cell wall, increases lipid content of the cell, alters the phospholipids of *S. cerevisiae*, and delays separation of the cells following nuclear division in *S. carlsbergensis*, only a decrease in the amount of phospholipids appears similar in *C. albicans*. However, the increased production of lipids and the changes in the fatty acids of the non-polar lipids indicates that a further investigation of the role of inositol in yeast lipid metabolism may prove valuable.

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GENETICS

Sex-linkage of the Glucose-6-phosphate Dehydrogenase Gene in Equidae

ALTHOUGH the number of chromosomes in the diploid set varies from seventeen to eighty, placental mammals as a whole comprise one uniform group with regard to total genetic content[1–3]. Diverse speciation from the common ancestor, proto-insectivores, was apparently accomplished by re-arrangement of linkage groups and mutations of individual genes. The *X*-chromosome, however, appears to have escaped rearrangement. The *X*-chromosomes of a great majority of placental mammals, including man, cattle, the mouse, the dog, the horse, and the donkey, have been found to be almost identical in absolute size, comprising about 5 per cent of the genome[4]. This suggests that the primitive *X*-chromosome of proto-insectivores has persisted in its entirety in various placental mammals.

Glucose-6-phosphate dehydrogenase (G6PD) deficiency and G6PD electrophoretic phenotypes are sex-linked in man. In order to establish whether this is true in other animals, we studied the electrophoretic mobility of G6PD in the Equidae.

To prepare the haemolysate, red cells were washed after removal of buffy coat and a freeze–thaw haemolysate was prepared in 0·01 M potassium phosphate buffer, *p*H 7·0, containing ethylenediamine tetraacetate (EDTA) and β-mercaptoethanol. This buffer, which is quite stable at 4° C, contained 5 mg EDTA disodium salt and 0·1 ml. β-mercaptoethanol in each 50 ml.

Gel was prepared from 65 g hydrolysed starch and 500 mg disodium ethylenediamine tetraacetate (EDTA), which were mixed well in 500 ml. of 0·01 M potassium phosphate buffer, *p*H 7·0, and heated. Before de-gassing, 5 mg of triphosphopyridine nucleotide (TPN) monosodium salt in 1·0 ml. water was added to the molten starch, and the slurry was poured into an eight-channel electrophoresis tray as described by Smithies[5]. The tray was allowed to stand at room temperature for about 6–8 h. The slotter was removed and 0·05 ml. of the haemolysate, representing a 1 : 100 dilution of red cells, was placed in each slot. White petrolatum–mineral oil mixture (50° C) was poured over the slots to a thickness of 2–5 mm and about 1 in. to both sides of the slots. The whole surface was then covered with 'Saran Wrap' and placed vertically in the tray. Electrophoresis was carried out in a cold room at 4° C for 16–17 h at a gradient of 6–7 V per cm. The reservoir solutions consisted of 0·1 M potassium phosphate buffer, *p*H 7·0. The cathode compartment also contained 5 mg TPN in 500 ml. buffer.

Staining solution was prepared by a modification of a previously described spot-test reagent for G6PD (ref. 6); 10 mg glucose-6-phosphate (G6P) dipotassium salt (Sigma), 2 mg 3(4,5-dimethyl thiazolyl 1-2) 2,5-diphenyl-tetrazolium bromide (MTT), 2 mg phenazine methosulphate (PMS), and 2 mg TPN were dissolved in 10 ml. 0·1 M *tris* HCl buffer, *p*H 8·0, and poured over the sliced gel. The gel was then kept covered at room temperature for 30 min.

When left at room temperature for 30 min after the staining mixture was poured, two distinct bands were seen for all the animals tested. A third band, more slowly moving, was apparent only after more prolonged staining.

We sampled nine horses (a Shetland pony, an Arabian, a thoroughbred, and six ordinary horses), five male and four female; six donkeys, three male and three female; eleven mules, four male and seven female; and two hinnies, one male and one female—a total of 28 animals. (A mule is the offspring of a male donkey and a female horse; a hinny is the offspring of a male horse and a female donkey.) The electrophoretic mobility of the G6PD of male and female horses was the same, irrespective of breed.

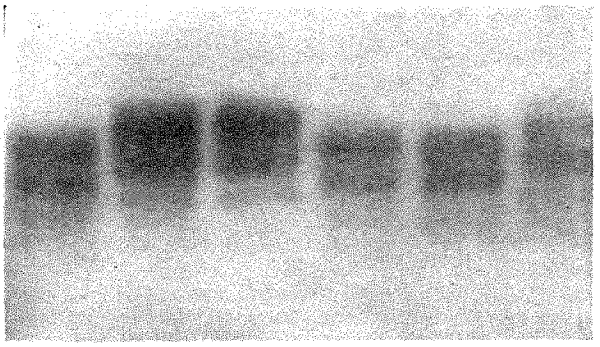


Fig. 1. The results of starch-gel electrophoresis of G6PD in Equidae. The following sources of enzyme were used: 1, male mule; 2, female donkey; 3, male hinny; 4, male horse; 5, male mule; 6, female donkey

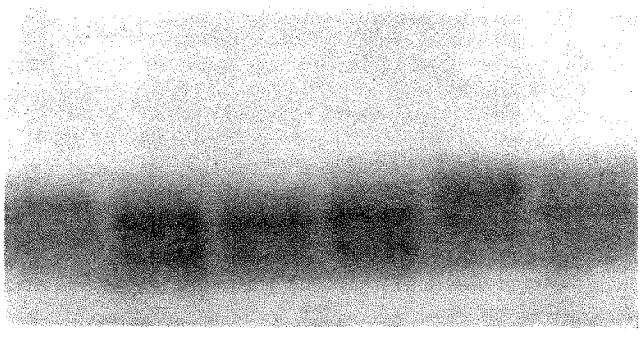


Fig. 2. The results of starch-gel electrophoresis of G6PD in an interspecies family group in Equidae. The following sources of enzyme were used: 1 and 5, male donkey (father); 2 and 4, male mule (son); 3 and 6, female horse (mother)

Male and female donkey enzyme had the same mobility, but the donkey enzyme bands were well ahead of the corresponding horse enzyme bands. All four male mules had G6PD bands identical with those from the horse. The male hinny had bands identical with those of the donkey (Fig. 1). Three of the female mules had G6PD bands like those of the donkey, two had bands like those of the horse, and two had bands of intermediate mobility. Female hinny bands resembled the horse bands. One family group was investigated. The results of electrophoresis of the G6PD of the father (donkey), mother (horse), and son (mule) are shown in Fig. 2.

In both types of crosses, the male offspring always showed the phenotype of the mother, verifying sex-linkage of the enzyme. The results of the electrophoretic determination on all twenty-eight animals are summarized in Table 1.

Sex-linkage of G6PD in man has been established by careful studies of pedigrees of kinships with G6PD variants which affect the electrophoretic mobility[7] or the activity of G6PD[8]. Young *et al.*[9] have presented data suggesting that G6PD is sex-linked in *Drosophila melanogaster*. However, since in this organism the *X*-chromosome comprises about 30 per cent of the genome, the presence of this gene on the *X*-chromosome may very well be fortuitous.

Table 1. G6PD PHENOTYPES IN EQUIDAE

Father	Mother	Offspring	Sex	No. of animals	Phenotype
Horse	Horse	Horse	♂	5	Horse
			♀	4	Horse
Donkey	Donkey	Donkey	♂	3	Donkey
			♀	3	Donkey
Horse	Donkey	Hinny	♂	1	Donkey
			♀	1	Horse
Donkey	Horse	Mule	♂	4	Horse
			♀	7	Horse, donkey, or intermediate

In view of the apparent morphologic stability of the *X*-chromosomes in mammalian species, it appeared to be of special interest to test the homology of sex-linked genes among various placental mammals. Haemophilia A and B, both sex-linked in man, have already been shown to be sex-linked in the dog as well[10,11]. A sex-linked mutation analogous to anhydrotic ectodermal dysplasia of man has been found in cattle[12]. Our finding that G6PD in the red blood cells of the donkey and the horse had different electrophoretic mobilities provided us with an opportunity for examining possible sex-linkage of the gene for this enzyme in Equidae.

If G6PD is sex-linked, the male interspecific hybrid receiving his only *X*-chromosome from his mother should manifest the same phenotype as the mother. As shown in Table 1, this is always the case. In the female hybrid, either the maternal, the paternal or a mixed phenotype might be expected, since it has been established that the locus for G6PD is involved in inactivation of the female *X*-chromosome[13–15].

This study clearly demonstrates the localization of the gene controlling G6PD phenotypes to the *X*-chromosomes in Equidae[16]. Investigations are under way to determine whether other interbreeding species may have different electrophoretic phenotypes, so that the possibility of sex linkage in such species may be explored.

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Amino-acid Substitutions in Haemoglobins and the Mutation Process

VARIOUS genetically determined human and animal haemoglobins differ in the amino-acid sequences of their polypeptide chains[1,2,6]. On the basis of the ribonucleic acid triplets published recently[4,5] all abnormal human haemoglobins which are due to single amino-acid substitutions can be explained by single base pair changes in the deoxyribonucleic acid. Comparing the normal human β- and δ-chains we found that only the exchange of threonine to glutamine in position 87 (1 of 10) cannot be interpreted by a single point mutation.

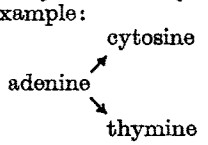
Comparison of the β-chains of man, gorilla, horse, and pig showed that only 4 (of 18) amino-acid substitutions cannot be due to a single base substitution.

Many of the amino-acid exchanges between the normal α- and β-chains, on the other hand, are most probably not caused by single point mutations.

Since the genetic code is a degenerated one, several codons for every amino-acid are possible[4,5]. On the grounds of base pair changes in haemoglobin genes (Table 1) some of these codons can be excluded for certain positions. Exclusions by abnormal haemoglobins are more certain than those based on comparison of β- and δ-changes or of β-chains of different species, since the occurrence of a single point mutation must be assumed.

The base pair changes can be divided into transitions and transversions[2]. Transitions are substitutions of a purine (pyrimidine) base by another purine (pyrimidine) in the DNA, whereas transversions are substitutions of a purine by a pyrimidine or vice versa.

Every base has only one possibility for a transition (adenine ⇌ guanine, cytosine ⇌ thymine), but two for transversions; for example:



Therefore we have altogether four possible transitions and eight possible transversions. If replacement was a random occurrence we should find twice as many transversions as transitions. Instead, however, there are more transitions than transversions (Table 2). The difference from that expected under an assumption of randomness is statistically significant ($P \ll 0{\cdot}0027$).

Table 1. RESTRICTION OF CODONS

Position and amino-acid	Possible codons	Impossible codons	Information
	(*a*) In the β-chain		
12 Thr	TGA, TGG	TGT, TGC	β→δ
16 Gly	CCA, CCG	CCT, CCC	β-Comparison Abnormal Hb
20 Val	CAT, CAC	CAA, CAG	β-Comparison
67 Val	CAT, CAC	CAA, CAG	Abnormal Hb
125 Pro	GGT, GGC	GGA, GGG	β→δ
126 Val	CAT, CAC	CAA, CAG	β→δ
70 or 76 Ala	CGT, CGC	CGA, CGG	Abnormal Hb
	(*b*) In the α-chain		
15 Gly	CCA, CCG	CCT, CCC	Abnormal Hb
22 Gly	CCA, CCG	CCT, CCC	Abnormal Hb
57 Gly	CCA, CCG	CCT, CCC	Abnormal Hb
	(*c*) In the δ-chain		
22 Ala	CGT, CGC	CGA, CGG	β→δ
116 Arg	GCA, GCG	GCT, GCC	β→δ

Table 2. UNEQUIVOCAL BASE PAIR CHANGES

Types of changes		Abnormal human haemoglobins	β→δ	β-comparison	
Transitions	A→G	—	—	1	
	G→A	3	—	—	
	T→C	5	1	3	
	C→T	11	1	2	Sum: 27
Transversions	A→T	1	1	2	
	T→A	1	1	—	
	G→C	2	—	—	
	C→G	2	—	—	
	A→C	—	—	1	
	C→A	—	1	—	
	G→T	1	3	—	
	T→G	2	1	—	Sum: 19

A special examination shows, on the other hand, that the four bases have about equal probabilities of being involved in the mutation process.

There are two possible explanations for the higher frequency of transitions: either amino-acid substitutions caused by transitions are less deleterious for the haemoglobin molecule than changes caused by transversions, or transitions really do occur more frequently because of steric similarity within the purines and the pyrimidines respectively.

The latter hypothesis is supported by the observation that transitions predominate in rare haemoglobin variants observed in recent populations more than in the δ- and β-substitutions which are characteristic of older populations. Arguments for this hypothesis will be presented in greater detail elsewhere[7].

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PSYCHOLOGY

Cross-modal Transfer of Conditional Discrimination Training in Monkeys

IN previous work monkeys were trained to discriminate accurately between two test-objects in one sense modality, say by touch. Their performance was then assessed when the same two objects were later presented for discrimination in another sense modality, say by vision. If their performance in the second (visual) modality was better than that of an appropriate control group of animals without previous (tactile) experience of these objects, cross-modal transfer of training has occurred. Recently the findings in such experiments and their implications for theories of perception and language were discussed in detail[1]. It was concluded that only in one investigation[2] with monkeys was there evidence of significant positive cross-modal transfer of training related to an individual discrimination habit. However, in that investigation[2], only one of four comparisons attained statistical significance. On replication with eight instead of six animals the same trend was found as before but no comparison reached statistical significance (personal communication from M. Wilson).

Different results were obtained when cross-modal transfer of a learnt principle (instead of a specific habit) was sought. Monkeys were trained on a series of thirty or more discrimination problems in the first sense modality. Their performance improved over this series of problems, even when nothing specific about the test-objects in one problem could be transferred to succeeding ones. The animals thus acquired a 'learning set' or, in the present terminology, learnt a principle in the first modality. When their performance was assessed on comparable problems in the second modality, a small but consistent cross-modal transfer effect (most recently measuring 3·5 per cent) was demonstrated[3] for this improvement at learning discrimination tasks.

In the present experiment we trained monkeys to perform accurately at a conditional discrimination task in one sense modality. Later we looked for transfer when the same task was given in another sense modality. With such conditional training the animal does not merely learn to make a particular stimulus-reward association (as it might be said to do in learning a specific discrimination habit): on some trials it learns to select the one test-object, on other trials it selects the second test-object, depending on the nature of a third 'conditional' feature possessed by both of the test-objects. Therefore in conditional training the animal learns a task having characteristics in common with both a principle and a specific habit.

Our subjects were eight adolescent rhesus monkeys, approximately matched in pairs. Animals 1, 2, 3, 4, 7 and 8 had participated (as animals *C*1, *C*2, Pre-str 1, Pre-str 2, *C*5 and *C*6, respectively) in an earlier investigation[4]. Animals 5 and 6 were untrained at the beginning of this experiment (except that each had learnt one tactile

Table 1. PERFORMANCE SCORES ON CONDITIONAL DISCRIMINATION TASK GIVEN IN TWO SENSE MODALITIES

Animal		1	2	3	4	5	6	7	8
Tactile									
Preliminary training to 20 correct in 30 trials, both heights	Trials	370	160	140	140	100	190	80	360
Alternately tall and short, to criterion	Trials	724	153	212	334	40	458	811	2,000*F*
	Errors	224	39	26	65	1	82	293	786
	Reversals	14	6	15	12	4	17	9	14
Reversal of polarity of reward									
Visual		Yes	No	Yes	No	Yes	No	No	
Percentage of correct responses in first 50 trials		48	50	50	64	50	54	66	
Alternately tall and short, to criterion	Trials	1,050	1,250	985	365	573	777	1,147	
	Errors	390	576	358	231	202	212	458	
	Reversals	16	8	12	7	13	23	13	
Reversal of polarity of reward									
Tactile		No	Yes	No	Yes	No	Yes	Yes	
Percentage of correct responses in first 50 trials		32	36	46	20	40	18	38	
Alternately tall and short, to criterion	Trials	1,849	380	322	909	260	890	965	
	Errors	773	205	108	301	98	319	416	
	Reversals	17	6	10	26	7	20	13	

Animals 1–4 were trained by one experimenter, animals 4–8 by another. The figures in the body of the table refer to the number of trials required to attain the criterion for conditional training stated by Chow (ref. 5); or the number of errors made in attaining this criterion; or the number of reversals made within one sense modality (that is, between tall and short rods) in attaining this criterion. The letter *F* for animal 8 indicates that it failed to meet the appropriate criterion within the stated number of trials.

object discrimination task, cylinder and sphere, in 230 and 280 trials, respectively). Training was carried out in standard apparatus[4] and with standard procedure for conditional tasks[5]. Forty trials were given daily, 5 days each week. The test-objects were four metal rods, set vertically on the lids of the food containers. The rods were either tall (4·8 cm) or short (1·1 cm); and also either fat (3·1 cm diameter) or thin (1·1 cm). For all animals two tall (or short) rods were presented concurrently, and choice of the thin (or fat) rod was rewarded. Each of the four pairs of animals received a different one of the four training conditions initially. Therefore, disregarding order of training, two pairs of animals learnt to select the thin rod if both rods were tall and the fat rod if both were short; the other four animals learnt to select the fat rod if both were tall, the thin rod if both were short. For tactile training in the dark, the animals were able to grasp the rods directly in pushing the lids; for visual training in the light, the rods were enclosed in standard transparent plastic cylinders. (Control procedures undertaken at the end of the main experiment demonstrated that all animals, except possibly animal 6, responded to the rods rather than to minor differences in the cylinders during visual training. Animal 6 gave ambiguous results.)

The sequence of training and the results are shown in Table 1. All animals were trained first in the dark, then in the light and finally once again in the dark. Following the design of an earlier investigation[2] the polarity of reward was reversed for one animal in each pair after changing from the first to the second sense modality, and for the other animal of that pair after changing back from vision to touch. With this design it was possible to compare 'reversal' animals with 'non-reversal' animals after two changes of sense modality. (Polarity of reward is said to be reversed when an animal that was trained in the first modality to select the thin rod if tall and the fat rod if short is trained in the new modality to select the fat rod if tall and the thin rod if short.) Animal 8 failed to reach the standard level of performance within 2,000 trials of training in the first sense modality. Therefore transfer results are available for only seven animals.

Various measures of performance have been used: (1) percentage of correct responses in the first fifty trials after a change in modality; (2) the number of trials required to reach criterion in the new modality; (3) the number of errors made in reaching criterion in the new modality. For the change from tactile to visual training (comparing three reversal animals with four non-reversal animals) there is no clear evidence of significant cross-modal transfer ($P = 0{\cdot}057$, 0·429 and 0·314 for the three stated measures, respectively). For the opposite change from vision to touch (comparing four reversal animals with three non-reversal animals) a similar result is found ($P = 0{\cdot}114$, 0·314 and 0·314, in all six comparisons using the Mann–Whitney test). Combining the two changes in sense modality and comparing the percentage of correct responses in the first fifty trials for six pairs of animals, there is again no significant difference between non-reversal and reversal animals ($P = > 0{\cdot}05$, Wilcoxon matched-pairs signed-rank test). A fourth measure of performance is shown in Table 1, namely reversals within one sense modality to criterion (that is, between tall and short rods); however, this measure correlates highly with the other measures, so that statistical analyses are not reported.

After completion of the main experiment, animals 2, 4, 6 and 7 were tested alternately or randomly (trial by trial) in the light and dark, but always using the rods without the plastic cylinders as for tactile training. Although opposite responses were required depending on the presence or absence of light (since the polarity of reward had been reversed for these four animals between visual and tactile training), all four animals performed at levels of 90 per cent correct responses or better without further training.

We have thus found no clear evidence of cross-modal transfer of conditional training in monkeys. It is noteworthy that we did find a weak and non-significant initial cross-modal effect for the first change of sense modality. With a similar measure (errors in the initial trials), Wilson and Shaffer[2] obtained a significant effect at this stage of their experiment. This effect is not so apparent in their or our results using other measures; nor is it evident using the same measure after the second change of sense modality in either study. Our negative findings confirm previous work with monkeys on specific discrimination habits acquired by vision and touch[6] or by vision and hearing[7]. Since significant transfer of learnt principles has been reported in monkeys[3] we conclude that learning a conditional task resembles more closely the formation of a discrimination habit than the acquisition of a principle. Moreover, in terms of brain function, we suppose that individual neural systems are concerned when the same test-objects are presented to the monkey for tactile and visual learning. These conclusions emphasize the important role of language as a cross-modal bridge in man.

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FORTHCOMING EVENTS

Monday, April 4

SOCIETY FOR GENERAL MICROBIOLOGY (at the Royal Institution, Albemarle Street, London, W.1), at 5 p.m.—Dr. W. Hayes, F.R.S.: "Genetic Transformation—a Retrospective Appreciation" (First Fred Griffith Memorial Lecture).

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. G. A. Allen: "Measurements on Semiconducting Material".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. K. W. Pepper. "The Challenge of Synthetics to Leather".

Tuesday, April 5

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (joint meeting with the Automatic Control Group of the Institution of Mechanical Engineers, at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. B. Adams and Mr. W. Bamford: "Mechanical Analogue/Digital Convertors".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. B. R. Webster: "Teaching by Television".

SOCIETY OF CHEMICAL INDUSTRY, COLLOID AND SURFACE CHEMISTRY GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Annual General Meeting, followed by Dr. L. Golberg. "Biological Attributes of Synthetic Surface-Active Compounds in the Animal Organism".

PLASTICS INSTITUTE, LONDON SECTION (at the Wellcome Buildings, Euston Road, London, N.W.1), at 6.30 p.m.—Symposium on "Powders".

SOCIETY FOR ANALYTICAL CHEMISTRY, AUTOMATIC METHODS GROUP (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 6.30 p.m.—Meeting on "Sample Handling Techniques".

Wednesday, April 6

COLOUR GROUP (GREAT BRITAIN) (at Imperial College, London, S.W.7), at 2.30 p.m.—Mr. I. J. P. James: "The Separate Luminance Colour Television Camera"; Mr. C. B. B. Wood: "Consideration in the Design of Colour Television Cameras"; Mr. W. N. Sproson: "Colorimetric Aspects of 3 and 4 Tube Colour Television Cameras".

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. M. G. Audley-Charles: "The Geology of Portuguese Timor", Dr. G. Y. Craig and Dr. G. Oertel: "Deterministic Models of Living and Fossil Populations of Animals".

ROYAL MICROSCOPICAL SOCIETY (at the Royal Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Prof. A. G. E. Pearse: "Developments in Enzyme Histochemistry".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

MASTER to teach Chemistry to Advanced and Scholarship Level—The Warden, St. Edward's School, Oxford (April 7)

LECTURER IN MATHEMATICS—The Principal, Avery Hill College, Bexley Road, Eltham, London, S.E.9 (April 8).

PRINCIPAL LECTURER (with an honours degree in botany and considerable teaching and research experience), and a LECTURER (with an honours degree in botany) to teach Botany—The Principal, West Ham College of Technology, Romford Road, Stratford, London, E.15, quoting Ref. RAW/JA/635/3 (April 8).

EXPERIMENTAL OFFICER (with a university degree or equivalent and preferably practical experience of the semiconductor field) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING IN THE FACULTY OF SCIENCE to assist research work on electro-luminescence in semiconductors and related phenomena—The Registrar, The University, Manchester, 13, quoting Ref. 54/66/Na (April 12).

LECTURER (preferably with a special interest in analytical chemistry) IN THE DEPARTMENT OF CHEMISTRY (Inorganic and Analytical)—The Secretary, The Queen's University, Belfast, Northern Ireland (April 13).

LECTURERS or ASSISTANT LECTURERS IN CHEMISTRY (there are vacancies in all branches of the subject, including new developments in chemical physics)—The Registrar, The University, Manchester, 13, quoting Ref 38/66 (April 13).

EXPERIMENTAL OFFICER (interested in the design and construction of equipment for a wide range of psychological experiments, and preferably a knowledge of electronics)—The Registrar, The University, Manchester, 13, quoting Ref. 58/66 (April 14).

EXPERIMENTAL OFFICER (with a university degree or equivalent qualification and preferably a knowledge of chemistry) IN THE DEPARTMENT OF PHYSICS OF THE FACULTY OF SCIENCE, to run an evaporation and target construction laboratory in the Nuclear Physics Section of the Department—The Registrar, The University, Manchester, 13, quoting Ref. 53/66/Na (April 14).

ASSISTANT LECTURER or LECTURER (with a registrable qualification in veterinary medicine or an honours degree in pharmacology, or a related subject) in the Department of Veterinary Pharmacology, Royal (Dick) School of Veterinary Studies—The Secretary, The University, Edinburgh (April 15).

LECTURER or SENIOR LECTURER (honours graduate, preferably with a knowledge of the economic aspects of tropical entomology) IN ENTOMOLOGY; and a SENIOR LECTURER (honours graduate, preferably with a doctorate and teaching and/or research experience at university or equivalent level) IN BIOCHEMISTRY at the University of Nigeria, Nsukka, Eastern Nigeria—The London Representative, University of Nigeria, 33 Craven Terrace, London, W.2 (April 15).

LECTURERS and ASSISTANT LECTURERS (with a good honours degree in physiology, biochemistry or related subjects, or with medical qualifications registrable in the United Kingdom) IN THE DEPARTMENT OF PHYSIOLOGY—The Registrar, The University, Manchester, 13 (April 15).

ASSISTANT LIBRARIAN (Grade II) (with a good honours degree and preferably professional qualifications and appropriate experience)—The Registrar, The University, Liverpool, quoting Ref. CV/494 (April 16).

SENIOR LECTURER IN MATHEMATICS for duties involving lecturing on pure mathematics including, if the lecturer is so qualified, lecturing in analysis or topology—The Secretary, The University, Aberdeen (April 16).

UNIVERSITY LECTURER or UNIVERSITY DEMONSTRATOR IN THE DEPARTMENT OF PHARMACOLOGY—T. D. Kellaway, Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge (April 16).

UNIVERSITY LECTURER (with at least a good honours degree in botany or agricultural botany, experience in teaching and research, and preferably with interests in, and experience of, genetics in relation to crop plant evolution) IN AGRICULTURAL BOTANY—The Secretary, School of Agriculture, University of Cambridge, Downing Street, Cambridge (April 16).

LECTURER or ASSISTANT LECTURER IN GEOLOGY at the University of the West Indies—The Inter-University Council, 33 Bedford Place, London, W.C.1 (April 18).

LECTURER or ASSISTANT LECTURER (with qualifications or interests in either climate and meteorology or the geography of planning) IN GEOGRAPHY—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (April 18).

LECTURER (preferably with a good honours degree in mathematics or statistics, with further academic qualifications in mathematical statistics and/or practical experience in the use of statistical theory) IN MATHEMATICAL STATISTICS, and an ASSISTANT LECTURER (preferably with a good honours degree in mathematics or pure mathematics with further academic experience in pure mathematics, particularly in the field of algebra) IN PURE MATHEMATICS—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (April 18).

EXPERIMENTAL OFFICER or SENIOR EXPERIMENTAL OFFICER (with a university degree or H.N.C. and preferably previous experience of electron microscopy) IN THE ELECTRON MICROSCOPE LABORATORY OF THE RHEUMATISM RESEARCH CENTRE IN THE FACULTY OF MEDICINE, to take part in an experimental programme of research in a rapidly developing branch of molecular biology—The Registrar, The University, Manchester, 13, quoting Ref. 56/66 (April 20)

ASSISTANT LECTURER IN ZOOLOGY—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (April 22).

ASSISTANT LECTURER or TEMPORARY ASSISTANT LECTURER in History and Philosophy of Science—The Secretary, The Queen's University, Belfast, Northern Ireland (April 22).

DEMONSTRATORS (with a good honours degree and approximately one year's research experience) IN PHYSICAL, ORGANIC OR INORGANIC CHEMISTRY—The Registrar, University College of Wales, Aberystwyth (April 22).

LECTURER (preferably with a research interest in numerical analysis, astrophysics, or fluid dynamics) IN APPLIED MATHEMATICS—The Registrar, King's College (University of London), Strand, London, W.C.2 (April 22).

ASTOR RESEARCH FELLOW IN ENGINEERING SCIENCE—The Warden, New College, Oxford (April 23).

CHAIR OF PHYSICAL METALLURGY—The Registrar, The University, Liverpool, quoting Ref. No. RV/485 (April 23).

DEMONSTRATOR/SENIOR DEMONSTRATOR in Inorganic Chemistry—The Registrar, The University, Newcastle upon Tyne, 2 (April 23).

ASSISTANT LECTURER IN STATISTICS (preferably in mathematical statistics or probability theory)—The Registrar, The University, Hull (April 25).

JUNIOR LECTURER IN ORGANIC CHEMISTRY—The Secretary, Trinity College (University of Dublin), Dublin, 2, Republic of Ireland (April 25).

SENIOR LECTURERS (2) and a LECTURER IN THE DEPARTMENT OF PHYSICS, University of Ibadan, Nigeria—The Inter-University Council, 33 Bedford Place, London, W.C.1 (April 27).

LECTURER (with a good honours degree in geography and either a higher degree or actively engaged in research leading to a higher degree) IN GEOGRAPHY at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, April 29)

READER IN CRYSTALLOGRAPHY (Chemistry Department) at Queen Mary College—The Academic Registrar, University of London, Senate House, London, W.C.1 (April 29).

UNIVERSITY LECTURER or ASSISTANT LECTURER IN ANIMAL NUTRITION in the School of Agriculture—The Secretary, The University, Aberdeen (April 29).

ASSISTANT LECTURER or LECTURER IN CHEMISTRY (the appointment may be made in any branch of organic chemistry)—The Registrar, The University, Sheffield (April 30).

BIOLOGIST (with mechanical ability) to work on fungus pathogens of crops, especially foliage diseases in controlled environments—The Secretary, Rothamsted Experimental Station, Harpenden, Herts, quoting Ref. 1052/84 (April 30).

CHAIR OF MECHANICAL ENGINEERING—The Registrar, The University, Sheffield (April 30).

LECTURER (honours graduate with a keen research interest in the field of automatic control, and interests in the theoretical or experimental aspects of modern control techniques) IN CONTROL ENGINEERING IN THE SCHOOL OF ENGINEERING SCIENCE—The Registrar, University College of North Wales, Bangor, North Wales (April 30).

LECTURER (man or woman) IN ORGANIC CHEMISTRY—The Registrar, University College of North Wales, Bangor, North Wales (April 30).

LECTURER or ASSISTANT LECTURER IN BIOLOGICAL CHEMISTRY—The Registrar, The University, Manchester, 13, quoting Ref. 71/66 (April 30).

LECTURERS or ASSISTANT LECTURERS (2) IN THE DEPARTMENT OF CHEMISTRY, one Inorganic and one Organic—The Secretary, Sir John Cass College, Jewry Street, London, E.C.3 (April 30).

PROFESSOR OF GEOLOGY at Rhodes University, Grahamstown, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, April 30).

PROFESSOR OF PLANT PHYSIOLOGY (Rice Breeding); an ASSOCIATE PROFESSOR or SENIOR LECTURER IN AGRONOMY (Oil Palm Research); an ASSOCIATE PROFESSOR or SENIOR LECTURER IN AGRONOMY/STATISTICS; a FARM DIRECTOR (Senior Lecturer or Lecturer Level); a SENIOR LECTURER or LECTURER IN AGRICULTURAL ECONOMICS (2 posts); a SENIOR LECTURER or LECTURER IN AGRICULTURAL ENGINEERING; a SENIOR LECTURER or LECTURER IN AGRICULTURAL EXTENSION; a SENIOR LECTURER or LECTURER IN ANIMAL SCIENCE (2 posts); a SENIOR LECTURER or LECTURER IN PLANT PATHOLOGY; a SENIOR LECTURER IN SOIL CHEMISTRY; LECTURERS IN BOTANY (2 posts); LECTURERS IN GEOGRAPHY (2 posts); and a LECTURER and an ASSISTANT LECTURER/RESEARCH ASSISTANT IN ZOOLOGY, at Njala University College, Sierra Leone—The Inter-University Council, 33 Bedford Place, London, W.C.1 (April 30).

SCIENTIFIC OFFICER (with a good honours degree in biology or in chemistry, and preferably with research experience in microbiology or

biochemistry) for general studies in microbial chemistry or on the ecology of micro-organisms inoculated into soil—The Secretary, Rothamsted Experimental Station, Harpenden, Herts, quoting Ref. 1051/67 (April 30).

UNIVERSITY DEMONSTRATOR (with a good honours degree in botany and a strong interest in cytology and genetics) IN BOTANY—Dr. S Smith, Department of Zoology, University of Cambridge, Downing Street, Cambridge (April 30).

ASSISTANT LECTURER (with special interests in inorganic chemistry) IN THE DEPARTMENT OF CHEMISTRY—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (May 1).

LECTURER (preferably with a special interest or experience in the field of small groups, or criminology, or social administration) IN SOCIOLOGY at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, May 2).

CHAIR OF MATHEMATICAL STATISTICS at the University of Western Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, May 7).

TEACHING FELLOWS (with a Ph D) IN THE DEPARTMENT OF INORGANIC AND STRUCTURAL CHEMISTRY—The Registrar, The University, Leeds, 2 (May 7).

ASSISTANT OR ASSOCIATE PROFESSOR in one of the following fields: plant biochemistry, morphogenesis, plant ecology and biophysics—Dr. G. H. N. Towers, Head of the Department of Botany, University of British Columbia, Vancouver, 8, Canada.

ASSOCIATE PROFESSOR IN PHILOSOPHY—Head, Department of Philosophy, Queen's University, Kingston, Ontario, Canada.

ASSOCIATE PROFESSOR OF SOCIOLOGY, and ASSISTANT and ASSOCIATE PROFESSORS OF GEOGRAPHY—The President, Lakehead University, Port Arthur, Ontario, Canada.

BIOCHEMIST (with at least a good honours degree) to study problems connected with the enzyme activity and metabolism of healthy and virus-infected leaves—The Secretary, Rothamsted Experimental Station, Harpenden, Herts, quoting Ref 1051/68.

CLIMATOLOGIST to assist with an expanding programme in microclimatology, bioclimatology and comparative climatology—Prof. B. J. Garnier, Department of Geography, McGill University, Montreal, P.Q., Canada.

HEAD OF THE MATHEMATICS DEPARTMENT—The Headmaster, Queen Elizabeth Grammar School, Wakefield, Yorks.

LECTURER IN WELDING TECHNOLOGY, and a LECTURER IN GENERAL METALLURGY—The Registrar, University of Strathclyde, George Street, Glasgow, C 1.

LECTURER OR ASSISTANT LECTURER IN SOCIOLOGY with special reference to criminology and contemporary sociological theory—The Secretary and Registrar, Bristol College of Science and Technology, Ashley Down, Bristol, 7, quoting Ref. CST 66/31.

POSTDOCTORAL FELLOW IN ORGANIC CHEMISTRY to join a team working on a major synthetic project—The Registrar, The University, Manchester, 13.

RESEARCH AND DEVELOPMENT OFFICER (with an honours degree in chemistry or one of the biological sciences and preferably some research experience on cereals) for duties which will involve liaison with Universities, Research Institutes, Trade Associations and cereal growers—The Establishment Officer (R. and D. Post), Home-Grown Cereals Authority, Ingram House, 13–15 John Adam Street, London, W.C.2.

RESEARCH OFFICER (graduate) for supervisory duties in the laboratories of the DEPARTMENT OF BIOCHEMISTRY AND AGRICULTURAL BIOCHEMISTRY—The Registrar, University College of Wales, Aberystwyth.

SECOND CHAIR OF CHEMISTRY—The Academic Registrar, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Ministry of Agriculture, Fisheries and Food. Fishery Investigations, Series II, Vol. XXIV, No. 2: The Determination of Natural Mortality and Its Causes in an Exploited Population of Cockles (*Cardium edule* L.) By D. A. Hancock and A. E Urquhart. Pp. iii+40+12 plates. (London: H.M Stationery Office, 1965.) 21*s* net. [271

Geological Survey and Museum. Summary of Progress of the Geological Survey of Great Britain and the Museum of Practical Geology for the year 1964—With Report of the Geological Survey Board. Pp. vi+117+9 plates. (London: H M. Stationery Office. 1965.) 13*s*. 6*d* net. [281

The Medical Research Council of Ireland. Annual Report for the year ended December 31, 1964. Pp. v+68. (Dublin: The Medical Research Council of Ireland, 1965.) 5*s* [281

Society for the Promotion of Nature Reserves. Handbook 1965—Forty-Eighth Annual Report. Pp. 96. (London: The Society for the Promotion of Nature Reserves, British Museum (Natural History), 1966.) 12*s*. 6*d*. [281

Ministry of Technology. National Physical Laboratory. Changing to the Metric System. Conversion Factors, Symbols and Definitions By Pamela Anderton and P. H. Bigg. Pp 46. (London: H.M. Stationery Office, 1965.) 3*s*. 6*d*. net [281

Bulletin of the British Museum (Natural History). Geology Vol. 11, No. 5: Some New British Albian Ostracoda. By P. Kaye. Pp. 215–253+11 plates. (London: British Museum (Natural History), 1965) 50*s*. [281

Spectrochemical Abstracts, Vol 10, 1963–64. By Ernest H. S. van Someren, F. Lachman and F. T. Birks. Pp. 109. (London: Hilger and Watts, 1965.) 63*s*. [281

The Library Association Pamphlet No. 25: Women and Librarianship: An Investigation into certain Problems of Library Staffing By Patricia Layzell Ward. Pp. 60. (London: The Library Association, 1966.) 14*s*. (L. A members 10*s*. 6*d*.) [281

Amgueddfa Genedlaethol Cymru—National Museum of Wales. Fifty-Eighth Annual Report 1964–65. Pp. 91+4 plates. (Cardiff. National Museum of Wales, 1965.) [281

Rowett Research Institute. Annual Report on Animal Nutrition and Allied Sciences, Vol. 21, 1965. Pp 83. (Bucksburn, Aberdeen: Rowett Research Institute, 1966) 10*s*; 1.60 dollars. [281

Northern Ireland: Ministry of Agriculture. Leaflet No. 99: Cabbage and Kale for Stock Feeding. Pp. 8. (Belfast: Ministry of Agriculture, 1965.) [281

Ministry of Agriculture, Fisheries and Food. Fishery Investigations, Series II, Vol. XXIV, No 3: Whiting of the Irish Sea and the Clyde. By D. J. Garrod and R. Gambell. Pp. iv+64. (London: H.M. Stationery Office, 1965.) 20*s*. net. [281

City of Leicester Museums and Art Gallery. 59th Report, April 1st, 1964—March 31st, 1965. Pp. 48+4 plates. (Leicester: City of Leicester Museums and Art Gallery, 1965.) [281

The West of Scotland Agricultural College. Research Bulletin No. 36: Comparison of Production from Twelve Varieties of Cocksfoot. By I. V. Hunt. Pp. 30. (Auchincruive, by Ayr: The West of Scotland Agricultural College, 1965.) 1*s*. [28

University of Aberdeen: School of Agriculture. Miscellaneous Publication No. 3: Index of Scientific and Economic Studies of Particular Significance to the Pig Industry of the United Kingdom, Part 4. By J. Ward. Pp. xviii+89. (Aberdeen: Department of Agriculture, The University, 1965.) 6*s*. [281

Philosophical Transactions of the Royal Society of London. Series A. Mathematical and Physical Sciences. No. 1098, Vol. 259 (20 January, 1966) The Measurement of the Specific Enthalpy of Steam at Pressures of 60 to 1,000 Bar and Temperatures of 400 to 700°C. By S. Angus and Prof. D. M. Newitt. Pp. 107–132. 10*s*.; 1.50 dollars. Series B: Biological Sciences. No 764, Vol. 250 (December 30, 1965): The Structure of the Nematocyst Thread and the Geometry of Discharge in *Corynactis viridis* Allman. By R. J. Skaer and L. E. R. Picken. Pp. 131–164+plates 12–21. 28*s*.; 4.20 dollars. (London: The Royal Society, 1965 and 1966.) [281

Other Countries

National Academy of Sciences—National Research Council. Weather and Climate Modification: Problems and Prospects. Vol. 1: Summary and Recommendations. Pp. xi+28. Vol. 2: Research and Development. Pp. xii+198. (Publication No. 1350.) (Washington, D.C.: National Academy of Sciences—National Research Council, 1966.) [311

New Zealand Forest Service: Forest Research Institute. Forestry Research Notes, No. 42: The Drying of Radiata Pine Timber Treated by the Oscillating-Pressure Method. By J. A. Kininmonth. Pp. 9. (Wellington: Government Printer, 1965.) [311

United States Department of Commerce: National Bureau of Standards Technical Note, No. 18–23: Quarterly Radio Noise Data, June–August, 1964. Pp. vi+90. (Washington, D.C.: Government Printing Office, 1966.) 50 cents. [311

Bulletin of the American Museum of Natural History. Vol. 131, Article 1: Termites (Isoptera) of Thailand. By Muzaffer Ahmad. Pp. 1–114. (New York. American Museum of Natural History, 1965) 2 dollars. [311

Anuario del Observatorio Astronomico de Madrid para 1966. Pp. 378. (Madrid: Observatorio Astronómico, 1965.) 50 pesetas. [311

Annals of the New York Academy of Sciences. Vol. 130, Article 2: Automation in Industrial Pharmaceutical Process and Quality Control. By E. Alburn and 68 other authors. Pp. 483–868. (New York: New York Academy of Sciences, 1965.) 9 dollars. [311

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Bulletin 122: Glacial Lake McConnell, and Surficial Geology of Parts of Slave River and Redstone River Map-Areas, District of Mackenzie. By B. G Craig. Pp. viii+33 (10 plates). 1 dollar Paper 64–34: Basal Mississippian Volcanic Rocks in Cape Breton Island, Nova Scotia. By Danford G. Kelley and William O. Mackasey. Pp. 10. 75 cents. Paper 65–33: Atomic Energy Developments and Future Uranium Requirements as Envisaged at the Third International United Nations Conference on the Peaceful Uses of Atomic Energy, Geneva, September 1964. Pp. v+17. 35 cents. (Ottawa: Queen's Printer, 1965.) [311

Tobacco Research Board of Rhodesia. Bulletin No. 9: The Production of Oriental Tobacco. Pp. 32. (Salisbury: Tobacco Research Board of Rhodesia, 1965) [311

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Land Research and Regional Survey, 1964–65. Pp. 98. (Canberra: Commonwealth Scientific and Industrial Research Organization, 1965.) [311

Institutt for Atomenergi, Kjeller. Kjeller Report No. 75: BIGG-II—a Multigroup Neutron Spectrum and Criticality Code. By O. P. Tverbak and J. M. Døderlein Pp 66 Kjeller Report No. 100: On the Liquid Scintillation Determination of Pu-239 in Pure and Contaminated Solutions. By K. Joon, P. A. Deurloo and I. A. Hundere Pp. 23. (Kjeller, Norway: Institutt for Atomenergi, Kjeller Research Establishment, 1965) [311

Institut Royal Météorologique de Belgique Bulletin Mensuel. Observations Géphysiques, Octobre, 1965. Pp 35. Observations Ionosphériques, Novembre, 1965. Pp. 26. (Uccle-Bruxelles: Institut Royal Météorologique de Belgique, 1965) [311

Annual Report of the Syntex Corporation for the fiscal year 1965. Pp. 24. (Panama: Syntex Corporation, 1966) [311

Publications de l'Institut National pour l'Étude Agronomique du Congo. Carte des Sols et de la Végétation du Congo, du Rwanda et du Burundi. 19: Maniema. A.-Sols. Notice Explicative de la Carte des Sols. Par M. Jamagne. Pp 132. (Bruxelles: Institut National pour l'Étude Agronomique du Congo, 1965.) [311

Australia: Commonwealth Scientific and Industrial Research Organization Annual Report of the Ore Dressing Laboratory, 1964–65. Pp. 8. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1965.) [311

Transactions of the American Philosophical Society. New Series, Vol 55, Part 9: Benjamin Franklin and Eighteenth-Century American Libraries. By Margaret Barton Korty. Pp. 83. (Philadelphia: American Philosophical Society, 1965.) 2 dollars. [311

National Academy of Sciences—National Research Council. Publication 1278. Survey of European Non-Conventional Chemical Notation Systems. Prepared by Donald E H. Frear. Pp. iv+78. (Washington, D.C: National Academy of Sciences—National Research Council, 1965) 3 dollars. [311

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SHIPS AND STRING AND SEALING-WAX

FOR most of the 'sixties, British industry has been dressed in sackcloth and ashes. One after another, important sectors of the British industrial economy have been examined in great detail by committees of all kinds. Most often, the reports of these proceedings have been sombre to read. Traditional and modern industries appear to have been equally open to criticism. The textile industry has taken a severe drubbing for several years, and is now undergoing yet another structural re-organization. Machine tools have been under constant fire. A few months ago the aircraft industry was closely, though unconvincingly, dissected by the Plowden Committee (*Nature*, 209, 1053; 1966). Less formally, the British computer industry is being kept under constant surveillance, though it is too soon to know whether the attempts of the British Government to concentrate development and manufacture will be successful or even well conceived. Two themes run through this spate of patient analysis. The structure of major industries is often unsuited to modern needs, commercial and otherwise. Then there is not enough research and development, and what there is tends to be used ineffectually. It is no wonder that the impression has grown up that British industry is falling quickly into decline. This is why it is such a great surprise that the report of the Geddes Committee on the British shipbuilding industry (Cmnd. 2937, H.M.S.O., 13*s*.) has turned out to be a cheerful and optimistic document.

This does not, of course, imply that all is well. By itself, the analysis of the Geddes Committee has much in common with recent tales of woe from other sectors of British industry. Products are technically sound, and sometimes excellent, but prices are often uncompetitive and delivery dates too long. This is why British shipyards are only just able to fill vacant slipways with new orders, and why the volume of production has been a diminishing proportion of the international market for ships. Relationships between shipbuilders and the suppliers on whom they rely, particularly the engine makers, are too diffuse, and complacent. The Geddes Committee hopes that reconstruction will somehow cure most of these ills. By gentle persuasion, and with the modest temptation of small supply of Government money, it hopes that the industry will coalesce into more effective units. So will British taxpayers. The Committee also hopes that the shipbuilding industry can somehow be persuaded to link its future with the steadily growing international demand for ships, and not simply to extrapolate into the future the depressing record of the past few years. The first signs are that shipbuilders are less optimistic, but—so infectious is the optimism of Geddes—they may yet be won round to believing that the future is worth having. The hallmark of the Geddes Report is the belief that the problems which have been so well identified can also, in due course, be solved. It will be interesting to see whether this is half the battle.

Meanwhile, this public scrutiny raises all the questions of how to determine what place some particular industry should have in the industrial economy of a nation, and how best science and technology may be harnessed to its ends. No amount of research and development could, by itself, make up for inadequate links between shipbuilders and those who supply them with steel or engines, for inexpert selling, bad labour relations and the lack of industrial training. It is particularly ironical that the Report should have been followed so quickly by a general increase of 4 per cent in the price of British steel. The Geddes committee was clearly surprised that, since the Second World War, one of the largest users of steel should have failed to negotiate a special price for a raw material which accounts for roughly a fifth of the price of a finished ship. Perhaps the price increasing, following so quickly on the Report, will persuade shipbuilders that their bargaining position is strong, not weak. Clearly there are limits to what science and technology can accomplish in unfavourable circumstances.

In reality, research and development in British shipbuilding are as much criticized as other parts of the industry, and rightly. Less than 1 per cent of turnover is spent on these activities, and even so, it is sometimes hard to tell where research ends and the design of particular ships begins. The co-operative research efforts which the shipbuilding industry could spare for engines were—according to Geddes—spent on steam turbines long after it had become likely that slow-speed Diesel engines were in fashion. (This does not mean that steam turbines will always languish, and there is even now a chance that slow-speed Diesels will be overtaken in performance by faster Diesels, even if shipbuilders will then have to provide more complicated reduction gearing.) Even now it is turning out to be extremely difficult for the British Government and industry to agree how best the development of competitive engines can be sponsored in partnership.

But this is only the bleak side of the picture. It is also plain that there has recently been a powerful enhancement of the industry's concern for research and of its ability to carry it out effectively. The National Physical Laboratory (which operates the most versatile model basin in Britain) is obviously hoping to find improvements of hull design for large ships as radical, and as valuable, as those which have led to the successful bulb-nosed trawler.

The study of the dynamic problems of ships at sea promises great benefits in the evolution of designs which economize in steel. Simplifying, and sometimes standardizing, the layout of ships may be well worth the study now being given to it. Automation will be increasingly used in working ships, especially as crews become more expensive. Systems for assembling steel plate are being blessed with some of the objective study they need, chiefly by the co-operative research associations for welding and shipbuilding. Hopes are running higher than for a decade. Problems abound—and Geddes asks for more money to solve them. Hopes of a flood of technical innovations are higher than for a decade.

How has all this come about? The most striking lesson to be learned from the Geddes Report is the great value of public discussion. The report on shipbuilding research in 1962 by the old Department of Scientific and Industrial Research (now merged in the Ministry of Technology) seems to have been particularly effective in giving the industry a grasp of the problem of designing modern engines. The sense of indignity engendered by a critical report can evidently work where common sense may fail. It is also plain how much can be done to revivify an industry by encouraging co-operative research financed chiefly by industry, but with backing from the Government. In British shipbuilding, the better spirit in the research associations is, so far, the most tangible sign that things may yet go well. The British Government, which has in the past tended to scorn the research associations it originally helped to create, ought to read with great care the passages in the Geddes Report on co-operative research.

TEACHING FOR EXPORT

MUCH good will come from the institution which the United Kingdom Ministry of Overseas Development and the Nuffield Foundation have chosen to call by the delicious name of CREDO ("Centre for Educational Development Overseas"). The intention is to provide a headquarters (in London) from which the use of new teaching materials developed in Britain and similar countries can be fostered in nations overseas. Inevitably, the first customers will be the nations known as underdeveloped, though it is only proper to remember that, in education, all nations are in some sense underdeveloped CREDO will work with materials and methods in all specialities, from English language to physics, though the greatest demand is likely to be for parts of the school curriculum in science. With luck, the institution should survive the three years covered by the first allocation of funds—especially if by then the Ministry of Overseas Development has the funds to sustain the good works it is always talking about.

The need for a mechanism for channelling educational aid to nations overseas should not, of course, obscure the difficulties of these operations. For one thing, it is necessary to avoid suspicions of neo-colonialism. Especially in dealings with African nations, CREDO will have to tread warily. It is understandable that many nations should fear that the old slogan about trade following the flag has now been replaced by a link between education and political influence. The sponsors of CREDO are mercifully seized of this danger. They seem also to acknowledge the difficulties of transplanting educational methods and materials from one environment to some other. The difficulty is that a sensitively designed curriculum is an intellectual and social entity in which should ideally participate whole communities of teachers. It follows that the best way to export teaching is to enable nations wishing to improve themselves to study, mimic and adapt those parts of practice elsewhere that seem to be desirable. By this test, CREDO will be most successful if it can avoid thinking of itself as an export agency; the ideal is that it should assist developing nations to create for themselves machinery by which curriculum development can be begun and then sustained.

IDEAS AND WORDS

Scientific Psychology

Principles and Approaches. Edited by Benjamin B. Wolman and Ernest Nagel. Pp. xv+620. (New York and London: Basic Books, 1965.) 70*s*.

ONCE upon a time philosophy gave birth to a large family of sciences. One by one they broke away from the maternal apron-strings. For psychology, however, the youngest of these, the breakaway proved a mixed blessing. No sooner did she walk alone than she found her footsteps dogged by seducers: behaviourism, reductionism, positivism, and a host of others.

In the face of these perils, and on the initiative of Dr. B. B. Wolman, the editor of *Scientific Psychology*, thirty scholars have been mobilized to advise on the defensive measures to be taken. At the outset, his co-editor, Ernest Nagel, counsels a return to the security of mother philosophy.

Dr. Wolman hopes that the compendium will establish a science of science, defined as the study, by scientific methods, of "systems of propositions which convey a certain kind of knowledge". This task he considers to be different from the psychology, sociology or history of science. Prof. Arne Naess takes up his challenge to create a metascience, and gives us a lively critique of the idea that scientists, in the course of their work, can be studied as if they were rats in mazes. For, he remarks, unlike the psychologist who himself constructs the mazes into which the rats are placed, the metascientist may have no idea of the 'maze' in which his 'rat' finds itself. That is, he may know little of the problems, goals, culs-de-sac or rewards of the scientist. Prof. Naess concludes that the metascientist cannot observe science from the outside. He must somehow participate in the labours of those who constitute his subject-matter. This participation is of special significance to psychologists because they have a similar relationship to their subject-matter, human beings.

Other contributors are concerned with philosophical or logical aspects of psychological issues. Outstanding among these are Jean Piaget and J.-B. Grize, who discuss the basis of genetic epistemology. This is a branch of psychology which traces, stage by stage, the natural development in the individual child of those mental operations which eventually come to grips with the essential concepts of mathematics and physical science. Piaget turns the tables on the editors by suggesting that a sound philosophy must rest on empirical psychology, rather than vice versa, for the concepts of mathematics and physics do not spring ready-made from the mind, nor are they abstracted from the objects of observation. They always presuppose a series of interactions between the thinking subject and the object of his thought. The study of child development thus poses questions which converge with the great problems of epistemology and the philosophy of the sciences: How is logic related to language? What is causality? Is number an intuitive concept? How is the idea of speed related to that of time? And so on.

Most of the contributors either limit themselves to philosophical problems as such or to particular psychological systems. Among the former are G. J. Warnock, who writes on logical analysis and the nature of thought, and D. M. Armstrong, who expounds a theory of perception. The latter are excessively represented by theorists of learning, including an admirable chapter by B. F. Ritchie, who is troubled about the vagueness of learning theory. Prof. Ritchie proposes two axioms for metascience. One relates to the scientist whose confidence in his theory is directly proportionate to the degree to which he cherishes it. The other applies to the scientist who on discovering a fact which contradicts his theory, revises his theory to render it untestable.

The chapters vary in quality. Some are written in an obscure prose exemplified by the title of the editor's final

chapter, "Principles of Monistic Transitionism", designed presumably to bring some unity to an otherwise loose ensemble.

JOHN COHEN

EVOLVING THE RULES OF WORKING TOGETHER

Industrial Relations in Engineering

By Arthur Marsh. (The Commonwealth and International Library of Science, Technology, Engineering and Liberal Studies.) Pp. xxiii+362. (London and New York: Pergamon Press, Ltd., 1965.) 30*s*. net.

IN the words of the author, the purpose of *Industrial Relations in Engineering* is to examine the present-day workings of industrial relations and to encourage trade unionists, managers and students of industrial relations to discuss the strength and weaknesses of the system.

The rate and pressure of technical change, particularly automation, which is likely to affect the jobs of many people, the need for a critical look at craft apprenticeships and retraining within industry and the impact of national planning, are all factors which stimulate interest and direct attention to what is undoubtedly the most important factor in the evolution of modern industrial life.

It would not be untrue to say that 'industrial relations' is, when brought down to factory level, where all negotiations begin, but another term for 'personnel management', the emphasis being placed on the aspect of employee relationship rather than executive policies and activities that are set up to foster good relations. Both are clearly allied to morale and good morale makes for effective work and economical operation.

Arthur Marsh in his study has drawn on his wide knowledge gained while developing extra-mural classes for industry at the University of Oxford, and quite logically his opening chapters deal with the historical background of trade unionism, the problem of defining engineering and the slow but steady emergence of Union organization and negotiation procedures from the early local committees to a national body in the form of a federation which is described in the Trades Union Congress Report (1947) as "a loose form of industrial unionism" and is a compromise between *ad hoc* working arrangements and a complete amalgamation of associated bodies.

In this role, the Confederation of Shipbuilding and Engineering Unions (C.S.E.U.) serves a very good purpose and prevents inter-union competition and overlapping.

The next chapter summarizes the general position and function of the C.S.E.U. and is followed by a description of the nature and origin of the managerial side of industrial relations. The role of the Engineering Employers' Federation, expressed in the simplest possible terms, is that it acts as the trade union for management.

The chapter dealing with the history and operation of the Engineering Employers' Federation is one of great interest, as it explains very clearly the reason for the defensive role undertaken in labour relations and why the local association structure of the organization has survived and continues. The comments of the author on the future of the Federation could be the subject for much comment and debate, but it is Chapter 8—"The Engineering System in Perspective"—which discusses the reaction of those inside and outside the industry to the many features in the Unions' and Federation's handling of disputes which is most important and gives scope for criticism and an urge to reorganize. The final paragraph of the chapter which deals with the influence exerted on industry by Government could quite easily have been considerably extended and perhaps in time this aspect of the subject will merit a volume to itself. The author mentions the Industrial Training Act, which is just beginning to bite and has received a mixed reception from employers but so far little published reaction from Unions.

The activities of the National Board for Prices and Incomes and the effect of restraint on prices without effective control of wages indicate that more Government pressure is essential if its policies are to be effective. Government influence is becoming a third factor in industrial relations, as since 1945 and especially since 1961 both Conservative and Labour Governments have found it necessary to intervene more directly in the affairs of industrial relations.

The latter part of the book is taken up with appendixes A to D, and these give much valuable information on the main negotiating bodies, procedures and agreements from the year 1898 to the 'packaged deal agreement' of December 22, 1964, and in a section labelled "Miscellaneous" there are notes on apprentice indentures, training of shop stewards, collective bargaining and procedures for dealing with redundancy.

There are all too few books written on industrial relations. This subject should be more widely known, understood and discussed: Arthur Marsh's book, although written primarily to advance teaching to a selected readership, can be of great interest to a broader section of the community—those who, while having no part in the engineering industry, are nevertheless affected by industrial disunity.

The arrangement of chapters and the details given in each will enable a reader to build up, step by step, a picture of the complex negotiating machinery and the code of behaviour evolved over a period of one hundred and fifty years of industrial growth to give Britain her present organization and procedure.

In this book we have all the history, the rules of the game, and an introduction to the characters and organization, and in studying it a reader can form his own opinion of a relationship which can affect us all, our work, our money, and our security and future.

L. E. JARMAN

INTRODUCTION TO NEUTRON RADIOGRAPHY

Neutron Radiography

Methods, Capabilities and Applications. By Harold Berger. Pp. vi+146. (Amsterdam, London and New York: Elsevier Publishing Company, 1965.) 50*s*.

HAROLD BERGER has produced an authoritative and clearly expressed book which provides an excellent survey of neutron radiography for the non-specialist reader and a thoroughly worth-while reference book for the worker in this comparatively new field. The value of *Neutron Radiography* to the specialist is considerably enhanced by a comprehensive and up-to-date bibliography.

After an introductory chapter in which the role of neutron radiography as complementary to X- and γ-radiography is reviewed, the author discusses the use of accelerators, radioactive sources and reactors as sources of neutrons for slow neutron radiography. Emphasis is placed on the use of reactors because of the higher fluxes available, and here the author could possibly be criticized for underestimating the potential use of low-energy accelerators which have the advantage of cheapness and mobility and which are gradually being developed to produce higher neutron yields. The comment has perhaps more relevance to the potential user in Britain where research reactors are less readily accessible to industrial research teams.

An excellent chapter deals with photographic methods for neutron detection, and here Harold Berger demonstrates to the full his ability to write for the specialist and non-specialist reader. His somewhat superficial treatment

of the nuclear processes involved may slightly irritate the nuclear physicist, yet they perform the essential function of providing the necessary background information to enable the non-specialist to understand and appreciate an excellent exposition of the practical techniques and relative merits of direct and transfer exposure methods. In a more speculative chapter dealing with non-photographic methods of neutron detection, the author concludes that neutron image intensifiers and multiwire spark counters are likely to be of future interest.

Practical considerations such as shutter design, the effect of collimation on resolution and the resolution, contrast and speed obtainable with various converter screen combinations using direct and transfer exposure techniques are discussed in Chapter 6, in sufficient detail to satisfy the potential user, while the following chapter on capabilities and limitations of the technique will be of more general interest. A chapter on applications is slightly biased towards applications in the reactor field, but here and elsewhere applications in metallurgy, biology and solid-state physics are adequately covered. Numerous excellent reproductions of neutron radiographs occur throughout the text.

Although clearly an enthusiast, Harold Berger is to be congratulated in preserving a well-balanced and objective point of view throughout, neither over- nor under-estimating the potential usefulness of this developing technique.

S. E. HUNT

RADIOACTIVITY OF THE HEAVY ELEMENTS

The Nuclear Properties of the Heavy Elements

By E. K. Hyde, I. Perlman and G. T. Seaborg. Vol. 1: Systematics of Nuclear Structure and Radioactivity. Pp. xv+406+xvii. 120*s*. Vol. 2: Detailed Radioactive Properties. Pp. xvi+407–1107+xlix. 200*s*. Vol. 3: Fission Phenomena. By E. K. Hyde. Pp. xvii+519+xxiv. 144*s*. (Englewood Cliffs, N.J., and London: Prentice Hall International, 1964.)

BEYOND thallium, alpha decay becomes an important factor determining the nuclear stability of the isotopes of each element. The twenty-two elements at present comprising this class are commonly called the heavy elements. Half are synthetic species; the remainder occur in the three natural radioactive decay series, although two of them only arise from minor decay modes. Three stable nuclides, isotopes of lead, are included, along with some three hundred radioactive nuclides and about twenty nuclear isomers of appreciable lifetime. Except for the forty-eight naturally occurring species, the investigation of which has been pursued in France since the early discoveries in radioactivity, the experimental exploration of this field has taken place predominantly in the United States. No one laboratory has a more distinguished record in the field than the E. O. Lawrence Radiation Laboratory in the University of California, Berkeley. This Laboratory was associated with the discovery of all the eleven synthetic elements.

Some of the wisdom and experience of this Laboratory has now been condensed into three volumes, jointly entitled *The Nuclear Properties of the Heavy Elements*. The first volume deals with the semi-empirical theoretical analysis of the nuclear data with which the Berkeley school has been so successful. The first two chapters provide the basic experimental data, the masses and nucleon separation energies and decay energy cycles linking all the known species. Problems arising in the selection of a compatible set of data are considered. The major portion of this volume is devoted to an account of the single independent particle and the unified models of the nucleus and their application to the interpretation of the large body of experimental data on decay processes and the excited states of these nuclei. This is followed by a detailed consideration of the alpha decay processes, their spectra and kinetics and interpretation in terms of hindrance factors. The fifth and final chapter deals with quite a different theme, the production of the synthetic heavy element nuclides. Each of the principal methods, bombardment with protons, deuterons or helium nuclei of moderate energy, bombardment with high-energy heavier nuclei and repeated neutron capture, is discussed in detail. The chapter finishes with a very brief account of cosmic nucleogenetic processes. Appendixes tabulate the electron binding and characteristic X-ray energies in these elements, Auger electron energies and fluorescent yields.

A noteworthy feature of this volume is the minimum of mathematical detail included in the portions dealing with nuclear models and the kinetics of alpha decay. Where appropriate, reference is made to earlier work of historical significance; for example, the pioneering work of Schintlmeister in this field is acknowledged.

The second volume contains a comprehensive account of the three hundred or more nuclides of these elements so far examined. For each nuclide treated the data recorded include the history of its discovery and first mode of preparation, the best alternative modes of preparation, a complete account of its properties, saving only fission properties which are reserved for the third volume, and the interpretation of these properties in terms of the shell and/or unified nuclear models.

The sequence chosen is at first a little unusual. The natural decay series and their members are treated first. This chapter contains a good deal of historical material and includes some topics, such as a brief account of assay methods for radium, that are not considered for the remaining species. There is also a substantial digression on nuclear geochronological procedures following the stable isotopes of lead.

Next, the six synthetic collateral decay series and the two $4n + 1$ series are considered. At the end of this chapter a very useful collection of reference alpha spectra of the members of both the natural and synthetic decay series appears. The isotopes of thorium, protactinium and uranium are then treated in order of mass number followed by a similar treatment, element by element, of the transuranium species. This final chapter, indeed, comprises about one-third of this volume. It describes the lighter isotopes of the natural elements not included in the previous chapters together with a good deal of discussion of the special features of these nearly spherical nuclei lying on a relatively flat part of the mass energy surface below the 126 neutron shell.

This grouping of the material inevitably leads to some measure of duplication and this is enhanced by an attempt to make each volume reasonably self-contained. Besides the subject and author indexes included in Volume 1, Volume 2 closes with similar cumulative indexes; in addition, it includes an isotopic index. The two volumes form a natural unit and indeed the pages in Volume 2 are numbered to follow those of Volume 1. The enormous volume of quantitative data contained is generally clearly presented and extensive use is made of graphical, tabular and other diagrammatic forms of presentation. Together, the two volumes contain more than two thousand references to original material. Many of these relate to unpublished work or to papers that have appeared in the profusion of official documents, reports, etc., which are often less readily available than the usual academic journals. For this reason alone these volumes will be one of the key works of reference for any library serving the nuclear scientist.

The third volume covers the phenomena of fission. It is surprising, in view of the substantial literature of the subject and the appearance of a number of good reviews covering particular aspects of fission, that this is the first monograph devoted exclusively to fission. It reminds one

that, despite the rapidly developing industry based on this phenomenon, the time that has elapsed since its discovery, and the considerable body of related research published every year, there is still no satisfactory theoretical account of fission. Nor are the experimental data yet so complete that one feels that nothing can be done which will add to our picture of the process.

This volume is divided into two parts. The first deals with low-energy fission, treating in succession the history, theory, cross-sections; mass, charge and kinetic energy distributions of the fission fragments; the characteristics of the prompt and delayed neutrons and the prompt gamma radiation. The second part deals with the more energetic fission processes, first in the region where compound nucleus formation is still important. A critical account is given of the substantial body of data on mass yield curves for these processes. A description of the high-energy fission processes follows, the results being interpreted in terms of the Serber model. A brief chapter on meson fission and a more extensive one on photo-fission complete the book. This volume is clearly the most comprehensive, authoritative and scholarly work on the fission process.

All three authors are experienced writers and the text generally reads very agreeably. As no doubt the price indicates, these volumes are well bound and produced. It is virtually impossible to avoid some typographical errors in such complex productions, but these are very few and far between. Taken together, the three volumes are a remarkable and invaluable contribution to nuclear science and will be essential volumes to all libraries covering this field.

A. G. MADDOCK

SOME ASPECTS OF MICROCIRCULATORY STUDIES

Microhemocirculation

Observable Variables and Their Biologic Control. By Prof. Elio Maggio. Pp. xiii+194. (Springfield, Ill.: Charles C. Thomas, 1965.) 16.50 dollars.

MICROHEMOCIRCULATION is, in revised form, the English publication of Prof. Maggio's work, *Micro-e-macro-emocircolazione*, published in Italy in 1962. According to the author, the script is designed for scientists interested in biology, teachers and students in medical schools, and physicians wishing to correlate clinical symptoms and signs with the results of experimental studies of microvascular problems.

Essentially the book is in six parts: (1) a historical survey of important macro- and micro-circulatory discoveries from the Hellenistic period to the present day; (2) methods of investigating the microcirculation; (3) a section consisting of two chapters devoted to a description of the distribution and structure of microvascular beds, their haemodynamics, transcapillary exchange and the chemical and nervous factors which may influence the size of microvessels; (4) the reaction of microvessels to pathological circumstances; (5) the response of vessels to tissue injury; (6) a short atlas of plates showing microvessels under normal conditions and their reactions to trauma and infection.

At a time when the emphasis on various parts of the medical curriculum is under review and the tendency is for reasonably abbreviated text-books to be prescribed, the place of any book, in the armament of the average student, which deals entirely with microcirculatory problems must be questioned. Nevertheless, the author must be congratulated in attempting to bridge the traditional schisms of teaching by considering the normal structure, function and pathology of microvessels in such short space. As a result, however, much is omitted and it is for this reason that the use of this book is largely extra-curricular, perhaps appealing to an undergraduate student wishing background reading during the preparation of a thesis, or providing a rapid synopsis of available methods for investigating some aspect of the microcirculation.

The historical survey is short but adequate, lucidly depicting the evolution of facts concerning the circulation and their separation from the various hypotheses of earlier centuries. The chapter dealing with methods of investigation serves only as an introduction to available techniques, no single method being considered in detail. Regrettably, when referring to histological techniques for validating observations made *in vivo*, the author fails to mention any but his own particular method, thus giving an unbalanced effect to the general discussion.

When considering the normal structure and distribution of capillary beds, the subject is considered so briefly that little information would be imparted to a student with little previous knowledge, the only microcirculation being described in any detail being that of the placenta. On p. 61 capillaries are defined, without qualification, as having a diameter of 2–18μ and are mentioned synonymously with sinusoids. These and other points would mislead the medical student who, in Britain, is concerned primarily with human anatomy. The present views on the control of microvessels by chemical mediators is rapidly reviewed, but the discussion of the part played by the nervous system is inadequate, presupposing considerable knowledge of neuroanatomy.

Two concluding chapters, very briefly, describe the elementary facts concerning responses of microvessels to inflammation, the role of mast cells and tissue response to injury. Brevity is often necessary, but it defeats its purpose here and it is impossible to give a coherent description of the mechanisms of blood coagulation in half a page and claim an adequate standard has been achieved.

An atlas of black-and-white illustrations reveals the value of various techniques in microvascular studies, the pictures of perivascular lining by mast cells, platelet adhesion to the endothelium and the formation of white thrombi in veins after experimental introduction of pathogenic organisms being impressive.

The majority of illustrations supporting the script are acknowledged from the works of other authors. Two electron micrographs (Figs. 25 and 26) are of poor quality, a better reproduction of an arterio-venous anastomosis (Fig. 24) is desirable, and Fig. 23, which shows the course of the spiral arteries and veins of the placenta, is reduced excessively. In style the publication has suffered during the translation, and phrases like "anatomo-physiologic variables of the microhaemocirculation" are a little frightening to the student. The bibliography is extensive enough to lead one into the subject deeply.

As a text on the normal structure, function and pathology of the microvessels, this book must be accounted a disappointment.

JOHN A. CLARKE

SIXTY YEARS OF TISSUE CULTURE

Cells and Tissues in Culture

Methods, Biology and Physiology. Edited by E. N. Willmer. Vol. 1: Pp. xiv+782. Vol. 2: Pp. xvi+809. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1965.) 168*s*. each volume.

"TISSUE culture is humanly important only in so far as it functions as a substrate for the growth of pathogenic or potentially pathogenic viruses."

Whether or not one agrees with this statement by Sir Francis Burnet (*Lancet*, i, 37; 1966) will depend on one's involvement in tissue culture and on the interpretation given to the words "humanly important". One way of assessing its validity might be to read the two volumes of *Cells and Tissues in Culture* which the editor, Dr. E. N. Willmer, describes as "an attempt to show what

has been achieved so far and to discuss where, when and how the technique may be most profitably employed". They are intended to sum up the contributions made by tissue culture to various biological fields during the 60 years since Ross G. Harrison showed that the method was capable of solving fundamental problems; at the same time, they provide an excellent guide for anyone contemplating the application of these techniques in a particular research programme.

The first volume is concerned with general matters apropos of cells and tissues in culture, including culture methods and media, the identification of cells *in vitro*, their motility and metabolism, cell division, and the actions of hormones and vitamins on cultured cells. The second deals with the techniques appropriate to the study of specific cells and tissues, and reviews the information already derived from their use, ranging from macrophages, lymphocytes and bone marrow, via bone and tooth, through muscle, nervous tissue and the special sense organs, to the culture of exocrine and endocrine glands and the gonads, with chapters on skin and the respiratory system.

A third volume is in preparation which will contain articles on various specialized, but important, topics such as malignant cells, antibody production *in vitro*, the use of tissue culture in radiobiology and virology, and plant tissue culture.

From their primary growth in the early years of this century, tissue culture techniques have now metastasized throughout the whole body of biological research, and as Dr. Willmer has called on the services of almost fifty contributors to give adequate coverage to these widely different topics, it is impossible for one individual to give a valid judgment on each and every chapter, and comment must be limited to a sample dictated by personal interests.

The opening chapter by Moscona, Trowell and Willmer is a necessarily brief account of the routine methods of tissue, cell and organ culture with comments on their advantages and drawbacks, sufficiently informative to satisfy the curiosity of the worker in other fields or to give the tyro an overall view leading him on to the standard books on techniques. The section on problems of cell identification by Willmer is a condensation of his classic *Cytology and Evolution*, and serves its purpose as a warning of the dangers of giving too precise a name to a particular variety of cell in culture. Abercrombie's chapter on the locomotion of cells considers the factors which may influence the movement of cells on the surfaces commonly available to them *in vitro*, and illustrates the difficulties involved in any attempt to explain events occurring *in vivo* on the basis of cellular activity in the totally abnormal environment of the culture.

The contributions in Volume 1 concerned with the carbohydrate, protein and nucleic acid metabolism of cultured cells cannot be critically assessed by a non-biochemist, but are clearly essential in a work of this kind.

The chapter on genetic cytology by Hsu must be mentioned, partly because it provides an outstanding example of the ability of tissue culture techniques to clarify a previously obscure field in biology and medicine, and also because the style of this author has enviable lightness of touch which makes a most complex topic enjoyable and easy to comprehend. There is little doubt that the recent rapid advances in the understanding of chromosomal aberrations owe as much to simple cell cultures as to the higher flights of molecular biology.

The remaining topics covered in the first volume—differentiation, cell aggregates, embryogenesis, the actions of hormones and vitamins—are all dealt with expertly by leading workers in these fields of tissue culture, and exemplify the value of the method as a means of isolating tissues and controlling their environment.

The second volume opens with an account by Jacoby of the cultural characteristics of the macrophage, a cell which is perhaps happier than most in the milieu of the culture flask or chamber and which is a rewarding subject for such investigation, since it is virtually a free-living organism within the body and readily adapts itself to new surroundings. The same cannot, alas, be said of the lymphocyte, which is notoriously reluctant to respond to the blandishments of the tissue culturist, dying rapidly *in vitro* however rich and succulent the medium offered to it. Trowell, who has made greater efforts than anyone to satisfy the requirements of this *prima donna* among cells, provides a thorough review of the somewhat negative information available.

One of the most successful applications of the organ culture method (as distinct from cell or tissue culture) has been in the growth of isolated rudimentary bones, which Fell and Robison used to such good effect; Chapter 4 in the second volume, by Biggers, collates the results of experiments based on this procedure—a further example of the value of isolating a tissue or organ so that viability, growth and differentiation can continue under closely controlled conditions. This is succeeded by a short discussion on the osteoclast by Hancox, whose use of the cell culture technique contrasts well with the preceding chapter.

The nervous system was not only one of the first tissues to be examined by *in vitro* methods, but has also continued to be one of the most intensively studied, and with the steady improvement in culture techniques the point has now been reached where myelination in culture can regularly be achieved, thanks to the groundwork carried out by biologists such as Margaret Murray. She sums up the situation with regard to nervous tissues in the ninth chapter of Volume 2, dealing with, *inter alia*, the stimulation of nerve fibre growth, the differentiation of neurone cytoplasm, myelination, and the culture of neuroglia. This chapter includes a section by Crane on the electrophysiological activity present in cultured neurones and neuroglial cells, which, when considered together with the light and electron microscopic evidence, implies a surprising degree of functional and structural organization in explanted nervous tissue.

Dr. Willmer has chosen his contributors well, and they have not let him down; the Academic Press have turned out the books to their usual high standard. Criticisms can only be minor and carping—there might, for example, have been a chapter on the application of the phase, interference and fluorescence microscopes, which could usefully have included more than a mere passing reference to the preparation of cultured tissue for electron mic[illegible] scopy. It was disappointing to find that few of the auth[illegible] have used phase-contrast photomicrographs, which c[illegible] have an intrinsic beauty, in their illustrations. Refer[illegible] to clones or 'pure' strains of cells are brief and scatte[illegible] and could preferably have been brought together in a separate chapter.

Dr. Willmer has already done much to correct the once widespread impression that tissue culture is a recondite and difficult procedure of doubtful value; this most recent product of his energy and enthusiasm should finally eradicate this belief and show that, irrespective of whether it is "humanly important", it is a valid biological tool capable of achieving preparations with an aesthetic appeal as well as great scientific potential.

J. A. SHARP

AMNESIA

Deranged Memory

A Psychonomic Study of the Amnesic Syndrome. By George A. Talland. Pp. xi + 356. (New York: Academic Press, Inc.; London: Academic Press, Inc. (Lond[illegible] Ltd., 1965.) 75*s*.

IN 1887, S. S. Korsakoff, a noted Russian psychiatrist, described a number of cases in which unusual psychical symptoms were associated with severe alcoholic ne[illegible]

pathy. In his view, the central feature of the disorder was "an extraordinarily peculiar amnesia, in which the memory for recent events, those that have just happened, is principally involved, whereas the remote past is remembered fairly well". His observations were abundantly confirmed by others, though it soon became clear that this curious disorder is not exclusively linked with chronic alcoholism and may be regarded as a reaction of the brain to a wide variety of pathological agencies. It was therefore christened the Korsakoff syndrome, by which name it has since been generally known.

In view of the widespread preoccupation with the cerebral basis of memory, it is odd that the Korsakoff syndrome has not attracted greater scientific interest. For here we see a remarkable dissociation between memory, which is often most severely impaired, and other psychological functions, such as intelligence, which are little, if at all, affected. So clear-cut a dissociation cannot, so far as is known at present, be produced experimentally in animals, and may perhaps reflect a special characteristic of the human brain.

Dr. Talland presents an excellent account of the Korsakoff syndrome from the psychological point of view. He is particularly good on the historical aspect, and has made a real effort to understand the laborious German work on the subject, which at its best is extremely penetrating, though at its worst turgid and quasi-metaphysical. Indeed, *Deranged Memory* is the first really adequate digest of the literature on the Korsakoff syndrome so far to appear in English.

Included also are the results of a considerable number of controlled psychological experiments which the author has himself carried out with Korsakoff patients, some of which have already been reported elsewhere. These experiments have two aims: first, to ascertain the general psychological status of the Korsakoff patient, and secondly, to throw light on the nature of the memory defect itself. As regards the first aim, Dr. Talland shows, as have others before him, that the defect of memory is seldom absolutely circumscribed. Minor abnormalities in perception, intelligence and reasoning can often be elicited by special tests, even if clinically inapparent. As regards the second aim, Dr. Talland is able to show that the memory loss cannot be exclusively ascribed to a failure of registration, of retention or of recall. All three phases of the memory process suffer, as does also associative learning. Indeed, all that one can really conclude from these experiments is that the rate of forgetting is enormously accelerated in Korsakoff patients, such that they become virtually unable to learn or to build up a coherent record of their present experience. As Sir Aubrey Lewis has well said, the differences between the amnesic and the normal state are quantitative rather than qualitative, and involve no factors not already known from the investigation of normal memory.

It is perhaps a pity that Dr. Talland pays so little attention to the neuropathology of the Korsakoff syndrome and its relation to the more strictly circumscribed defects of memory that are now known to result from bilateral lesions in the hippocampal region of the brain. For, in these cases, one may see a relatively pure memory disorder, lacking the confabulation and disorientation so characteristic of the Korsakoff state. Indeed, the latter is unlikely to prove altogether simple, from the psychological point of view, and almost certainly involves defects in insight and judgment which are not necessarily linked with, let alone derived from, the peculiar disorder of memory. These additional defects may well derive from subcortical brain damage, which has been established in many typical cases of alcoholic Korsakoff syndrome.

This interesting and unusual book may be commended to psychologists and others with an interest in memory and its disorders, It is well organized and clearly written, and will long remain a valuable source book. Unfortunately the price is rather high.

O. L. ZANGWILL

RESPONSES OF INDIVIDUALS AND ENVIRONMENTS

Psychobiological Approaches to Social Behavior

Edited by P. H. Leiderman and D. Shapiro. Pp. xv + 203. (London : Tavistock Publications, Ltd., 1965.) 48*s*.

A SYMPOSIUM, sponsored jointly by the Department of Psychiatry of the Harvard Medical School and the U.S. Office of Naval Research, was held in 1963 to bring together individuals concerned with an increasingly significant field of psychological investigation, the impact of social and group environment on the emotional and bodily responses of man. It seems so apparent that psychological functioning must be influenced by the social environment that it is almost startling to note that the social psychological background is rarely specified in physiological investigation. *Psychobiological Approaches to Social Behavior* brings together data which would normally be spread through the literature of several disciplines. Among the interactions of the different life sciences which were described by various authors are the sensitivity of psychoendocrine systems to social and physical environment; plasma-lipid responses to leadership, conformity and deviation; muscular tension; physiological activation or psychological act ?; the interaction of the cognitive and physiological determinants of emotional state; and the experimental exploration of the et-epimeletic or care-soliciting behavioural system.

Bringing together the proceedings of this symposium for publication in book form has been entrusted to Prof. P. H. Leiderman and Prof. D. Shapiro, well known for their work showing how social factors in small group interaction influence the galvanic skin potential. In a concluding chapter they discuss the implications for future research of the investigations reported and rightly suggest that there has already been established a methodological and conceptual framework for fruitful exchange between the psychological and the social sciences. The exchange will almost certainly include a wide extension of suitable mathematical techniques. Future research should proceed from those studies which attempt to manipulate social variables by pre-experimental selection and arrangement of individuals. In the words of the editors, "small groups of two, three and four subjects could be studied under the varying effects of social approval and disapproval, success and failure, co-operation and competition, formal and informal social settings, conditions of isolation, and varying social relationships. Groups selected for personality type, patient category, occupational group, sex and ethnicity could be included in these different social environments". Other investigations could begin with the study of the socialization of physiological response levels during the course of maturation; useful information concerning individual and family structure could be obtained by physiological studies of family interaction during the course of development. Another area of research which would have an important bearing on psychosomatic medicine is the degree to which visceral responses can be learned or unlearned under different conditions of social reward. Equally, as the editors show, the relationship between pharmacological agents and social behaviour is almost unexplored. "A social situation is the only appropriate testing ground for assessing the value of a drug in influencing an individual's adaptation to his social environment." Other lines of research which are indicated from this fascinating integration of social and biological factors would stem from human evolution, genetics and ecology. The editors and authors could fairly claim that this report of their symposium is not only an important and exciting book but provides guidelines for much inter-disciplinary co-operation between workers in the psycho-biological fields of to-morrow.

T. H. HAWKINS

Mach Bands

Quantitative Studies on Neural Networks in the Retina. By Dr. Floyd Ratliff. Pp. xii+365. (San Francisco, London and Amsterdam: Holden-Day, Inc., 1965.) 15.25 dollars.

THIS interesting and unusual book contains three main portions. The first is devoted to a most fascinating biography of Ernst Mach along with a description of his major contributions in the fields of philosophy, physics and biology. Ratliff shows how these interests in diverse fields were intertwined and gives them a unity.

In the middle portion of *Mach Bands* the author lucidly describes Mach's original discovery of a most remarkable visual phenomenon—now called Mach bands. If the eye is presented with a sudden change in contrast gradient, such as occurs with the diffuse shadow of an edge, the subject perceives a bright band where the gradient is negative and a dark band where the gradient is positive. Mach could foresee that this was an important property of the visual nervous system and that it would enable the visual system to transmit information about the presence of contours. Almost a century had to pass before Ratliff and other neurophysiologists demonstrated the existence of this effect in single nerve fibres passing from the eye to the brain. Ratliff successfully attempts to integrate Mach's original psychophysical observations with our modern knowledge of neurophysiology and discusses the relevance of these findings to present-day theories of how we perceive detail.

The final portion of the book is a translation of the six relevant papers that Mach published between 1865 and 1906. In view of the present research interest in this field, these translations will form a most convenient reference source.

This book can be thoroughly recommended to all experimental psychologists and physiologists interested in the visual system, and to all those who have to use, or be aware of, some of the strange properties of the visual sense.

F. W. CAMPBELL

The Biliary System

Edited by Dr. W. Taylor. (A Symposium of the NATO Advanced Study Institute.) Pp. xxv+712. (Oxford: Blackwell Scientific Publications, 1965.) 135*s*. net.

THE Biliary System is a report of a symposium held in Newcastle in the autumn of 1963 under the auspices of the North Atlantic Treaty Organization. The conference was attended by some ninety authorities on liver function from all over the world, and a high proportion of these presented communications to the conference.

The subjects were classified in eight main divisions commencing with structural and functional relationships of the liver and including various metabolic and clinical aspects of the subject. All the contributions are well written and illustrated and each one constitutes either an important new contribution to the subject or a valuable summary of the existing situation. Most of the papers have a short summary or conclusion at the end, but it would have been an improvement if this had been insisted on in each case.

With such a variety of papers it would be invidious to select any for special mention, but I was particularly impressed with the sections on biliary secretion in man and with that on bilirubin metabolism. The unexpected difference in the intestinal absorption of conjugated and unconjugated bilirubin is specially interesting in its relation to the biological purpose of conjugation, and the problem of cholestasis is well reviewed. New information on congenital, non-haemolytic jaundice is contributed by several authors.

The work concludes with a series of short summarizing articles by Prof. Sheila Sherlock, Dr. R. W. Brauer, Prof. W. H. H. Andrews and Dr. A. E. Doisy which give a very valuable perspective of the conference and should, perhaps, be read first. The book is extremely well produced and the whole production is a tribute to the organizing powers of Dr. W. Taylor, who acted as director of the conference; it should certainly be read by all interested in any aspect of liver function and represents good value at the price.

N. F. MACLAGAN

Advances in Ecological Research

Vol. 2. Edited by J. B. Cragg. Pp. xi+264. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1964.) 57*s*. 6*d*.

THIS is a continuation of a series designed to provide biologists with a balanced view of recent developments in the ecological investigation of plants and animals. The volume consists of four substantial articles. In the first, M. E. Solomon considers the processes involved in the natural control of insects and mites, placing emphasis on the results of practical studies of population dynamics, the type of data required for the study of natural control and their analysis. Under the title "The Use of Statistics in Phytosociology", Lambert and Dale deal with the problem of putting the investigation of vegetation on an objective basis by the use of appropriate statistical techniques. The third article, by Bray and Gorham, is a report of their study on litter production in forests of the world. In this they discuss the components of litter, the factors affecting litter-fall and the seasonal changes in the standing crop of leaves. They conclude with a consideration of leaf litter as an index to net production. The final and by far the largest article is by Heslop-Harrison, who reviews the development of concepts and methods in genecology during the preceding forty years.

Without exception the papers are well and attractively written and together constitute an important and stimulating contribution to the modern science of ecology.

K. JONES

Preparation and Bio-Medical Application of Labelled Molecules

Edited by J. Sirchis. (Proceedings of a Symposium sponsored by the Radiation Chemistry and Radioelements Centers of the Universities of Bologna, Padua and Rome; the Italian National Research Council; and the European Atomic Energy Community, Venice, August 23–29, 1964.) Pp. xviii+502. (Brussels: European Atomic Energy Community, 1964.) n.p.

THIS is a compendium of information on the chemistry of isotopic labelling. Procedures are described for labelling specifically with radio-carbon and tritium a wide variety of substances at present of interest. The list includes several α-amino-acids, γ-amino butyric acid, actinomycin, indan, *cis*-octenoic acid and nicotine. Recoil labelling and labelling by heterogeneous exchange on gas chromatographic columns are discussed in several articles, and also side reactions occurring during Wilzbach exchange labelling with tritium. Biosynthetic procedures are described for straight-chain hydrocarbons, terpenes, phytosterols, furocoumarins and plant hormones.

More than half the book is concerned with results of biological application of labelled substances. Winchell *et al.* (Berkeley) contribute an outstanding article on the kinetics of iodine-131 plasma proteins in dog lymph and Pinter (Baltimore) does the same for dog kidneys. Major articles by Tolbert *et al.* (Boulder) and Fish *et al.* (San Francisco) deal with results of metabolic studies based on measuring expired $^{14}CO_2$. Biancho *et al.* (Milan) attempt to correlate haem and globin specific activities, and Benassi and Piccotti (Turin) report on the incorporation of tritiated thymidine into ascites tumour cells. The articles are of a generally high standard, but any thread of continuity throughout the volume is scarcely perceptible.

A. S. MCFARLANE

CURARE SENSITIVITY IN NEONATALLY THYMECTOMIZED MICE

By DR. MICHAEL E. BLAW*, PROF. ROBERT A. GOOD†, and DR. RAYMOND D. A. PETERSON‡

Pediatric Research Laboratories of the Variety Club Heart Hospital, and the Division of Neurology, University of Minnesota, Minneapolis

THE correlation of anatomical changes in the thymus with myasthenia gravis has offered hope for a better understanding of the pathogenesis of this disease. Thymectomy has been beneficial in some myasthenic patients, but its failure to alter the disease consistently has tended to obscure the relationship of the thymus to myasthenia gravis. Strauss[1] found that antibodies directed against the *A*-band of striated muscle exist in many patients with myasthenia gravis, but it has not been possible as yet to reconcile these findings with the apparent physiological or anatomical abnormalities that occur at the myoneural junction. Other stigmata of an 'autoimmune' process, however, have been observed in patients with myasthenia gravis, such as circulating anti-nuclear antibodies, antibodies reacting against heart muscle, and against thymic epithelial cells, in addition to the occasional association of myasthenia with diseases considered to be autoimmune, such as ulcerative colitis, hepatitis and lupus erythematosus[2–5]. Following the demonstration that the thymus played an important part in the development of the immune system, Adner *et al.*[6] investigated the capacity of myasthenic patients to manifest delayed hypersensitivity. They found such patients to be quantitatively deficient in this functional indicator of the thymus-dependent lymphoid tissue[7,8].

The occurrence of transitory myasthenia observed in some babies born of myasthenic mothers has suggested that a substance with an action similar to curare may be circulating in the blood of myasthenic patients. However, transfer studies with sera from myasthenic patients utilizing normal human adults and experimental animals as recipients have not yet revealed such a substance[9].

On the basis of these observations of a close association between myasthenia gravis and (1) histological changes in the thymus; (2) an abnormality in function of the immune system; (3) a circulating curare-like substance in the blood, we postulated that the thymus and the thymus-dependent lymphoid tissue might, indeed, be abnormal in patients with myasthenia and that perhaps neonatally thymectomized mice, also deficient in thymus-dependent lymphoid development[10,11], would be more sensitive to curare. In this view the lymphoid system might have, either as a primary or secondary function, the capacity to detoxify certain natural toxins such as curare.

Albino Swiss mice were thymectomized within 24 h of birth and returned to the mother for suckling. Beginning after 4 weeks of age this strain of mice undergoes a rapid wasting disease following the depletion of the lymphoid tissue produced by neonatal thymectomy[12]. At the time that these investigations were carried out the mice were active and healthy in appearance, although their lymphoid tissues were known to be morphologically and functionally deficient. Between 3 weeks and 4 weeks of age the mice were assayed for sensitivity to curare by assessing the loss of the righting reflex according to the technique of Wolf[13]. *d*-Tubocurarine was injected intraperitoneally and the dose calculated on a μg/kg basis. Each animal was tested for loss of righting reflex every 1–2 min. The animal was placed on its side and if unable to attain the upright position within 15 sec the response was considered positive. All operated animals, whether they had a positive or negative response, were autopsied to determine whether the thymus had been completely removed. Animals with any evidence of incomplete thymectomy were not included in the study. Unoperated 3–4-week-old Swiss albino mice served as controls.

A total of 156 thymectomized and 261 control animals were assayed. The dose at which 50 per cent of the animals would be expected to show a positive response (ED_{50}) was 475 μg/kg in the control group, whereas the ED_{50} was 310 μg/kg in the thymectomized mice. The 99 per cent confidence interval for the control group was 396 μg/kg to 570 μg/kg and 248 μg/kg to 388 μg/kg for the thymectomized animals. The data from this experiment are tabulated in Table 1. Probit analysis of the data by the method of Litchfield and Wilcoxon[14] is shown in Fig. 1. The results are significant beyond the $P = 0{\cdot}01$ level and strongly support the contention that mice which are deficient in their thymic-dependent lymphoid tissue are more sensitive to the myoneural blocking effect of curare than are controls.

Table 1. SENSITIVITY OF NEONATALLY THYMECTOMIZED MICE TO *d*-TUBOCURARINE

Dose (μg/kg)	Controls: No. of animals	Controls: Per cent loss of righting reflex	Neonatally thymectomized: No. of animals	Neonatally thymectomized: Per cent loss of righting reflex
101–150			17	0
151–200			16	25·0
201–250	21	0	12	33 3
251–300	22	13·6	17	23·6
301–350	21	14·3	13	69·2
351–400	20	20·0	13	53·8
401–450	21	42·8	11	72·7
451–500	26	42·4	16	62·5
501–550	22	68 0	10	80·0
551–600	20	70·0	14	78 6
601–650	21	62·0	10	90·0
651–700	20	70·0		
701–750	27	76·0		
751–800	20	95 0		

The increased sensitivity to *d*-tubocurarine manifested by neonatally thymectomized mice might be related to the wasting disease that affects these animals after 4 weeks, although at the time the assays were carried out the animals were healthy in appearance, not lymphopenic, and normally active, though their lymphoid cells and tissues are considered to be abnormal by immunological

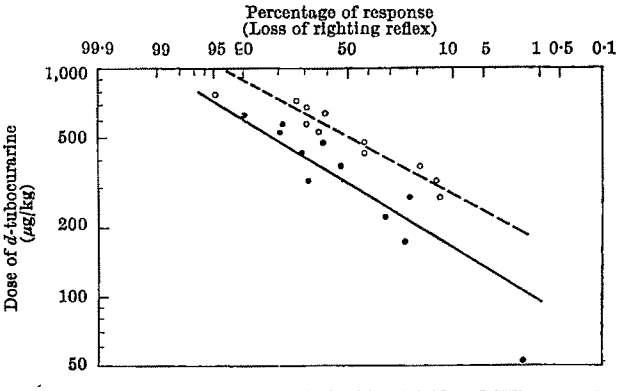


Fig. 1. Probit analysis by the method of Litchfield and Wilcoxon of response to *d*-tubocurarine. ○, Control; ●, thymectomized

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‡ Established investigator, American Heart Association.

criteria[10–12]. Nevertheless, the incipient wasting disease might have some effect on the myoneural junction, a defect not evident clinically but reflected in the extreme vulnerability to curare. This is difficult to rule out entirely.

Pharmacological studies[15–18] on the distribution and excretion of ^{14}C-labelled *d*-tubocurarine dimethyl ether and other forms of curare in normal animals indicate that 90 per cent of an intravenous dose disappears from the blood within 2 min. Within 24 h up to 91 per cent of the material can be recovered in the urine. Most of the excreted curare in the urine is chemically unchanged and still biologically active. Although paralysis may last but 15 min, within the same 15-min period only 10 per cent of the material is found in the urine. The conclusion drawn from these investigations is that curare is rapidly complexed in the body but slowly released and excreted by the kidneys. Observations of the clearance and excretion of radioactively labelled curare by normal and neonatally thymectomized mice are being made to test the hypothesis of a defect in the complexing of curare in the thymectomized animals.

As our investigations relate to the pathogenesis of myasthenia gravis, the initial hypothesis has gained support. In the human baby most evidence suggests that, although the thymic system as measured by its immune function is qualitatively intact, it is quantitatively deficient[19]. The occurrence of transitory myasthenia in babies of myasthenic mothers may depend, at least in part, on this quantitative deficiency of the thymic component of the lymphoid system and the consequent deficient ability of the baby to complex a postulated humoral substance which has crossed the placental barrier from the mother. The alternative, of course, is the more popular view that the mother produces an auto-antibody which damages the myoneural junction or interferes with its function.

In conclusion, these data demonstrate that neonatally thymectomized mice are more sensitive to curare than control mice when assayed by the loss of righting reflex. It is suggested that the thymus and its thymus-dependent lymphoid tissue may function as a system which, in addition to its immune function, acts in detoxification of certain naturally occurring toxins.

We thank Dr. Nelson Goldberg for his help in the statistical analysis.

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A NATURALLY OCCURRING FRAGMENT RELATED TO THE HEAVY CHAINS OF IMMUNOGLOBULIN G IN NORMAL HUMAN URINE

By Dr. M. W. TURNER and Dr. D. S. ROWE

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In recent years there has been considerable progress in the elucidation of structural, metabolic, and antibody features of the immunoglobulins. It is now known that these proteins are comprised of two varieties of polypeptide chains called heavy and light chains[1], and that the heavy chains differ in the four main classes of immunoglobulins—IgG, IgA, IgM and IgD (refs. 2–4). There are two types of light chains, type *K* and type *L*. Both types are common to all classes of immunoglobulins.

Previous investigations on normal human urine have demonstrated free light chains of both types[5,6] as well as intact IgG and IgA[6,7]. The presence of light chains, and their pathological counterparts, the Bence Jones proteins, is important to considerations of immunoglobulin biosynthesis and metabolism, and a demonstration of other fragments would be of similar interest. This article describes the finding of another immunoglobulin component in normal human urine. This component is related to the heavy polypeptide chains of IgG.

Large volumes of urine were collected from two healthy adult males and stored at 4° C in the presence of 0·1 per cent sodium azide. The urine samples were dialysed overnight against running tap-water, centrifuged to remove cellular debris and then lyophilized. The lyophilized products were reconstituted in 0·2 M *tris*-hydrochloric acid buffer (*p*H 8·0) to 1/200 and 1/120 of their original volumes respectively. Insoluble material was removed by high-speed centrifugation. In the analyses to be described both samples yielded similar results.

The reconstituted urine samples were fractionated by gel filtration on columns of 'Sephadex *G*-200' (ref. 8), using 0·2 M *tris*-hydrochloric acid + 0·2 M sodium chloride (*p*H 8·0) buffer for the elution. A representative elution pattern is shown in Fig. 1. Proteins were eluted from fraction 20 to fraction 60. Albumin was eluted in the region of fraction 40. Appropriate fractions were concentrated four-fold by ultra-filtration, and Ouchterlony gel-diffusion analyses[9] were carried out using antisera specific for the polypeptide chains indicated. IgM and IgD were not detected. IgA was detected in several of the early fractions. Fractions from two regions reacted with the antiserum to the γ-heavy chains of IgG. The activity in fractions 30–36 was due to the presence of intact IgG molecules as has been previously demonstrated[7].

These fractions gave a reaction of identity on Ouchterlony analysis with serum IgG. Fractions 51–58 also precipitated with the antiserum. These fractions showed a reaction of partial identity with serum IgG. The antiserum used in these tests was prepared by immunizing rabbits with the heavy chains of IgG isolated by the method of Franěk and Zikán[10]. On Ouchterlony analysis this antiserum reacted strongly with heavy (γ) chain preparations and also weakly with light chain preparations. Before it was used, the antiserum had been absorbed with the Fab fragments of IgG. No reactivity with light chains could then be detected.

Fig. 1 also shows the distribution of the two types of light chains in the separation. It will be noted that both type *K* and type *L* chains were detected in fractions from the same region as the material reacting with the antiserum apparently specific to the γ-heavy chains. Thus it was of importance to establish whether the reactivity with this antiserum was, in fact, due to heavy chain determinants in these fractions or to some hitherto undetected anti-light chain antibodies in the antiserum.

It is clear from the observations of Nachman and Engle[11] that light chains separated from normal pooled human gamma globulin contain antigenic determinants not detectable in the intact native molecule. These hidden determinants can also be demonstrated in Bence Jones proteins. Antibodies to such determinants could have been present in the anti-heavy chain antiserum used in this investigation, and might not have been completely removed by absorption with Fab. To eliminate this possibility, two further absorptions of the antiserum were carried out. The original antiserum was absorbed to slight antigen excess by a light chain preparation from normal IgG, and by a mixture of type *K* and type *L* Bence Jones proteins. Neither of these absorptions affected the reactivity of the antiserum with the urinary proteins. It is still precipitated with fractions 30–36 and 51–58. The protein in these latter fractions was again found to be antigenically deficient as compared with intact IgG (Fig. 2).

These findings imply that the low molecular weight protein in fractions 51–58 was antigenically deficient not only with respect to intact IgG, but also with respect to the γ-heavy chains of IgG. Ouchterlony analysis (Fig. 3)

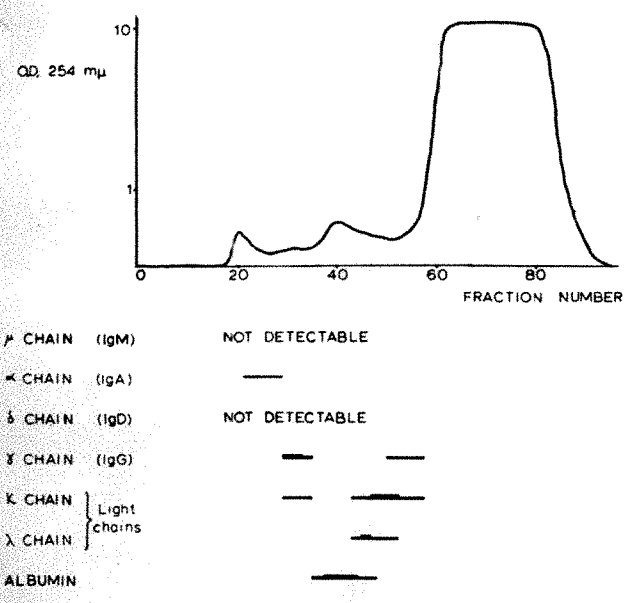


Fig. 1. Elution pattern of gel-filtration of a normal urine concentrate on 'Sephadex G-200'. Optical densities were measured at 254 mμ by a recording spectrophotometer. The final large peak did not contain protein. Shown below as horizontal bars are the distribution ranges of fractions precipitating on Ouchterlony analysis with antisera specific to the indicated immunoglobulin polypeptide chains, and to serum albumin

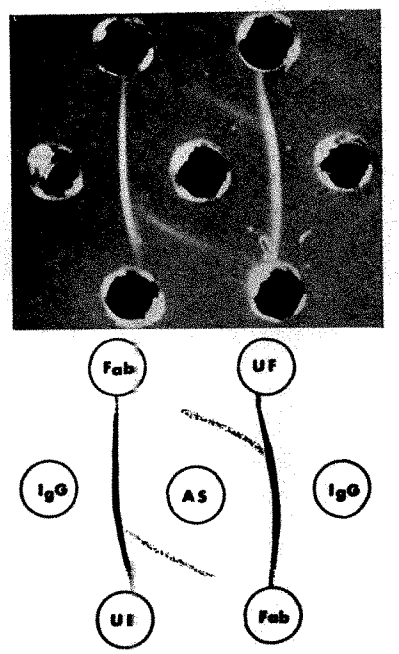


Fig. 2. Ouchterlony analysis showing reaction of partial identity between the low molecular weight urine fractions 51–59 (*UF*) and IgG (1 mg/ml.). The antiserum (*AS*) was specific for γ-heavy chains and failed to react 4 mg/ml., chromium concentration 0·1 mg/ml

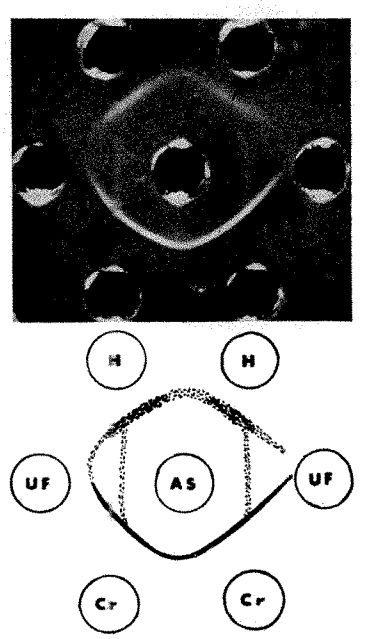


Fig. 3. Ouchterlony analysis showing reaction of partial identity between the low molecular weight urine fractions 51–58 (*UF*) and heavy chains (*H*) prepared from serum IgG. A similar relationship is seen to the abnormal protein (*Cr*) from a patient with heavy-chain disease. The antiserum (*AS*) was specific to γ-heavy chains. Heavy-chain concentration 4 mg/ml., chromium concentration 0·1 mg/ml.

showed that this was the case; a γ-chain preparation formed a clear spur over the low molecular weight urine protein.

Franklin, Lowenstein, Bigelow and Meltzer[12] have described a plasma cell dyscrasia in which there was an excessive production of protein related to γ-heavy chains. It has been shown[13] that this material is not the complete heavy chain, but is similar to the Fc fragment produced by papain digestion of IgG. It was possible that the low molecular weight urine protein was a normal counterpart of this pathological protein. However, the Ouchterlony analysis shown in Fig. 3 excludes this possibility. Using

the antiserum specific to heavy chains, the low molecular weight urine protein was antigenically deficient with respect to the pathological 'heavy chain' protein (Cr) (kindly supplied by Dr. Franklin).

The low molecular weight urine protein was then considered in relation to the fragments of IgG produced by papain digestion. Papain in the presence of cysteine yields two fragments, Fab and Fc, distinguishable by immunoelectrophoresis (Fig. 4). The relationship of these fragments to the peptide chains of the molecule has been elucidated by Cohen[14]. Fab contains the light chains and part of the γ-heavy chains; Fc contains the remainder of the γ-chains, and its antigenic determinants are specific to the γ-chains. Thus, antisera which are Fc specific are also γ-chain specific. The previous results suggest that the low molecular weight urine protein is related but antigenically deficient with respect to Fc.

Using appropriate antisera papain digests of IgG can be shown to contain an additional component which is antigenically deficient with respect to Fc. This fragment has been previously termed F′ (ref. 15) but will here be referred to as Fc′ being consistent with current immunoglobulin nomenclature[3]. Fc′ is distinguished by its anodal mobility on immunoelectrophoresis (Fig. 4).

The relationship between the low molecular weight urinary protein and Fc′ has been determined by comparative immunoelectrophoresis (Fig. 4). These two components appear to be antigenically identical. They are also identical with respect to electrophoretic mobility (Fig. 5). This figure shows that the urinary light chains, although eluted in the same 'Sephadex' fractions, are clearly distinct from Fc′ by their electrophoretic mobility as well as by their antigenic determinants. This indicates that the Fc′ determinants and the light chain determinants are present on different molecules in the urine. No fragments comparable in their electrophoretic mobility and antigenicity with Fab or Fc components were detected in any urine fraction.

Various low molecular weight γ-globulins in plasma and urine have recently been described[16–18]. These proteins, which migrate in the γ-globulin electrophoretic region, are clearly distinct from Fc′ which is of α-globulin mobility. In addition fragments antigenically related to Fc- and γ-chains have been described[6], but these were not identified as Fc′.

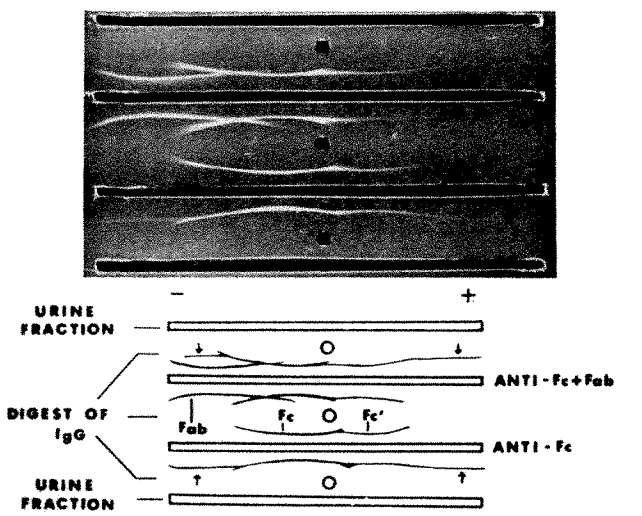


Fig. 4. Comparative immunoelectrophoresis. The three centre wells were filled with papain digest of IgG and electrophoresis was performed. Antisera were then placed in the centre troughs and the low molecular weight urine fractions 51–58 in the outer troughs. The antisera reacted with the urine fractions to produce straight lines indicated by the arrows. These lines fuse with the Fc′ line of the papain digest (antigenic identity), partially fuse with the Fc line (partial identity) and are independent of the Fab line (non-identity)

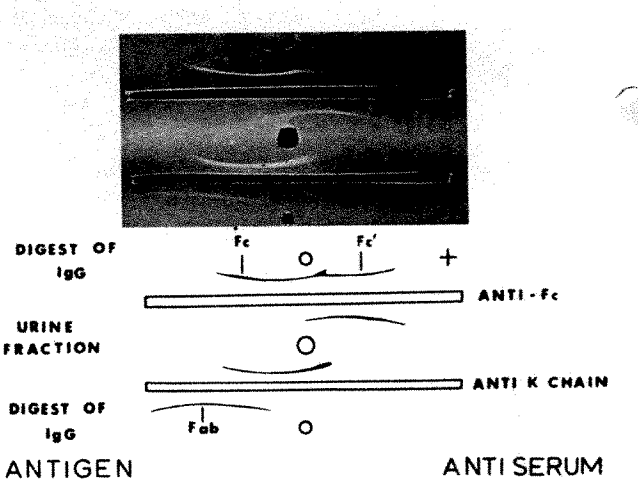


Fig. 5. Immunoelectrophoretic analyses showing that the electrophoretic mobilities of Fc′ from urine and Fc′ in a papain digest of IgG are identical. However, Fc′ differs in its electrophoretic mobility from urinary light chains in the same urine fractions as revealed by the antiserum to kappa light chains. There is no evidence of material with the antigenic and electrophoretic properties of Fc or Fab in the urine fraction

In a study of 'heavy chain disease' Takatsuki and Osserman[19] demonstrated Fc′ as a constituent of the abnormal proteins from four cases, but Fc′ was not detected in the fifth. In a study of myeloma proteins the same authors had similar findings, G-myeloma proteins of class *C* showed Fc′ after treatment with papain and cysteine, whereas in G-myeloma proteins of class *Z* Fc′ were not detectable. Fc′ may therefore be part of some IgG molecules, but not of others.

The Fc′ of normal urine may represent an independently synthesized chain, comparable with light chains. Such material might be present in the plasma and readily excreted in the urine on account of its low molecular weight; it might also be found in larger amounts in serum and urine of patients with certain disorders of immunoglobulin synthesis. Alternatively, Fc′ may be the result of enzymatic degradation of some IgG molecules present in urine. Poulik and Schuster[20] have shown that Fc′ is not produced from IgG by digestion by papain alone, but requires in addition the presence of cysteine. Reducing agents which may be present in urine might, in conjunction with proteolytic enzymes, lead to the separation of Fc′. However, it was notable that other fragments of enzymatic degradation, such as Fc and Fab, were not detected in these studies.

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F'c FRAGMENT OF IMMUNOGLOBULINS

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PAPAIN digestion of IgG immunoglobulins yields three main fragments which can be separated by chromatography[1-4]. Two different immunological determinants were localized on the Fab and Fc fragments and afterwards related to the *L* and *H* polypeptide chains of the immunoglobulins[5,6]. The presence of an additional non-dialysable fragment (F') was detected with some antisera[2,7,8]. Starch-gel electrophoresis of the papain digests revealed a marked heterogeneity of the Fc fragments[9-11] of IgG immunoglobulins and IgG myeloma globulins. A group of fast migrating components was found in such digests which were designated as F'c components[12,13]. Isolation and characterization of these components were therefore undertaken.

Cohn fraction-II (Fr-II), IgG gamma globulins of single individuals, γG and γA myeloma globulins as well as free urinary *H*-chain[14] (generous gift of Dr. E. C. Franklin) were digested in papain according to my earlier method[9]. Trypsin digests were also prepared according to Schrohenloher[15]. Papain digests of γ M and cryomacroglobulins as well as of IgG globulins of chimpanzees, rhesus monkeys and slow loris monkeys were also studied.

Agar-gel electrophoresis and immunoelectrophoresis patterns of representative digests are shown in Fig. 1. The F'c component represents only a small portion of the digests as shown by staining (Fig. 1*a*). The precipitin lines developed with several antisera containing anti-Fc and anti-F'c antibodies indicate that the F'c fragment is deficient in at least one antigenic determinant with respect to the Fc fragment (Fig. 1*c*, *d*, *e* and *f*) and the spur formed is less defined in the case of the free *H*-chain (Fig. 1*g* and *h*). The F'c fragment produced by trypsin digestion migrates with lesser mobility than the papain-F'c fragment (Fig. 1*d* and *h*).

Starch-gel electrophoretic and immunoelectrophoretic analysis identified the F'c fragment as migrating immediately behind the 'brown-line' in the discontinuous system of buffers[16]. Three components were readily separated (Fig. 2*A*, *D* and *E*). The fastest of the components is present in the lowest concentration and the slowest in the highest concentration (Fig. 2*A*, *D* and *E*). A fourth, slowly migrating component can be demonstrated when isolated F'c components are analysed at higher concentrations (4–5 per cent). The F'c components obtained by digestion with trypsin differ in mobilities and amounts from those present in papain digests; the free '*H*-chain' of Franklin (Cr type) provides excellent material for such studies (Fig. 2*F*). F'c components were not demonstrated in papain or trypsin digests of γ A myelomas (Fig. 2*B*, *C*), γ M globulins and cryomacroglobulins, but were demonstrated in IgG globulins of the non-human primates. Despite immunological cross-reactivity between the F'c of human and chimpanzee, the electrophoretic mobility of the chimpanzee F'c was considerably less than that derived from human IgG.

Two methods were used to isolate the F'c fragment. The F'c components, fast migrating sub-components of the Fc fragment and a portion of the undigested IgG globulins are not adsorbed on carboxymethyl-cellulose when 0·01 M acetate buffer, *p*H 5·4, is used. This unadsorbed fraction, when rechromatographed on DEAE-cellulose (0·05 M phosphate buffer, *p*H 7·8, followed by 0·2 M phosphate buffer, *p*H 7·8), affords three peaks and

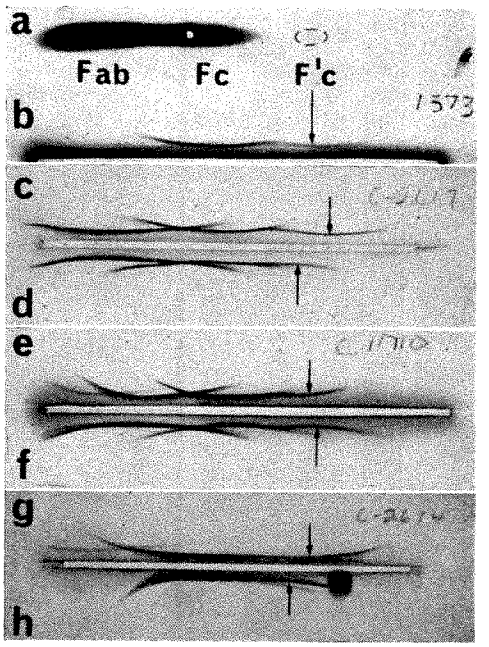


Fig. 1. Agar-gel electrophoresis and immunoelectrophoresis of papain and trypsin digested IgG immunoglobulins. (*a*) Agar-gel electrophoresis of Cohn Fr-II-papain digested stained with amido black 10*B*; (*b–h*) agar-gel immunoelectrophoresis of papain and trypsin digested Cohn Fr-II, γ G myeloma globulins and urinary free *H*-chain (Franklin), (*b*, *c*, *e*, *f* and *g*) papain digested; (*d*, *h*) trypsin digested. Protein: (*b*, *c* and *d*) Fr-II; (*e*) γ G myeloma CA; (*f*) γ G myeloma TA; (*g* and *h*) free urinary *H*-chain. Antisera contain antibodies against the Fab, Fc and F'c fragments. Arrows indicate the position of the F'c fragments

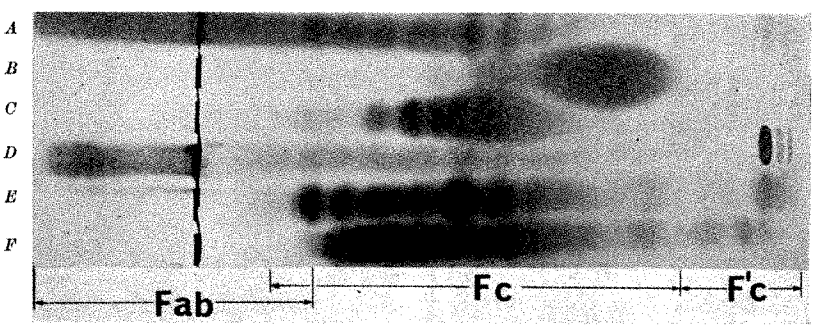


Fig. 2. [Starch-gel electrophoresis of digests of immunoglobulins. Papain digests: *A*, Fr-II; *B*, γ A myeloma LA; *C*, γ A myeloma BR; *D*, γ G myeloma CA; *E*, free *H*-chain. Trypsin digest: *F*, free *H*-chain

pure F'c fragment is present in the tail portion of the third peak. Kunkel's starch-block electrophoresis[17] is an alternative method to obtain the F'c fragment in pure state (barbital buffer). Ultracentrifugal analysis of the pure F'c fragment (obtained by either method) has shown that this fragment sediments much slower (2·1*S*) than the Fc fragment (3·5*S*). This fragment constitutes about 10 per cent of the intact molecule. With Fr-II digested for 18 h tests for the presence of Gm groups are so far inconclusive.

Mode of production of the F'c was also studied. Human Fr-II was digested with papain in the presence and absence of cysteine for 18 h. The F'c fragment was not produced in the absence of cysteine as shown in Fig. 3*G'*. After this period of time the sample lacking cysteine (*G'*) was divided into two parts and to one part was added cysteine (final concentration 0·01 M) (Fig. 3*a*) and to the other was added fresh papain and cysteine (final concentration of cysteine 0·01 M) (Fig. 3*b*). The mixtures were incubated at 37° C and samples were taken at intervals for immunoelectrophoretic analysis. F'c component was detectable after 2 h (Fig. 3*b*, 2 h) and fully developed after 24 h of incubation (Fig. 3*a* and *b*, 24 h). Thus cysteine in addition to papain is necessary to produce the F'c fragment.

Little attention has been given in the past to F'c fragment in papain digests. This failure is primarily due to its low concentration and the need of special antisera for its detection. Consequently, it is not accounted for in the present models of the gamma globulin molecule.

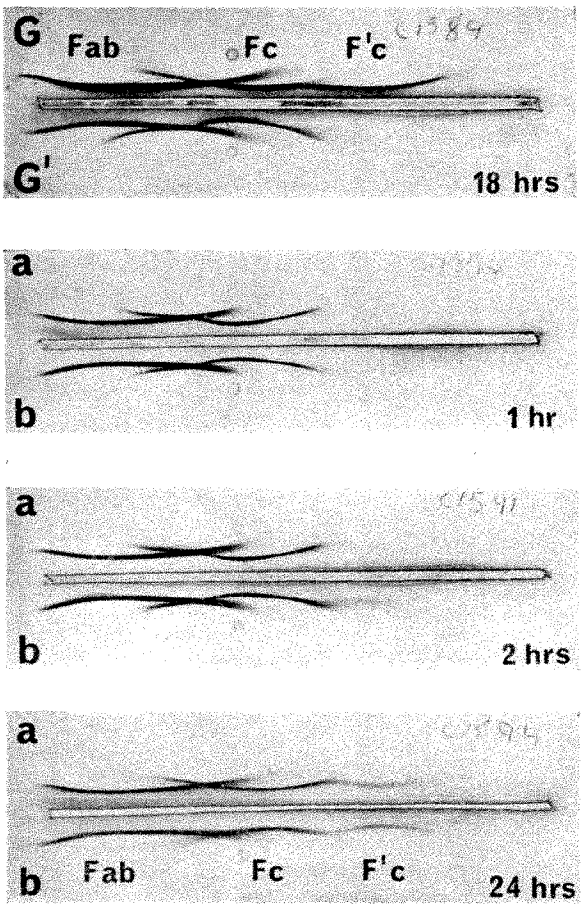


Fig. 3. Immunoelectrophoretic patterns demonstrating the role of cysteine in the development of F'c fragment during papain digestion of Cohn Fr-II. *G*, Digestion in the presence of cysteine, 18 h; *G'*, digestion in the absence of cysteine, 18 h; *a*, cysteine added to *G'*; *b*, cysteine and papain added to G'. Antiserum contains antibodies against Fab, Fc and F'c fragments

The findings presented here show that F'c is derived from the Fc fragment. This notion is supported by the release of this fragment after papain digestion of the free urinary *H*-chain. Since the latter lacks the *A* piece, and consequently a portion of the *H*-chain, this possible source of the F'c fragment can be eliminated. It is generally assumed that papain cleaves peptide bonds near the middle of the *H*-chain. This reaction is probably coupled with reduction of an inter-*H*-chain disulphide bond by the reagents used for activation of the papain. Because there is perhaps only one such bond[18] the prospective region of the *H*-chain from which the F'c fragment may be derived can be anticipated. This region may have a unique amino-acid sequence since the Fc fragment formed contains from nine to eleven separable sub-components[9]. These are of about equal molecular weight, but differ significantly with respect to charge. Their number, mobilities and concentration of each are identical in IgG immunoglobulin and all γ G myeloma so far tested. The simplest explanation of this type of heterogeneity would be by process of sequential cleavage. All these sub-components carry a common antigenic determinant and are structurally complex as previously shown[9,19]. Since the F'c fragment is also heterogeneous (four components) a common origin of both fragments can be envisaged. It has been demonstrated that the Fc fragment obtained by papain alone (in the absence of cysteine) can be separated into four sub-components which migrate with different mobilities than those obtained by digestion with papain in the presence of cysteine[13]. Furthermore, F'c fragment is not formed (compare Fig. 3) under identical conditions in the absence of cysteine[13]. Thus it is feasible that the sub-components produced by papain in the absence of cysteine still contain at least one disulphide bond. When such is reduced, the F'c fragments are formed, the remaining portions of the sub-components rendered more susceptible to papain and additional sub-components derived. A search for the SH-groups of the F'c fragment should be profitable provided it is not destroyed during the reactions.

The F'c fragment carries at least one antigenic determinant in common with the Fc fragment. The fragment is immunologically active even after reductive cleavage in the presence of urea and can be further separated into a number of components in urea starch-gel. The biological functions have not been investigated as yet. As already mentioned, testing for the presence of Gm groups proved disappointing. This may be attributed to the length of time of digestion (18 h). Further tests are under way. The low sedimentation value and lack of hexose provide further evidence that F'c fragment is a definite entity.

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USE OF CHARGED THIOL REAGENTS IN INTERPRETING THE ELECTROPHORETIC PATTERNS OF IMMUNE GLOBULIN CHAINS AND FRAGMENTS

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THE immune globulins of serum consist of light and heavy polypeptide chains[1,2]. When the light chains are separated and examined by electrophoresis in alkaline starch-gel containing 8 M urea, they form a pattern of equally spaced bands[3] as shown in Fig. 1*a*, *h*. Bence Jones proteins, which appear in the urine of patients with multiple myeloma, are known to be relatively homogeneous light chains[4] and each in electrophoresis forms mainly a single band, corresponding to one or other of the normal light-chain bands. Considerable differences in amino-acid sequence have been found (within a stretch of the N-terminal half) between different Bence Jones proteins[5–7]. This suggests a simple explanation for the origin of the banding patterns of the light chains. Variation in the proportion of basic and acidic residues of light chains will result in variation in electrophoretic mobility. If light chains in the electrophoresis medium are of almost identical size and shape, chains with identical net charge should travel as a single band, even where they differ in amino-acid composition. Thus, even a very heterogeneous population of chains would yield only a limited number of well-defined bands in electrophoresis, if variations in residues partially ionized at the gel *p*H are relatively infrequent. This explanation predicts that chains in neighbouring bands will only differ by a single charge. This can be tested, because if a single carboxyl group can be added to a light chain it should alter its electrophoretic pattern by a single band spacing. In the following experiment, such a shift was in fact observed.

The charge increment was added to several Bence Jones proteins by carboxymethylation of a cysteine residue with iodoacetate. It was necessary first to reduce them partially with 2-mercaptoethanol as Bence Jones proteins exist partly as disulphide-bonded dimers[8], and even unreduced monomers have a cysteine residue blocked with a lone half-cystine[9]. After treatment with 0·15 M 2-mercaptoethanol for 1 h in *p*H 8·2 *tris* buffer, part of each preparation was then made 0·3 M in iodoacetate, and the remainder was made 0·25 M in iodoacetamide. After 3 h at 0° C, the preparations were dialysed against cold saline and then examined by electrophoresis in starch-gel containing glycine buffer, *p*H 8·5, 0·05 M with 8 M urea. In Fig. 1 comparison of the relative positions of the two preparations of a type *K* Bence Jones protein (Rad.), *b* and *d*, with normal light chains, *a* and *h*, shows that carboxymethylation has altered the position of the main band and faint neighbouring bands by a single-band spacing. The extent of carboxymethylation was confirmed by estimating in a Spinco amino-acid analyser the amount of carboxymethyl-cysteine liberated after hydrolysis for 24 h in 6 N hydrochloric acid at 106° C *in vacuo*. 1·02 moles of carboxymethyl-cysteine were recovered per 20,000 g, and 3·5 half-cystine residues were also recovered. Thus a single charge per chain had been introduced, resulting in a mobility shift of a single-band spacing. Confirmation of the nature of light chain banding was obtained by introducing carboxyl groups randomly into Bence Jones proteins by prolonged treatment with iodoacetate at *p*H 10 after reduction. Treated thus, Rad. generated a pattern of bands, Fig. 1*c*, perfectly mimicking those of normal light chains.

Among the type *L* proteins tested was one (Pet.) which altered its position after carboxymethylation by two spacings (Fig. 1*e* and *g*) suggesting that two cysteine residues per chain had been carboxymethylated. Gel-filtration in 1 M propionic acid in a 'Sephadex *G*-100' column showed that 75–80 per cent of Pet. chains exist as disulphide-bonded dimers. The remaining monomer chains also have one sulphydryl radical which cannot be titrated due to the fact that it is linked to a half-cystine[9]. Yet by spectrophotometric titration of Pet. with *p*-chloromercuribenzoate before reduction, a further 0·8 sulphydryl group per chain was estimated. No such titratable groups were found in any of the other Bence Jones proteins examined. This suggests that Pet. chains must have two cysteine residues which are not involved in intra-chain bonds, as compared with the single cysteine residue more common in light chains. To confirm this, a sample of Pet. was treated with iodoacetate to charge the free sulphydryl group, then with 2-mercaptoethanol and iodoacetamide to liberate and to block the second sulphydryl group (Fig. 2*c*). With a second sample the sequence of iodoacetate and iodoacetamide treatments was reversed to charge only the sulphydryl group released after reduction (Fig. 2*a*). The effect was, as predicted, to

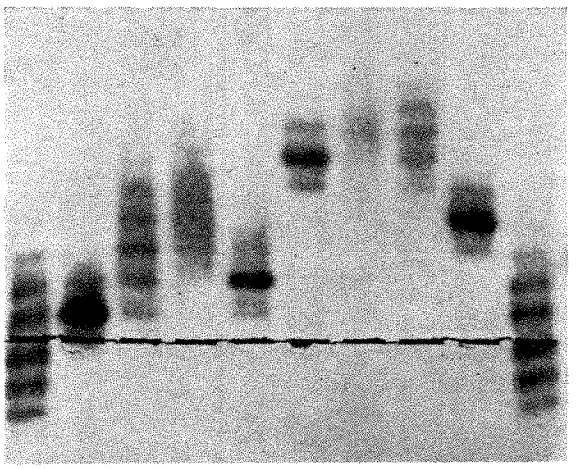


Fig. 1. Urea starch-gel patterns: (*a* and *h*) normal human light chains; (*b*) Rad. reduced and iodoacetamide treated; (*c*) Rad. randomly carboxymethylated; (*d*) Rad. reduced and iodoacetate treated; (*e*) Pet. reduced and iodoacetate treated; (*f*) Pet. randomly carboxymethylated; (*g*) Pet. reduced and iodoacetamide treated

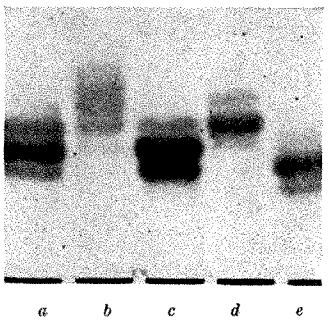


Fig. 2. Comparison of treated Pet. samples: (*a*) iodoacetamide treated, reduced and iodoacetate treated; (*b*) randomly carboxymethylated; (*c*) iodoacetate treated, reduced and iodoacetamide treated; (*d*) reduced and iodoacetate treated; (*e*) reduced and iodoacetamide treated

produce in each case a shift of only a single spacing in the pattern. The earlier pair of preparations demonstrating the full double shift is shown in Fig. 2*d* and *e* for comparison. It can be seen that part of the sample (in the slowest band of Fig. 2*c*) has failed to alter its behaviour after treatment with iodoacetate. Thus some Pet. chains have no reactive sulphydryl groups before reduction, as had, in fact, been indicated by the *p*-chloromercuribenzoate titration. Two observations suggest that these are dimer chains. Milstein found that after performic oxidation of

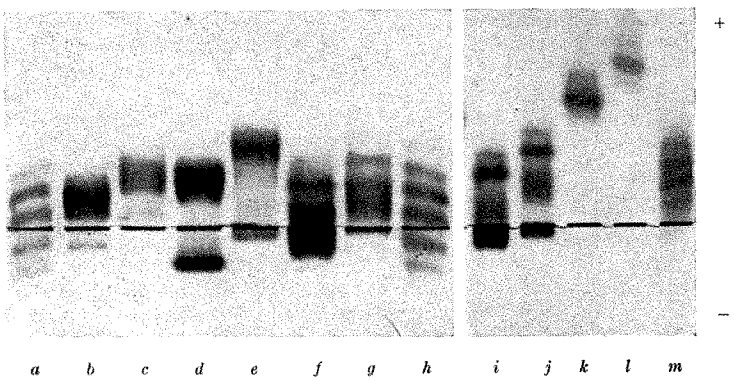


Fig. 3. Effect of carboxymethylation on patterns of myeloma chains: (*a*, *h* and *m*) normal human light chains. IgM Hu. reduced and (*b*) iodoacetamide treated; (*c*) iodoacetate treated. IgM Ma. reduced and (*d*) iodoacetamide treated; (*e*) iodoacetate treated. IgG Tu. reduced and (*f*) iodoacetamide treated; (*g*) iodoacetate treated. IgG Wr. reduced and (*i*) iodoacetamide treated; (*j*) iodoacetate treated. Bence Jones Pet. reduced and (*k*) iodoacetamide treated; (*l*) iodoacetate treated. Lower bands in IgM patterns and upper bands in IgG patterns are light chains

bonded Pet. dimers, 0·24 residues of cysteic acid were released per chain[9] (Milstein referred to Pet. as 'X'). Also, investigations at present being carried out to locate the free sulphydryl group, using radio-labelled iodoacetate, indicate less labelling of dimer than of monomer chains.

One of the advantages of the aforementioned method of estimating sulphydryl groups is that, in heterogeneous systems, each component which can be electrophoretically resolved can be titrated separately without previous purification. Other methods tend only to give a mean value. In this way the method has proved useful in looking at the constituent chains of myeloma proteins. In Fig. 3 are shown examples of two macroglobulin (IgM) and two IgG preparations. Each preparation had been reduced and treated with iodoacetamide or iodoacetate, as described earlier. In each case the light chain band was moved forward by a single spacing, indicating the release of a single light-chain sulphydryl group by reductive cleavage. No light chains released from the immune globulins examined so far have exhibited a multiple shift resembling that of Bence Jones Pet.

In those samples where the heavy-chain sulphydryl groups released by reduction were carboxymethylated, these heavy chains also, of course, differed in mobility from the corresponding chains treated with iodoacetamide (Fig. 3). When milder conditions of carboxymethylation were used (Fig. 4 ii) employing 0·2 M iodoacetate for 1 h, although all heavy chains reacted, as judged by their altered position, the light chains were only partially carboxymethylated. This relatively slow reaction of the light chain cysteine may result from charge repulsion due to its being, or neighbouring, the C terminal residue[10]. These conditions were also inadequate for Bence Jones protein carboxymethylation, and were replaced by the more vigorous treatment described earlier.

Fortunately in some cases the heavy chains were resolved into banded patterns. We have seen such banding in IgA, IgG and IgM heavy chains from myeloma proteins. In Fig. 4 (i) the patterns of heavy-chain preparations from one fast IgG (Re.) and two slow IgG (Tu. and Wr.) myeloma proteins all resolve into bands. Neighbouring bands are separated by rather less than half the light chain spacing. Normal human IgG heavy-chain preparations do not resolve into bands, but those of heavy chains isolated from slow bovine IgG, seen in Fig. 4 (iii), are similarly spaced relative to bovine light chains.

In Fig. 4 (ii) the first two patterns are of reduced macroglobulin. Here the heavy-chain bands are evident, and their spacing is even closer than that of IgG heavy chains, being a third of the light-chain spacing. The effect of iodoacetate has been to preserve the band pattern, but move it forward four spacings. Since similar treatment has been shown to carboxymethylate four cysteines per IgM heavy chain[11,12], this strongly suggests that heavy chains in neighbouring bands, like light chains, differ by a single electronic charge. This would be consistent with the fact that the spacings of light chains and heavy IgG and IgM chains are in inverse relation to their molecular weights, which are respectively approximately 20,000, 50,000 and 70,000 (refs. 12–15). Support for this relation comes from examining the Fab fragment of IgG (ref. 16), with a molecular weight of 42,000 (ref. 13), formed by treatment with papain. This important fragment carries in antibodies a single antigen-binding site. Composed of a light chain with a molecular weight of 20,000, and part of the heavy chain (Fd fragment)[17], with molecular weight of 22,000, its band spacing resulting from net single charge differences should be half that of light chains. Reductive cleavage to light chains and Fd fragments should then convert the pattern to normal light-chain spacing. That this change is in fact observed may be seen in Fig. 5 (i), where the patterns of three bovine Fab preparations from different IgG components are shown before and after reduction. Rabbit Fab preparations also form band patterns[18], and it is remarkable that they have a similar spacing to light chains. That is to say, each fragment with a molecular weight of 40,000 must differ from that in a neighbouring band by two charges, resembling in this the behaviour of symmetrical light-chain dimers. This could only result from allied variations in both light chain and the Fd region of the heavy chain, restricted in both chains of each molecule to those having parallel effects on mobility. Evidence that the more acidic rabbit Fab fragments yield more acidic Fd fragments and light chains has been presented in an earlier communication[18]. It is not clear what biosynthetic mechanism could lead to such parallel variations, and it is clearly important to confirm the observation in other species. A similar preliminary finding for chromatographically purified human Fab fragments from the two slow IgG myeloma proteins Tu. and Wr. is shown in Fig. 5 (ii). As in the rabbit,

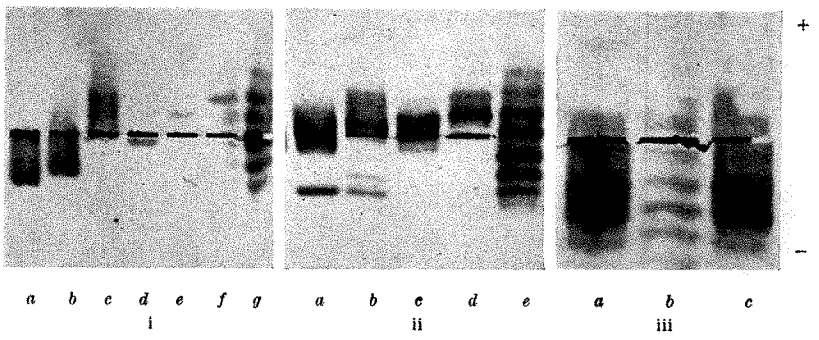


Fig. 4. Electrophoretic patterns showing heavy chain bands. (i) Separated human chains: (*a*) Wr. heavy chain; (*b*) Tu. heavy chain; (*c*) Re. heavy chain; (*d*) Wr. light chain; (*e*) Tu. light chain; (*f*) Re. light chain; (*g*) normal human light chains. (ii) Reduced myeloma proteins: IgM Ma. reduced and (*a*) iodoacetamide treated; (*b*) iodoacetate treated. IgG Re. reduced and (*c*) iodoacetamide treated; (*d*) iodoacetate treated; (*e*) normal human light chains. (iii) Separated chains of bovine IgG2: (*a* and *c*) heavy chain; (*b*) light chain

reduced Fab fragments from slow human IgG globulins resolve electrophoretically into a more acidic light chain, and a less acidic Fd heavy-chain fragment. In the two myeloma patterns shown, the lowest bands are Fd, the upper bands light chains, and some remaining intact Fab is detectable midway between them. The different positions of the relatively homogeneous Fd bands confirm that the Fd region is truly variable, rather than subject to variable papain splitting. Considering the many alternative pairs of Fd fragments and light chains which might in principle have combined to form Fab fragments having the observed mobility difference, the parallelism in fact found here in mobility difference between Fab fragments, light chains and Fd fragments is interesting. No general conclusion can, however, be drawn until many more reduced Fab preparations from the same heavy-chain sub-class have been compared.

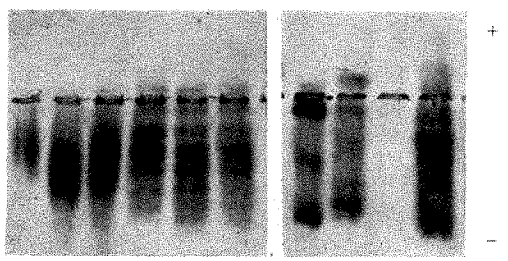


Fig. 5. Reduction of bovine and human Fab fragments. (i) Bovine Fab patterns: (*a*) Preparation from IgG2 and (*d*) same after reduction and iodoacetamide treatment; (*b*) preparation from slow IgG1 and (*e*) same after reduction and iodoacetamide treatment; (*c*) preparation from fast IgG1 and (*f*) same after reduction and iodoacetamide treatment. (ii) Human Fab preparations reduced and iodoacetamide treated: (*a*) Wr.; (*b*) Tu.; (*c*) normal IgG. Lowest bands are Fd fragments, upper bands are light chains. Fd fragments of (*c*) form diffuse unbanded zone

More recently we have been using electrophoresis in agar-gel cooled with petroleum ether[19] to measure mobility changes. This has the advantage of yielding results in an hour, and requiring very little material. For example, it was possible to confirm the titration of Pet. with *p*-chloromercuribenzoate by following the mobility change, using a total of only 10 μl. of 1 per cent Pet. solution. Analytical ultracentrifugation showed that the Bence Jones proteins studied travel as dimers in the aqueous buffer used in the agar-gel. Rad. dimers begin to dissociate at concentrations of less than 0·2 per cent, and the dissociation curves of the native protein and of reduced Rad. treated with iodoacetate or iodoacetamide are almost identical. This suggests that although cysteines that are capable of reacting with the carboxymethyl radical can come sufficiently close to form a disulphide bridge, they are so placed away from the hydrophobic surfaces involved in dimerization that, following carboxymethylation, no forces which could make the dimer unstable are introduced with the ionized groups. The mobility relationships observed for reduced Bence Jones protein in urea starch-gels hold also in aqueous buffers in agar gel. Fig. 6 shows, for example, the agar-gel patterns of Pet. and Rad. separated by three times the spacing distance observed for a single charge increase per chain in carboxymethylated Rad., just as they were in urea starch-gel. This implies that no charged residues become exposed when passing from the folded dimers in agar-gel to the monomer[18] partially unfolded in 8 M urea starch-gel. This concurs with the finding that buried ionized side-chains are absent from the models of myoglobin, lysozyme and all the known haemoglobin variants[14,15].

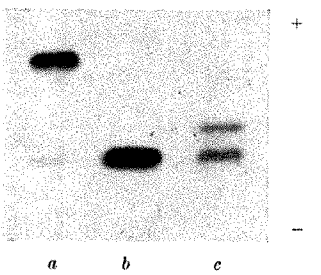


Fig. 6. Agar-gel electrophoretic patterns of Bence Jones proteins. Reduced and iodoacetamide treated: (*a*) Pet.; (*b*) Rad.; (*c*) Rad. reduced and partially iodoacetate treated

I thank Dr. D'A. Kok and Dr. P. Lachmann for samples of urine and serum, and J. Herbert for technical assistance.

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TRANSPLANTATION IMMUNITY: LOCALIZATION IN MOUSE SERUM OF ANTIBODIES RESPONSIBLE FOR HAEMAGGLUTINATION, CYTOTOXICITY AND ENHANCEMENT

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IMMUNE reactions to transplantation antigens result in several effects on cells bearing the corresponding antigens. Since Gorer developed, with Mikulska, the well-known immune haemagglutination test in 1954[1], a great deal of work has been done to detect various antibody responses in mice exposed to allogeneic antigens. Mouse antisera reacting specifically with transplantation antigens have been further studied for their cytotoxic effect, in the presence of complement, on lymphoid and tumoral cells from the immunizing strain[2]. Another biological effect is known, in the field of transplantation, to be produced by specific immune sera on tumoral[3] and even normal[4] tissues—the enhancement or facilitation phenomenon.

In this preliminary work, we have attempted to ascertain whether these three immunological effects on target cells bearing the corresponding transplantation antigens are attributable to one and the same immunoglobulin acting under different conditions, or if two or more immunoglobulins are involved. In the latter case, it would be relevant to know whether each one is responsible for one of the three biological effects.

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Sixty female mice of the B_{10}–D_2 line were immunized by a course of injections with A/Jax spleen cells and their serum pooled after the last booster. The pool was divided into two parts: one was investigated without further modification; the second was fractionated by separative electrophoresis into twenty-six fractions. Haemagglutination of A/Jax erythrocytes and cytotoxicity against A/Jax spleen cells were studied both for the complete serum and for the individual electrophoretic fractions. Haemagglutination titre was expressed as the last dilution giving a clear positive agglutination. Cytotoxic power was expressed as the percentage of cells unable to exclude trypan blue after standard incubation with fractions diluted 1/1 and 1/2, plus guinea-pig complement; a total of one hundred cells was counted for each dilution.

Because of the limited amounts, fractions were paired and eight of the 'double fractions' of different electrophoretic mobility were assayed for enhancing properties in eight groups of mice: a volume of 0·1 ml. of complete serum or of serum fractions was injected intravenously into B_{10}–D_2 males in order to test passive enhancement of sarcoma I.

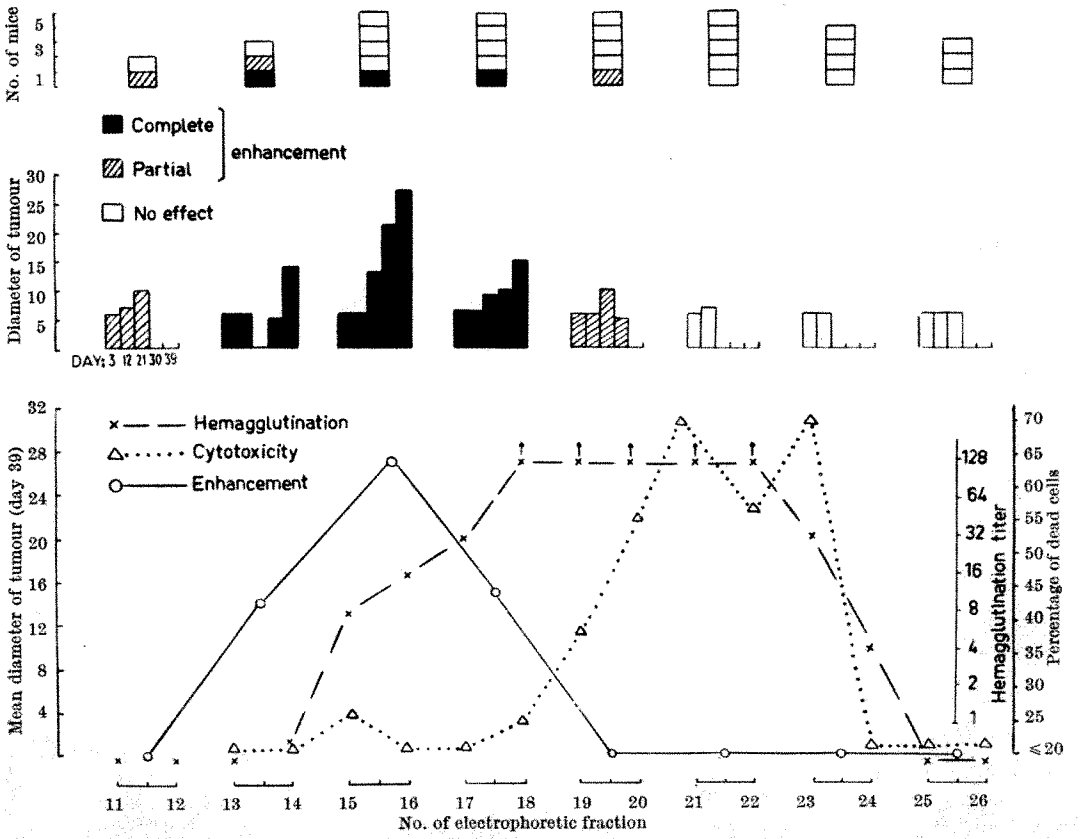


Fig. 1. Biological properties of antibodies: electrophoretic separation. Fractions were examined individually for haemagglutination and cytotoxicity; they were pooled in pairs for the study of enhancing properties. 'Complete' enhancement of a tumour indicates that it resulted in the death of the recipient allogeneic mouse; 'partial' enhancement indicates a tumour of maximum diameter ⩾9 mm; 'no effect' indicates tumours with a maximum diameter ⩽7 mm. The mean diameter of the most conspicuous tumour was taken in each group on days 3, 12, 21, 30 and 39. The mean diameter on day 39 was used for the evaluation of the degree of enhancement because three 'enhanced' mice died (from the tumour) after this period of time. In cytotoxicity tests, the percentage of dead cells in each preparation was taken as an expression of the intensity of cytotoxicity; the base line of 20 per cent is superior or equal to controls and to the inactive fractions

Twenty-four hours later, these mice received a single subcutaneous injection of 10^7 ascites sarcoma I cells, carried by the indigenous *A/Jax* strain. In order to localize the different immunoglobulins in the sequence of fractions an immunoelectrophoretic study of the paired fractions was undertaken with rabbit antisera giving specific precipitation lines with four mouse immunoglobulins: IgG, IgM, IgA and IgY (refs. 5 and 6).

The intact, non-fractionated pool of B_{10}–D_2 anti-A sera had a haemagglutination titre of 1 : 2,050, a cytotoxic power giving 31 per cent of cell death on A lymphoid cells and an enhancing capacity resulting in lethal growth of five tumours out of fourteen implanted in fourteen mice (while the normal rate of take in control mice within the same conditions is, in our experience, lower than 1 out of 30).

Electrophoretic fractionation of the pool and examination for the haemagglutinating, cytotoxic and enhancing activities of the fractions lead to the results expressed in Fig. 1. Fraction 11 is the fastest of the fractions studied for biological activity and fraction 26 the slowest. The anti-A haemagglutinating activity extends from fraction 15 to fraction 24 with a maximum between 18 and 22. The anti-A cytotoxic activity (with added guinea-pig complement) is found in the slow part (from fraction 19 to fraction 23, with a maximum between 20 and 23), while the 'anti'-A enhancing-facilitating activity is found in the fast part (from fraction 13+14 to fraction 17+18, with a maximum at fraction 15+16), even before the appearance of detectable haemagglutinating activity. In view of the small number of mice submitted to the action of each pair of fractions, it was felt necessary to require several criteria for enhancement: proportion of animals with a tumour growing over a certain average (8 mm); mean diameter of tumours in a given group of mice; growth curve of individual tumours and deaths of mice from growing tumours. These criteria have been taken into account in Fig. 1.

The paired fractions still available after enhancement experiments were submitted to immunoelectrophoresis using two rabbit antisera active against the four immunoglobulins known to be present in mouse serum. The results, summarized in Table 1, suggest a possible association of cytotoxic antibodies with IgG and of enhancing antibodies with IgY (if not with IgM). However, the fact that the more cytotoxic fractions (21 and 22) and the more enhancing fractions (15+16) were no longer available for immunoelectrophoretic analysis prevented us from gaining a final answer.

Table 1. TYPES OF IMMUNOGLOBULINS PRESENT IN THE ELECTROPHORETIC FRACTIONS STUDIED FOR BIOLOGICAL ACTIVITY

Type of immunoglobulin	Presence in the following electrophoretic fractions					
	11+12	13+14	15+16	17+18	19+20	21+22	23+24	25+26
IgG	−	±	++	+++	++	+
IgA	−	±	+	+	−	−
IgM	±	+	−	−	−	−
IgY	+	+++	+	+	−	−

The intensity (± to +++) represents a semi-quantitative evaluation. It is based on (1) the inverse distance between the trough and the precipitation line and (2) the intensity of the precipitation line. Fractions 15+16 and 21+22 were not available for analysis.

The main result of this investigation resides in the electrophoretic separation of three biological activities involved in transplantation immunity reactions; cytotoxic activity in the slower fractions, enhancing activity in the faster fractions (almost without detectable overlapping) and haemagglutinating activity in almost the whole spectrum. These findings are reminiscent of the description of the γ_1 (fast) and γ_2 (slow) types of IgG described in guinea-pig[7] and in mouse[8] sera.

In both instances, the slow fractions were capable of haemagglutination and complement fixation while the fast fractions were capable of haemagglutination, incapable of complement fixation and capable of another biological activity: conferring anaphylactic sensitivity to the same species or conferring immunological protection to the target cells. To go further would require the identification of the responsible immunoglobulins. Our results, although suggestive, do not allow firm conclusions in that respect since, while the higher concentration of IgY is in the enhancing fractions, the same fractions also contain the three other immunoglobulins.

We thank Dr. Jean Maillard for help in fractionating the serum and Dr. G. Hermann for providing the anti-mouse immunoglobulin sera and for helping us to interpret the immunoelectrophoretograms.

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PRODUCTION OF GRAFT-VERSUS-HOST DISEASE IN THE RAT AND ITS TREATMENT WITH CYTOTOXIC AGENTS

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CYCLOPHOSPHAMIDE (CY) is a potent immunosuppressive drug in the mouse[1], rat[2–4] and man[5]. We have noted also that hybrid adult mice injected with parental, allogeneic or xenogeneic immuno-competent cells following a preliminary injection of CY will show a graft-versus-host reaction[6]. The present report presents preliminary observations on the induction and treatment of a graft-versus-host reaction in the CY pretreated rat.

Female Lewis and *BN* rats, age 10–12 weeks, were used as recipients and donors, respectively. Recipient animals were given a single intraperitoneal injection of 100 mg/kg of CY (about 60 per cent of the LD_{50} for 30 days observation) 4 h prior to the intravenous injection of allogeneic cells. The animals were observed daily and weighed periodically.

Groups of Lewis rats were injected with CY and 4 h later were given graded doses of nucleated bone marrow, blood or spleen cells suspended in Tyrode's solution. The incidence of dermatitis and mortality observed over a 30-day period is recorded in Table 1.

Lewis rats given 64×10^6 nucleated *BN* spleen cells, or 32×10^6 blood cells, intravenously regularly developed a fiery red and oedematous dermatitis involving the ears, paws, tail and to a lesser extent the snout, characteristic of graft-versus-host disease in the rat[7–9], 7–9 days after cell transfer. The dermatitis, with rare exception, persisted until death of the animal or from 10 to 30 days (15 day average) in animals that recovered. After 4–5 days, the dermatitis frequently evolved to desquamation and hair loss (particularly about the snout). In a few animals splinter-like haemorrhages were seen in the skin of

* Leukemia Society Scholar.

Table 1. GRAFT-VERSUS-HOST DISEASE IN CY-PRETREATED LEWIS RATS INJECTED WITH *BN* CELLS

Donor tissue	No. of nucleated cells × 10^6 injected	Fraction of animals with dermatitis†	Mortality (30 days)
Bone marrow	128·0	3/9	3/9
Bone marrow	256·0	7/9	3/9
Blood	8·0	4/17	1/17
Blood	16·0	4/8	2/8
Blood	32·0	8/9	6/9*
Spleen	32·0	3/8	1/8
Spleen	64·0	45/45	37/45*
Saline	—	0/27	3/27

* Mortality greater ($P < 0.01$) than control by the χ^2 test.

† Incidence of dermatitis significantly greater ($P < 0.01$) in all groups as compared with the control by the χ^2 test.

Table 2. EFFECT OF DRUG TREATMENT ON GRAFT-VERSUS-HOST DISEASE IN LEWIS RATS

Drug	Dose (mg/kg)	Time of drug injection after *BN* spleen cell transfer (days)	Fraction of animals with dermatitis	Mortality (30 days)
MTX	0·05	2, 3, 5	10/10	9/10
MTX	0·20	2, 3, 5	3/9*	3/9*
MTX	0·50	2, 3, 5	0/10*	4/10*
MTX	0·05	7, 8, 9, 10, 11	10/10	7/10
MTX	0·20	7, 8, 9, 10, 11	8/8	7/8
MTX	0·50	7, 9, 11	8/8	8/8
Cort.	20·00	7, 8, 9, 10, 11	8/8	7/8
CY	5·00	2, 3, 5	7/7	2/7*
CY	10·00	2, 3, 5	10/18*	2/18*
CY	5·00	7, 8, 9, 10, 11	7/8	8/8
CY	10·00	7, 9, 11	7/8	4/8
Cells alone		—	45/45	37/45
No cells		—	0/27	3/27

*Incidence of dermatitis or mortality significantly less ($P < 0.05$) than the control by the χ^2 test.

the ear. Diarrhoea occurred occasionally, but was not a common clinical manifestation. The rats rapidly lost about 15–20 per cent of their original body-weight by 15–20 days after cellular transfer. The few animals destined to survive regained their original body-weight over the next 10–14 days. The majority of animals died 10–20 days after the injection of *BN* cells. Further mortality was only rarely seen when animals were observed for periods up to 60 days. This syndrome of pronounced weight loss and dermatitis resulting in high mortality was not seen in CY-injected rats that received saline, syngeneic spleen cells, or heated allogeneic spleen cells.

The data in Table 1 indicate that nucleated cells derived from bone marrow, although less effective than spleen and blood, were capable of inducing graft-versus-host disease. Furthermore, the data suggest that nucleated cells from peripheral blood were more potent than those from spleen in causing the disease. Finally, the incidence of dermatitis and/or mortality was dependent on the dose of cells injected for blood and spleen.

Table 2 records the results of intraperitoneal injections of cortisone (Cort.), methotrexate (MTX) and *cyclo*phosphamide (CY) on the incidence of dermatitis and mortality in CY-pretreated Lewis rats injected with 64×10^6 *BN* spleen cells.

The entries marked with an asterisk in Table 2 indicate treatments that had a beneficial effect on the course of the disease. Methotrexate was able to reduce both the incidence of dermatitis and mortality when begun 2 days after the cell transfer. Treatment with this agent initiated 7 days after cell transfer did not lower mortality nor did it appear to alter noticeably the subsequent course of the dermatitis. *Cyclo*phosphamide reduced the mortality and incidence of dermatitis (the latter only at the higher dose) when begun 2 days after cell transfer. In addition, CY given 7, 9 and 11 days after the injection of *BN* cells appeared to reduce the severity of the dermatitis. The observed reduction in mortality, however, was not significant ($P > 0.05$) when compared to the control by the χ^2 test. Cortisone in the one dose schedule used was ineffective in reducing the mortality or the subsequent course of the dermatitis.

None of the surviving animals from the foregoing groups was a chimera—as tested by the rejection time of full thickness *BN* skin grafted 60 days after the induction of the disease. A more detailed investigation regarding the duration of the chimeric state in animals such as these is at present being carried out.

The graft-versus-host disease described here is easily produced in the adult rat with drug pretreatment which in itself entails very little mortality. Dermatitis is striking and regular in appearance and provides a valuable guide as to the course of the disease. For these reasons, it is felt that this system is particularly well suited for the investigation of therapy directed toward modifying graft-versus-host disease in the adult rat.

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A POSSIBLE HOMEOSTATIC MECHANISM IN COMPLEMENT FIXATION: INHIBITION OF THE ACTIVITY OF FIXED C'_1 BY THE FIXATION OF C'_{3a}

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FOR the study of the reactivity of conglutinin and immuno-conglutinins with fixed guinea-pig complement[1] alexinated intermediates at various stages of the complement reaction sequence were prepared as outlined in Fig. 1.

It was of course necessary to confirm that the intermediates formed did show the reactivity expected of them. The test systems used are outlined in Table 1 and are discussed more fully elsewhere[1].

There is one very surprising result in Table 1. The intermediate EAC'_{143a} failed to react with C'_2 to give an intermediate lysing with C'_{EDTA}.

Two explanations for this lack of reactivity were considered. First, the C'_{H62} may contain the C'_1 inhibitor as described by Lepow and Leon[3]; secondly, the fixation of C'_{3a} itself may give rise to inhibition of the reaction with C'_2.

The first explanation is less likely in that the C'_1 inhibitor was reported to be destroyed at 63°. It was, however, tested by decaying EAC'_{142} to EAC'_{14} at 37° for 75 min prior to reaction with C'_{H62}. In these conditions C'_1 inhibitor should still react with C'_1 whereas C'_{3a} will not be bound. It was found that EAC'_{14} treated with C'_{H62} reacted with C'_2 at the same dilution as untreated EAC'_{14}; a finding that was regarded as excluding the participation of a C'_1 inhibitor.

It was, therefore, thought likely that the fixation of C'_{3a} itself was responsible for the inhibition. Now, it is shown in Table 1 that the concentrated *R4* reagent did lyse EAC'_{143a}. The C'_4 in this intermediate thus seems to be

fully active, and the inhibition must, therefore, be of C'_1. This conclusion was supported by testing with the *R*4 reagent after absorption with 5 mg/ml. of an antigen–antibody precipitate (ovalbumin–rabbit anti-ovalbumin) for 15 min at 37°. This treatment removes C'_1 from the *R*4 but—in the absence of C'_4—has little effect on the other C′ components. This reagent produced lysis of EAC'_{14} but again failed to lyse EAC'_{143a}.

Table 2 summarizes these results.

Thus when C'_{3a} is bound on to EAC'_{142} and the C'_2 then allowed to decay, the intermediate formed will no longer react with further C'_2. It has been shown that this inhibition affects C'_1 rather than C'_4 activity and that it is not due to the presence of a C'_1 inhibitor which will react with EAC'_{14}. One is, therefore, led to the conclusion that the fixation of C'_{3a} leads to the inhibition of the activity of the C'_1 already bound. It has not so far been determined whether the C'_1 is actually eluted from the alexinated complex or if it is inactivated *in situ*. The inhibition does not appear to extend to the fixation of further C'_1 since the fixed C'_4 is still active.

This inhibition can be pictured as a feed-back mechanism for regulating the extent of 'long-term' complement fixation at a particular complement-fixing site. When C'_1 has been inactivated no more C'_2 can be fixed so that, when the C'_2 already fixed has decayed, no further fixation of C'_3 components will occur.

The fixation of C'_{3a} is known to be biologically a highly significant step in complement fixation, the EAC'_{1423a} intermediate being that participating in phagocytosis[4],

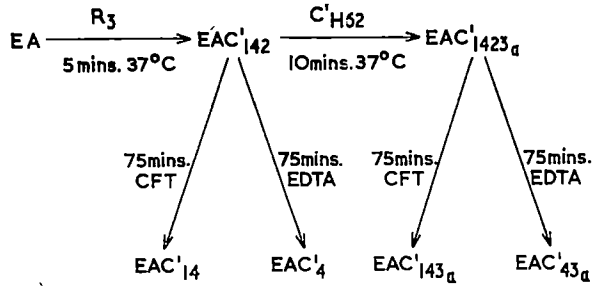


Fig. 1 Preparation of alexinated intermediates. EA, Sheep erythrocytes sensitized with rabbit antibody; R_3, 'Zymoxan'-absorbed guinea-pig serum; C'_{H52}, guinea-pig serum heated at 62° C at *p*H 8·5 for 20 min[3], CFT, complement fixation test diluent (Oxoid), EDTA, 0 01 M EDTA in saline, *p*H 7·2

Table 1. CHARACTERIZATION OF ALEXINATED INTERMEDIATES

Test system	Test for presence on cell of	Alexinated intermediate: EA	EAC'_{142}	EAC'_{14}	EAC'_4	EAC'_{1423a}	EAC'_{143a}	EAC'_{43a}
Lysis with 1/40 *R*4	C'_4	0	100	100	100	100	100	100
Lysis with C'_{EDTA}	C'_{42}	0	100	0	0	100	0	0
Lysis with C'_2 followed by C'_{EDTA}	C'_{14}	0	*	100	0	*	0	0
Lysis with 1/400 *R*4	C'_{43a}	0	0	0	0	100	0	0
Conglutinin	C'_{3a}	–	–	–	–	++	++	++

0, No haemolysis; –, no conglutination; 100, > 50 per cent haemolysis; ++, strong conglutination; *, test not applicable because intermediates lyse with C'_{EDTA} *per se*; *R*4, guinea-pig serum treated with 0 1 M ammonia at 37° C for 45 min; C'_{EDTA}, 1/50 guinea-pig serum in 0 01 M EDTA saline; C'_2, fraction of guinea-pig serum prepared by chromatography on DEAE cellulose[1].

Table 2. FURTHER CHARACTERIZATION OF ALEXINATED INTERMEDIATES

Test system	Test for presence on cell of	Alexinated intermediate: EAC'_{14}	EAC'_{14} treated with C'_{H52}	EAC'_4	EAC'_{143a}
Lysis with 1/40 *R*4	C'_4	100	100	100	100
Lysis with 1/20 *R*4 absorbed with antigen antibody complexes	C'_{14}	100	100	0	0
Lysis with C'_2 followed by C'_{EDTA}	C'_{14}	100	100	0	0
Conglutinin	C'_{3a}	–	–	–	++

The symbols are used as in Table 1

immune adherence[5], and conglutination[6]. A homeostatic mechanism regulating the extent of fixation of this component would, therefore, make good biological sense.

It should, perhaps, also direct attention to the *in vivo* significance of 'long-term' complement fixation, that is, those processes which continue after the initial rapid reaction sequence leading to haemolysis and which have received virtually no attention in the past.

I thank Mrs. Diana Elias for her assistance, and Dr. R. R. A. Coombs and Mr. P. M. Henson for their advice. The work was supported by a grant from the Nuffield Foundation.

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COAGULATION OF PRIMATE BLOOD BY RUSSELL'S VIPER VENOM

By CHRISTINE HAWKEY and C. SYMONS
Nuffield Institute of Comparative Medicine, The Zoological Society of London

IN a comparative investigation of haemostatic mechanisms in captive wild animals, it has been found that the clotting system of non-human Anthropoidea is essentially similar to that of normal human beings. There is, however, one respect in which non-human primate blood differs from that of all other mammals so far investigated, namely, in the reaction with venom from *Vipera russelli*. This preliminary communication reports delayed clotting time in non-human primates with the Russell's viper venom (RVV) associated with normal prothrombin times. No previous reports of species differences in reaction to RVV have been found.

The coagulant activity of RVV is due to an esterase in the venom, which splits plasma factor X to form an active derivative[1–3]. This activated factor X, which is not neutralized by anti-venom, brings about rapid conversion of prothrombin to thrombin in the presence of phospholipid, factor V and calcium ions. Its action is independent of the presence of factors VII, VIII, IX, XI and XII. In order to mask the effect of variable amounts of phospholipid of platelet or alimentary origin, an optimum amount of cephalin or lecithin is included in laboratory tests with RVV. A mixture containing equal volumes of normal citrated human plasma, RVV('Stypven', Burroughs Wellcome) in 0·25 per cent ovolecithin and M/40 calcium chloride gives a clotting time of between 8 and 10 sec at 37° C. Reduction of the concentration of prothrombin, or of factors V or X, results in prolongation of this time.

Venous blood was collected into 1/10 volumes of 3·8 per cent trisodium citrate from ten monkeys approximately 15 min after tranquillizing with an intramuscular injection of 'Phencyclidine' (2 mg/kg). Platelet-poor plasma was either tested immediately or stored in polystyrene containers at −35° C until tested. Table 1 shows RVV clotting times and prothrombin times[4]. In all monkeys the RVV clotting time was greater than in humans, whereas in all other mammals tested the RVV clotting time was less than 10 sec. Prothrombin times

measured for the same plasma samples were all within the normal human range, indicating levels of prothrombin and of factors V, VII and X comparable with those in humans. This was confirmed by two-stage prothrombin tests[5] and assays of factors V (ref. 6), VII (ref. 7) and X (ref. 8), all of which gave normal results. Monkeys belonging to the family Cercopithecidae show the most marked prolongation with RVV. Fig. 1 indicates the relationship between prothrombin time and RVV clotting time in a variety of animals, demonstrating the prolongation of RVV clotting time in non-human primates.

As prolongation of the RVV clotting time cannot be explained by deficiencies of clotting factors, the possibility was considered that monkey blood contains a venom inhibitor. The presence of such an inhibitor was tested for as follows. Human serum, or serum from *Cercopithecus ascaneus schmidti*, was incubated with equal volumes of RVV in ovolecithin at 37° C. At intervals, a sub-sample of this mixture was added to normal human substrate plasma and the clotting time recorded after recalcification. The results given in Table 2 show that the ability of RVV to clot human plasma is not impaired by incubation with monkey serum, thus excluding the presence of an inhibitor active *in vitro*.

The results of this experiment also indicate that RVV reacts normally with monkey factor X, producing activated factor X which will rapidly convert human prothrombin to thrombin. Substitution of monkey plasma for human substrate plasma in the second stage of the test, however, results in prolongation of the clotting times (Table 2). This suggests that the reaction between RVV-activated factor X and monkey prothrombin and/or factor V is the limiting feature. Further experiments with purified clotting factors are required to confirm this.

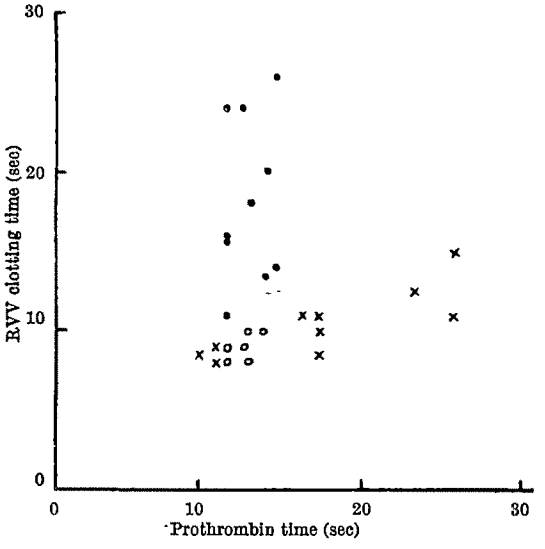


Fig. 1. Differential prolongation of RVV clotting times in non-human primates. ○, Normal humans; ●, non-human primates; ×, other mammals

Table 1. COMPARISON OF PROTHROMBIN TIMES AND RVV CLOTTING TIMES IN PRIMATES

Plasma	Clotting time (sec) Human brain extract	*Monkey brain extract	Russell's viper venom
Normal human	12–14 5	14	8–10
Platyrrhina			
Cebus capuchina	12	12	16
C. apella	12	11	11
Ateles sp.	12	12	16
Catarrhina			
Cercopithecidae			
Macaca maurus	12	14	24
M. mulatta	13	13	24
Cercocebus torqualis	13·5	15	18
Cercopithecus pygerythrus	15	14	26
C. ascaneus schmidti	14	13	20
Pongidae			
Pan satyrus	15	—	14
Pan satyrus	14	—	13·5

* Prepared from fresh brain of *Cercopithecus ascaneus schmidti*.

Table 2. TWO-STAGE TEST FOR INHIBITION

Serum in incubation mixture	Substrate plasma	Clotting time (sec) after incubation for 0	5 min	10 min
Human	Human	9	9·5	10
Monkey	Human	9	10	10
Human	Monkey	17	18 5	20

The fact that all other coagulation tests carried out on the monkeys under discussion have given results within normal human limits shows that the coagulation system—an important part of the haemostatic mechanism—is well developed in primates. The reduced reaction with RVV, which has been demonstrated *in vitro*, may also be of significance *in vivo* and, as snakes are natural enemies of monkeys, may have evolved as a result of natural selection.

This work was supported by a grant from the British Heart Foundation. We thank O. Graham-Jones for collection of blood samples and G. F. Nevill for technical assistance.

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NEWS AND VIEWS

Microbiology in the Queen's University of Belfast: Prof. K. B. Fraser

DR. K. B. FRASER has been appointed to the chair of microbiology in the Queen's University of Belfast, in succession to Prof. G. Dick (*Nature*, 207, 916; 1965). Dr. Fraser graduated M.B., Ch.B. in 1940 in the University of Aberdeen, and after a distinguished war record, including the award of the Military Cross, he returned in 1946 to Aberdeen where he took up a career in microbiology, eventually becoming a senior lecturer in the Department of Bacteriology. In 1961 he moved to Glasgow and joined the staff of the newly built Institute of Virology as a senior lecturer in virology and an honorary consultant virologist, and has remained in this post until his present appointment. Dr. Fraser's interest in virology began in 1951 when he spent a year working with Sir MacFarlane Burnet in Melbourne. He was responsible with Sir MacFarlane for much of the early work on the genetic recombination of influenza virus, and on his return he continued with his well-known study on virus virulence factors. His interest in genetics was continued during a further period in 1959 as a Research Fellow with Prof. Pontecorvo in Glasgow. Dr. Fraser's other main interest is in the field of immunology and he has been a pioneer in the applications of immunofluorescent techniques in experimental virology. During the past few years in the Institute of Virology in Glasgow, Dr. Fraser and his group have been working on incomplete growth cycles of myxoviruses, and on early stages of polyoma virus growth and transformation. In addition to his distinguished research, Dr. Fraser's ability as a teacher is well known to both

medical and science students in Aberdeen and Glasgow, and also to postgraduates who have attended the recent courses in basic virology at Glasgow.

Director of the Forest Products Research Laboratory: Mr. J. Bryan

MR. J. BRYAN, director of the Forest Products Research Laboratory of the Ministry of Technology, retired from the public service on December 31, 1965. With the exception of a short period with Dorman Long, Ltd., early in his career, he devoted the whole of his working life to the study of timber and the service of the Forest Products Research Laboratory, which he joined virtually at its inception in 1926. He formed and led the section working on wood preservation and later became head of the Timber Mechanics Section. He took charge of all engineering investigation in the Laboratory in 1945 and was appointed assistant director in 1954 and director in 1962. An engineer by training and inclination, with a keen interest in natural products and processes, he was well fitted to assist in the transformation of timber from a craft to an engineering material and the establishment of a scientific basis for wood technology. During his period of service with the Laboratory he saw great advances on both these fronts and he saw the Laboratory develop into a leading world authority on timber and its use. His career spanned most of the life to date of the Forestry Commission, with which he worked in close co-operation, especially when its plantations began to reach the age when timber properties could be tested. He was for some years chairman of the joint Committee on Research into Home-Grown Timbers of the Department of Scientific and Industrial Research and the Forestry Commission.

Mr. T. A. Oxley

MR. T. A. OXLEY has been appointed director of the Ministry's Forest Products Research Laboratory, Princes Risborough, in succession to Mr. Bryan. The appointment took effect from March 1. Mr. Oxley was born in Hampstead, where he attended the Haberdashers Aske's School, and graduated with honours from the Imperial College of Science and Technology in 1933. After spending 2 years on postgraduate research, he worked for 3 years as a demonstrator at Imperial College. He then joined a group in the Department of Zoology at Imperial College, investigating, as a grain-specialist, pest infestation of stored products. This group later formed the Pest Infestation Laboratory of the former Department of Scientific and Industrial Research, where Mr. Oxley became head of the biology section and later of the grain-storage section. He was appointed assistant director of the Tropical Products Institute in 1959. For the past 5 years Mr. Oxley has been a deputy commissioner, scientific and engineering directorate, of the Civil Service Commission.

Council for National Academic Awards: Recognized Degree Courses

THE first report of the Council for National Academic Awards covers the year ended September 30, 1965, and records the establishment of three Boards of Studies for Arts and Social Studies, Science and Technology, and Research Degrees, as well as of eighteen Subject Boards (Pp. 33. *Recognised Degree Courses*. List No. 2. Pp. 6. London: Council for National Academic Awards, 1965). The Council considered 67 courses in business studies, science and technology proposed by Colleges to lead to the Council's degrees; arrangements have been made to award the Council's degrees to holders of either the Diploma in Technology or Membership of the College of Technologists who wish to convert their awards. At the beginning of the 1965–66 academic year, 4,073 students were enrolled in 89 courses leading to the Council's degrees, including 129 first-year students in five courses leading to a B.A. degree in business studies. Of fifty-seven courses in science and technology considered by the Council, seventeen were approved to start in 1965 and a further seven to start in 1965 or 1966 subject to satisfaction on outstanding points; thirty-three were rejected. Five out of ten courses in business studies were approved to start in 1965; two were approved to start in 1966 subject to satisfaction on several points; three were rejected. Of the 4,073 students enrolled, 1,052 were in mechanical and production engineering, 978 in electrical and electronic engineering, 493 in civil engineering, 410 in chemistry, chemical technology and industrial chemistry, 359 in physics and physical electronics, and 220 in aeronautical engineering. Appendixes give the membership of the various Boards of Studies.

Sixth-form Teaching

THE Schools Council is in the unenviable position of having to foster change in the British educational system without treading on the corns of its sponsors—principally the local authorities responsible for education systems, organizations of professional teachers and the Department of Education and Science. In the circumstances it is inevitable that it should spend a good deal of its energy flying kites, talking of innovation and then waiting to see whether the result is delight or revolution. This seems to have been the spirit in which the Schools Council organized a conference in Nottingham a year ago to parade before a small group of teachers some recent suggestions as to how the pattern of science teaching in the sixth forms of British secondary schools may, in the near future, change. A record of this conference has now been published (*Science in the Sixth Form*, H.M.S.O., 4*s*. 6*d*.).

Outside the United Kingdom it may seem strange that British schools should regard as daring an experiment in which some of the material now taught to senior secondary pupils under the separate labels of physics and chemistry should be combined in a single course called physical science. Indeed, the proposal is only meaningful in the context of the British sixth form, where most students aiming at university or higher education follow courses in three specialized subjects, and where schools are urged, sometimes vainly, to devote a third of the time available to broader pursuits (including physical education). The most compelling argument for physical science, advanced at Nottingham, is that such a course would make it possible for those going on to science departments at universities to know something of the biological and the physical sciences, and to learn some mathematics as well. At present it is all too common that students intending to go on to university are required to choose, at sixteen or so, on the particular discipline in which they intend to graduate 5 years later, and that they should promptly direct their studies to that end. A course in physical science would make it possible to postpone this choice for 2 years and—giddy thought—might even allow some intending scientists to read a little history.

Prospects for Change

A group of a dozen schools is likely to try out such a course in the years immediately ahead. The value of the experiment will obviously depend on whether British universities are willing to give it a fair wind. Nothing said at the Nottingham conference can be used to predict how things will go, but when the results of the first experiment are available for study, the universities are bound to worry a great deal, in part legitimately, before deciding what their attitude will be. It would be a great, though uncovenanted, benefit if one consequence were the fuller involvement of British universities in the creative evolution of the school curriculum.

A more radical proposal given an airing at the Nottingham conference was for a pattern of sixth-form study

made up from 'minor' as well as 'major' courses. On paper, a minor course would take half the time of a major course. Theoretically, a student could choose between a concentrated pattern of studies of the kind now common and a much broader and more liberal education. The Schools Council is obviously keen on the concept, though it is bound to be a long time before the universities and the schools are thoroughly convinced. In the hope of showing that its proposals are practical, the Schools Council is working hard to design teaching time-tables in which major and minor courses are intertwined. In time, no doubt, there will be practical experiments in schools. Success could bring a new pattern of sixth-form science teaching within a decade. Certainly the talk that there is bound to be of this proposal will sharpen discussion of the four-year university course.

Essays on Humanity

IN his *Two Essays on Humanity*, K. W. Monsarrat is concerned first with "Man's Thoughts" and then with "Man's Health" (Pp. 19. Liverpool: Henry Young and Sons, 1965). In his first essay he elaborates the thesis that the most precious of all man's neural abilities is the ability to form validity judgments and to exert his powers accordingly. He emphasizes the need for recognition that the word 'mind' represents an abstract idea of the individual's thought-producing activity by that part of his cell-structure which the microscopist terms his brain-cortex, and refers to the clinical evidence that changes in the blood content as the blood-stream passes through glandular structures may affect the production of thought. In the second essay, he criticizes the development and organization of the health service, arguing that a really comprehensive scheme has been prevented by the party structure of Parliament assisted by the short-sightedness of the British Medical Association, which, like Parliament itself, twice failed to grasp the opportunity, originally presented by the report of the Dawson Committee in 1920. In consequence, he maintains that the family doctor has been forced to choose between reducing the standard of his care of his patients, submitting to financial straits which mean relative poverty, abandoning his profession or emigrating.

British Acoustical Society

THE Acoustics Groups of the Institute of Physics and the Physical Society and the Society of Acoustic Technology in the United Kingdom have spawned a new society—the British Acoustical Society—which is intended to cover the "rapid growth of interest and discovery in the various branches of acoustics in Britain". The new society hopes to bring some simplification to a field of study in which a number of separate societies and sub-groups have sprung up in recent years. It will be concerned with "all aspects of sound, hearing and vibration", and will be engaged in problems of great public interest such as aircraft and traffic noise, the design of concert halls and the industrial uses of sound, including ultrasonics. The planning of the new society has been done by a committee under Sir Gordon Sutherland as chairman, and the new society will be managed provisionally by a council with Dr. A. J. King as chairman and Prof. E. J. Richards as vice-chairman. There is to be an inaugural meeting during May 5–6 at the Department of Physics of the Imperial College of Science and Technology, London, S.W.7. Further information can be obtained from Dr. R. W. B. Stephens at that address.

Chemistry and Metabolism of Lactic Acids

Chemistry and Metabolism of L- *and* D-*Lactic Acids* contains a series of papers presented at a conference held in November 1964 (*Ann. N.Y. Acad. Sci.*, 119, Article 3. By N. R. Alpert and 45 other authors. Pp. 851–1165. New York: New York Academy of Sciences, 1965. 6 dollars). The objects of the conference, as stated by the co-chairmen, were to demonstrate the need to use specific isomers in metabolic studies rather than the D,L-mixtures frequently used, and to bring together present knowledge on lactic acid. The first of these objectives is worthwhile, since investigations with stereospecific enzymes have shown that the information gained by using racemic substrates is often misleading, and many of the papers in this volume amply show that a great deal more information can be obtained using the individual isomers of lactic acid. The large amounts of information available on some aspects of the subject make the second objective difficult to attain: the material available on the lactic dehydrogenases could in itself fill a volume. The volume opens with a paper on the chemistry of lactic acid, followed by a series of papers dealing with specific enzymes concerned in the metabolism of the lactic acid isomers and other hydroxy acids. The study of the metabolism of the lactic isomers then proceeds from these specific enzymes to metabolic pathways in micro-organisms, tissue preparations, isolated organs and the intact animal. The final section of the volume consists of papers on the medical aspects of lactic acid metabolism. This section contains a paper on the metabolism of the lactic acid isomers in human brain in both normal and mental patients, followed by four papers on lactic acid levels in the blood during acidosis, shock, neonatal life and hyperventilation. The volume represents a useful survey of the various aspects of lactic acid chemistry and metabolism, in which condensation of the subject-matter due to space limitations is largely offset by lists of references at the end of each paper. As such it can be recommended to anyone interested in the important aspects of this field, and also to anyone tempted to use racemic mixtures in enzymatic or metabolic investigations.

Occurrence of *Rana ridibunda* in a Hot Spring near Edremit (Turkey)

ATILLA ÖZALPAN, of the Department of General Zoology, University of Istanbul, has written to the Editor as follows: "It is unusual to find vertebrate species in hot springs. For this reason, we have found it worth-while to report the occurrence of *Rana ridibunda* in a hot spring near Edremit (Balikesir, Turkey). The temperatures of different parts of the hot spring are shown in Fig. 1.

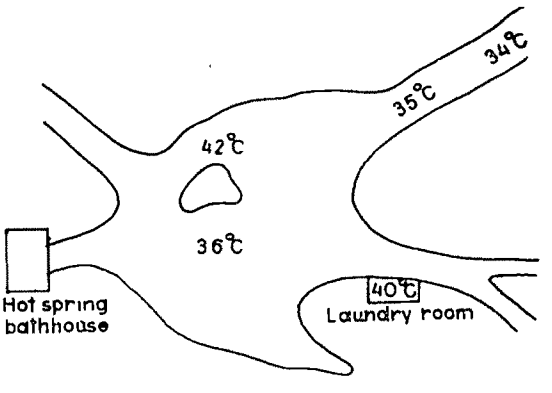


Fig. 1. The hot spring (Derman) near Edremit

"On April 23, 1965, many small metamorphosed individuals and tadpoles of different sizes were collected from the hot spring. The larvae and the frogs appeared to have no particular preference for a special temperature in the hot spring. They were found in its coldest and hottest regions. It seems that the frogs are adapted not only to the temperature of the water but also to its salinity. The physiological basis of this phenomenon is being worked out in our laboratory and will be published elsewhere."

University News: Bradford Institute of Technology

DR. D. W. MATHIESON, reader in pharmaceutical chemistry in the University of London, has been appointed professor of pharmaceutical chemistry.

Strathclyde

DR. W. C. BOWMAN, reader in pharmacology in the School of Pharmacy, University of London, has been appointed professor of experimental pharmacology in the University of Strathclyde. Dr. Bowman's major research interest is in the mechanism of action of drugs which interfere with synaptic and neuro-effector transmission.

Sheffield

PROF. G. J. HOIJTINK, professor of chemistry in the University of Amsterdam, has been appointed to the chair of physical chemistry. Prof. R. McWeeny, professor of theoretical chemistry in the University of Keele, has been appointed to the chair of theoretical chemistry.

Swansea

MR. J. OLIVER, senior lecturer in the Department of Geography, has been appointed to the newly established second chair of geography in the University College of Swansea.

Awards and Appointments

DR. PEYTON ROUS has received the Paul Ehrlich and Ludwig Darmstaedter Prize, which is the highest medical award of West Germany.

PROF. G. PUPPI has been elected chairman of the CERN Scientific Policy Committee, in succession to Prof. L. Leprince-Ringuet.

DR. D. H. WHIFFEN was appointed superintendent of the Division of Molecular Science at the National Physical Laboratory, Teddington, Middlesex, as from April 1.

H. B. STEWART, JUN., formerly chief oceanographer of the Coast and Geodetic Survey, has been appointed head of the New Institute for Oceanography of the U.S. Environmental Science Services Administration

Announcements

THE U.S. National Academy of Sciences and the Academy of the Socialist Republic of Romania have announced a three-year exchange programme, providing for visits of up to one year by individual scientists of the two countries to lecture and to conduct research. The new programme is the third to go into effect this year between the U.S. Academy and one of its sister institutions in Eastern Europe.

A SYMPOSIUM on "Terpenoids in Plants", organized by the Phytochemical Group, will be held in the University College of Wales, Aberystwyth, during April 12–14. Further information can be obtained from Mr. A. H. Williams, Research Station, Long Ashton, Bristol.

A ONE-DAY symposium on "The Environmental Stability of Low-voltage Contacts" will be held at the Luton College of Technology on April 13. Further information can be obtained from Dr. M. G. Parker, Luton College of Technology, Park Square, Luton, Bedfordshire.

THE second international conference on "Electron and Ion Beams" will be held in New York during April 17–20. Further information can be obtained from the Secretary, Institute of Metals Division, Metallurgical Society of A.I.M.E., 345 East 47th Street, New York.

A JOINT meeting of the Microchemical Methods Group and the Scottish Section of the Society for Analytical Chemistry will be held in the University of Edinburgh during April 15–17. Further information can be obtained from the Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

A ONE-DAY colloquium on "Winning of Chemicals from Flames" will be held by the British Section of the Combustion Institute in the University of Sheffield on April 15. Further information can be obtained from Prof. W. G. Parker, Department of Chemistry, College of Advanced Technology, Birmingham 4.

A SEMINAR on "The Dynamic Role of Molecular Constituents in Plant–Parasite Interaction", sponsored jointly under the United States–Japan Co-operative Science Program by the National Science Foundation and the Japanese Society for Promotion of Science, will be held in Gamagori during May 15–21. Further information can be obtained from Dr. C. J. Mirocha, Department of Plant Pathology and Physiology, University of Minnesota, St. Paul.

THE NIGHT SKY IN MAY

All times are in Universal Time

MOON

New Moon 20d 10h
Full Moon 4d 21h

CONJUNCTIONS WITH THE MOON

Venus 16d 17h, 2° N.
Mars — —
Jupiter 22d 23h, 3° S.
Saturn 15d 09h, 3° N.

PLANETS

Times of rising (*R*) and setting (*S*) during the month

Name	*R*/*S*	Beginning	Middle	End	Mag.	D_θ (10^6 miles)	Zodiacal position
Mercury	—	Unfavourable for observation			—	116	Aries
Venus	*R*	3h 20m	2h 50m	2h 25m	−3·6	91	Pisces
Mars	—	Unfavourable for observation			—	230	Aries
Jupiter	*S*	23h 40m	22h 55m	22h 10m	−1·5	551	Gemini
Saturn	*R*	3h 30m	2h 30m	1h 35m	+1 4	941	Aquarius

D_θ is the distance of planet from the Earth on the 15th of the month

OCCULTATIONS OF STARS BRIGHTER THAN MAGNITUDE +6 AT GREENWICH

Star	*R*/*D*	Time	Mag.
α Lib	*R*	4d 21h 35·9m	+2·9
7 Vir	*D*	28d 22h 41·0m	+5·2
82 Vir	*D*	30d 21h 56·8m	+5·2

(*D*, disappearance; *R*, reappearance)

OTHER PHENOMENA

1d 18h, Venus 1° N of Saturn.

4d, Penumbral eclipse of Moon:

Moon enters Penumbra	19h 07m	Beginning of eclipse visible in Eastern Europe, Africa, Asia, Australia,
Mid eclipse	21h 12m	end of eclipse visible Western Europe, Africa and South America.
Moon leaves Penumbra	23h 17m	

20d, Annular eclipse of Sun:

Eclipse begins	6h 50m	Visible Brazil, Atlantic, North and Central Africa, Europe, Asia.
Central eclipse begins	7h 54m	
Central eclipse at local apparent noon	9h 51m	
Central eclipse ends	11h 23m	
Eclipse ends	12h 26m	

SCIENTIFIC RESEARCH IN INDIA

THE Survey and Planning of Scientific Research Unit of the Council of Scientific and Industrial Research, New Delhi, has issued a survey of scientific research in Indian universities, based primarily on the annual reports of some twenty-five universities, and covering twelve scientific subjects*. Forty-eight universities have scientific departments, and nine sent reports for the full 10 years (1952–62); some of the new universities did not send annual reports. The survey gives the total number of papers published by the university departments each year since 1952–63, and the distribution by subject and by periodicals, with statistics of authors. In the period covered, the number of Indian universities has grown from twenty, with 108,722 students in science faculties, to fifty-five, with 1,386,374 in science faculties.

In agriculture, Allahabad, Andhra, Annamalai and Calcutta appear to have active research departments; at Calcutta and Banaras most of the emphasis is on agricultural botany, and at Annamalai on pathology; animal husbandry and veterinary science are receiving little attention. Most of the universities except Aligarh, Jabalpur, Jadavpur, Mysore, Saugar and Karnatak are active in botanical research, notably at Allahabad, in the physiology and ecology of angiosperms and plant pathology due to fungi. The morphology of angiosperms is being studied at Andhra, the physiology and ecology of angiosperms at Annamalai, cytogenics of angiosperms and other groups at Calcutta, morphology and physiology of angiosperms at Delhi and Utkal, and plant pathology due to fungi at Madras. In chemistry, 589 papers came from Andhra, 438 from Calcutta, 374 from Delhi, 360 from Lucknow, 265 from Banaras, 236 from Jadavpur, 234 from Utkal and 216 from Madras. In Delhi, Lucknow, Calcutta, Banaras, Annamalai and Poona, the emphasis was mainly on organic chemistry; at Allahabad on physical chemistry; inorganic work came chiefly from Allahabad, Andhra, Banaras, Jadavpur and Utkal. Only Calcutta was concerned with research in industrial chemistry and work on analytical chemistry was mostly insignificant. In engineering, 80 per cent of the papers published were in chemical engineering and only in Banaras was there any research in metallurgy.

There is considerable research activity in geology at Andhra, Calcutta, Jadavpur and Lucknow, while in mathematics the contribution has come mainly from Lucknow, Calcutta, Delhi and Banaras. Out of 95 papers from Banaras, 63 were published in 1961–62, the theory of numbers and theory of functions accounting for 60 per cent of the total. Those from Banaras were concerned mainly with relativity and theory of functions; from Lucknow, with algebra, theory of functions and mechanics; from Delhi, with geometry and mechanics; from Calcutta, with algebra and geometry; and from Allahabad, with the theory of functions. Research in the medical sciences was most active at Lucknow, followed by Andhra, Bombay, Delhi, Nagpur and Poona. Medicine and pharmacology, with some pathology and physiology (Lucknow), physiology and medicine (Bombay), physiology (including biophysics and biochemistry) (Calcutta), and pathology, anatomy, medicine and gynaecology (Andhra) were the chief fields. Only at Andhra, Banaras and Panjab was effective research in progress in pharmacology, about 90 per cent of papers from Andhra and 70 per cent from Banaras being in chemotherapy.

Most universities were consistently active in physics, especially nuclear physics (Bombay and Delhi); theoretical physics (Madras, Annamalai and Delhi); spectroscopy (Andhra); electronics (Andhra and Calcutta); microwaves and acoustics (Andhra); crystallography, biophysics and mechanics (Madras). Bombay and Calcutta have published a few papers on cosmic rays. Calcutta, Baroda, Bombay and Poona, with Karnatak (since 1958), have published most of the research papers in statistics, largely on the design of experiments, theory of inference and descriptive statistics. Only at Utkal, Kerala and Sri Venkateswara has there been any research in veterinary science, but Lucknow, Madras, Andhra, Baroda, Delhi, Allahabad, Annamalai and Sri Venkateswara have active research departments in zoology. Cytogenetics appears to be neglected except at Allahabad. Of the Council's total research grants to universities of 8·8 million rupees in 1958–65, 45 per cent was for chemical research, 22 per cent for physical research and 11 per cent for biological research.

* India: Council of Scientific and Industrial Research. Survey Report No. 6: *Scientific Research in Indian Universities*. By U. Sen, A. Rahman, S. P. Gupta, A. K. Roy, S. Malik, S. H. M. Husaini, S. Das Gupta and Y. K. Agarwal. (New Delhi: Council of Scientific and Industrial Research, 1965.)

FUNCTIONAL ARCHAEOLOGY

THE value to the prehistorian of experimentation with archaeological material has long been recognized. The list of materials submitted to testing for methods of production and for possible functions is a long one, ranging from the working of flint and stone to the firing of Romano-British pottery in reconstructed kilns, and this article draws attention to two recent experiments in functional archaeology.

Among the first archaeologists to consider the possibilities of this approach was Sir John Evans, who compared his prehistoric flintwork with that produced by his nineteenth-century contemporaries at Brandon in Norfolk and in other countries as well. In Scandinavia, Jorgensen and Steensberg have in recent years directed experiments with flint axes to see how efficiently these could fell trees, and the next stages in primitive cultivation, burning and sowing of seed, have also not been neglected. In Britain, ploughing experiments with reconstructed Danish ards have recently been carried out, and these may be of real assistance in interpreting the traces of ploughing recovered from such sites as Gwithian in Cornwall. The Council for British Archaeology has also carried out experimental work on storage pits to see how efficiently these basketry-lined pits preserved grain. In Ireland the cooking experiments on Bronze Age sites are quite well known, and seem to have been successful both in function and in the taste of the finished product. Many other experiments involving the reconstruction of larger-scale

monuments, defensive ramparts, standing stones and wattle-and-daub houses could be mentioned, but the present article emphasizes the experimental techniques involving the functions of actual or duplicated artefactual material. A considerable amount of experimental work has been carried out on the casting of copper and bronze products, and on the firing of Romano-British pottery, but these have been concerned mainly with production techniques, while it is the functional aspect that is particularly relevant at the moment. Recent work on this aspect has included the testing of the capabilities of sheet metal and leather shields to stand up to sword and spear blows, the determination of the range of musical notes obtainable from both Danish and Irish Bronze Age horns, the function of Upper Palaeolithic and early Mesolithic harpoon-heads, and, most recently, two papers in *Man* (65, 1965) which advance new interpretations of the function of well-known Palaeolithic artefacts. In one (No. 148) Dr. M. D. W. Jeffreys attempts to show that the handaxe, that characteristic tool of the widespread African Chelles-Acheul and European and western Asian Abbevillian and Acheulean industries, was not in fact a handaxe, but was a handbolt or missile. He defines a handaxe as an elongated tool with a pointed and a blunt end, bifacially worked with a cutting edge all the way around which shows no sign of wear. Because of the cutting edge, Jeffreys believes that such axes could not have been held in the hand, and he argues that these objects were hurled, the blunt end aimed at the quarry and thrown with the cutting edge horizontal to the ground. The weight distribution he thinks would cause the missile to revolve in such a way that the sharp-pointed end would move at great speed. In my opinion, experimental throwing of handaxes seems to disprove this theory; handaxes exhibit immense variation in their form, and many of the type with one pointed end have a rounded butt deliberately left unretouched, presumably to provide a safe grip. That different shapes and sizes of axes were designed for different functions seems without question, but it is incorrect to call all these tools handbolts.

The second paper (No. 143) is a contribution by Leon Underwood and is concerned with Upper Palaeolithic perforated batons. These, often decorated, have been interpreted in the past as leather-working tools, arrow straighteners, or generally as emblems of authority and ceremony. Underwood argues that these batons were used as spear-throwers on the basis of analogous forms in Eskimo throwers. The latter have a prepared grip and a nipple to engage the end of the spear shaft. On not a single Palaeolithic baton has the end survived sufficiently to provide the slightest hint of a nipple or hook, and apart from this hypothetical appendage, there is really no point of comparison between the batons and the Eskimo throwers other than the hole or holes. That such batons, if provided with hooks, would function as spear-throwers is not disputed, because Underwood has provided models to show this. But there are a few complete Palaeolithic batons, and these show no sign of the hook nor of a bound-on hook. In any case it would seem strange if the development of the baton as a spear-thrower, from Aurignacian times through the Magdalenian, did not become abruptly altered by the apparently quite rapid appearance of the true spear-thrower in mid-Magdalenian times; in fact, there is a high degree of consistency in baton development over some 15,000 years. Underwood has made a number of provocative statements in his paper; although some of these are improbable, his ingenuity may nevertheless stimulate further work of this experimental kind.

J. M. COLES

THE TEACHING OF PARASITOLOGY

PARASITOLOGY is taught in schools, in agricultural and biological departments of universities and technical colleges and in schools of medicine and veterinary medicine. Parasitologists are employed in a wide range of research establishments and many people trained in parasitology have found employment outside this field. The British Society for Parasitology felt that a meeting on the teaching of this subject would bring together those who taught and those who employed parasitologists and that all would profit from an informal discussion of common aims and problems. Accordingly, a colloquium was held at the Imperial College of Science and Technology, London, on January 10.

The form of the colloquium itself was an experiment as a large number of people were expected to attend and the usual formal meeting was quite unsuitable as a basis for the informal discussion which was envisaged. It was eventually decided that four speakers should briefly discuss particular topics of a general or controversial nature and that the participants should then divide up into small groups to discuss the papers and a list of topics prepared by the organizers. Each group was to be led by a rapporteur and at the end of the group discussions the participants would reassemble and listen to summaries from the rapporteurs and discuss them. In advance, each participant received a summary of the replies to a questionnaire which gave some idea of how much and what kind of parasitology was being taught in Great Britain. Questionnaires had been sent to biology or zoology departments of universities, schools of medicine and veterinary medicine and certain colleges of technology. Forty-one biology or zoology departments in universities were approached; thirty-five replied, and of these twenty-nine ran some sort of course in parasitology and twenty-three of these planned to expand their courses during the next 5 years. Eighteen of the twenty-two medical schools replied and twelve of these ran parasitology courses, three of which will be expanded. All six veterinary schools replied and four of these ran courses, one of which is to be expanded. Seventeen technical colleges were sent questionnaires and twelve replied; of these, nine ran courses and all planned some expansion. In all, fifty-four separate departments run eighty-three courses in parasitology each year and a considerable further expansion is envisaged. These courses are very varied and range from one or two lectures to postgraduate courses lasting a whole academic year. The content of the courses is also varied but, in general, the parasitology taught to the veterinary students is applied, while that taught to all other students ranges from wholly systematic to wholly experimental, although most courses cover all aspects of the subject to some extent. Other information obtained from the questionnaire dealt with the nature and content of parasitology courses and was intended to act as a stimulus for discussion.

Eighty-six people attended the colloquium, which opened under the chairmanship of Prof. B. G. Peters, who outlined the nature, aims and procedure of the meeting. Prof. W. E. Kershaw spoke in general terms about the teaching of parasitology and more particularly about the M.Sc. course at the University of Liverpool. The second speaker was Dr. H. D. Crofton, who discussed the different problems involved in teaching science and veterinary students. The latter, he said, regarded

any course as an interruption in the path of their chosen career and thought they could obtain most of the parasitology they needed from the publications of the pharmaceutical firms. The method of teaching different types of students should vary considerably, and scientists could benefit from an ecological approach. D. A. Denham spoke from the point of view of someone who had recently been taught parasitology. He criticized the tendency to teach this subject as an extension of a course on systematic zoology; in other words, parasites rather than parasitology. Nothing was more disheartening to a student than boxes of stained slides, and many must have been put off the subject by this approach. The parasites of parasitology, he said, were those who used this easy way of teaching. The last speaker was Dr. S. R. Smithers, who spoke as one working in a research establishment as a 'consumer' of parasitologists. Dr. Smithers argued that a parasitologist ought to be something else as well, an immunologist, a pathologist or a taxonomist, for example. Specialization ought to be something that came after an undergraduate career. An ideal situation would be one in which an undergraduate received instruction in a number of interlinked disciplines including parasitology.

The meeting then broke up into five groups in order to discuss the teaching of parasitology informally, and reassembled at the end of the day to summarize the findings.

The topics discussed and the views aired in the discussion groups were many and varied and it would be impossible to summarize them all in a short space. It was generally felt that something about parasites ought to be said at schools, but that there was no need for a separate course in parasitology. The use of parasites other than those in the standard text-books was desirable especially as many are easily obtained from animals used for dissections, whereas few schools have the facilities to show pupils the living parasites required by most syllabuses. Plant nematodes are a particularly good introduction to experimental parasitology as their use requires no licence. The teaching of undergraduates was discussed at length and the only common conclusion reached was that there are many ways of teaching parasitology and this variety cannot do anything but good for the subject.

It was felt that veterinary students required a course which was vocational in nature and that, unless they were being specifically trained for work in the tropics, parasites such as trypanosomes need only be mentioned in passing. During such a course a student should be introduced to the wider field of parasitology, but this should not be the main object of the course. Medical students receive very little training in parasitology and it was generally agreed that a short course is useful and even one lecture is better than none, but that long courses would serve no particular purpose. The requirements of science students are quite different from those of medical and veterinary students, and the various opinions expressed ranged between wide extremes. General agreement was reached that parasitology ought to be mentioned early on in an undergraduate course in order to stimulate interest in the subject. This need not take the form of specific lectures but could be incorporated into zoology or physiology courses. Parasitic examples could be used to illustrate a variety of biological principles, and the way in which this was done could encourage interested students to look further into the subject.

What was generally agreed was that a sound biological background was necessary before parasitology could be studied in depth. The general feeling was that formal courses in parasitology should be left until the final undergraduate or first postgraduate year, by which time the students would have covered the necessary background. The type of course offered to final year students varies considerably from university to university, and this variation provided the basis for further discussion. No single type of course could equally be applicable in all departments and it was felt that a student needed instruction not only on the parasites and their interactions with their hosts but also on the disciplines which impinge on parasitology. A number of courses run at present take the form of a review of the parasitic protozoa, platyhelminthes, nematodes and arthropods, with brief mention of their physiology, biochemistry and ecology. It was regretted that only too often a parasite is regarded as something in a taxonomic key rather than as a living organism. It was agreed that some systematics must be taught and the more that is covered in the pre-parasitology years the less needs to be done in the final year. Students of parasitology should be able to use keys, and some training in this was thought to be necessary, although no agreement as to the extent of such training could be reached. Some felt that emphasis on difficult but basic characters, such as the bursae of nematodes, would be preferable to a general coverage of a number of taxonomic points and others felt the reverse. What was agreed, however, was that there is, and always will be, a need for people who can identify parasites.

Considerable discussion ranged around other possible approaches to the teaching of parasitology, especially the experimental approach, although few participants actually had much experience of such courses. The main difficulty seemed to be a lack of possible experiments, but most people were willing and able to devise suitable exercises based on their own first-hand experience, so the difficulty is really one of communication. It was agreed that the British Society for Parasitology would provide a useful service if it collected together a number of tried experiments and exercises and distributed them to those involved in teaching. Individual projects undertaken by students were also thought to be useful in the teaching of the subject and difficulties which might arise could be overcome by the exchange of information.

Postgraduate courses were discussed by all the groups and the opinions expressed varied considerably. The general feeling was that such courses were useful but that they could easily become white elephants. The courses really needed were those which brought several disciplines together and were of a vocational nature. There seemed no point in beginning a postgraduate course simply to assimilate material pushed out of an undergraduate course, nor was there any point in such a course if it was merely an expansion of one given to undergraduates. The problem of financing students for such courses was raised and difficulties had been encountered. The general feeling was that the initiation of postgraduate courses should not be undertaken lightly but that well-planned courses of a high standard and of a vocational nature could contribute much to the field of parasitology.

Other aspects of the teaching of parasitology discussed included the merits of sandwich courses, which were regarded as being very useful and in some ways better than the usual undergraduate courses. The expansion of this type of teaching might have a considerable impact on the future of the subject. The training of technicians should be mainly vocational but it was felt that those hoping to work in the tropics should be taught some basic science and, in particular, the use of keys.

All the participants at the colloquium had the opportunity to learn and to preach, and most did both, so the meeting served its purpose. Among the concrete proposals which arose were suggestions that the British Society for Parasitology should consider collecting not only the experiments in parasitology already mentioned but also lists of teaching materials and teaching aids such as films and film strips. It remains to be seen whether or not this meeting will have any impact on the teaching of parasitology, but there is no doubt that the subject will continue to be taught and no pessimism was felt concerning the future employment of the students in these courses.

F. E. G. Cox

BIOGEOGRAPHY OF SOVIET ASIA

EXPLORATIONS of the vast territory extending from the Urals and the Caspian Sea to the Pacific Ocean date back more than a century and have been intensified in the recent decades, but their results are scattered in many regional publications, either botanical, or zoological, rarely both. Two recent books, each dealing with a little-known part of Soviet Asia, provide much more comprehensive biogeographical information than is reflected in their titles*.

Middle Asia is not a political, but a physico-geographical unit comprising southern Kazakhstan, Uzbekistan, Tadzhikistan, Turkmenia, north-eastern Iran, Northern Afghanistan and the Tian-Shan system. Kryzhanovsky's book, in its introductory chapters, provides a concise history of exploration in this region, summarizing the views of botanists and zoologists on the present state and the origins of its vegetation and fauna. Equally concise and clear accounts are given of the palaeogeography of the region from the Upper Cretaceous to the Quaternary, emphasizing its continuous and gradually increasing aridity, except in the geologically younger ranges of the Tian-Shan; the main thesis is that the Middle Asian arid landscapes are much more ancient than those of the northern Africa in the west and the Central Asia in the east; fluctuations of climate and violent orogenetic processes favoured radiating evolution within the region of predominantly xerophilous flora and fauna. The main chapters of the book are based on a thorough analysis of the insect fauna in relation to the distribution of genera and species both within the region and elsewhere. This is followed by descriptions of the populations of various landscape types—sand deserts, sandy plains, submontane ephemeral steppes, mountains, river valleys and cultivated lands. In each case the background conditions of existence of plants and animals are discussed in relation to their previous evolutionary history and the resulting extremely intricate pattern of strongly contrasting elements is made clear. The concluding chapters present broad views of the present composition of the terrestrial fauna (not of insects only) and of the main events leading to its formation; the significant conclusion is that Middle Asia should be regarded as the most ancient and important centre of origin of the desert fauna and flora of the northern hemisphere, a view advanced by some Russian biogeographers in the past and opposed by others, but greatly strengthened by the overwhelming evidence so masterfully presented by Kryzhanovsky.

The book by Kurentzov deals with another fascinating and relatively little-known area, the Soviet Far East. The author's own field-work there extended over many years and he adopted an attractive method of taking the reader over a variety of landscapes, from the lush deciduous forests in the southern valleys, where many subtropical plants and animals are found; forests of cedar harbouring the Amur tiger; oak forests on mountain slopes; conifer taiga still higher up, with the peculiar flora and fauna of bare outcrops; semi-arid lands of Dauria, etc. Vivid descriptions of landscapes and of their animal (mainly insect) population are given and the typical representatives of the fauna of each ecological type are illustrated. A competent analysis of the fauna and of its chequered history, in relation not only to the surrounding parts of Asia but also to North America, concludes the book.

A feature of both books, which is characteristic of the Soviet approach, is that they include detailed cartographic division of the areas in relation to their natural resources, thus combining scientific description with the potential planned development. The wealth of the factual material, condensed from extensive, mainly Russian, literature, makes both books outstanding as the sources of up-to-date knowledge of the two regions of Asia which are among the less known to western biogeographers.

BORIS UVAROV

* *The Composition and Origin of Terrestrial Fauna of Middle Asia*, by O. L. Kryzhanovsky (Moscow–Leningrad, 1965), 419 pp., 24 figs. *The Zoogeography of the Amur Region*, by A. I. Kurentzov (Moscow–Leningrad, 1965), 155 pp., 80 figs.

VIRUS NOMENCLATURE

IF it is accepted that viruses have characteristics that distinguish them from animals, plants and bacteria, it follows that their nomenclature cannot be ruled by any of the existing international codes of nomenclature. Taking this into consideration and recognizing the need for an internationally adopted system of virus nomenclature, the International Association of Microbiological Societies (IAMS) has taken initial steps to set up an International Committee of Nomenclature of Viruses (ICNV) to meet for the first time at the Eighth International Congress of Microbiology being held next July in Moscow. Affiliated societies of microbiology have been invited to appoint members to serve on the committee.

A provisional Committee of Nomenclature of Viruses (PCNV) appointed by the International Association of Microbiological Societies met in Paris and prepared a document containing recommendations to be considered by the main meeting in Moscow (*Ann. Inst. Pasteur*, 5, 625; 1965). In these recommendations the basis for a code of virus nomenclature is suggested. The main points of these recommendations can be summarized as follows: (1) Viruses of animals, plants and bacteria should be gathered into the single phylum *Vira*. (2) The phylum should be divided into sub-phyla, classes, orders, families, genera and species, these divisions being by necessity arbitrary and not equivalent to taxa of other phyla. (3) Genera should have Latin or Latinized Greek names ending in *virus*. Each genus is typified by a well-authenticated species to be deposited in international culture collections. (4) Each family should be named after a type genus to which the suffix *idae* is added. (5) The code should not include a law of priority. A list of *nomina conservanda* in which virus names in general use are maintained whenever possible is submitted for approval by the International Committee of Nomenclature of Viruses. (6) Taxa designated after personal names, anagrams, siglas, hybrids of names or nonsense names should not be acceptable. (7) New names to be adopted must be submitted to the International Committee of Nomenclature through its Judicial Commission.

The question of virus classification as opposed to virus nomenclature is not ruled by the code and it is open to virologists individually or in groups to put forward proposals in this respect. Making use of this privilege, the provisional Committee suggests a system of virus classification conforming to the principles laid down in the proposed code.

Four characters, namely (*a*) type of nucleic acid, (*b*) symmetry of the virion, (*c*) presence or absence of envelope and (*d*) dimensions, are adopted in this hierarchical order

to divide the phylum into sub-phyla, orders, classes, families and genera.

The recommendations of the provisional Committee of Nomenclature were recently presented for discussion at a well-attended meeting of the Virus Group of the Society for General Microbiology held in London. That the proposals received considerable criticism is perhaps not surprising in view of the strong and even emotional feelings usually generated by matters of taxonomy. Critics fell largely into three groups:

(*a*) Those who thought that at our present state of ignorance viruses cannot be classified on a rational basis and therefore no attempts should be made to devise a uniform system of nomenclature until more information becomes available.

(*b*) A more important group of speakers pointed out a number of inaccuracies of fact and interpretation contained in the proposals and criticized the choice and hierarchical arrangement of criteria adopted for the classification system.

(*c*) Last, but not least, a number of speakers objected to the use of Latin names and to the adoption of a classification based on a fixed set of characters arranged in an arbitrary hierarchy. This group, represented mainly by plant virologists but supported by some eminent animal virologists, offered as an alternative a system based on Adansonian principles in which viruses would be designated by vernacular trivial names followed by a cryptogram containing information available or not on a number of unweighted characters (Gibbs *et al.*, *Nature*, 209, 450; 1966).

It is difficult to imagine a system of virus nomenclature which will please all virologists, but it is hoped that discussions such as the one reported here will lead to proposals acceptable by a sizable majority.

H. G. PEREIRA

PROBABLE MODE OF LANDING OF *LUNA* 9

By PROF. T. GOLD

Center for Radiophysics and Space Research, Cornell University, Ithaca, New York

FROM the data available through the news media concerning *Luna* 9 one can make some guesses as to its mode of landing which are of interest in a discussion of the lunar surface that is implied. Apparently, the spacecraft consisted of two parts: a bus of about 1·5 tons and a 2-ft. diameter sphere of 220 lb. It would seem that the sphere had no propulsion or attitude control units aboard but was merely the container for the experimental equipment. The bus contained all the proximity radar, the propulsion and attitude control units and all the associated electronics required to achieve the soft landing; that is, the low velocity relative to the lunar surface. The sphere was separated from the bus when its propulsion and control function was over—that is, in the final free-fall period.

Such a technique has several advantages in simplicity over one where the entire vehicle lowers itself on to the surface to stand erect for the purpose of the experimentation and telemetry:

(1) *Cancellation of the horizontal component of velocity.* In the *Luna* 9 technique it will be permissible, and possibly even advantageous, for the sphere to approach the surface with a horizontal component of velocity several times larger than the vertical. The radar control of the landing thus avoids the necessity of high precision in the detection and cancellation of this component, which is one that must be more difficult to control than the vertical. One supposes, therefore, that experiments will have been carried out of dropping the sphere in various ways on a variety of surfaces to decide what the permissible tolerance in the two components of velocity would be. On most surfaces the horizontal components of velocity could be between five and ten times greater than the vertical for a given permissible amount of damage or a given value of peak deceleration. One has to suppose, therefore, that the tolerances on the landing mechanism of the bus were such as to leave the sphere finally at impact with five to ten times as much horizontal as vertical velocity. We have no knowledge what the total velocity was beyond the statement that it was 'several metres per second'.

(2) *Absence of attitude control.* No attitude control is required in the final phase of the descent when all the active manoeuvre of the bus is finished. The sphere can be separated in some arbitrary way to skip, bounce and roll over the surface and finally come to rest. Its internal weight distribution may be such as to favour the desired way up and the subsequent opening of its four petals will assure that no extremely skew position can be maintained. It is obviously very unlikely that the sphere would lie precisely upside-down when the petals open so that they would fail to erect it.

(3) *Freedom from sinkage.* Such a method of landing will have appeared attractive in view of the unknown strength of the lunar surface. The loading should clearly be greatly reduced by abandoning all heavy rocket and control equipment and relying on a comparatively light sphere of a diameter comparable perhaps to that of the bus. Secondly, a large horizontal velocity at the impact of the sphere would tend to make the initial impact one of skipping like a stone on water, rather than one in which digging-in could occur. For the velocity tolerances that would be likely, and for as flat an angle of approach as given by the ratio of horizontal to vertical velocity of between five and ten, such a sphere would indeed skip, even on water, and certainly on most suggested types of lunar surface. The place where the sphere finally comes to rest in this mode will not have suffered any impact loading, and the compaction will only be that appropriate to the lunar weight of the sphere, which it would seem is 40 lb.

If indeed this were the mode of landing of *Luna* 9 then it would be clear why no impact information has become available that reflects on the strength of the lunar surface. The sphere has no attitude control and probably no internal knowledge of attitude in its tumbling phase. Even if accelerations were available for telemetry no meaningful integration of the accelerations could be carried out beyond gaining some idea of the length of time for which the sphere skipped and rolled. The small effect on the vertical component of acceleration which any compressibility of the surface might have made can then obviously not be sorted out when there is no independent directional information available in that phase. It is thus probable that nothing more can be said than that a 2-ft. sphere pressing on the surface with a force of 40 lb. did not sink in more than a few inches. It is not quite clear what depth of sinkage the television pictures would have revealed but possibly as much as 6 in. would have gone unnoticed. The bearing pressure is then as low as 0·12 lb. per in.^{2}. Most proposed types of lunar surface material would maintain that.

The bus is not visible in the panoramic view obtained by *Luna* 9. The panorama unfortunately shows the near ground over less than half the way around only because the vehicle did not in fact erect itself sufficiently accurately.

The track that the sphere made on the surface would be likely to be from the direction of the bus and that track is thus also not on the available panorama. The other possibility, that the bus is not visible because it sank out of sight, but that on the other hand on the same surface the sphere left no visible track, is clearly most unlikely. The experimentation initially proposed then probably included a study of the visible track, and it was only the unfortunate angle in which the sphere came to rest that made this impossible.

The subsequent movement of Luna 9. It appears that between the picture-taking sequences, a movement occurred of unknown origin in which the camera position was changed by several inches. It was suggested in the Press that the experimenters involved had no knowledge of the origin of this movement and did not attribute it to any activity within the vehicle.

Earthquakes are clearly outside the range of reasonable explanations since a degree of such activity would be required that would have shown up in other ways.

On any dry, terrestrial ground, and in the absence of wind, it would seem very improbable that an object after coming to rest would make significant movements in a period of days. The observation must thus be taken to be a very significant one that implies properties of the lunar surface quite unlike those of terrestrial surfaces.

In whatever position the vehicle came to rest and whatever stresses, such as by spring-loaded panels, were then in effect, it would be necessary to discover some process with a time constant in the range of hours, to allow the movement to be triggered off. The immediate dynamical time constants of the settling would be in the neighbourhood of seconds only. Plastic time constants in dry rock are many orders of magnitude too long. Thermal heating and cooling could have the right time constants, but the displacements resulting are so small that one would need to suppose the vehicle to be extremely delicately poised for such effects to trigger off a substantial movement.

It is thus quite a puzzle to discover some effect that would have been going on for a period of hours after the landing which, when it reached a certain stage, allowed a sudden resettling of the vehicle. Time constants of the same nature as those in an hour-glass may provide an explanation. If the material of the lunar ground were composed of small rock particles, slightly adhering to each other, it would be possible for there to be a lot of sub-surface hollow spaces. In particular, many small secondary impacts at rifle bullet speeds would make long tunnels at all angles through such a material. The structure that results would be the statistical equilibrium between the compacting effects of some types of impact and the tunnel-digging effects of others. On such a surface it would then not be too unlikely for the added weight of the vehicle to result in many little slides and collapses in internal cavities, which gradually reduce the strength of the ground and eventually let it settle. The time constants, as in an hour-glass, would be those of the dynamical time constants of falling grains but multiplied by the number of grains that had to fall in sequence to produce the effect.

While this explanation can be taken as a suggestion only, the fact that the ground tends to give way after some period of time under an additional load has probably to be taken seriously.

SIGNIFICANCE OF INCLUSIONS IN CARBONACEOUS METEORITES

THE possibility has frequently been suggested and discussed that all meteorites which have become available for observation have originated from the same parent body, or from a comparable set of parent bodies, of asteroid dimensions. Such a theory is supported by hitherto observed instances of interbrecciations between: siderite–pallasite; siderite–mesosiderite; siderite–chondrite; siderite–achondrite; mesosiderite–chondrite; mesosiderite–achondrite; chondrite–achondrite (for a summary see refs. 1 and 2).

That such a common origin exists has not yet been proved through actually observed associations of those meteorites which could be included in the 'main carbon-rich groups', with more than 0·2 per cent carbon (namely, (*A*) carbonaceous chondrites; (*B*) ureilites; (*C*) enstatite chondrites).

The thirty or so carbonaceous chondrites which have so far been recorded have been sub-divided by Wiik[3] into three types; the carbon + water + sulphur ranging between 24 and 30 per cent for 'type I'; 12 and 24 per cent for 'type II'; 3 and 12 per cent for 'type III'. Recent investigations have shown[4] that 'type I' stones consist of more than 90 per cent low-temperature ground mass of hydrated silicates and carbonaceous matter, of grain-size less than 1μ, in which are embedded globular or rounded particles, varying in diameter from 5 to 50μ, of magnetite, glass, olivine, troilite and other high-temperature minerals. I have interpreted[4,5] these to be 'microchondri', that is, solidified 'fog' particles from an incandescent cloud, as often they reveal exsolution cores. In the 'type II' stones, the ground mass is reduced to about 60 per cent and larger chondrules, that is, 'raindrops', are present in addition to the 'microchondri'. Most of these are, however, of the 'bunch of grapes' type, and still reveal the original microchondrules. These apparently became compacted in a viscous or sticky state. Within 'type III' stones the ground mass is reduced to about 35 per cent, and the majority of the chondrules are 'fully coalesced'; the latter stones grade into the non-carbonaceous chondrites. The three hitherto known ureilites contain between 0·5 and 1 per cent carbon, and they consist of what appear to be squashed single crystals or granular aggregates (possibly of 'microchondrules') of silicates, with concentrations of carbonaceous matter and diamonds along the grain boundaries. The ten enstatite chondrites contain 3–10 per cent carbon + water + sulphur, and, roughly speaking, have a petrographical structure similar to the 'type III' carbonaceous stones or the ordinary chondrites. More detailed descriptions of the carbon-rich groups of meteorites are to be found elsewhere[2,4,6,7].

This article describes some recently observed examples of inhomogeneities, inclusions and interbrecciation, in meteoritic materials of which at least one sample belongs to the foregoing 'carbon-rich' groups.

Inclusions

(1) *Orgueil*

Inclusion A. In the course of preparation of material for analytical work during 1962, I carefully separated some 3 g of the stone, obtained from a 20-g crust-less fragment from the Museum in Paris. An unusually hard, angular inclusion was found, which on analysis revealed a rather higher percentage of troilite plates and magnetite microchondrules than was present in the rest of the stone; however, the petrographical structure remained within the range of the 'type I' stones. An essentially similar type of angular inclusion was observed in a thin section of the Orgueil meteorite[8].

Inclusion B. I observed in a thin section of a rough-surfaces specimen of the Orgueil material from the Paris Museum an inclusion of about 1·5 × 1·0 mm cross-section. This had an approximately globular habit (in fact, in outline, it had the shape of a jug) and in cross-section appeared to consist of some eight 'bunch-of-grapes'-type

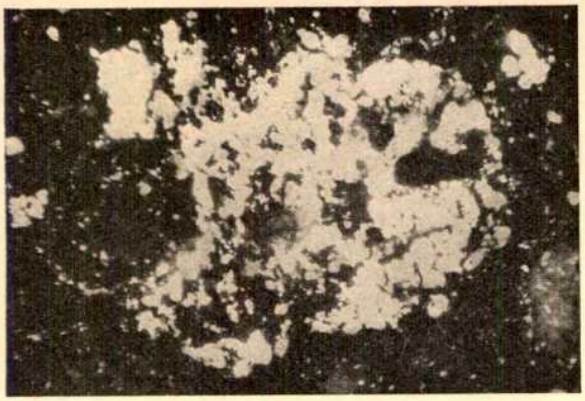


Fig. 1. Group of chondrules forming a small inclusion of 'type II' carbonaceous chondrite, within a thin section of Orgueil from the Museum of Paris. The two smaller chondrules on the lower left-hand side of the microphotograph reveal concentrical zoning of transparent (glass and crystalline silicate) and opaque (magnetite, troilite and nickel-iron) microchondrules. These rather rare features, however, were also observed by me in the 'type II' Cold Bokkeveldt, and the 'type III' Vigarano, and Mokoia. Ordinary light (×*c.* 45)

chondrules, and a larger coalescent mass of troilite within the largest of them (Fig. 1). The individual microchondrules were magnetite, troilite, glass and single crystal grains of a material as yet unidentified, but which appeared somewhat corroded and displayed a birefringence similar to that of olivine. The chondrule assemblage within the inclusion was comparable with that of 'type II' stones, in which similar groups of chondrules of up to 10 mm cross-section frequently appear, without much ground mass.

Inclusion C. In the course of dissecting a sample (see inclusion *A*), a hard, grey fragment, weighing 0·13 g, was obtained which, so far as could be deduced from the small quantity of substance available, closely resembled the pidgeonite chondrites—this was so both from the point of view of chemical composition (carbon, 0·17 per cent; sulphur, 2·26 per cent; hydrogen, 0·05 per cent), mineral composition and petrographical structure. When a powdered sample of the inclusion was examined, iron-rich olivine and pidgeonite were found together with the usual opaque minerals, including some nickel–iron. These would seem to have originated from major chondri, but a smaller olivine chondrule with the rather unusual feature of a 50μ diameter gas inclusion was also observed (see ref. 7).

(2) *Mighei*

A perfectly angular inclusion of about 4 cm × 2·5 cm surface area was found in one of the 'type II' Mighei stones from the Moscow Museum. The fragment in question is considerably lighter than the main mass, and an investigation using a binocular microscope revealed a chondrule assemblage characteristic of 'type III' stones.

(3) *Sharps*

The examples described so far illustrate fragments of meteorites of lower volatiles and lower carbon contents, included in hosts of higher carbon content. In contrast, an approximately brick-shaped 'type I' fragment with well-defined straight boundaries was discovered by Dr. K. Fredriksson (Smithsonian Museum, Washington) in the Sharps meteorite. The meteorite is at present being studied in detail by Dr. Fredriksson, and so this account will be restricted to a few preliminary observations. Both the elemental composition and the petrographical structure of the host material appear to be transitional between 'type III' carbonaceous chondrites and pidgeonite chondrites. The elemental composition of the inclusion according to Dr. Fredriksson is: carbon, 5·9 per cent; sulphur, 2·5 per cent; water, 2·5 per cent—the percentage of carbon being rather greater, and the sulphur and hydrogen lower than the range of the 'type I' stones at present known. Owing to the oxidizing conditions indicated by the mineral assemblage of the stone (and carbonaceous chondrites in general), it is not surprising that a proportion of the sulphur is found to be in a relatively volatile state as the free element and as sulphates, whereas the bulk of the carbon is in a relatively non-volatile state as graphite, amorphous carbon and highly polymerized organic molecules, the hydrogen, of course, being volatile. For this reason, we would expect that any thermal metamorphism would have resulted in a depletion of sulphur and hydrogen without a comparable depletion in carbon; this proved to be the case for the inclusion in question. The high carbon-level suggests that the inclusion was, in its original state, a material even richer in carbon, hydrogen and sulphur than our presently known 'type I' stones. That the inclusion entered the host when the latter was still hot is clearly indicated by the bands of 'redistribution' brought about by distillation of the carbonaceous matter. Such 'redistributions' follow, in a concentrical fashion, the margins of the inclusion and even those of the minor host inclusions or protrusions within the inclusion (see Fig. 2). Such a phase of thermal metamorphism is also indicated by the rather markedly crystalline nature of the ground mass, and by certain strain

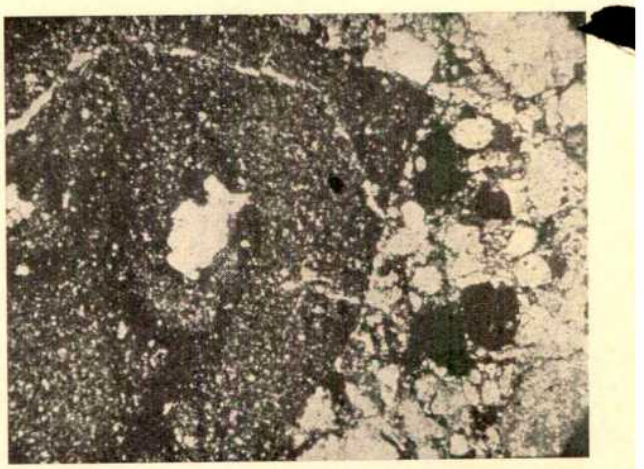


Fig. 2. Part of a 'type I' inclusion from a thin section of Sharps meteorites from the Smithsonian Museum, Washington. Note the marked change of statistical petrography between the inclusion and the host, and the dark carbon-rich zones which are approximately parallel to the margins, and to the margins of the two smaller inclusions of the host, within the inclusion proper. Ordinary light. (×*c.* 26)

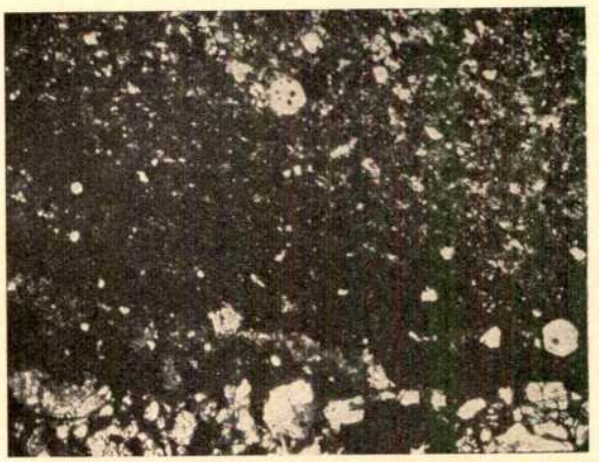


Fig. 3. Detail of an inclusion from Sharps meteorite. The boundary of the host runs along the base of the microphotograph. Note carbonaceous band parallel to the boundary, and also: (*A*) possibly strained glass cored microchondrule at the bottom right-hand side with a very low (about 0·05) diameter of core : diameter of microchondrule, ratio, which is characteristic of the 'type I' stones in general (ref. 4); (*B*) multicore olivine microchondrule, with troilite and nickel–iron cores, at top centre; (*C*) several glass and crystalline silicate microchondrules at top centre and left centre. Ordinary light. (×96)

anisotropy of some of the microchondrules of glassy appearance. On the other hand, microchondrules of unstrained glass, and cored microchondrules of crystalline silicate (most likely olivine), do exist (Fig. 3), which makes the similarity between the statistical petrography of 'type I' stones and the inclusion in question very close indeed.

(4) *Renazzo*

The features of the inclusion observed in the Sharps meteorite are essentially repeated, within a rather squashed inclusion, with some flow structures, in a thin section of Renazzo from the Smithsonian Museum, Washington (Figs. 4 and 5). The flow structure and inclusions of some chondri in this indicate a more intensive metamorphism, and this impression is strengthened by the more recrystallized nature of the ground mass (compare Figs. 2 and 3 with Figs. 4 and 5). The presence of individual glass and magnetite microchondrules is most significant (Fig. 6) as, in my experience, these are exclusively characteristic of 'type I' stones.

(5) *Cold Bokkeveldt*

The foregoing examples illustrate associations of meteorites within the chondrite–carbonaceous chondrite series. I found in one of the sections of Cold Bokkeveldt meteorite at the British Museum (Natural History) some angular material (perhaps squashed or fragmented large globular type chondrules) reminiscent of typical ureilite structure. An entire section from the same source, however, reveals an exclusively ureilite-type structure with large single crystal grains of clinopyroxene and globular patches of olivine, troilite and nickel–iron, obscured by carbonaceous matter, graphite and possibly diamonds along the boundaries. This structure is characteristic of the Goalpara ureilite, which can be classified as 'type I ureilite'. In the case of Novo Urey and Dyalpur, both the clinopyroxene and the higher melting olivine grains are coalesced into single crystals; these can be classified as 'type II ureilites', as they were formed at higher temperatures. A specimen of Cold Bokkeveldt exhibited in the Museum in Vienna is a breccia revealing numerous angular fragments of dark ureilite in the lighter matrix of the 'type II' stone.

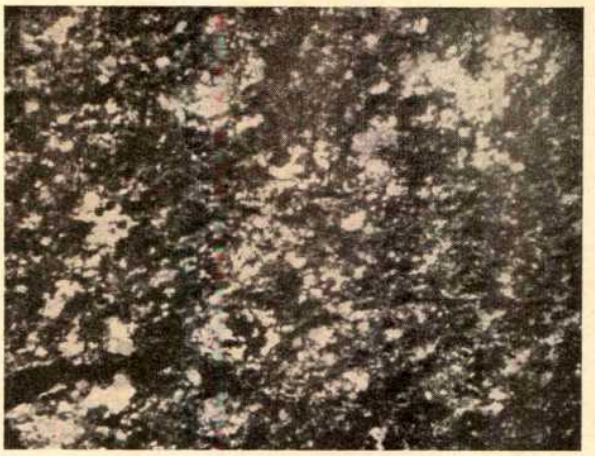


Fig. 6. Ground mass of 'type 1' inclusion in Renazzo. Note magnetite microchondrules and groups of partially fused magnetite microchondrules, which, from my previous experience, are quite exclusively characteristic for 'type 1 carbonaceous chondrites. Ordinary light. (×304)

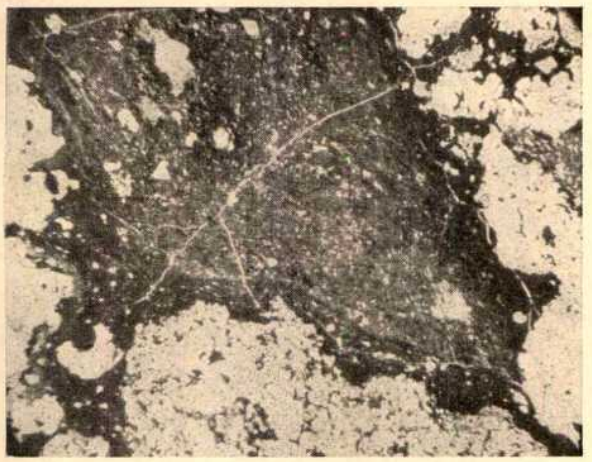


Fig. 4. Inclusion of a 'type 1' stone from a thin section of Renazzo, from the Smithsonian Museum, Washington. Note indications of extensive thermal metamorphism of inclusion; compared with Sharps meteorite (Fig. 2), there some flow structure is evident, and the mass seems to be partially squeezed in between the gaps of chondrules. Carbon enriched zones are present, similarly to those of Sharps meteorite, parallel to the margins and also surrounding a group of included chondrules from the host, along the upper left centre of the microphotograph. Ordinary light. (× c. 26)

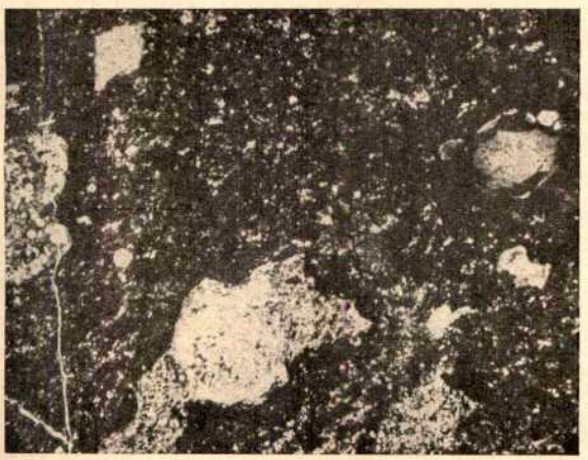


Fig. 5. Detail of the inclusion in Renazzo meteorite shown in Fig. 4. Note almost continuous opaque (carbonaceous) bands surrounding the two inclusions of the host along left half of the microphotograph, and also large glass microchondrule along right and several small glass microchondri along left centre. Ordinary light. (×96)

(6) *Mokoia*

With the exception of an obvious impact contaminant resin[9], no inclusions were observed in this meteorite, although Briggs and Kitto[10] reported a range of 0·5–2·1 per cent carbon in the distinct portions of the samples analysed. The total volatiles of eight different grains from the specimen in the Paris Museum were determined, and a range of between 2·58 per cent and 13·52 per cent was obtained—giving an average of 4·36 per cent. (The standard procedure for determining total volatiles of the stones consisted of heating finely ground powder which had been dried in a vacuum desiccator for 10 min to 1,000° C in a nitrogen atmosphere.) Compared with this, five determinations of total volatiles carried out on the Murray meteorite material only gave a range of 12·4–17·2 per cent. It would seem from the results of a minimum of three total volatile determinations that Mokoia may be the most inhomogeneous stone of all those so far investigated, with properties grading from 'type III' to 'type II' within different portions of the stone, even when material is taken from well beneath any known surface crust.

(7) *Kainsaz*

The co-existence of chondri and fragmented chondri with partially interlocking aggregates of well-formed olivine and other silicate crystals was noted in the 'type III' Kainsaz and, to a smaller extent, in the 'type II' Cold Bokkeveldt. The latter structures are generally similar to those in the chondritic-type achondrites, although major inclusions of achondritic matter have not so far been observed.

Future Potentialities of the Study of Associations between Diverse Types of Meteorites

It appears from the literature that research has, on the whole, been restricted to the more obvious cases of markedly brecciated stones. It is possible that much useful information could be obtained through a systematic search for, and study of, the less-evident associations and inhomogeneities, within both the carbonaceous and non-carbonaceous meteorites.

An unexpected example in support of this view became available to me during a recent field study at the village of Barwell, Leicestershire, with members of the University and Museum of Leicester and the British Museum. We were carrying out an investigation of the impact area of the shower of olivine–bronzite chondrites which fell there during December 24, 1965. I had an opportunity there of making a preliminary inspection of about 50 lb. of the material. It was observed that individual stones varied in shade from very light to medium dark grey; only further investigations could reveal whether these differences are caused by any variation of the percentage of carbon (which is expected to be within the 0·0*X* range for the type of stone in question) or by some other chemical or physical (porosity, etc.) factors. One specimen contained a globular olivine aggregate, 0·5–0·75 in. in diameter, which is unusually large for a chondrule-type structure: certainly it deserves further detailed study.

Such studies could well include an investigation of any chemical interaction between inclusion and host and a search for possible zoning, as well as the chemical and petrographical characterization of 'variable' stones (such as Mokoia and Barwell) which show apparently smooth transitions in properties without any sign of brecciation. In any investigation of these and other problems, the application of some of the more recently developed experimental techniques should prove particularly useful for the detection of changes in the carbonaceous complex—particularly so spot tests for the distribution of major and minor trace elements, infra-red and ultra-violet spectroscopy, gas chromatography and mass spectrometry.

Some Theoretical Considerations

The associations described here clearly point to a common parent body for at least some of the following types of meteorites; (1) Type I–type III carbonaceous chondrites–chondrites (Orgueil, Sharps). (2) Type I–type III carbonaceous chondrite (Renazzo). (3) Type II–type III carbonaceous chondrite (Mighei, Modoia). (4) Type II carbonaceous chondrite–ureilite (Cold Bokkeveldt). (5) Type II carbonaceous chondrite–achondrite (Cold Bokkeveldt). (6) Type III carbonaceous chondrite–achondrite (Kainsaz).

This covers the main range of carbonaceous and non-carbonaceous stony meteorites, with the exception of certain rarer varieties. In view, however, of the very considerable chemical and petrographical uniformity within the 'carbonaceous chondrite–chondrite series', it is likely that, even in cases where associations have not so far been observed in our studies of inclusions, the stones in question originate from identical or very similar parent bodies.

Before any profitable analysis of the possible mechanisms of the emplacements of the fragments can be undertaken, some genetical implications must be considered of the interrelations between volatile and non-volatile constituents of the 'carbonaceous chondrite–chondrite series' (ref. 4). It seems to me that the most likely interpretation of these is that, at the highest temperature stage of its cosmological history, the parent body had an incandescent cloud mantle, covered by cooler clouds of cosmic dust. The 'type I' stones are probably derived from the close-to-surface zones, where mild volcanic activity—pulsation through de-gassing—may have caused a limited amount of intermixing of the cool cosmic dust with spray particles from the marginal zones of the incandescent cloud and volatile condensates. This would explain the small spray diameters and high percentage of glass that was rapidly quenched.

Stones containing less volatile material would be derived from increasingly deeper zones of the parent body, and would have had less access to cosmic dust. They would have received greater quantities of spray particles from the more crowded and hotter portions of the incandescent cloud mantle and, furthermore, would have been subjected to a greater degree of heating subsequent to the final cohesion of the material of the stones from the incandescent clouds. Ureilites, and other types of achondrites, may represent localized spray condensates—that is, 'lavas'—which formed from the highly degassed or impact-shocked portions of the incandescent clouds. Alternatively, the features could be explained as being due to a more localized close-to-surface 'ash eruption'-type activity—the stones rich in volatiles coming from the regions farthest from the erupting vent. It is a possibility that the original vertical zoning, through the 'incandescent cloud mantle' model effect, was afterwards modified somewhat by localized volcanic eruptions and intrusions.

The previously observed shift of the volatilization curves towards lower temperatures with the increase of total volatiles content of the stones seems to favour the 'incandescent cloud' model (as discussed in ref. 11). Certain features of the inclusions, however, indicate occasional localized volcanic activity. Thus, the presence of higher volatile, thermally metamorphosed fragments in Sharps and Renazzo can be considered to have arisen from an upsurging column of dense incandescent cloud with perhaps partially solidified spray particles which may have engulfed low-temperature fragments near the surface. The exceptionally large gas bubble in the chondrule of 'Inclusion *C*' of Orgueil seems to indicate a sudden release of pressure; it is probable that this was due to the rapid ascent of the fragment in the course of eruptive activity.

The fact that all the inclusions observed so far have well-defined straight boundaries (with the possible exception of inclusion *B* of Orgeuil) excludes, of course, any possibility of water transportation and is an additional evidence for the essentially 'volcanic breccia'-type origin of the structures. However, in the terrestrial pyroclastics, some rounding of the individual fragments is not uncommon, particularly where more extensive and explosive volcanic activity has occurred. The absence of any sign of rounding, even in such soft material as that included in Sharps, indicates that the volcanic activity was of a mild character and of short duration, as would be expected for a parent body of relatively small dimensions.

Finally, the apparent absence of association between the carbonaceous chondrite and ureilite groups of high carbon stones, on one hand, and the enstatite chondrites, on the other, should be considered. The oxidized iron : reduced iron ratio of carbonaceous chondrites and possibly that of ureilites is the highest in all meteoritic matter. This would suggest that within the close-to-surface zones of the parent body, from which these stones are believed to originate, there was a somewhat hydrogen-depleted, oxidizing assemblage of gases escaping and forming a temporary atmosphere. The high carbon percentage, associated with an extremely low oxidized iron : reduced iron ratio of the enstatite chondrites, may indicate that these stones are derived from the close-to-surface zones of relatively larger parent bodies, in which the de-gassing products, throughout the entire period of de-gassing, remained more reducing as a result of the retention of a higher proportion of hydrogen, methane, and other reducing gases, due to a more intensive gravitational field. If the foregoing considerations are correct, then we should not expect any signs of association between the carbonaceous chondrite–ureilite group of 'oxidized' high volatile stones, and the enstatite chondrite group of 'reduced' ones. It is very likely, however, that a more detailed search would

reveal interbrecciation between enstatite chondrites of different types.

Finally, the anomalously high carbon : sulphur ratio of 2·36 of the inclusion of Sharps is significant in view of the mean of 0·59 for Type I carbonaceous chondrites. This may be explained as due to preferential escape in the course of the thermal metamorphism of the fragment of sulphur as sulphur vapour and sulphur dioxide, and the retention of amorphous carbon and graphite under the somewhat oxidizing conditions that prevailed. The even higher carbon : sulphur ratio for the ureilites of about 6·0 can be explained by the same mechanism, if more intensive heating is postulated: in this connexion analytical data regarding the ureilite-type fragments in Cold Bokkeveldt would be of considerable interest. On the other hand, de-volatilization under more reducing conditions would cause essentially the reverse trend, as then the bulk of sulphur is present as non-volatile ferrous sulphide and the carbon forms mainly volatile hydrocarbons. It is possible that the latter explanation would account for the low sulphur : carbon ratio of 0·06 for the enstatite chondrites and the even lower value (of the order of 0·01) for the ordinary chondrites.

These observations and considerations would seem to indicate that the very broad range of sulphur : carbon ratios of meteorites can be derived through de-volatilization of primordial matter (cosmic dust) of approximately uniform composition under differing temperatures and reducing or oxidizing conditions. Detailed investigations of the composition of inclusions of carbon, sulphur and other volatile elements or inclusions may give us further significant information in assessing the relative importance of the parameters, including the time involved.

I would like to thank Prof. J. D. Bernal, Birkbeck College, London (where much of the work was actually carried out), for encouragement, and Prof. J. Orcel (Paris), Prof. K. Strokay (Budapest), Dr. Henderson and Dr. B. Mason (Smithsonian Institution, Washington), Dr. H. Scholler (Museum of Vienna), Dr. H. B. Wiik (Helsinki), and Dr. G. F. Claringbull (British Museum (Natural History)) for specimens and the opportunity to study thin sections. I also thank Dr. K. Fredriksson (Smithsonian Institution, Washington) for information about his discovery of the inclusion in the thin sections in Sharps meteorite and for placing these at my disposal.

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I CONSIDER the studies contained in Prof. Mueller's article to be of great importance. Much has been shown by examining the single meteorite stone, but this work, for the most part, has consisted of investigations on the stone as a whole attributed to various classes. What Prof. Mueller has done in this way is to examine meteorites containing other types of meteorite as inclusions, thus demonstrating that they must have been derived from the same parent body.

I would like to direct particular attention to the study, by Dr. Fredriksson, of the Sharps meteorite and its bearing on the origin of carbonaceous meteorites 'type I'. At present, only preliminary observations have been made: in my opinion their bearing is conclusive; the Sharps meteorite contains at least one large inclusion of a 'type I' carbonaceous stone.

Two features of this observation call for particular attention. First, the inclusion is an angular fragment with generally defined straight boundaries against the surrounding chondrite. This indicates that it is a fragment broken from a larger, already formed, carbonaceous meteorite which has been incorporated, subsequent to its breaking up, in the then unconsolidated chondrite stone. Secondly, the fragment, as it is found, shows evidence of contact metamorphism, presumably in bands following the outline of the fragment as well as those of the included chondrules. This shows that at the time of inclusion the chondrules were hotter than the carbonaceous material although not so hot as to cause excessive degassing or boiling away of the volatile constituents.

It is clear that further investigation would throw much light on the origin of both types of meteorite. Prof. Mueller has put forward plausible accounts of these with which I am inclined to agree, but more may come out of a more detailed study. This should include a micro-chemical analysis point by point both inside and outside the inclusion to check how far the material had been altered and how far the contact metamorphism has affected the parent body. For this, electron probe investigations might be very useful and also electron spin resonance and nuclear magnetic resonance.

Some such studies will undoubtedly be carried out and it would be appropriate for much of it to be done in Britain, for the British Museum (Natural History) holds the most extensive collection of meteorites in the world. The personnel and apparatus do not yet exist in Britain for such studies to be carried out on an adequate scale. Unless such resources and manpower can be made available, a field of great promise in the age of spacial exploration of our planetary system could well be lost.

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ESTIMATION OF ZINC-65 BACKGROUND LEVELS FOR MARINE COASTAL WATERS

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WITH the increased possibility of a nuclear accident near or in the marine coastal environment a more accurate estimate of background radioactivity levels for elements such as zinc-65 is needed. Estimates of zinc-65 on the Pacific coast are complicated by the uncertainty of the contribution resulting from the Columbia River outflow. The average rate of input from this source is 30 c. zinc-65 and 700 c. chromium-51 per day[1]. Even at these relatively high levels dilution with sea water soon reduces the concentration to such a degree that direct measurement in sea water is impossible. Chemical concentration of zinc-65 in sea-water samples has generally

Table 1 COLUMBIA RIVER SAMPLES

Location	Animal	Concentration in dry tissue						
		Type of tissue	Zn p p m.	^{65}Zn pc./g	^{65}Zn/Zn pc./mg	Cr p p m.	^{51}Cr pc./g	^{51}Cr/Cr pc./mg	Dry/fresh wt. %
Ecola Beach, Oregon	*Mitella polymerus*	Stems	270	30·0	111	—	1·21	—	25 9
		Bodies	1,490	142	96				14·7
		Embryos	1,190	206	173				8·9
	Pisaster ochraceus	HC	188	64·3	342	7·3	5·62	770	31·2
	Mytilus californianus	Soft	220	61·1	278	1·2	1 70	1,420	17·5
North Jetty, Illwaco, Washington	*Pisaster ochraceus*	HC	168	92·9	553	10 0	20 1	2,000	27 4
		♀G	281	220	782	7·5	18·0	2,400	13·2
		♂G	68	38·6	567	12·3	34·4	2,640	13 5
North Head, Illwaco, Washington	*Mitella polymerus*	Stems	670	129	192	3·6	6·98	1,940	20 8
		Bodies	2,090	395	189	14·0	17·0	1,260	17·4
		Embryos	800	227	284	—	—	—	23·1
	Pisaster ochraceus	HC	214	119	555	7·6	26·0	3,450	30·9
		♀G	450	230	512	4·3	14 6	3,390	17·7
		♂G	48	36	750	10·0	37·4	3,740	16·5
	Mytilus californianus	Soft	230	162	706	1·8	17 9	10,000	14·6
Willapa Bay, Washington	*Crassostrea gigas*	Soft	745	89 5	120	—	—	—	20 4

Stems, peduncle including skin and ovaries in various states of development. Bodies, digestive and male reproductive system excluding shell. HC, hepatic caecum. G, gonad male or female Embryos, contents of the mantle cavity.

met with failure[2]. It has been demonstrated, however, that several marine species concentrate zinc so well that the determination of zinc-65 in tissues of these animals is relatively simple.

This article presents data on zinc and zinc-65 for marine tissues collected near to and remote from the Columbia River and correlates these data into a more accurate estimate of that level of contamination by zinc-65 which should be considered background.

During August of 1964 samples of *Mitella polymerus* (the goose barnacle), *Pisaster ochraceus* (the common coastal starfish) and *Mytilus californianus* (the California mussel) were collected from locations at and near the mouth of the Columbia River. These species were chosen for study since they can be obtained in large quantity at practically any location on the Pacific coast. A sample of *Crassostrea gigas* (oyster) was included for comparison with previous investigations of the Willapa Bay region. All tissues were dried by lyophilization before analysis. The normal sample size was 40–70 g of dry tissue. The radioisotope concentration of each sample was determined with the total-body counter at this University. (We thank Dr. N. S. MacDonald and his staff for the counting of all samples included in this report.) The stable element concentrations were determined with conventional optical emission and X-ray fluorescence techniques.

Table 1 outlines the data for zinc and includes, where reasonable counting accuracy could be maintained, data for chromium. Chromium-51 is the major γ-emitting isotope present in the river water as it passes Vancouver[1]. This element is added to the reactor cooling water as part of the purification process[3].

The present zinc-65 levels in *M. californianus* are generally in good agreement with values from previous studies[4,5], as is the value for the Willapa Bay oysters. It can be noticed that although the activity levels of the tissues of a species may vary by as much as sevenfold the specific activity is constant to within a factor of 1·4 and, as expected, serves as a relatively satisfactory index of the contamination level.

The specific activity of chromium decreases rather markedly in the order mussel, starfish and barnacle. This may indicate a food chain of mussel—starfish . . . barnacle with an equivalent hold-up time between starfish and mussel of between 40 days and 50 days as estimated from the radiation decay of chromium-51. The much lower, 2–3 times, zinc-65 and chromium-51 specific activity value for barnacle suggests that this animal receives much of its food from an area of lower contamination than its place of residence. Quite possibly this includes organic debris washed in from some distance seaward. In any event, it suggests that specific activity data must be applied cautiously in an area of rapidly changing levels such as are found in the Columbia River region.

An estimation of the contribution of these relatively high zinc-65 levels to the much lower values (17–200 pc./kg wet tissue) observed by Folsom[2] in the San Diego region (*c.* 1,200 miles) can be approached from several points of view. First, data reported by Watson[4] suggest that the zinc-65 concentration decreases in proportion to the 1·23 power of the distance from the river mouth. Extrapolation of these data to 1,200 miles indicates a concentration in *M. californianus* of 300–800 pc. zinc-65/kg wet tissue (1,900–5,000 pc./kg dry). A second estimate results from extrapolating the salinity data of Steffánson and Richards[6]. The decline in freshwater concentration indicated by these data agrees well with the decline in zinc-65 concentration observed for *M. californianus* along the Oregon coast. An extrapolation of these data indicates a zero contribution from the Columbia River at distances greater than 700 miles.

The general agreement between the extrapolation of Watson's data and the observations in the San Diego region seem to suggest that the Columbia River is the prime contributor to zinc-65 on the Pacific coast. However, determination of zinc and zinc-65 in the Atlantic oyster, *Crassostrea virginica*, carried out by Skauen and Rankin[7] during 1960 to 1963, indicates that the background level in the oyster is 30–50 pc. zinc-65/kg fresh tissue. The general agreement of this level with the San Diego data would suggest that this level of contamination is most likely due to fall-out or possibly nuclear submarine activity.

In order to resolve this last-mentioned point, several 'background level' samples were collected from the East coast, the Southern California coast and the Salton Sea. The Salton Sea sample should represent fall-out activity only. These data are presented in Table 2. The activity-levels again vary considerably while the specific activity-levels of zinc-65 are relatively constant. The higher specific activity for the Long Island Sound sample may indicate an effect due to nuclear submarine activity, but more likely indicates a greater contribution owing to fall-out washed from the surroundings as is possibly the case for the Salton Sea sample.

It is interesting to compare the zinc concentrating ability of the two types of barnacles by comparing their zinc

Table 2. 1964–65 BACKGROUND VALUES

Location	Animal	Concentration in dry tissues				
		Type of tissue	Zn p p.m.	^{65}Zn pc /kg	^{65}Zn/Zn pc /mg	Dry/fresh wt %
La Jolla, California	*Mitella polymerus*	Stems	150	92	0 61	30 2
		Bodies	910	684	0·75	18 4
	Mytilus californianus	Soft	91	35	0·39	23 1
Malibu, California	*Pisaster ochraceus*	HC	160	22	0 14	
Chesapeake Bay	*Crassostrea virginica*	Soft	10,200	456	0 05	7 60
Long Island Sound	*Venus mercenaria* "little neck"	Soft	214	276	1 29	20 4
Salton Sea	*Balanus amphitrite saltonensis*	Soft	193	516	2·68	18 6

concentration with that determined for their marine environment—0·86 μg zinc/l. for the Salton Sea and 6·4 μg zinc/l. at La Jolla. The body portion of *M. polymerus* was compared with the morphologically similar soft tissue dissected from *B. amphitrite saltonensis*. The concentrating factors were 26,000 and 44,000, respectively. These values are about an eighth of the value for *C. virginica* under similar conditions[8] but are essentially the same as the concentrating ability of *C. gigas*.

These data indicate that the present level of zinc-65 from fall-out has established a specific activity for this isotope which is relatively constant in the coastal environment at 0·05–2·7 pc. zinc-65/mg zinc. In order to compare this constancy with data from previous investigations the average zinc concentration for several foods was determined and applied to the 1958 and 1959 data of Perkins[9] and Murthy[10]. These data are shown in Table 3. Here again the relative constancy of the specific activity values is striking. Since approximately six years separate the data of these two tables, it is most likely that the data in Table 2 represent the fall-out from 1961 and 1962 tests while the data of Table 3 result from pre-1959 tests.

Table 3. 1958–59 BACKGROUND VALUES

Food	Reference	Concentration in fresh tissue: ^{65}Zn pc./kg	Zn p.p.m.	^{65}Zn/Zn pc./mg	Collection date
Clams	Murthy (ref. 10)	40	44	0·92	May '58
Oysters	Murthy (ref. 10), Perkins (ref. 9)	164	710	0 23	'58, '59
Eggs	Murthy (ref. 10)	6	13·5	0·44	Aug. '58
Milk	Murthy (ref. 10)	4	5·1	1 29	'59
Tomatoes	Perkins (ref. 9)	7·2	3·4	0·21	'59

Folsom[2] has demonstrated that manganese-54 is concentrated by as much as 6,000-fold in phytoplankton and by 2,000-fold in *M. polymerus* stems. Manganese-54 as fall-out debris was produced principally by the U.S.S.R. 1961 test series and no appreciable new input of manganese-54 has occurred since 1961 (ref. 11). During this investigation several samples contained sufficient manganese-54 to permit accurate determination. These results are presented in Table 4. As with zinc-65, the specific activity values of manganese-54 are more constant than the corresponding radioactivity levels.

Table 4. MANGANESE AND MANGANESE-54 IN DRY TISSUE

Location	Animal	Type of tissue	Mn p.p.m.	^{54}Mn pc./kg	^{54}Mn/Mn pc./mg
Ecola Beach, Oregon	*Mitella polymerus*	Stems	114	1,700	14·9
North Head, Illwaco, Washington	*Mitella polymerus*	Stems	154	2,230	14·5
Willapa Bay, Washington	*Crassostrea gigas*	Soft	59 1	1,060	18·0
La Jolla, California	*Mitella polymerus*	Stems	37 9	243	6·4

The concentrating ability of *M. polymerus* tissues for zinc and manganese may be used to estimate the late 1964 levels of zinc-65 and manganese-54 in Southern Californian sea water. For manganese-54 we may use *M. polymerus* stems to arrive at a sea-water value of 37×10^{-15} c. manganese-54/l. ($243 \times 0{\cdot}302/2{,}000$). For zinc-65 the body portion of the animal is more useful, yielding an estimate of $4{\cdot}5 \times 10^{-15}$ c. zinc-65/l. ($684 \times 0{\cdot}184/26{,}000$). If we use the specific activities shown in Tables 2 and 4 together with the average concentration of these two elements in sea water[12], two similar estimates are obtained, that is, 13×10^{-15} c. manganese-54/l. ($2 \times 10^{-3} \times 6{\cdot}4$) and $5{\cdot}8 \times 10^{-15}$ c. zinc-65/l. ($10 \times 10^{-3} \times 0{\cdot}58$). By a similar approach the zinc-65 concentration of the Salton Sea would be $2{\cdot}3 \times 10^{-15}$ c. zinc-65/l. ($0{\cdot}86 \times 10^{-3} \times 2{\cdot}68$).

The estimated values for manganese-54 in sea water are quite similar to those reported by Folsom when the sampling time difference is corrected for radioactive decay and for the seasonal changes in rate of fall-out as indicated by local air samples and the sea-water samples reported by Folsom. The apparent magnitude of this seasonal variation is sixfold with the minima occurring in mid-winter[13].

To estimate the concentration of radioelements such as zinc-65 in sea water by biological concentration alone, one must have accurate knowledge of the concentrating ability of the tissue or animal involved. However, if knowledge of the stable element concentration is available it is only necessary to select tissues known to concentrate the element of interest to arrive at an accurate value for the mean sea-water concentration. Present analytical techniques make it relatively easy to obtain accurate analysis for elements such as zinc, manganese and chromium in sea water and in biological tissues.

Should it not be possible to include a stable element determination a tissue of relatively constant concentration could be used. Of the tissues reported here, only two have been found to be of constant concentration, namely, the starfish hepatic caecum and the male gonad. The hepatic caecum contains 54 ± 20 μg zinc/g fresh tissue which is $0{\cdot}29 \pm 0{\cdot}11$ per cent zinc when converted to ash at 510° C. The male gonad contains $9{\cdot}0 \pm 1{\cdot}9$ μg zinc/g fresh tissue which is $0{\cdot}043 \pm 0{\cdot}008$ per cent when ashed. These values result from approximately fifty samplings conducted at several locations along the Pacific coast during the past few years.

To evaluate the background contamination level of zinc-65 on the Pacific coast, namely that resulting from the Columbia River plus that resulting from fall-out, it would seem essential to include analysis for manganese-54 to support that portion of zinc-65 assigned to fall-out and analysis for chromium-51 to evaluate the contribution from the Columbia River. (The relatively high and apparently constant level of chromium in the starfish male gonad together with the relatively low zinc value would suggest that this tissue would be particularly suited for the biological concentration of chromium-51 in the presence of zinc-65.) Even though the half-life of chromium-51 is only 27·8 days as opposed to 245 days for zinc-65 the effective concentration of chromium-51 would exceed that of zinc-65 for some 150 days and apparently is not removed as easily from the river plume as is zinc-65 (ref. 14). At present it seems reasonable to assume that there is no significant contribution to the Southern California coastal zinc-65 levels from the Columbia River and that essentially all the present level results from fall-out.

A similar approach, although not requiring the determination of chromium-51, would certainly improve the evaluation of excessive contamination in areas such as New London, Connecticut[7], where by external sampling it was apparently impossible to resolve the contribution from a nuclear submarine 'incident' from that which may have resulted from fall-out from the U.S.S.R. tests until the activity-level had subsided from a 250-fold increase to near background and approximately 18 months had elapsed.

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EFFECTS OF X-IRRADIATION ON THE METABOLIC CHANGES ACCOMPANYING PHAGOCYTOSIS

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IT is well known that irradiated animals are generally more susceptible to infection than non-irradiated animals. A number of different host defence mechanisms against infection may fail to protect the irradiated animals—a major one being the ingestion and subsequent destruction of micro-organisms by the phagocytic cells. The effects of ionizing radiation on phagocytosis have been intensely investigated, but many reports are contradictory[1–3]. Some of these contradictions seem to depend on dosage, experimental animals and to a large extent on the assay procedures.

Particle uptake by polymorphonuclear neutrophils (PMN) is known to be accompanied by a number of metabolic changes in the phagocytes. For example, an increase in the rate of oxygen consumption and glycolysis and a large stimulation of the oxidation of glucose-1-^{14}C and formate-^{14}C have been reported to accompany phagocytosis by guinea-pig exudate PMN[4,5]. Furthermore, a variety of antimicrobial agents and hydrolytic enzymes are also known to be released from cytoplasmic granules of PMN during phagocytosis[6–7].

An investigation of the established phagocytic and metabolic alterations found in PMN following irradiation might not only shed light on possible mechanisms of host susceptibility but also might provide further insight into the phagocytic process. Thus, this initial study is directed towards establishing some baseline effects of irradiation on leucocytic metabolism in the absence and presence of particles. This article reports the results gained from this investigation. (A preliminary report has already been published[8].)

Guinea-pigs of both sexes weighing 300–400 g were used throughout these experiments. All common chemicals used were of reagent or analytical grade and obtained commercially. Radioactive substances were purchased from New England Nuclear Corporation, Boston, Massachusetts. Polystyrene spherules of 0·81μ diameter were obtained from the Difco Laboratories, Detroit, Michigan.

Peritoneal exudates were essentially prepared according to previously described procedures[4]. This in outline consisted of injecting intraperitoneally a 12 per cent sterile suspension of sodium caesinate into guinea-pigs. 12–18 h later the animals were killed, the peritoneal cavity was exposed and the exudate was aspirated. The peritoneal cavity was rinsed once with ice-cold Krebs–Ringer salt solution buffered with 2 mM phosphate buffer of *p*H 7·4 (wash solution). The exudate and washings (usually from three animals) were pooled and the PMN separated by centrifugation (110*g* for 4 min), washed twice with wash solution and brought up to desired volume. The amount of cellular material in the suspension was estimated by measuring the optical density of a suitably diluted suspension at 600 mμ. From the optical density either the number of cells present or the amount of cellular phosphorus was calculated from a pre-calibrated curve.

Oxygen consumption measurements were made by using conventional Warburg manometric techniques. 6-ml. microflasks with 1-ml. reaction mixture containing about $5{\cdot}7 \times 10^7$ PMN (100γ cellular phosphorus) were used. The reaction was terminated by immersing the flasks in crushed ice. After aliquots had been removed from the reaction mixture for microscopic examination the flasks were tightly stoppered and 0·1 ml. of a 30 per cent solution of trichloroacetic acid kept in the side-arm was tipped into the flask to precipitate protein and release bound carbon dioxide.

Phagocytosis, as monitored by light microscopy, was measured by an independent worker from coded slides. One hundred cells were counted from each sample.

For the assay of $^{14}CO_2$ the contents of the centre wells were transferred quantitatively to centrifuge tubes containing carrier potassium carbonate to yield a convenient weight of barium carbonate (16 mg). Barium carbonate was precipitated from the solution by the addition of 0·1 ml. of 2 M ammonium chloride followed by the addition of 0·5 ml. 20 per cent barium chloride. The barium carbonate precipitate was washed once with freshly distilled water and once with acetone. It was then resuspended in 0·5 ml. of acetone and transferred quantitatively to aluminium planchets with the aid of more acetone in order to gain an even spread. Self-absorption corrections were made from barium carbonate absorption curves prepared for use with the counter[9].

Lactate estimations were made from protein-free aliquots of the reaction mixture, at three different dilutions, by the method of Barker and Summerson[10]. Total and differential white blood cell counts were made by conventional techniques from blood obtained from the marginal ear vein.

Irradiation was carried out with a Picker X-ray therapy unit operated at 280 kVp. The cell suspensions were irradiated in thin-walled small glass test-tubes. At a target distance of 12 cm, the dose-rate was about 1,500 r./min with no added filtration. Whole-body irradiation was carried out in plastic cages at a target distance of 75 cm. The half-value layer was 3·4 mm of copper with an added Thoreus No. 3 filter and the dose-rate was 20 r./min. All doses were measured in air using a calibrated Victoreen dose-meter.

In vitro irradiation of exudate guinea-pig PMN with X-ray doses up to 50,000 r. had little or no measurable effect on the phagocytic and metabolic functions of these cells. Attempts to demonstrate an effect of X-rays by irradiating the cell suspension in 100 per cent oxygen atmosphere and/or preincubating the cells in order to deplete endogenous substrates were also not successful.

Preliminary experiments on whole-body X-irradiation of guinea-pigs established an $LD\ \frac{30}{60}$ of about 200 r. Total and differential counts were made on the peripheral white blood cells throughout the 30-day period. The animals developed leucopenia 2 days after being irradiated with 500 r. and 5–6 days after 100 r. which was persistent for 3–4 weeks. Deaths following irradiation usually occurred at, or soon after, the peak of leucopenia (about 500 white blood cells/mm^3). As the leucopenia progressed, the white blood cell differential shifted to a higher percentage of lymphocytes and monocytes.

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The effects of high dose (1,500 r.) whole-body X-irradiation on the phagocytic and the related abilities of the casein-induced exudate PMN to oxidize glucose-1-^{14}C and formate-^{14}C were studied. Two different experimental conditions were set up: In one, six animals were injected with casein and, 18 h later, all were irradiated. The cells were collected and examined 1 h after irradiation. In another, six different animals were exposed to X-irradiation and 6 h later casein was injected. The resulting exudates were collected 18 h later for investigation. Exudate PMN obtained from non-irradiated animals were included and acted as our controls. A sub-optimal ratio of particles to phagocytes (100 : 1) was used with the hope that any alterations in the phagocytic–metabolic activity might be more readily detected. The results of these experiments are presented in Table 1. It is seen that the PMN induced after the high dose of X-irradiation phagocytized poorly and also exhibited a lowered phagocytic–metabolic rate. The experiment was repeated using cells which had been pre-incubated for a 2-h period. The particles and the labelled substrates were then tipped in from the side-arm and all the parameters shown in Table 1 were re-examined for a further 30 min. The results obtained after this period of pre-incubation were similar to those reported in Table 1 (for this reason they are not shown), though the metabolic rate was reduced by about 20 per cent in all groups.

Experiments conducted with low dose of whole-body X-irradiation revealed that a decreased phagocytic ability and metabolic activities of the PMN could be demonstrated on the ninth and third post-irradiation day, with 50 r. and 100 r. respectively. Some decrease in metabolic activity was evident in the groups exposed to 100 r. as early as 2 days after irradiation (Table 2). The reaction was carried out for a longer period of time (60 min) in order to determine whether the phagocytic and metabolic activities of the PMN collected from irradiated animals declined faster.

It would seem that the changes observed in the metabolism and phagocytosis of exudate PMN obtained from irradiated animals are related to the time elapsed after exposure to irradiation in addition to the dose of irradiation. Thus the period of investigation was extended to 30 days with a group of thirty-six animals exposed to 100 r. Respiration, glycolysis and production of radioactive carbon dioxide from glucose-1-^{14}C, glucose-6-^{14}C, succinate-1-^{14}C and formate-^{14}C were measured in the absence, and presence, of particles. PMN obtained from non-irradiated animals were used as controls with each experiment. Some of the results are summarized in Fig. 1.

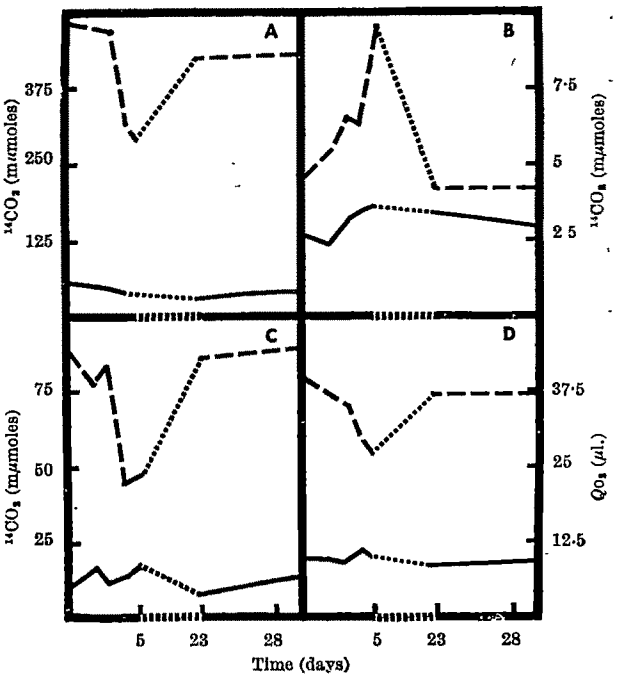


Fig. 1. Effects of whole-body irradiation on resting and phagocytizing PMN. See Table 1 for details. Control (non-irradiated) values are on X-axis: solid lines and broken lines represent resting and phagocytizing cells, respectively. *A*, $^{14}CO_2$ production from glucose-1-^{14}C; *B*, $^{14}CO_2$ production from glucose-6-^{14}C; *C*, $^{14}CO_2$ production from formate-^{14}C; *D*, oxygen consumption

Random deaths of six of the irradiated animals (out of thirty-six) occurred between the fifteenth and twenty-fifth day after irradiation. Between the fifth and fourteenth day after irradiation, the guinea-pigs did not respond to the challenge with casein. Very thin exudates, containing mostly the injected casein, was found in the peritoneal cavity. However, despite the severe leucopenia with a high percentage of mononuclear white blood cells that is seen as a result of X-irradiation, the exudate

Table 1. EFFECTS OF HIGH DOSE WHOLE-BODY X-IRRADIATION (1,500 r.) ON THE METABOLISM OF PMN IN THE ABSENCE AND PRESENCE OF PARTICLES

Groups	Substrates	Q (μl.)		$^{14}CO_2$ (mμmoles)		Lactate (μmoles)		Particle entry
	Particles	−	+	−	+	−	+	
Non-irradiated:	Glucose-1-^{14}C	8·5	30·8 (362)	54·9	447 (814)	3·4	3·1	+ + +
	Formate-^{14}C	8·7	26·9 (309)	7·5	51·2 (683)	1·4	1·5	+ + +
Irradiated with induced PMN in peritoneal cavity (18 h before irradiation)	Glucose-1-^{14}C	8·4	30 6 (364)	62·2	476 (765)	3 5	3 3	+ + +
	Formate-^{14}C	9·2	28·9 (314)	8·6	57·1 (664)	1 4	1·7	+ + +
Irradiated and PMN induced (18 h after irradiation)	Glucose-1-^{14}C	11·2	29 9 (267)	71·9	355 (494)	2·9	2 5	+ +
	Formate-^{14}C	11·5	21 1 (183)	19·4	46·8 (241)	1·1	1·2	+ +

All values are for 30-min reaction period and standardized for 100 cellular phosphorus—average of duplicate reaction flasks Particles used were 0·81μ polystyrene at a particle to PMN ratio of 100. (Figures in parentheses refer to the percentage of stimulation observed due to phagocytosis.) + + +, + +, +, are used to denote the efficiency of phagocytosis—approximately 90, 60, and 30 per cent of the PMN having twenty or more particles per cell respectively.

Table 2. EFFECTS OF LOW DOSE WHOLE-BODY X-IRRADIATION (50 r. AND 100 r.) ON THE METABOLISM OF PMN IN THE ABSENCE AND PRESENCE OF PARTICLES

Conditions			(10 mM) Glucose-1-^{14}C			(10 mM) Glucose-6-^{14}C			(2 mM) Formate-^{14}C			(10 mM) Succinate 1-4-^{14}C			Phago-cytosis
Dose	Post irradiation day	Particles	Q (μl.)	$^{14}CO_2$ (mμmoles)	Lactate (μmoles)	Q (μl.)	$^{14}CO_2$ (mμmoles)	Lactate (μmoles)	Q (μl.)	$^{14}CO_2$ (mμmoles)	Lactate (μmoles)	Q (μl.)	$^{14}CO_2$ (mμmoles)	Lactate (μmoles)	
Normal animals	—	−	22·0	134·4	6·2	22·0	5·4	6·2	26·8	25·2	2 3	28·1	71·6	3·2	
50 r.	5	+	48·2	930 0	5·8	48·2	9·3	5·8	49·7	172·0	2·7	51·6	68·2	4·0	+ + +
		−	25 1	110·2	4·6	22·5	7·4	4·3	27·2	27·6	1·5	29·0	71·9	1·7	
50 r.	9	+	36·8	782 0	4·2	35·4	8·6	3·8	41·7	95·4	1·8	43·9	62·5	2·0	+ + +
		−	16·7	105·6	5·2	16·1	6 7	5·0	20·8	16·8	2·1	22·3	65·4	2 0	
100 r.	2	+	33·0	470·0	4·6	35 1	8·4	4·8	48·2	110·0	2·5	54·6	64·4	2 8	+ +
		−	20·9	148·0	5·3	21·0	7·2	5·4	23 0	39·0	2·3	25·0	66·0	2·6	
100 r.	3	+	36·0	778·0	5·3	38·1	16·5	4·8	40·2	216·0	2·7	47 3	65 0	3·0	+ + +
		−	17 5	166 2	4 2	16·9	9·0	4·5	20 4	52·0	1·9	19·3	87·0	2·4	
		+	23 0	516·0	4 6	27·9	11·5	4·2	31 7	178·2	2·4	33 5	88·5	2·6	+ +

All values are for 60 min reaction period. See Table 1 for additional details.

differential remained the same throughout the experimental period (that is, more than 95 per cent PMN).

It can be seen from Fig. 1 that the phagocytic capacity of the exudate PMN decreased between the third and twenty-second day after irradiation. Simultaneous inhibition of the phagocytic respiration and oxidation of glucose-1-^{14}C and of formate-^{14}C are observed. We also detected a significant decrease in lactate production (from 3·5 to 2·3 μmoles/100γ cellular phosphorus/30 min on the fifth day after irradiation) However, the oxidation of glucose-6-^{14}C was significantly stimulated following X-irradiation. Succinate oxidation was stimulated slightly during this period. Although the phagocytic ability appeared to be normal by the twenty-third day after irradiation, the metabolic properties of these PMN were not completely normal throughout the 30-day observation period.

The stimulation of the oxidation of glucose-6-^{14}C and the concurrent inhibition of the oxidation of glucose-1-^{14}C due to X-irradiation results in a significant change in the ratio of these two carbon atoms of glucose oxidized by PMN. This alteration occurs both with phagocytizing and resting cells, and causes a decrease of more than 65 per cent in the ratio on the fifth day after irradiation (Table 3).

Lethal effects of whole-body irradiation have been ascribed[11] to at least three major syndromes: (*a*) neurological at doses of more than 30,000 r.; (*b*) gastrointestinal at doses between 1,000–30,000 r.; (*c*) haemopoietic at doses of a few hundred r. The haemopoietic syndrome, which occurs at moderately low doses, is characterized by bone marrow aplasia leading to infection, haemorrhage and anaemia. The increased susceptibility to infection, which is the major cause of the ensuing death, suggests that the host–parasite interaction has altered.

A considerable amount of work has been undertaken on the effects of irradiation on phagocytosis. However, to the best of our knowledge, no reports have appeared which describe the effect of irradiation on the metabolic activities of the phagocytizing cell. Our findings in the present experiments are essentially in agreement with those of previous workers. For example, Wagner *et al.*[12] found a 20 per cent inhibition of respiration with doses of 20,000–100,000 r. with no measurable effect on the rate of glycolysis. Levy and Ketchel[13] have shown that amoeboid movements of leucocytes are not inhibited unless doses of 200,000 r. were given.

The possible effects of X-irradiation on the exudate PMN under *in vivo* conditions were investigated by exposing guinea-pigs containing a pre-induced exudate in the peritoneal cavity (casein injected 16 h prior to irradiation) to 1,500 r. The phagocytic and metabolic activities of these cells were similar to those obtained from non-irradiated animals. However, the same amount of irradiation decreases the phagocytic and metabolic properties of PMN-induced 6 h post-irradiation. These results would indicate that the influences of irradiation on the leucocyte metabolism are due to an indirect effect.

Simultaneous studies on the leucocyte levels in the peripheral blood indicate that an irradiation effect is evident prior to the development of a pronounced leucopenia. Thus a single dose of 100 r. decreases the phagocytic and associated metabolic functions of exudate PMN between the third and twenty-third days following irradiation. However, leucopenia is noticed on the sixth day after irradiation, with the peak occurring about the tenth day. It should also be noted that the effect is accentuated with time. Recovery to the normal phagocytic ability is evident on the twenty-third day. Harris[14] reported the occurrence of leucopenia between the fourth and twenty-second day in guinea-pigs exposed to 150–170 r. whole-body irradiation from a cobalt-60 source.

Table 3. GLUCOSE-1-^{14}C AND GLUCOSE-6-^{14}C OXIDASE ACTIVITIES OF IRRADIATED AND NON-IRRADIATED PMN DURING PHAGOCYTOSIS

Conditions	Particles	$^{14}CO_2$ (mμmoles) produced from Glucose-1-^{14}C	$^{14}CO_2$ (mμmoles) produced from Glucose-6-^{14}C	Ratio C_1/C_6	Particle uptake
Non-irradiated (controls)	−	55	2·4	23	
	+	490	5·8	85	+ + +
Irradiated-100 r. (5 days post-irradiation)	−	46	5·4	8·6	
	+	290	9·6	30	+

See Table 1 for details.

Many authors have reported the existence of a general correlation between the peripheral granulocyte count and susceptibility to infection (see review by Cronkite and Brecher[11]). However, Shechmeister and Fishman[15] have demonstrated a decreased migration of blood leucocytes of rats and rabbits which could not be ascribed to leucopenia or plasma factors. They suggested that the phagocytic and digestive functions of rat leucocytes following whole-body irradiation are altered[16]. Donaldson *et al.*[17] have demonstrated that mice and rabbit peritoneal macrophages show a suppression of intracellular digestion of chicken erythrocytes and yeast cells following whole-body irradiation both by *in vitro* and *in vivo* tests. Our metabolic studies with the use of radioactive substrates, aided by microscopic observations, tend to support the hypothesis of a functionally impaired phagocyte. These results are especially interesting in view of the fact that the differential count of the cells used for investigation remained constant throughout the experimental period (more than 95 per cent PMN). Thus together with impaired particle uptake, PMN isolated from irradiated guinea-pigs show decreased glycolytic and respiratory rates, and decreased rates of oxidation of glucose-1-^{14}C and formate-^{14}C. All these parameters are known to be stimulated during phagocytosis[4,5]. However, the significance of these stimulations is still obscure. It has been suggested that the increased lactate production during phagocytosis causes the granules of PMN to lyse and release a variety of hydrolytic enzymes and antibacterial agents due possibly to an intracellular decrease in *p*H (refs. 6 and 7). Thus the decreased lactic acid production following irradiation could be responsible for the decreased phagocytic activity and a subsequent loss in the killing ability of the PMN. Also, the formation of hydrogen peroxide by the increased oxidation of reduced triphosphopyridine nucleotide has been demonstrated and invoked to act as the antimicrobial agent[18]. The decreased formic oxidase activity by PMN of irradiated animals might again suggest an altered bactericidal activity. Experiments designed to examine these possibilities are at present being carried out.

It has been shown that PMN preferentially oxidize glucose-1-^{14}C to a significantly greater extent than glucose-6-^{14}C, resulting in a high C_1/C_6 ratio for glucose utilization[4]. It is interesting that this ratio decreased about 33 per cent following whole-body irradiation both in the resting and phagocytizing PMN. This was due to both decreased glucose-1-^{14}C oxidation and increased glucose-6-^{14}C oxidation (Table 3). Since the oxidation of glucose-6-^{14}C and glucose-1-^{14}C involves DPN and TPN as co-factors, respectively, it is interesting to speculate on the part that the altered oxidized-reduced DPN/TPN ratio could play in the phagocytic ability of these cells.

Finally, it should be noted that essentially all the metabolic parameters investigated are affected by doses of irradiation as low as 100 r., and that these parameters do not completely return to the normal state as late as 30 days after irradiation. However, the phagocytic ability of the cell appears normal as early as 21 days after irradiation. These results suggest that the PMN, despite altered metabolism, is able to 'phagocytize normally' only because the cell has now lost its selective membrane permeability and that it is now possible for it to take up particles indiscriminately. This hypothesis is amenable to experimentation and it is actively being pursued.

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HIGH-PERFORMANCE HYDROCARBON FUEL CELLS WITH FLUORIDE ELECTROLYTES

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IT has been reported that aqueous caesium carbonate and bicarbonate are satisfactory fuel-cell electrolytes for use with carbonaceous fuels such as ethylene[1] and methanol[2]. An attempt to extend the use of the carbonate and bicarbonate electrolytes to include saturated hydrocarbon fuels showed that the fuel cell performance was significantly lower with paraffins than with the olefines and partially oxygenated hydrocarbons such as methanol[3].

The effects on the fuel-cell performance of fuel gas flow rate, electrolyte flow rate, and operating temperature, and the shape of the current density-voltage curve for a platinum-black anode all indicated a diffusion-limited process for the fuel in the electrolyte phase[3] (for example, in the meniscus or electrolyte film in the pores of the electrode). This led to the conclusion that the product of solubility and diffusion coefficient for the fuel in the electrolyte was probably very low. With this conclusion in mind, an attempt was made to increase the solubility of the hydrocarbons in the caesium-salt electrolyte by replacing some of the carbonate anion by fluoride, since hydrofluoric acid is known to enhance the solubility of saturated hydrocarbons in aqueous solution[4] and since the fluoride anion is probably not adsorbed[5,6].

The apparatus used in the present work consisted of a 'Teflon' fuel cell[2] enclosed in a forced-convection air thermostat. The electrodes were porous, 'Teflon'-bonded platinum black supported on 45-mesh platinum gauze and had an active geometric area of 11·4 cm². The electrocatalyst content of the electrodes was 51 mg/cm², with a specific area of 20 m²/g. (The procedure for preparing the electrodes has already been described[9].) The starting electrolyte was an 87 weight per cent aqueous solution of caesium carbonate, prepared according to methods previously described[7,8]. Baker and Adamson reagent grade 48 per cent hydrofluoric acid was added (see later). The hydrocarbon fuel was Matheson instrument grade propane of 99·5 per cent minimum purity, and the oxygen was electrolytic grade, 99·6 per cent minimum purity.

The electrical characteristics of the anode, the cathode, and the cell as a whole were measured on a resistance-free basis with the aid of an interrupter[2,10]. The potentials of the anode and cathode were measured against a reversible hydrogen electrode in the same electrolyte and at the same temperature. Current density-voltage data were taken at steady state, usually 2–5 min after a change in current.

The fuel cell was operated at 150° C, where the best performance was observed using propane as the fuel and caesium carbonate as the electrolyte. The anode performance under these conditions was 6·7 m.amp/cm² at an anode versus hydrogen reference potential of 0·5 V (E_{ar} = 0·5 V). The electrolyte was then drained from the cell (total electrolyte volume in the circulation system was 200 cm³) and 25 cm³ of 48 per cent hydrofluoric acid was added. This converted a fraction of the original caesium carbonate to caesium fluoride. The electrolyte was then concentrated to a composition corresponding to a boiling point of 160° C, and returned to the fuel cell. After the current density-voltage data had been obtained using a given electrolyte mixture, an electrolyte sample was removed for analysis, and hydrofluoric acid added as before.

Some of the results obtained from this series of experiments are shown in Fig. 1. The numbers on the curves indicate the number of additions of hydrofluoric acid that had been made to the original caesium carbonate electrolyte. Noteworthy is the change in the shape of the current density-voltage curve from that of a diffusion-limited anode reaction (additions 1–6) to that of an electrochemical rate-determining process (Nos. 8–13), the latter rate being an exponential function of the anode overvoltage. These results indicate that the product of the solubility and diffusion coefficient for propane in the electrolytes resulting from more than six additions of hydrofluoric acid is significantly greater than in the initial caesium carbonate electrolyte.

The effect of electrolyte composition on the rate of the anodic oxidation of propane on platinum black at 150° C and at an anode versus hydrogen reference potential of 0·5 V is shown in Fig. 2. Fig. 2 was prepared from the results of the chemical analyses of the electrolyte samples and from the current density-voltage data including those in Fig. 1. Fig. 2 shows a striking increase of the rate of

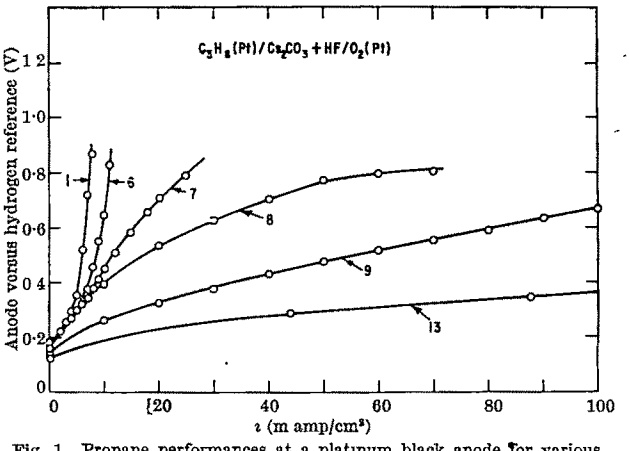


Fig. 1 Propane performances at a platinum black anode for various numbers of additions of hydrofluoric acid to the original caesium carbonate electrolyte The numbers on the curves indicate the number of additions of hydrofluoric acid made. Cell No. 8058-20. T = 150° C

anodic oxidation of propane with increasing F^-/Cs^+ ratio amounting to a factor of almost a hundred. Additions of hydrofluoric acid required to produce a value of F^-/Cs^+ in excess of 3·3 were not made because they would have reduced the atmospheric boiling-point of the electrolyte to a value of less than 160° C. The break in the curve corresponds closely to the *p*H 7 point rather than to the equivalence point (which is slightly alkaline), suggesting that an acidic electrolyte is necessary for improved performance.

The results in Figs. 1 and 2 indicate that at least two factors are responsible for the improvement in the current density at a given anode overvoltage: the solubility-diffusion coefficient product for propane in the electrolyte was increased to a point where dissolved fuel diffusion no longer limited anode performance; further increases in the acidity of the electrolyte beyond that necessary for improved mass transport rates resulted in further increases in the electrochemical rate constant.

The current density-voltage relationships for the anode versus reference, cathode versus reference and cathode versus anode are shown in Fig. 3 for the electrolyte composition that gave the best performance. A current density of 400 m.amp/cm² was obtained at nearly 0·2 V (resistance-free). The maximum power density for this cell (80 mW/cm²) is, to the best of my knowledge, higher than that for any previously reported direct hydrocarbon fuel cell operated under any conditions. This suggests that there may be some specific effect associated with the fluoride anion, resulting in an improvement in performance over that available using other strong aqueous acids.

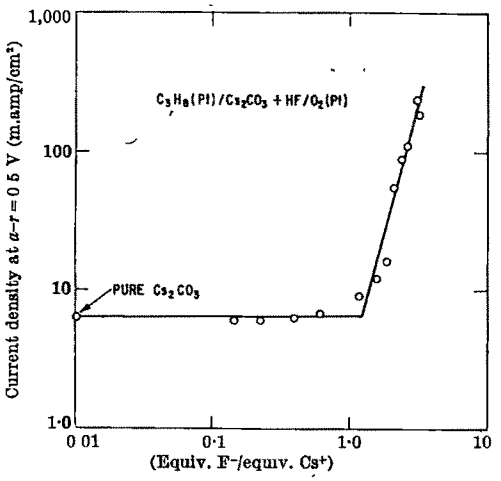


Fig 2. Effect of the ratio of fluoride to caesium (by equivalents) on the current density of a propane anode at a potential of 0·5 V versus reversible hydrogen. Cell No 8058-20. $T=150°$ C

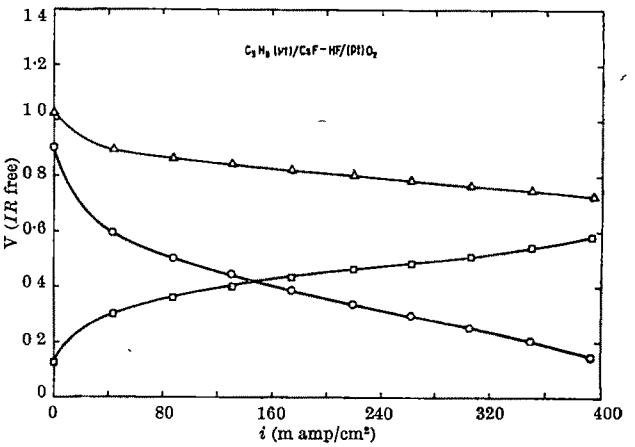


Fig 3. Current density voltage curves for a propane anode (□) and an oxygen cathode (△) versus reversible hydrogen, and the anode versus cathode (○), all on a resistance-free basis. Electrolyte: caesium fluoride, 29·3 mole per cent; hydrofluoric acid, 60·9 mole per cent; water, 9·8 mole per cent. Cell No. 8058-20. $T=150°$ C

It can be concluded that the ternary system caesium fluoride–hydrofluoric acid–water comprises a family of electrolytes suitable for high-performance saturated hydrocarbon fuel cells. Two effects differentiate this system from the caesium carbonate–water system: increased hydrocarbon solubility and diffusivity, and a greater overall rate for the electrochemical oxidation. Comparison of the present results with those for propane using other strong acid electrolytes leads to the suggestion of specific differences related to anion effects.

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AGGREGATION OF GUINEA-PIG PLATELETS BY ADENOSINE DIPHOSPHATE

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THE aggregation of human, pig, and rabbit platelets caused by adenosine diphosphate (ADP), and its inhibition by adenosine are now well documented (refs. 1–8). Cuthbertson and Mills[3] showed that rat platelets aggregate in response to ADP, and that adenosine fails to inhibit ADP in this species; this has recently been confirmed[9].

By use of the turbidimetric method of Born[10] it is possible to record continuously changes in optical density of platelet-rich plasma caused by platelet aggregation, and by its spontaneous reversal. The optical density of citrated platelet-rich plasma of the aforementioned species decreases almost immediately on addition of suitable concentrations of ADP. This decrease is progressive for 3–4 min, after which the optical density remains constant for 20–30 sec, and then starts to increase toward the initial level, which is attained in 6–8 min; this 'recovery' is due to disbanding of the platelet aggregates[10], caused, it is assumed[10,11], by the degradation of ADP with the consequent formation of breakdown products which inhibit ADP.

This article describes results of the investigation of ADP-induced aggregation of guinea-pig platelets. Platelet-rich plasma was obtained from male Hartley strain guinea-pigs as previously described for the rat[9]. Platelet aggregation was investigated by the method of Born[10].

Experiments were performed at 23° C; concentrations of drugs refer to those in citrated platelet-rich plasma. Experiments were performed at least four times, each with a different pool of platelet-rich plasma (14–20 guinea-pigs for each pool).

Platelet aggregation was proportional to the concentration of ADP, as shown in Fig. 1. The extent of platelet aggregation caused by a given concentration of ADP varied somewhat in different pools of platelet-rich plasma. Guinea-pig platelets responded to concentrations of ADP lower than those found necessary by Born and Cross[2] to aggregate human and pig platelets in citrated plasma. Near-threshold concentrations of ADP resulted in a typical aggregation curve—a decrease in optical density followed by a return to, or near, the initial level (Fig. 1*A*). Slightly higher concentrations of ADP resulted in a biphasic aggregation curve; the decrease in optical density caused by ADP was followed by the usual increase toward the initial level, but this was interrupted by a plateau, and, after that, by a second phase of decreasing optical density (Fig. 1*B*). With still higher concentrations of ADP, the curve was one of progressive decline in optical density (Fig. 1*C*). Even with the higher concentrations of ADP, the optical density returned to, or near, the initial level, but not until approximately 30 min after the addition of ADP. ADP-induced aggregation of guinea-pig platelets was not inhibited by adenosine (Fig. 2). Epinephrine and 5-hydroxytryptamine (5-HT), both at 10^{-5} M, did not cause aggregation of guinea-pig platelets.

The results show that guinea-pig platelets are more responsive to the aggregating action of ADP than are the platelets of man and pig[2], and that adenosine does not inhibit ADP-induced aggregation of guinea-pig platelets. Born and Cross[12] reported that cat platelets in citrated plasma respond to lower concentrations of ADP than do human and pig platelets, and that ADP-induced aggregation of cat platelets is not inhibited by adenosine. Examination of the aggregation curves of Born and Cross with cat platelets (Fig 1 of ref. 12) shows that at concentrations of ADP slightly above threshold, a biphasic response was obtained, similar to that described here with guinea-pig platelets. Comparison of their figure with Fig. 1 of this article shows that cat and guinea-pig platelets are approximately equal in their responsiveness to ADP. The greater responsiveness to ADP of cat and guinea-pig platelets could be the result of a greater inherent sensitivity to ADP of these platelets. Another possibility is suggested by the notion that the spontaneous dispersal of ADP-induced platelet aggregates is the result of ADP degradation, and the concomitant formation of ADP inhibitors (namely, adenosine and adenosine monophosphate)[10,11]. Since exogenous adenosine does not inhibit ADP in cat or guinea-pig plasma it might be argued that the greater responsiveness to ADP of cat and guinea-pig platelets is due to the ineffectiveness of adenosine as an inhibitor of ADP (this argument would be based on the assumption that adenosine is a product of ADP degradation in cat and guinea-pig plasma). But adenosine also fails to inhibit ADP-induced aggregation of rat platelets[3,5], yet the aggregation curves obtained with rat platelets resemble those obtained with human[3] and rabbit platelets[3,5], but not those obtained with cat[12] and guinea-pig platelets. Cuthbertson and Mills[3], working with heparinized rat plasma and citrated rabbit plasma, found that rat platelets were more responsive to ADP than were rabbit platelets. Spaet and Zucker[13] have shown that human platelets are more responsive to ADP in heparinized plasma than they are in citrated plasma. It is possible, therefore, that the findings of Cuthbertson and Mills are due to the effects of the anticoagulants they used. It thus appears that differences in responsiveness to ADP and in the shape of aggregation curves obtained with cat and guinea-pig platelets, on one hand, and with human, pig, and rat platelets, on the other, are not determined solely by whether or not adenosine inhibits ADP.

As mentioned earlier, spontaneous dispersal of ADP-induced platelet aggregates may be due, in part, to degradation of ADP. An apparent increased sensitivity of platelets to ADP might, therefore, be a manifestation of increased stability of ADP in the plasma. To investigate this possibility in the guinea-pig, ADP, 3×10^{-7} M, was incubated in guinea-pig platelet-rich plasma for various time intervals before the commencement of stirring. The results are shown in Fig. 3. When stirring was started immediately after the addition of ADP, a typical response was obtained (Fig. 3*A*). When ADP remained in contact with platelet-rich plasma for 3 min before stirring, there was a loss in activity (Fig. 3*B*), and between 6 and 9 min activity was not evident (Figs. 3*C* and 3*D*). There is thus an apparent paradox in that, on one hand, within 9 min after the addition of ADP to platelet-rich plasma in the absence of stirring, the nucleotide was inactivated

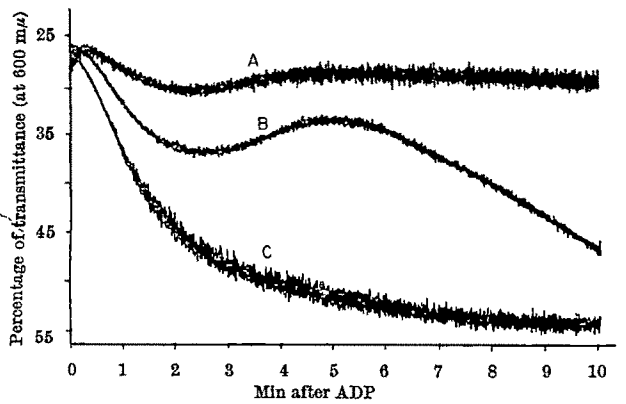


Fig. 1 Changes in optical density of platelet-rich guinea-pig plasma caused by ADP added at zero time in concentrations of: (*A*), 2×10^{-7} M; (*B*), 3×10^{-7} M; (*C*), 5×10^{-6} M. In this and in succeeding figures the ordinate was calibrated in transmittance units with the colorimeter

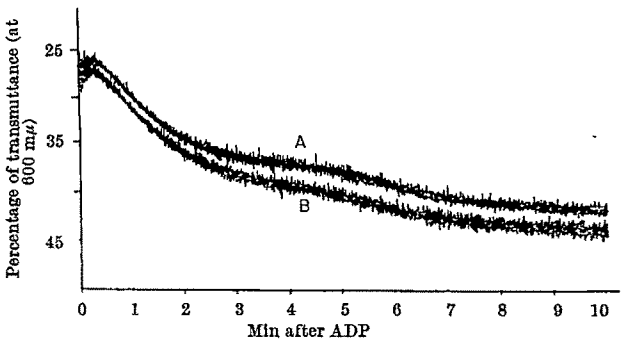


Fig. 2. Changes in optical density of platelet-rich guinea-pig plasma caused by ADP alone, and with adenosine. ADP (3×10^{-7} M) added at zero time to plasma which had been incubated 10 min at 23° C with: (*A*), 0·154 M sodium chloride, (*B*), 1×10^{-5} M adenosine

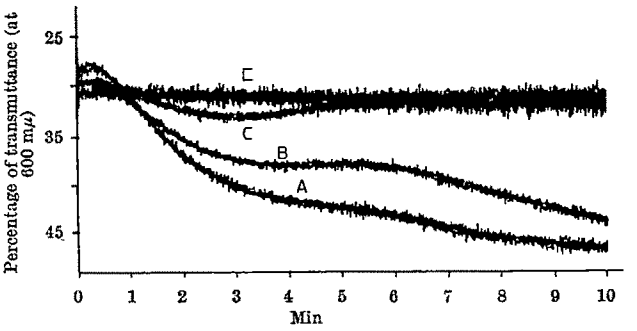


Fig. 3. Changes in optical density of platelet-rich guinea-pig plasma caused by ADP. Stirring was started at zero time in samples of plasma which had been incubated at 23° C with ADP 3×10^{-7} M for following times. (*A*), 0 min; (*B*), 3 min; (*C*), 6 min; (*D*), 9 min.

to the extent that there was no detectable platelet aggregation on subsequent stirring; on the other hand, when the same concentration of ADP was added with immediate stirring, platelets were still aggregating 10 min after the addition of ADP (Fig. 3*A*). One obvious explanation of this result is that ADP inactivation is curtailed when platelets aggregate, compared with its inactivation in the presence of non-aggregated platelets. The inactivation of ADP in platelet-rich plasma of the rabbit and rat has recently been investigated in the manner described here[9]. In both these species the time course of ADP inactivation is in accord with expectation based on the shape and time course of the aggregation curves, assuming, of course, that inactivation of ADP is contributory to disbanding of platelet aggregates. In these two species there is thus no evidence to indicate that the rate or extent of ADP inactivation differs according to whether or not platelets aggregate. The question as to whether or not such differential inactivation of ADP occurs in guinea-pig plasma cannot now be settled.

The biphasic nature of the aggregation curve, obtained with suitable concentrations of ADP in guinea-pig platelet-rich plasma, suggests another possible mechanism for the apparent greater stability of ADP in the presence of aggregated guinea-pig platelets. Near-threshold levels of ADP did not result in a biphasic curve (Figs. 1*A* and 3*C*), but concentrations slightly above threshold did (Figs. 1*B*, 2*A*, 3*A*), while still higher concentrations resulted in a progressively declining curve which was not interrupted by a plateau (Fig. 1*C*). Consideration must be given to the possibility that aggregation of guinea-pig platelets is associated with the release from such platelets of an aggregating substance. The results are now discussed hypothetically, on the basis of this possibility. Near-threshold levels of ADP result in the aggregation of only a small proportion of the platelets, and hence the release from them of a small amount of aggregating substance. The platelet aggregates disband, due, presumably, to the inactivation of ADP, and the optical density of the plasma returns to the initial level (Fig. 1*A*). With higher concentrations of ADP, more platelets aggregate as indicated by a greater decrease in optical density (Fig. 1*B*). The optical density remains constant for about 30 sec (from 2 to 2·5 min, Fig. 1*B*), indicating that aggregation and disbanding of aggregates are proceeding at equivalent rates. The optical density then starts to increase as disbanding of aggregates exceeds aggregation—'recovery' has started. However, aggregation has proceeded to the extent that sufficient aggregating substance has been released from the platelets to start a second phase of aggregation. Once again the optical density remains constant as the rate of aggregation equals that of disbanding (Fig. 1*B*, at 5 min); finally, aggregation exceeds disbanding, and the optical density decreases again. With still higher concentrations of ADP, sufficient numbers of platelets have aggregated to result in the release of aggregating substance early enough to prevent disbanding of the aggregates; the aggregation curve is thus one of progressive decline (Fig. 1*C*).

It is thus possible to account for (1) the increased responsiveness to ADP of guinea-pig platelets; (2) the peculiar shape of the aggregation curves; (3) the apparent greater stability of ADP in the presence of aggregated platelets, on the basis of the hypothesis that ADP-induced aggregation of guinea-pig platelets is associated with the release of an aggregating substance from these platelets. Assuming the release of such a substance, the question is raised as to its identity. ADP is the only known physiological substance which directly causes platelets to aggregate. In those species where platelets are induced to aggregate by epinephrine and 5-HT[14,15], thrombin[16], collagen[17], tri-ethyl-tin[18], latex particles[19] and fatty acids[20], evidence indicates that such aggregation is caused by ADP released from platelets as a result of their interaction with these agents. Guinea-pig platelets did not aggregate in response to epinephrine or to 5-hydroxytryptamine. It thus seems likely that if an aggregating substance is released from guinea-pig platelets it has a direct aggregating action, and may, therefore, be ADP. Spaet and Zucker[13] were unable to detect release of ADP from human platelets which had been caused to aggregate by addition of ADP; this is in accord with the shape of the aggregation curve obtained with human platelets[2].

I thank Miss Martha Williams and Mrs. Diane Konick for their technical assistance.

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AGGREGATION OF RABBIT AND GUINEA-PIG PLATELETS BY OXIME ESTERS

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PLATELET aggregation induced by thrombin[1,2], fatty acids[2], collagen[3] and latex particles[4] is possibly the result of the action of these substances in causing the release from platelets of the aggregating agent adenosine diphosphate (ADP)[6]; indirect evidence suggests that the same mechanism may be responsible for aggregation induced by epinephrine and 5-hydroxytryptamine[6], and tri-ethyl-tin[7]. It has now been found that 2-acetylfuran methoxycarbonyloxime (2-AFMO) induces aggregation of rabbit and guinea-pig, but not of rat platelets; results of investigation of the action of this and related compounds on platelet aggregation are reported here.

The preparation of platelet-rich plasma from male New Zealand white rabbits and the use of Born's[8] turbidimetric method of investigating platelet aggregation have been described[9]. Platelet-rich plasma from male Hartley guinea-pigs and Sprague–Dawley rats was prepared as described for the rabbit. Platelet-poor plasma was prepared by centrifugation of platelet-rich plasma at 10,000*g* for 20 min. Compounds were dissolved in 0·154 M sodium chloride solution and added in a volume of 0·04 ml. to 4·0 ml. of plasma except where noted otherwise. All concentrations are those of the compounds in plasma. Experiments were performed at room temperature (23° C), except where noted otherwise.

2-AFMO (5×10^{-4}–5×10^{-5} M) caused a decrease in the optical density of rabbit and guinea-pig platelet-rich but not of platelet-poor plasma, in which a stirrer was

rotated at 1,000 r.p.m. The optical density of platelet-rich plasma was not changed by the addition of 2-AFMO in the absence of stirring. Clumps appeared when 2-AFMO was added to stirred platelet-rich plasma, but not when it was added to platelet-rich plasma without stirring, or to platelet-poor plasma. The clumps were identified as platelet aggregates by microscopic observation. Stirring platelet-rich plasma did not cause detectable clumping or significant decrease in optical density. On the basis of these results, it is concluded that the decrease in optical density of stirred platelet-rich plasma in the presence of 2-AFMO was due to platelet aggregation induced by the compound.

Platelet aggregation induced by 2-AFMO in rabbit and guinea-pig platelet-rich plasma was proportional to the concentration of the compound, and at lower concentrations the aggregates began to disperse in less than 5 min (Figs. 1 and 2). The extent of platelet aggregation in response to a given concentration of the compound varied somewhat with different pools of platelet-rich plasma. Following the addition of 2-AFMO to platelet-rich plasma, there was a delay of 40–60 sec before the platelets aggregated (Figs. 1 and 2). This delay in the response occurred consistently in all samples of rabbit and guinea-pig platelet-rich plasma, and at all concentrations at which the compound was tested. The shapes of the aggregation curves obtained with guinea-pig platelets were similar to those previously obtained when guinea-pig platelets aggregated in response to added ADP[10]. Near-threshold concentrations of 2-AFMO resulted in a decrease in optical density followed by a return to the initial level in approximately 5 min (Fig. 2*A*). Slightly higher concentrations of 2-AFMO resulted in a biphasic aggregation curve (Fig. 2*B*). Still higher concentrations of 2-AFMO induced a progressive decline in optical density (Fig. 2*C*). Even with the higher concentrations of 2-AFMO, the optical density returned to, or near, the initial level within 30 min following the addition of 2-AFMO. 2×10^{-3} M 2-AFMO did not induce aggregation of rat platelets in six different pools of platelet-rich plasma.

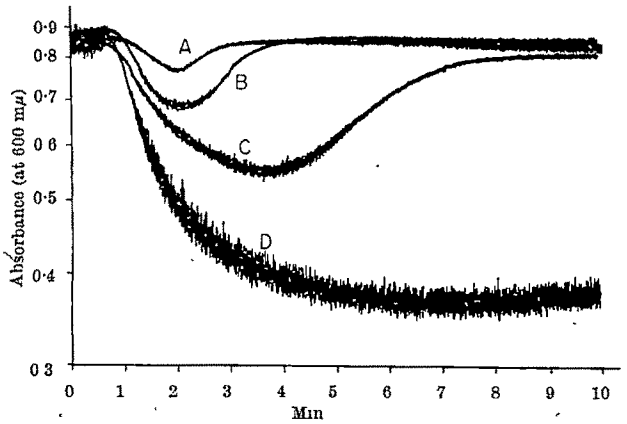


Fig. 1. Changes in optical density of rabbit platelet-rich plasma caused by 2-acetylfuran methoxycarbonyloxime (2-AFMO), added at zero time. (*A*), 5×10^{-5} M, (*B*), 1×10^{-4} M; (*C*), 2×10^{-4} M; (*D*), 4×10^{-4} M

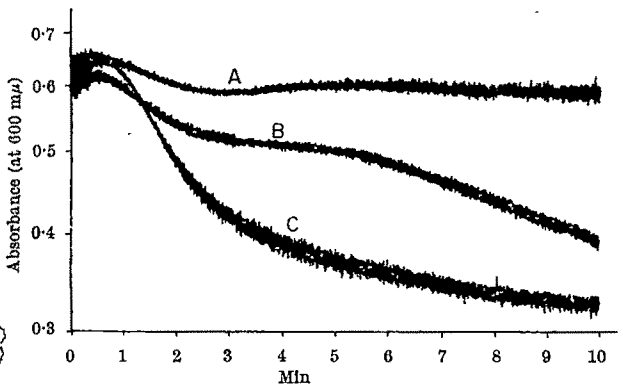


Fig. 2. Changes in optical density of guinea-pig platelet-rich plasma caused by 2-AFMO added at zero time: (*A*), 6×10^{-5} M; (*B*), 8×10^{-5} M; (*C*), 1×10^{-4} M

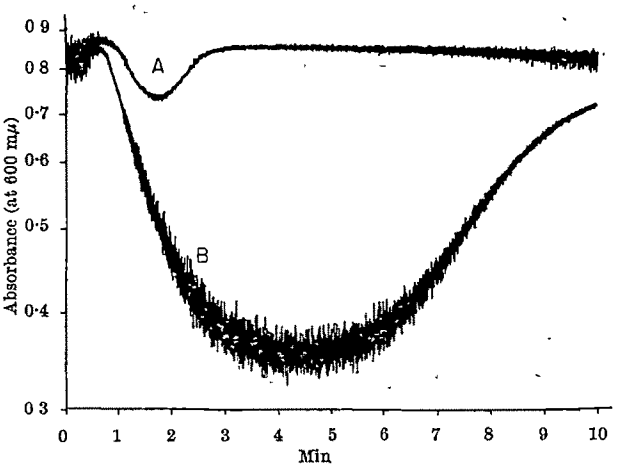


Fig. 3. Changes in optical density of rabbit platelet-rich plasma caused by 2-AFMO (2×10^{-4} M) alone, and in the presence of adenosine (1×10^{-5} M). 2-AFMO added at zero time to plasma which had been incubated 10 min with: (*A*), adenosine, (*B*), 0·154 M sodium chloride solution

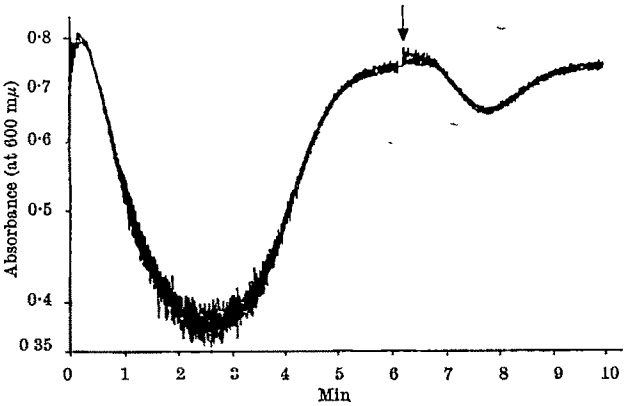


Fig. 4. Changes in optical density of rabbit platelet-rich plasma caused by ADP (1×10^{-6} M) added at zero time, and by 2-AFMO (2×10^{-4} M) added at ↓

10^{-5} M adenosine inhibited aggregation of rabbit platelets induced by 2×10^{-4} M 2-AFMO (Fig. 3). This inhibition was surmountable by a tenfold increase in concentration of the aggregating agent. The aggregation of guinea-pig platelets induced by 10^{-4} M 2-AFMO was not inhibited by 10^{-5} M adenosine. Following spontaneous reversal of strong aggregation of rabbit platelets induced by 2×10^{-4} M 2-AFMO, a second addition of the compound at the same concentration caused only slight aggregation. Following reversal of strong aggregation of rabbit platelets caused by 10^{-6} M ADP, only slight aggregation was induced by 2×10^{-4} M 2-AFMO (Fig. 4); conversely, weak aggregation was caused by 10^{-6} M ADP when it was added to rabbit platelet-rich plasma following the reversal of strong aggregation induced by 2×10^{-4} M 2-AFMO (Fig. 5).

Control responses to 2×10^{-4} M 2-AFMO and to 10^{-6} M ADP were obtained with rabbit platelet-rich plasma (Fig. 6). 2×10^{-3} M 2-AFMO was incubated in platelet-rich and in platelet-poor plasma for 5 min at 23° C, without stirring. Samples of 0·4 ml. from each were then tested for platelet aggregating activity in separate 4·0 ml. portions of platelet-rich plasma. The sample from platelet-poor plasma caused barely detectable platelet aggregation after a delay of about 60 sec, but that from the platelet-rich plasma caused strong platelet aggregation (Fig. 7); moreover, the latter occurred virtually immediately, in contrast to the typical delay which preceded aggregation

induced by 2-AFMO when this was added directly to the control sample of platelet-rich plasma (Fig. 6). The loss of activity of 2-AFMO following its incubation in rabbit platelet-poor plasma was further investigated. Platelet-poor plasma was heated for 30 min at 60° C, followed by centrifugation to remove coagulated protein. 2×10^{-3} M 2-AFMO was then incubated in a sample of the clear supernatant for 5 min at 23° C; another sample of the supernatant was similarly incubated with 0·154 M sodium chloride solution. When 0·4 ml. of the 2-AFMO incubation mixture was added to 4·0 ml. rabbit platelet-rich plasma, strong platelet aggregation occurred after a delay of approximately 60 sec. A 0·4 ml. portion of the sodium chloride incubation mixture did not cause platelet aggregation.

Compounds related to 2-AFMO were investigated in rabbit platelet-rich plasma for aggregating activity, and for inhibition of ADP- and 2-AFMO-induced platelet aggregation. 2-Acetylfuran, 2-acetylfuran oxime, acetone *o*-benzoyloxime, and 3-carbomethoxyisoxazole-5-aldehyde-acetyloxime, each at 5×10^{-4} M, did not induce platelet aggregation, nor did they inhibit aggregation induced by 10^{-6} M ADP or by 5×10^{-4} M 2-AFMO. 2-Acetylfuran acetyloxime, like 2-AFMO, induced aggregation of rabbit platelets; the concentration range which resulted in proportional responses was 5×10^{-4}–5×10^{-5} M.

Gaarder *et al.*[5] demonstrated that ADP causes aggregation of human platelets. Other agents have since been found to induce aggregation of platelets of some, but not all, the species in which they have been investigated. For example, epinephrine, norepinephrine and 5-hydroxytryptamine (5-HT) induce aggregation of human platelets, whereas rabbit platelets are not responsive to the catecholamines, and their behaviour to 5-HT is not predictable[11]; it has recently been reported that guinea-pig platelets are not aggregated by epinephrine or by 5-HT[10]. In contrast to this species difference in responsiveness of platelets to these amines, ADP causes aggregation of platelets of all species which have been investigated. As mentioned earlier, there is evidence to support the hypothesis that aggregation in response to the addition to platelet suspensions of agents other than the nucleotide is mediated by ADP released from platelets as a consequence of their interaction with these agents (refs. 1–4, 6, 7).

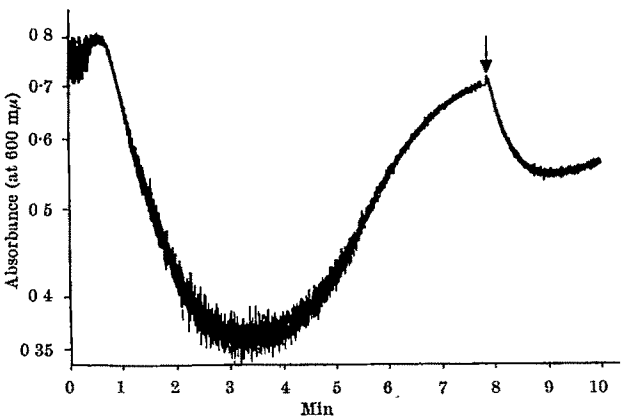


Fig. 5. Changes in optical density of rabbit platelet-rich plasma caused by 2-AFMO (2×10^{-4} M) added at zero time, and by ADP (1×10^{-6} M) added at ↓

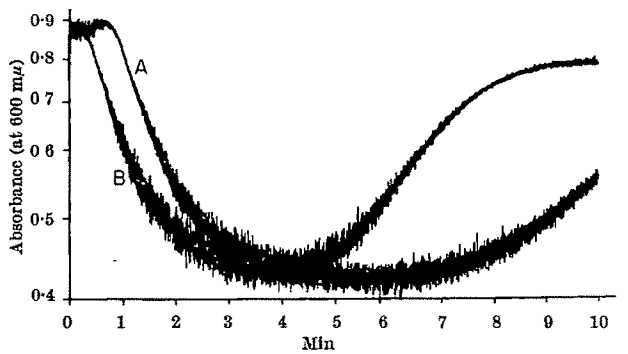


Fig. 6. Changes in optical density of rabbit platelet-rich plasma caused by: (*A*), 2×10^{-4} M 2-AFMO; (*B*), 1×10^{-6} M ADP. Compounds added at zero time. Plasma samples used in experiments illustrated by this figure and by Fig. 7 were taken, for each experiment, from the same pool of plasma

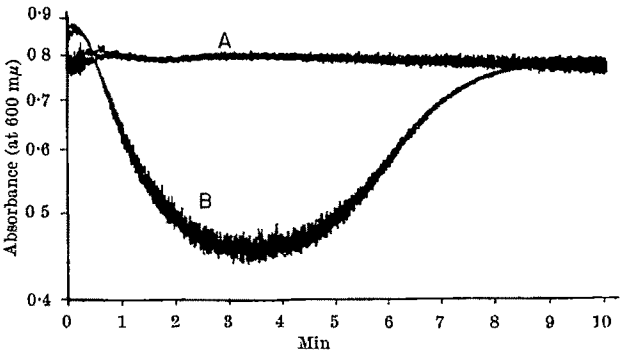


Fig. 7. Changes in optical density of rabbit platelet-rich plasma caused by 0·4 ml. samples of: (*A*), rabbit platelet-poor plasma, (*B*), rabbit platelet-rich plasma, both of which had been incubated 5 min with 2-AFMO (2×10^{-3} M) then added to 4·0 ml. platelet-rich plasma at zero time

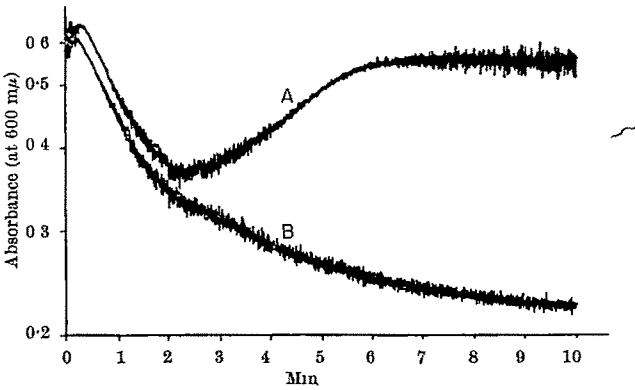


Fig. 8. Changes in optical density of guinea-pig platelet-rich plasma caused by ADP (2.5×10^{-7} M) added at zero time, followed 2 min later by: (*A*), 10^{-3} M hydroxylamine·HCl, (*B*) 0·154 M sodium chloride solution

It is possible that the aggregation of rabbit and guinea-pig platelets induced by 2-AFMO is mediated by ADP released from the platelets. 2-AFMO-induced aggregation of rabbit platelets was inhibited by adenosine, which, as Born and Cross[12] first demonstrated with human plasma, and Regoli and Clark[13] then showed with rabbit plasma, inhibits ADP-induced platelet aggregation. But adenosine did not inhibit 2-AFMO-induced aggregation of guinea-pig platelets; it has been reported that adenosine fails to inhibit aggregation of guinea-pig platelets caused by ADP[10]. Further implication of ADP as the mediator is provided by the observation that rabbit platelet-rich but not platelet-poor plasma, following incubation with 2-AFMO, contained an aggregating factor which, like ADP, acted without the delay in onset of aggregation which was characteristic of 2-AFMO when it was added directly to stirred platelet-rich plasma. The weak response of rabbit platelets to 2-AFMO when this was added following reversal of aggregation caused by ADP, and vice versa, resembles the response of rabbit platelets to repeated additions of ADP[9]. These results do not establish that 2-AFMO-induced platelet aggregation is mediated by ADP released from platelets but they are compatible with such a mechanism of action.

Rabbit platelets aggregated in the presence of 2-acetylfuran acetyloxime, and at the concentrations investigated this compound was approximately equal in effectiveness to 2-AFMO. Rabbit platelets were not aggregated by 2-acetylfuran oxime, a hydrolysis product common to both aggregating agents, which could form in rabbit plasma as a consequence of its esterase activity[14]. The ineffectiveness of both 2-acetylfuran and its oxime derivative suggested that the oxime ester function was necessary for aggregation. However, both acetone *o*-benzoyloxime and 3-carbomethoxyisoxazole-5-aldehydeacetyloxime were ineffective.

We are not yet able to speculate on the mechanism of interaction of platelets and the oxime esters. Oximes are known to reverse the inhibition of cholinesterase by organophosphorus compounds[15], and hydroxylamine is less active in this respect[16]. We found that hydroxylamine inhibited ADP-induced platelet aggregation in each of the three species investigated in the present study. When 10^{-5} M hydroxylamine was added to platelet-rich plasma 2 min after the addition of ADP, there was immediate cessation of aggregation and dispersal of aggregates. Fig. 8 shows this effect with guinea-pig platelets. It is possible that inhibition by hydroxylamine was due to nucleophilic attack at the terminal phosphate bond of ADP.

We thank Miss Martha Williams and Mrs. Diane Konick for their technical assistance.

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POSSIBLE MECHANISM OF AGGREGATION OF BLOOD PLATELETS BY ADENOSINE DIPHOSPHATE

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ALTHOUGH the ability of adenosine diphosphate (ADP) to produce platelet aggregation has been recognized for several years[1], the mechanism of this reaction has not been defined. Several hypotheses offered to explain this phenomenon have implied platelet binding of the ADP molecule resulting in aggregation through participation of this molecule in an intercellular bridge[2–4] or by alteration of the platelet's surface charge[5,6]. Alternatively, Spaet[7] has proposed an energy-yielding reaction involving breakdown of ADP as the cause of platelet aggregation. Recent experiments in our laboratory have revealed serious objections to these hypotheses and have suggested a different explanation for the action of ADP.

Isotopic experiments utilizing ADP-8-^{14}C and ADP-^{32}P have shown that in citrated platelet-rich plasma there is stepwise dephosphorylation of ADP to AMP and then to adenosine, which is incorporated by the platelet[8]. The dephosphorylation is primarily a plasma reaction and does not require the presence of platelets[7,8]. Since no radioactivity is recovered in the platelets after addition of ADP-^{32}P or AMP-^{32}P, it appears that these compounds do not bind to the platelet but are only incorporated after dephosphorylation. Afterwards the platelet-bound adenosine is rephosphorylated, the ^{14}C label eventually appearing in platelet adenosine triphosphate (ATP)[8].

In platelet-rich plasma, ADP-^{14}C-induced platelet aggregation is regularly accompanied by incorporation of radioactivity by the platelets and is inhibited by agents such as EDTA or arginine methyl ester, which inhibit incorporation of the ^{14}C label[9].

Washed platelets resuspended in heated plasma or in a buffered electrolyte solution aggregate strongly with ADP, if an acid- or ammonium sulphate-precipitate of plasma or serum is added. However, in these systems, platelet incorporation of radioactivity with ADP-^{14}C is strongly inhibited[8]. Thus, the binding of labelled nucleotide is not vital to platelet aggregation by ADP.

The breakdown of ADP to AMP and of AMP to adenosine in platelet-rich plasma is blocked by 0·01 M potassium cyanide (Table 1), which also strongly inhibits the incorporation of radioactivity by platelets after ADP-^{14}C. However, cyanide does not block platelet aggregation but in fact facilitates it and may retard disaggregation (Fig. 1). Thus, breakdown of ADP is not required for platelet aggregation.

Table 1. INHIBITION OF DEPHOSPHORYLATION OF ADP OR AMP BY CYANIDE

	ATP	ADP	AMP	Adenosine
(1) ADP-8-^{14}C	1	10	15	10
(2) ADP-8-^{14}C + 0 01 M potassium cyanide	0	31	2	0
(3) AMP-8-^{14}C	2	2	14	14
(4) AMP-8-^{14}C + 0 01 M potassium cyanide	0	0	33	1

Incubation of 10^{-6} M ADP-8-^{14}C or 10^{-6} M AMP-8-^{14}C in citrated platelet-rich plasma for 10 min at 37° C, followed by extraction with 10 per cent trichloroacetic acid and paper chromatography as previously described (ref. 8). Tracings were obtained by scanning the strips in a Vanguard 880 'Autoscanner', and the curves were identified by comparison with suitable standards and their area measured by planimetry. The figures above represent the area (in cm^2) under each curve.
Results of similar experiments in platelet-free plasma were qualitatively identical, except that no ATP was identified in the extracts.

Previous experiments with ^{14}C- or ^{32}P-labelled ATP have shown that ATP is converted to ADP in plasma and then follows the same degradation pathway outlined above. The dephosphorylation of ATP to ADP is not inhibited by 0·01 M potassium cyanide, but the subsequent breakdown of ADP is blocked (Table 2), and platelet incorporation of radioactivity after incubation with ATP-8-^{14}C is prevented. Aggregation of platelets by

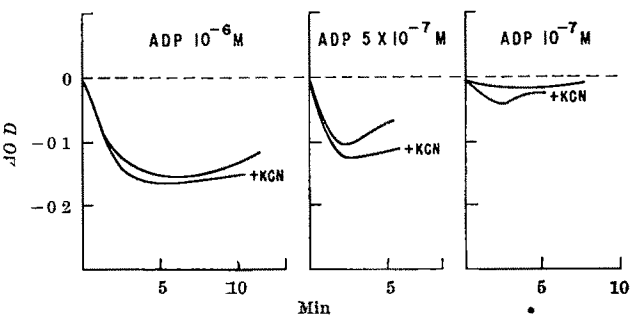


Fig. 1. Platelet aggregation with ADP (turbidimetric technique of Born (ref. 2) and O'Brien (ref. 10)). 0·01 M cyanide facilitates aggregation and, at higher concentrations of ADP, retards disaggregation

ADP is inhibited by preliminary addition of ATP and is reversed by ATP added after maximal aggregation has occurred (Fig. 2). This effect of ATP is not blocked by cyanide. Thus, the inhibition of ADP-aggregation by ATP does not require binding of the ATP molecule to the platelet.

Table 2. EFFECT OF CYANIDE ON DEGRADATION OF ATP

	ATP	ADP	AMP	Adenosine
(1) ATP-8-^{14}C	5	9	17	11
(2) ATP-8-^{14}C + 0·01 M potassium cyanide	10	40	0	0

Incubation of 10^{-6} M ATP-8-^{14}C in platelet-rich plasma and treatment as in Table 1. Similar results were obtained in platelet-free plasma.

The most likely explanation for these observations is the following hypothesis: Platelets require an active energy-supplying metabolic process to remain unsticky, the adhesive form being the low-energy state in a system analogous to the contracted state of striated muscle. Preservation of size and shape of the platelet and other cells requires an active metabolic process. This process involved splitting of ATP to ADP by an ATPase located in the platelet membrane. In the presence of added ADP, the breakdown of ATP by this enzyme is blocked by product inhibition. Addition of ADP to platelet-rich plasma inhibits an active process, disrupting the regulatory membrane system by which platelet 'non-stickiness' is maintained. That ADP causes platelets to swell has been shown by Zucker and Borrelli[11]; it is probable that sphering of the platelet exposes receptive sites on the platelet surface permitting secondary bridging with fibrinogen[12–14] or in appropriate conditions with other proteins[6]. This secondary bridging phenomenon probably involves a divalent cation bond, because it can be split by EDTA. EDTA also produces platelet swelling[15], perhaps by blocking a divalent cation-dependent membrane ATPase.

We have observed strong ATPase activity of intact platelets resuspended in a buffered electrolyte system containing calcium and magnesium (Fig. 3). Similar data have recently been reported by Robinson *et al.*[6], and White and Krivit[17] have localized ATPase activity in the platelet membrane by histochemical techniques. The activity is inhibited by adenosine diphosphate (Fig. 3) but not by 10^{-2} M potassium cyanide or 10^{-3} M ouabain. Very weak activity was also found in a red cell suspension and in platelet-free citrated plasma.

The action of a membrane ATPase which splits substrate ATP on the outer surface of the cell membrane is unusual, but 'ecto-ATPase' activity has been demonstrated in neuroglia and other cells[18]. Furthermore, the platelet is an unusual cell, unique in its origin as a cytoplasmic fragment with an external surface that was originally buried within the cytoplasm of the megakaryocyte. The activity of the calcium–magnesium-dependent membrane ATPase of platelets is depressed by temperature decrease, which is also known to cause platelets to swell[15] and to aggregate[19]. It is possible that

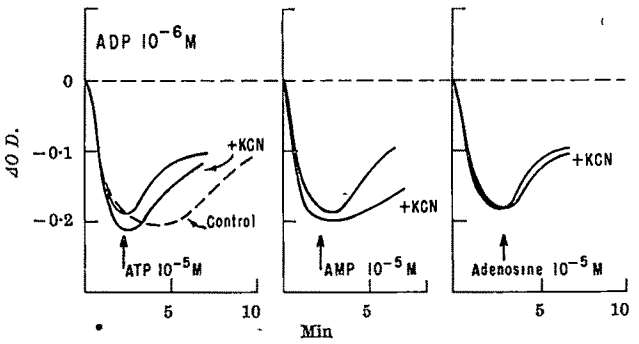


Fig. 2. Disaggregation by ATP, AMP, and adenosine of platelets aggregated by ADP. The effect of AMP is prevented by 0·01 M cyanide; the effect of ATP and of adenosine is not

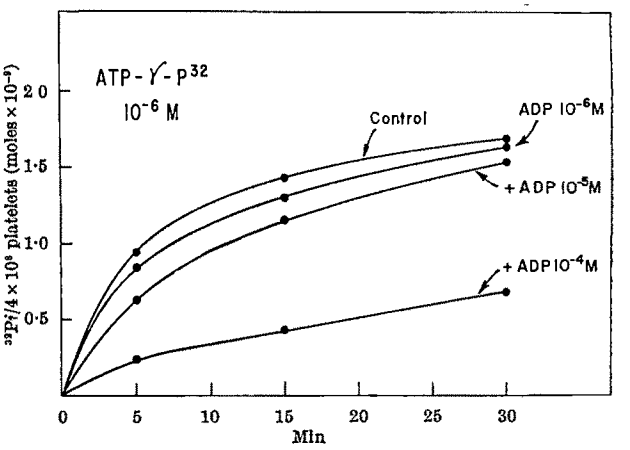


Fig. 3. Release of inorganic phosphorus-32 from ATP-γ-^{32}P. Washed platelets were re-suspended in Ringer's solution containing 1/10 volume of 3·8 per cent tri-sodium citrate and 0·005 M magnesium chloride, buffered to pH 7·5, and were incubated at 20° with 2×10^{-9} moles of ATP-γ-^{32}P. After the reaction was stopped with trichloroacetic acid, inorganic phosphate was determined by a modification of the method of Martin and Doty (ref. 16)

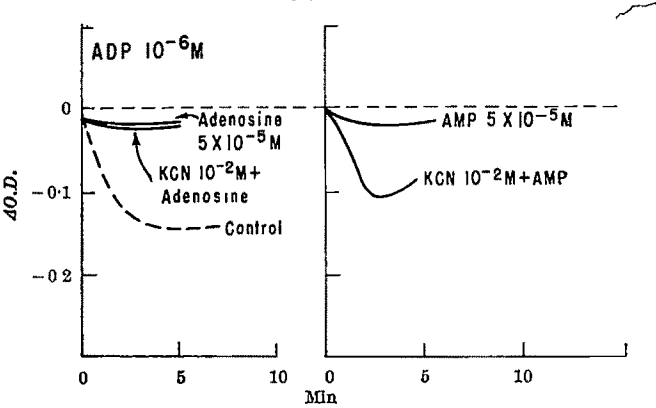


Fig. 4. Inhibition of ADP-induced platelet aggregation by AMP and adenosine added 10 min before the ADP. The inhibitory effect of AMP is impaired by cyanide; the effect of adenosine is not

this enzyme is thrombosthenin, a calcium–magnesium dependent ATPase isolated by Bettex-Galland and Lüscher[20] from an unknown locus in platelets. The known contractile properties of this protein could account for the change of platelet shape and development of stickiness resulting from the addition of ADP.

It is likely that disaggregation following aggregation produced by ADP results from degradation of ADP, which allows membrane ATPase activity to resume. Increasing temperature favours disaggregation[10] by favouring dephosphorylation of ADP by plasma enzymes and by increasing the membrane ATPase activity.

The inhibitory effect of ATP on platelet aggregation can be explained by its role as the natural substrate for the postulated membrane ATPase. One might argue that ATP could inhibit or reverse platelet aggregation by complexing ionized calcium. This explanation is unlikely, for we found citrate, which has calcium complexing ability equal to that of ATP[21], to be 100 times less potent than ATP as an inhibitor of ADP action.

Adenosine is known to inhibit ADP-induced platelet aggregation, and this effect increases with time[2]. It is not blocked by cyanide (Fig. 4). When added to ADP-aggregated platelets, adenosine causes them to disaggregate (Fig. 2), a reaction which is not opposed by cyanide. 0·01 M potassium cyanide is a very weak inhibitor of the incorporation of adenosine-^{14}C by platelets[8].

AMP inhibits the aggregating effect of ADP[2] and causes disaggregation when added to ADP-clumped platelets (Figs. 4 and 2), but these effects are blocked by cyanide, which blocks conversion of AMP to adenosine and prevents platelet incorporation of radioactivity after AMP-^{14}C

is added to platelet-rich plasma. It appears that AMP must be converted to adenosine before it can inhibit platelet aggregation in response to ADP. ADP, AMP and adenosine or their degradation products apparently compete for sites on the platelet[8], and it has been proposed[2] that this accounts for the inhibition of ADP-induced platelet aggregation by AMP and adenosine. Alternative explanations are possible, such as one which involves synthesis of ATP from platelet adenosine, a reaction which is not blocked by cyanide and which might be expected to favour the membrane ATPase reaction described here and to increase with time, as does the inhibitory effect of adenosine and AMP. This platelet synthesis of ATP from adenosine is not blocked by 5×10^{-4} M 2,4-dinitrophenol (ref. 8) and therefore probably occurs by a route other than oxidative phosphorylation.

In summary, this hypothesis is proposed for further study. Addition of adenosine diphosphate to platelet-rich plasma leads to breakdown of the ADP through stepwise dephosphorylation to adenosine which is then incorporated into the platelets. This reaction is not crucial to platelet clumping but is probably important for ultimate dispersal of the platelet aggregate. The presence of ADP in the platelet environment inhibits the splitting of ATP to ADP, a reaction which otherwise occurs through platelet membrane ATPase, the function of which is customarily to keep the platelet in an unsticky state and to maintain the cell size and shape. Inhibition of this ATPase by its normal product, ADP, leads to exposure of adhesive sites and permits platelet aggregation through intermedate secondary bridges containing calcium, fibrinogen, or other proteins.

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EFFECT OF TRYPSIN ON *Escherichia coli* AND RABBIT RETICULOCYTE RIBOSOMES

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WE have previously reported experiments which suggest that ribosomal aggregates from heart muscle are stabilized by interaction between nascent peptide chains[1]. Treatment of the ribosomal assemblage with a proteolytic enzyme, either chymotrypsin or trypsin, as well as with ribonuclease is required to release monomers; neither enzyme alone is completely effective. Similar results have been reported for the chick embryo polysomes that synthesize collagen[2], and for the ribosomal aggregates isolated from lymph nodes that are active in γ globulin synthesis[3]. Myofibrillar proteins, collagen and γ globulin are all composed of sub-units having a strong tendency for spontaneous interaction. It was concluded, therefore, that an interaction between newly synthesized polypeptide chains might in certain circumstances contribute to the stability of polysomes.

Liver and reticulocyte polysomes, in contrast to those from muscle, connective tissue and lymph nodes, are completely converted to monomers, or into Mg^{2+}-stabilized dimers, by ribonuclease treatment[4,5]. Moreover, Williamson and Schweet have shown that treatment of ribosomes from reticulocytes with puromycin leads to the release of peptide chains but not to breakdown of the ribosome aggregate unless energy is added[6]. Since mild trypsin treatment of liver[1,7,8] and reticulocyte[8] polysomes also leads to their breakdown, it would appear that proteolytic enzymes affect the ribonucleoprotein complex not only by causing the release of nascent peptide chains, but also by an effect on the structure of the ribosomes.

To determine the means whereby proteolytic enzymes alter the state of aggregation of ribosomes, a systematic investigation of the effect of mild proteolysis on ribosomal structure and function was undertaken. It is the purpose of this article to report the results of that investigation. Relatively mild tryptic digestion caused an inhibition of the binding by ribosomes of polyuridylic acid. Perhaps, secondary to this effect, the binding of aminoacyl-*s*RNA to ribosomes and the incorporation of ^{14}C-labelled amino-acid into protein were also inhibited. Trypsin also caused a dissociation of *E. coli* and reticulocyte monomers into their sub-units; trypsin-treated sub-units failed to recombine to form 70*S* particles when incubated in the presence of an adequate concentration of magnesium. The results of trypsin treatment of ribosomes and polysomes emphasize the importance of the protein moiety of the ribosome in the binding of artificial, and probably also of natural, messenger RNA, and in the Mg^{2+}-requiring association of ribosomal sub-units. Trypsin appears then to disrupt polysomes not only by removal of nascent peptide chains, but also by disturbing the aminoacyl-*s*RNA- ribosome-*m*RNA[9] complex through damage to the ribosomal protein.

E. coli strain *B* was grown in an enriched medium[10] and collected in the exponential phase of growth. Ribosomes were isolated, pre-incubated and washed as described by Nirenberg and Matthaei[11]. Reticulocytes were obtained from anaemic New Zealand albino rabbits by cardiac puncture after treatment of the animals with 2·5 per cent phenylhydrazine. The polyribosomes and monomers were isolated according to the method of Arnstein *et al.*[12].

Assay of the binding of ^{14}C-phenylalanyl-*s*RNA to ribosomes was carried out with 'Millipore' membrane filters as described by Nirenberg and Leder[13]. The 70*S* ribosomes and the 50*S* sub-units adhere quantitatively to the membrane, but approximately 30 per cent of the 30*S* sub-units pass through. Determination of incorporation of ^{14}C-phenylalanine into protein by ribosomes was according to the method of Nirenberg and Matthaei[11]; incorporation was terminated by the addition of 5 per cent

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Table 1. EFFECT OF TRYPSIN ON BINDING AND INCORPORATION OF ^{14}C-PHENYLALANYL-*s*RNA BY *E. coli* AND RABBIT RETICULOCYTE RIBOSOMES

Experiment	*E. coli*			Rabbit reticulocytes					
				Monomers			Polysomes		
	Control (c.p.m.)	Trypsin (c p.m)	Decrease (%)	Control (c p.m.)	Trypsin (c.p.m.)	Decrease (%)	Control (c.p.m.)	Trypsin (c.p.m)	Decrease (%)
^{14}C-PHENYLALANYL-*s*RNA									
Binding	981	578	42	1,703	598	65			
Release	665	639	4	332	199	40			
INCORPORATION OF ^{14}C-PHENYLALANINE									
Without poly U	139	117	21	190	139	27	2,460	980	60
With poly U	5,126	527	90	5,442	685	88	5,417	1,006	82

E. coli ribosomes were pre-incubated for 45 min at 35° C with the complete system for assaying amino-acid incorporation into protein[11]. The medium (medium I) used to measure ^{14}C-phenylalanyl-*s*RNA binding to ribosomes contained in 0 1 ml.: 2–4 × 10^3 c.p.m. ^{14}C-phenylalanyl-*s*RNA; 10 μmoles *tris*-HCl buffer *p*H 7·6; 2 μmoles magnesium acetate; 8 μmoles ammonium chloride; and 20 μg polyuridylic acid. Incubation was for 15 min at 35°. The medium (medium II) used to assay ^{14}C-phenylalanine incorporation into protein by *E. coli* ribosomes contained 0·1 ml.: 5 × 10^4 c.p.m. ^{14}C-phenylalanine; 5 μmoles *tris*-HCl buffer *p*H 7 6, 1 5 μmoles magnesium chloride; 5 μmoles potassium chloride; 0·75 μmoles mercaptoethanol; 0·1 μmole ATP; 0 2 μmole phosphoenolpyruvate; 0·025 μmole GTP; 2 5 μg pyruvate kinase; 0·1 mg *E. coli* 105,000*g* supernatant protein; and 20 μg polyuridylic acid when indicated. For ^{14}C-phenylalanine incorporation into protein by reticulocyte ribosomes, the system was the same except that the magnesium chloride concentration was 8 mM; 10 mM glutathione was used in place of the mercaptoethanol; and 1 mg of reticulocyte '*p*H 5 enzymes' was used. Incubation was for 15 min at 35° when ribosomal nomomers were used and 5 min at 35° C when polysomes were used. In each assay, approximately 2 $O.D_{260}$ mμ of ribosomes were present. Results are expressed as c.p m. of ^{14}C-phenylalanine bound or incorporated per $O.D_{260}$ mμ of ribosomes in 30 min at 0° C. To test for release of bound ^{14}C-phenylalanyl-*s*RNA, trypsin treatment followed incubation with ^{14}C-phenylalanyl-*s*RNA. The values are the average of duplicate determinations.

Table 2. A COMPARISON OF THE ABILITY OF *E. coli* RIBOSOMES TO SYNTHESIZE ^{14}C-POLYPHENYLALANINE AFTER TRYPTIC TREATMENT IN THE PRESENCE OR ABSENCE OF PREVIOUSLY ADDED ^{14}C-PHENYLALANYL-*s*RNA AND POLY U

System	C.p.m./$O.D_{260}$	Decrease (%)
Ribosomes (control)	2,148	
Ribosomes pre-treated with trypsin, then ^{14}C-Phe-*s*RNA and poly U added	1,101	51·5
Ribosomes (control)	1,930	
Ribosomes incubated with ^{14}C-Phe-*s*RNA and poly U first, then trypsin treated	915	52·5

^{14}C-phenylalanyl-RNA and polyuridylic acid were bound to *E. coli* ribosomes either before or after trypsin treatment as indicated. The incubation conditions for binding were as in Table 1. Trypsin was added at a concentration of 0 5 μg/$O.D_{260}$ ribosomes and the reaction terminated, after incubating for 30 min in ice by a three-fold excess of soybean trypsin inhibitor.

Ribosomes thus pretreated were incubated for a second time in a mixture containing energy and 105,000*g* supernatant enzymes (medium II, Table 1) for 10 min at 35° C

trichloroacetic acid, and 1 mg of bovine serum albumin was added. The specific circumstances of the experiments are given in Tables 1 and 2 and Figs. 1 and 2. The precipitate was washed with cold and hot trichloroacetic acid on a 'Millipore' membrane filter. The membranes and the protein were dissolved in Bray's solution and radioactivity was determined with 49 per cent efficiency in a 'Tricarb' liquid scintillation counter.

Trypsin and soybean trypsin inhibitor were purchased from Worthington Biochemical Corporation; ^{14}C-labelled and non-radioactive polyuridylic acid were purchased from Miles Chemical Corporation; ^{14}C-L-phenylalanine, having a specific activity of 333 mc./mmole, was obtained from New England Nuclear Corporation. *E. coli* stripped *s*RNA (General Biochemicals) was charged with ^{14}C-phenylalanine and nineteen ^{12}C-amino-acids[14]; the product had a specific activity of 1–1·6 × 10^5 c.p.m./mg *s*RNA.

Treatment of ribosomes with 5 μg trypsin for 30 min at 0° C leads to the release of less than 0·3 per cent of 280 mμ absorbing material. (In each case the concentration of trypsin is in μg of enzyme per optical density unit (at 260 mμ) of ribosomes.)

The effect of trypsin on the following ribosomal properties was studied: (1) the dissociation of monomers into sub-units; (2) the reassociation of sub-units; (3) the binding and release of ^{14}C-polyuridylic acid; (4) the binding and release of ^{14}C-phenylalanyl-*s*RNA; (5) the incorporation of ^{14}C-phenylalanine into protein. In each of the tests the effect of trypsin was completely blocked by the prior addition of a three-fold excess of soybean trypsin inhibitor. Neither trypsin inhibitor nor DFP trypsin caused a change in ribosome function. No ribonuclease activity was detectable in the trypsin.

(1) *Dissociation of monomers into sub-units.* There was no appreciable breakdown of *E. coli* ribosomes to 50*S* and 30*S* sub-units when treated for 30 min at 0° C with 0·5–5 μg of trypsin (Fig. 1*A*), or for 5 min at 25° (Fig. 2). When 10 μg of trypsin was used, however, the 70*S* peak was somewhat decreased in height and broadened, indicating slight breakdown of 70*S* particles (Fig. 1*A*, curve 3). Rarely, a ribosomal preparation was unusually sensitive to trypsin, and complete disruption into 50*S* and 30*S* sub-units occurred with 2 μg trypsin.

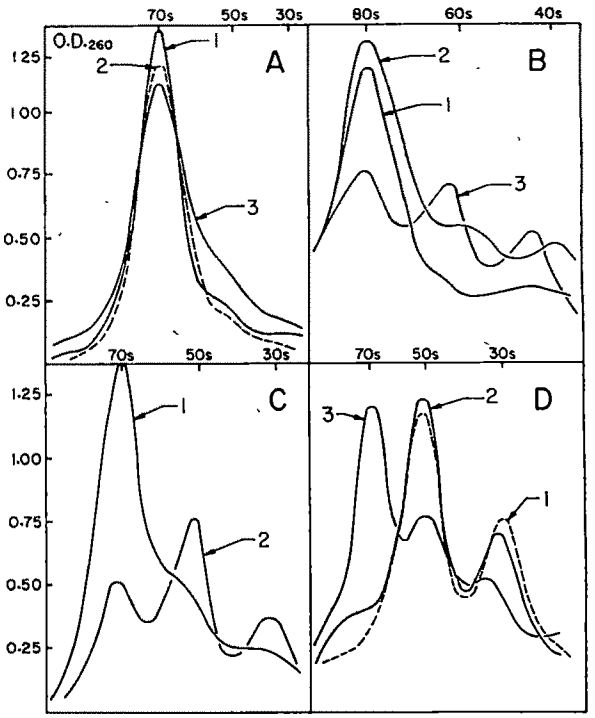


Fig. 1. Sucrose gradient centrifugation of ribosomes treated with trypsin. The concentration of ribosomes is given as the absorption at 260 mμ. A 0·1-ml. portion of ribosomes containing 2–3 $O.D_{260}$ mμ was analysed on a 5–20 per cent linear sucrose gradient prepared with the same buffer as that in which the ribosomes were suspended. The gradient was centrifuged at 39,000 r.p.m. in the *SW* 39 rotor of the model *L*2 Spinco centrifuge for 90 min at 0°. The sedimentation profiles were recorded with a Gilford spectrophotometer, model 2000. Ribosomes or ribosomal sub-units were exposed to trypsin for 30 min at 0°, following which soybean inhibitor was added in three-fold excess. *A*, *E. coli* ribosomes: curve 1, control; curve 2, treated with 0 5 μg trypsin/$O.D_{260}$ mμ; curve 3, treated with 10 μg trypsin/$O.D_{260}$ mμ. *B*, Reticulocyte monomers purified by differential centrifugation: curve 1, control; curve 2, treated with 0·7 μg trypsin/$O.D_{260}$ mμ; curve 3, treated with 10 μg trypsin/$O.D_{260}$ mμ. *C*, Dissociated and reassociated *E. coli* ribosomes. Ribosomes were dissociated by dialysis for 3 h against 5 × 10^{-3} M *tris*-HCl buffer *p*H 7·3, 10^{-4} M magnesium chloride, and were reassociated by overnight dialysis against 5 × 10^{-3} N *tris*-HCl buffer *p*H 7·3, 1·75 × 10^{-2} M magnesium chloride, 8·6 × 10^{-2} M potassium chloride, and 6 × 10^{-3} mercaptoethanol. Trypsin treatment followed re-association. Curve 1, No trypsin treatment; curve 2, treatment with 5 μg trypsin/$O.D_{260}$mμ *D*, Effect of tryptic treatment on reassociation of 50*S* and 30*S* sub-units. Curve 1, *E. coli* ribosomes dissociated into 50*S* and 30*S* sub-units by dialysis against low Mg^{2+}; curve 2, 50*S* and 30*S* sub-units treated with 5 μg trypsin/$O.D_{260}$ mμ followed by re-association; curve 3, 70*S* ribosomes treated with 5 μg, trypsin/$O.D_{260}$ mμ followed by dissociation and re-association

The 'trypsin resistant' ribosomes could be transformed into 'sensitive' ones by dissociating them to sub-units in 10^{-4} M Mg^{2+}, and then reassociating the sub-units in 10^{-2} M Mg^{2+}. The reconstituted ribosomes were extremely sensitive to trypsin, as little as 0·5 μg of enzyme now causing their dissociation (Fig. 1*C*). The reason for this is not clear; it may have been the result of the release of a ribosomal component (*m*RNA, *s*RNA or the nascent peptide chain) during dissociation; or the sub-units may not reassociate in a manner identical to their original structure.

(2) *Reassociation of sub-units.* Trypsin-treated ribosomes may be dissociated by dialysis against a low concentration of Mg^{2+}, and reassociated to 70*S* particles by dialysis against buffer containing 10^{-2} M Mg^{2+} (Fig. 1*D*, curve 3). The reassociation was, however, 20–30 per cent less complete when the ribosomes had been treated with trypsin (compare Fig. 1*D*, curve 3, with Fig. 1*C*, curve 1). If instead of the 70*S* monomers the sub-units obtained by dialysis were exposed to trypsin, no reassociation occurred (Fig. 1*D*, curve 2). The sub-units were far more sensitive to tryptic digestion than the intact ribosome, perhaps because the association to form 70*S* monomers

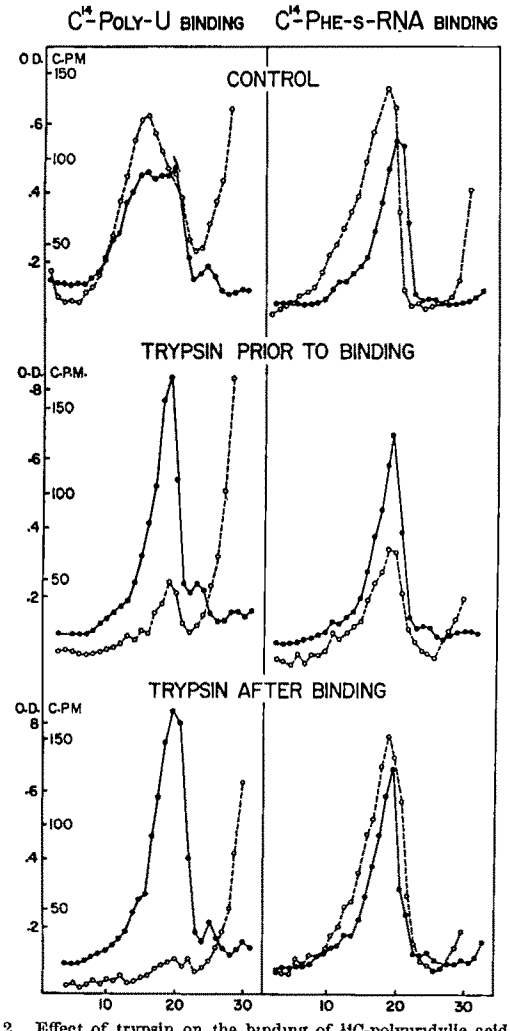


Fig. 2. Effect of trypsin on the binding of ^{14}C-polyuridylic acid and ^{14}C-phenylalanine-*s*RNA to *E. coli* ribosomes. The ribosomes were prepared as described in Table 1. The conditions for assay of the binding of ^{14}C-polyuridylic acid to ribosomes were identical to those of Suarez and Nathans[19]. The 0·2 ml. reaction mixture contained 2×10^4 c.p.m. of ^{14}C-polyuridylic acid (specific activity 0·183 μc./mg), and 7 $O.D._{260}$ mμ of ribosomes. The conditions for measuring the binding of ^{14}C-phenylalanyl-*s*RNA to ribosome were described in Table 1. Ribosomes were treated with 5 μg of trypsin per $O.D._{260}$ mμ for 5 min at 25° C, and 15 μg of trypsin inhibitor was then added. Density gradient centrifugation was carried out as described in Fig. 1. The tubes were punctured and 10-drop fractions were collected and processed as described before[1]. ●—●, Optical density at 260 mμ; ○ - - - - ○, c.p.m.

partly protects the sites required for cohesion of the sub-units.

Similarly, 70*S* ribosomes isolated from *Streptococcus pyogenes*[15] and liver[16] are relatively resistant to ribonuclease, but become sensitive to the enzyme when dissociated to their sub-units.

Reticulocyte ribosomes react to trypsin treatment in a manner similar to that of *E. coli* ribosomes except that they are more sensitive to the enzyme. As little as 0·7 μg of trypsin at 0° C for 30 min causes considerable increase in the numbers of 60*S* and 40*S* particles (Fig. 1*B*). Yeast ribosomes also are split into sub-units by treatment with papain for 12 min at 37° C (ref. 17).

(3) *Binding and release of ^{14}C-polyuridylic acid.* The binding of ^{14}C-polyuridylic acid to *E. coli* 70*S* ribosomes was decreased by 75 per cent after treatment with 5 μg of trypsin for 5 min at 25° C (Fig. 2). The results accord with those of Kaji and Kaji[18]. The ^{14}C-polyuridylic acid bound to 70*S* ribosomes by incubation for 20 min at 0° C was completely released by treatment with trypsin. The amount of ^{14}C-polyuridylic acid bound to *E. coli* ribosomes was not affected by the presence of aminoacyl *s*RNA. Furthermore, the addition of aminoacyl *s*RNA (20 μg *s*RNA per $O.D._{260}$) did not prevent the trypsin-induced release of polyuridylic acid from the ribosome. Trypsin did not alter the sedimentation (7*S*) of ^{14}C-polyuridylic acid in a linear sucrose density gradient.

(4) *Binding and release of ^{14}C-phenylalanyl sRNA.* Treatment of 70*S* ribosomes from *E. coli* with 0·5, 5 or 10 μg of trypsin caused a decrease in the polyuridylic acid directed binding of ^{14}C-phenylalanyl-*s*RNA to the ribonucleoprotein particles (Table 1 and Fig. 2*A*). Analysis of the ribosomes on sucrose gradients disclosed that the decrease in binding of ^{14}C-phenylalanine-*s*RNA occurred even though less than 10 per cent of the ribonucleoprotein particles were split into sub-units. Ribosomes from *E. coli* and reticulocyte were treated with trypsin and centrifuged in a sucrose gradient; the 70*S* particles isolated from the gradient showed a reduction of ^{14}C-phenylalanyl-*s*RNA binding similar to that of the whole preparation. Thus, the inhibition by trypsin of the binding of aminoacyl-*s*RNA to ribosomes does not seem to be solely a consequence of the formation of sub-units.

When *E. coli* ribosomes to which aminoacyl-*s*RNA was bound were treated with 0·5 or 5·0 μg of trypsin, no release of radioactivity occurred (Table 1, Fig. 2).

Trypsin decreased the amount of ^{14}C-phenylalanyl-*s*RNA bound to reticulocyte monomers, as was also the case with *E. coli* ribosomes (Table 1). About 40 per cent of the ^{14}C-phenylalanyl-*s*RNA bound to reticulocyte ribosomes was removed by subsequent treatment with 5 μg trypsin in contrast to the results obtained with *E. coli* ribosomes (Table 1).

The effect of trypsin on ^{14}C-phenylalanyl-*s*RNA binding and on ^{14}C-polyuridylic acid binding to ribosomes was not of the same degree. Although the binding of charged *s*RNA required polyuridylic acid, trypsin doses which result in more than an 80 per cent reduction of polyuridylic acid binding led to only a 50 per cent decrease in *s*RNA binding. Moreover, trypsin caused the release of all the polyuridylic acid bound to ribosomes from *E. coli*, whereas little or no charged *s*RNA was liberated. The results may be accounted for by transfer of the aminoacyl *s*RNA from a ribosomal site requiring polyuridylic acid to another site where it is bound directly to the ribosome. A binding site of this type has been suggested by Nirenberg and Leder[13].

(5) *Incorporation of ^{14}C-phenylalanine into protein.* Incorporation of ^{14}C-phenylalanine into protein by either *E. coli* or reticulocyte ribosomes was greatly impaired by trypsin treatment (Table 1). The stimulation by polyuridylic acid of phenylalanine incorporation into protein by ribosomal monomers was nearly abolished by trypsin, as might have been predicted from the fact that the enzyme

effected a decrease in the binding of polyuridylic acid. The incorporation of ^{14}C-phenylalanine into reticulocyte polysomes containing natural messenger RNA is affected to a lesser degree (Table 1). Trypsin had less effect on the incorporation of ^{14}C-phenylalanine into monomers in the absence of polyuridylic acid. It would appear that the attachment of natural messenger to ribosomes is more resistant to trypsin than is polyuridylic acid. Trypsin was still effective when ^{14}C-phenylalanyl-*s*RNA and polyuridylic acid were added prior to the proteolytic enzyme (Table 2).

The experiments indicate that the binding of polyuridylic acid to ribosomes is markedly diminished by alteration or removal of one or more ribosomal peptides. The proteolytic treatment was mild, as indicated by the unchanged sedimentation constant and the absorption ratio (280 mμ : 260 mμ) of the monomers. To what extent the ribosome protein participates in the binding of polyuridylic acid and natural messenger RNA is not known. It is possible that a specific configuration or structure of the ribosomal protein is required for the binding of messenger RNA to the ribosomal RNA. Had the prior association of polyuridylic acid and *s*RNA with the ribosome protected against proteolytic destruction of the binding site, this possibility would have been reinforced.

There was less reduction of ^{14}C-phenylalanyl-*s*RNA binding to ribosomes by trypsin than, and may well be secondary to, the inhibition of polyuridylic acid binding. The binding of that aminoacyl *s*RNA to ribosomes is dependent on the presence of natural or artificial *m*RNA. The release of template RNA from the ribosome could then secondarily cause the loss of aminoacyl *s*RNA. The observation that ^{14}C-phenylalanyl *s*RNA bound to ribosomes in the presence of polyuridylic acid is not released by tryptic digestion despite the release of bound polyuridylic acid is difficult to explain. The aminoacyl *s*RNA may be transferred to another binding site which is not dependent on the presence of polyuridylic acid. Although the effect of trypsin on phenylalanyl-*s*RNA binding can be adequately explained as secondary to a decrease in polyuridylic acid binding, an additional effect on ribosomal structure to alter an aminoacyl RNA binding site cannot be ruled out.

Similarly the inhibition of ^{14}C-phenylalanine incorporation into protein by trypsin could be secondary to the effect of the enzyme on the binding and release of polyuridylic acid. If so, the inhibition by trypsin of amino-acid incorporation into protein observed with reticulocyte polysomes, and with *E. coli* and reticulocyte monomers, suggests that natural *m*RNA is also released by proteolytic action. The polyuridylic acid directed amino-acid incorporation of ^{14}C-phenylalanine was in all cases more sensitive to tryptic action than incorporation directed by natural messenger. Thus, natural *m*RNA bound to ribosomes may be more resistant to release by trypsin than is bound polyuridylic acid. Possibly, this is an example of more effective protection of those binding sites from tryptic digestion by the bound *m*RNA than by polyuridylic acid.

An additional site of action of tryptic digestion is required to account for the enhanced formation of sub-units from *E. coli* ribosomes. It also appears that the peptide configuration required for this Mg^{2+}-linked association of sub-units is exposed when ribosomes are dissociated into 50*S* and 30*S* sub-units. These sub-units are much more sensitive to tryptic digestion than are 70*S* ribosomes.

The disruption of polyribosomes from *E. coli* or reticulocytes by proteolytic enzymes would seem then to be the result of an alteration of the binding site for *m*RNA; the *m*RNA is released and then the ribosomal assemblage breaks down. Inasmuch as complete dissociation of *E. coli* and reticulocyte ribosomes was obtained by ribonuclease, it is unnecessary to postulate an interaction between nascent polypeptide chains for the stabilization of the polyribosomes. The failure of proteolytic enzymes to affect the polysomal structure from rabbit skeletal muscle reported by Breuer *et al.*[20] may be due to a relative insensitivity of the messenger binding site in that preparation.

However, where ribonuclease does not completely disrupt the polyribosomal structure, as in the case of the ribosomes concerned with the synthesis of myofibrillar protein, collagen or γ globulin, it is possible that an interaction between ribosomal-bound polypeptide chains containing the sub-units of the protein participates in stabilization of the structure.

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TRANSPIRATION RESISTANCE AND ENERGY BUDGET OF *Populus sargentii* LEAVES

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THE energy budget of a plant leaf is intimately coupled to the leaf temperature and resulting physiology including photosynthesis, respiration, transpiration and other processes. The transpirational loss of water represents a loss of energy and is therefore part of the energy budget of a leaf. The energy budget may be written as follows, if storage and metabolic energy are neglected:

$$Q_{abs} = R_l + C - LE \quad (1)$$

where Q_{abs} = total amount of solar, skylight, and thermal radiation absorbed by the leaf; R_l = re-radiation from the leaf surface; C = convection from the leaf to the air; L = latent heat of vaporization of water, a quantity which is slightly temperature-dependent and the units of

* This work was carried out while the senior author was a participant in the Undergraduate Research Program in Engineering at the University of Colorado, Boulder.

which are cal g^{-1}; and E = transpiration rate. Each of these quantities may be evaluated from the following relationships:

$$R_l = \varepsilon \sigma T^4 \text{ cal cm}^{-2} \text{ min}^{-1} \quad (2)$$

where T_l is the leaf temperature; σ is the Stefan–Boltzmann radiation constant and ε is the emissivity of the leaf surface, taken to be 0·97.

$$C = 6{\cdot}5 \times 10^{-3} (V/D)^{1/2} (T_l - T_a) \text{ cal cm}^{-2} \text{ min}^{-1} \quad (3)$$

where V is the wind speed in cm sec^{-1}; D is the significant leaf dimension in the direction of air flow, in cm; T_l is the leaf temperature; and T_a is the air temperature in °C.

$$E = \frac{{}_s d_l(T_l) - d_a(T_a)}{R} \text{ g cm}^{-2} \text{ min}^{-1} \quad (4)$$

where ${}_s d_l(T_l)$ is the density of water vapour at the mesophyll cell walls lining the substomatal cavity of the leaf assuming saturation at the leaf temperature T_l, $d_a(T_a)$ is the density of water vapour in the free air in g cm^{-3}, and R is the diffusion resistance of the leaf to water vapour in min cm^{-1}. This equation can be rewritten in terms of the relative humidity (r.h.) of the air, and the water vapour density of the air at saturation ${}_s d_a(T_a)$:

$$E = \frac{{}_s d_l(T_l) - \text{r.h.}_s d_a(T_a)}{R} \text{ g cm}^{-2} \text{ min}^{-1} \quad (5)$$

The assumption that the mesophyll cell walls lining the substomatal cavity are saturated with water may be incorrect. If the water content is below saturation within the substomatal cavity then the calculated value of R is larger than the true value and it must then be recognized that only an 'apparent, or fictitious' resistance is determined. However, the apparent resistance is useful if saturation is assumed whenever it is reintroduced into equation (5) to compute a transpiration rate. Also, the apparent resistance will be related rather closely to the actual resistance.

For a leaf in its natural environment, the energy budget, equation (1), is constantly solved by adjustments in leaf temperature and transpiration rate. For given environmental conditions—air temperature, relative humidity, wind speed, and radiation intensity—the transpiration resistance of a leaf governs how freely the leaf transpires and thereby determines whether the leaf temperature is relatively high or low. Once the transpiration resistance of a leaf is known, then the energy budget equations can be solved to give the predicted leaf temperature and transpiration rate for any given environmental conditions. Conversely, by measuring the leaf temperature and evaluating the energy budget for the leaf, one can show that a measured transpiration rate is a valid, actual rate. This will be demonstrated by the observed results.

In this investigation we observed the transpiration rate of leaves from a *Populus sargentii* tree and from this determined the diffusion resistance of the leaves by solving for R in equation (5). Each leaf was excised from the branch with its petiole under water to prevent air from blocking the tracheids. It was then sealed into a potometer chamber, and placed in the test chamber of a controlled-environment wind tunnel, where it was subjected to a steady wind speed and illumination. The temperature and relative humidity of the air were continuously monitored, and by threading a 40-gauge thermocouple through the leaf we also recorded the leaf temperature. The leaf surface was in each case at an angle of 45° to the wind direction. The light source was a 650-W '*DWA*' movie lamp.

The transpiration resistance, leaf temperature, air temperature, T leaf − T air, relative humidity, and the various components of heat loss from the leaf, are shown in the figures for four different leaves. In theory, the net radiation absorbed should remain constant with time, and thus the net heat loss should also be constant. In the figures a slight upward trend can be seen, and small fluctuations occur in the net heat loss term. The upward trend results from a tendency of the wind tunnel to become heated with use; its walls grow warmer and the thermal radiation load on the leaf increases accordingly. The fluctuations may have occurred as the storage term varied about zero, or they may have been due in part to experimental error in reading the potometer.

Fig. 1 shows an example of the typical behaviour of leaves observed in this investigation. The light was filtered by 10 cm of water to remove most of its heat; the result at the surface of the leaf was 2,120 ft.-candles of visible light with only 0·32 cal cm^{-2} min^{-1} net radiation from the lamp.

The initial low value of resistance may have been caused by uptake as the petiole and leaf cells adjusted to the osmotic concentration of the incoming tap water. Note that while the air temperature was greater than the leaf temperature, the value of (T leaf − T air) follows the resistance quite closely. Thus, when resistance is highest, transpiration is lowest and the temperature difference is algebraically at its highest (near zero). When the resistance drops, transpiration increases, and (T leaf − T air) drops to a low value (near − 3° C). Note also that because the leaf is cooler than the air, the convection term is actually a heat gain for the leaf, and is a negative heat loss. The steep drop in transpiration resistance at about 1,030 occurred, of course, as the stomates opened.

The leaf of Fig. 2 was illuminated with 313 ft.-candles, but since the light was not filtered, the heat load from the lamp was 1·1 cal cm^{-2} min^{-1} at the surface of the leaf. Because of the higher incoming radiation, this leaf was warmer than the air and the convection term was positive. Note that the (T leaf − T air) difference was positive, and that for this case also it increased and decreased with the resistance.

For leaves under low illumination, the resistance tended to vary erratically as in the example of Fig. 3. This leaf received 35 ft.-candles from the lamp, and 0·4 cal cm^{-2}

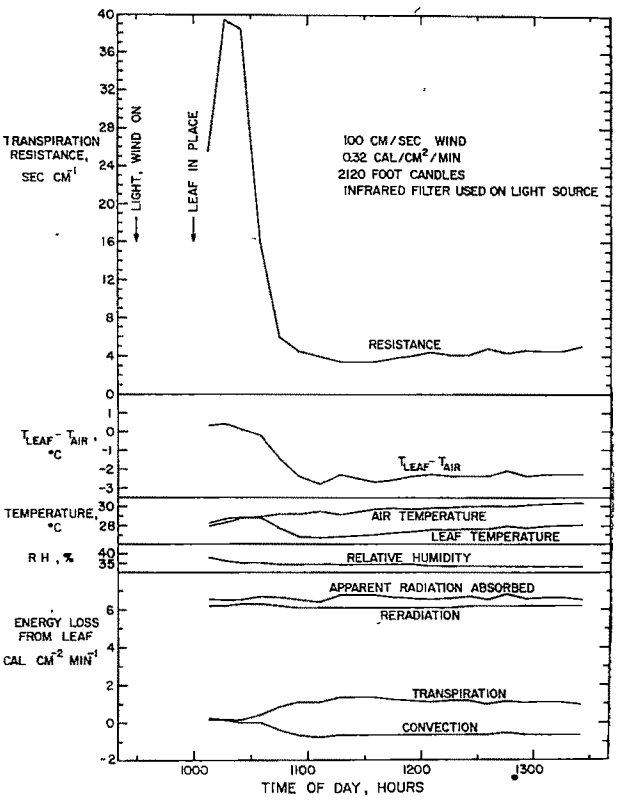


Fig. 1. Energy parameters for a cottonwood leaf under high illumination and low thermal radiation

min^{-1} at its surface. The transpiration rate was sufficient to keep the leaf temperature below that of the air, and the convection term was therefore negative.

Data for the most interesting leaf observed are presented in Fig. 4. It behaved normally until about 1520 hours, but then its resistance began to oscillate with increasing amplitude. It is striking that, while the convection and transpiration terms of the energy balance varied widely, the net energy loss remained fairly constant (within limits, as already discussed). This was so even though the leaf temperature was also quite variable.

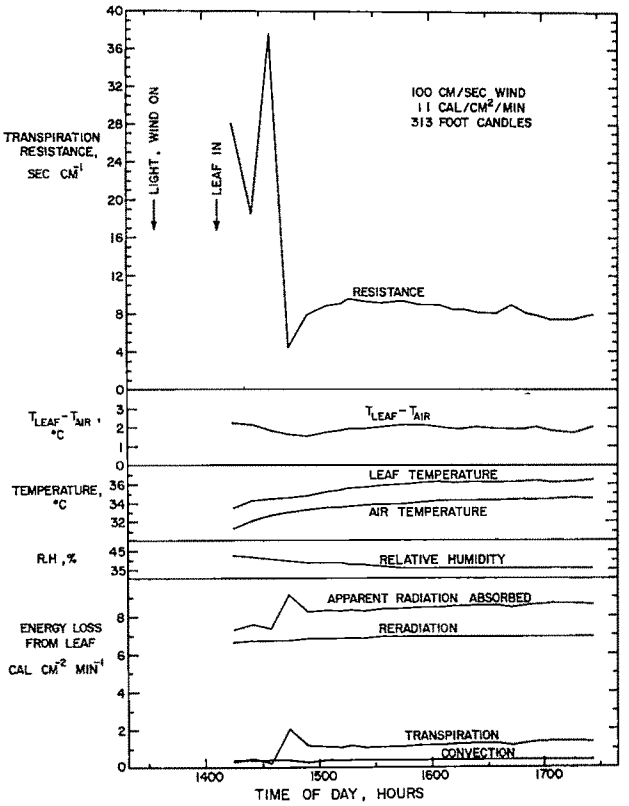


Fig. 2. Energy parameters for a cottonwood leaf under moderate illumination and high thermal radiation

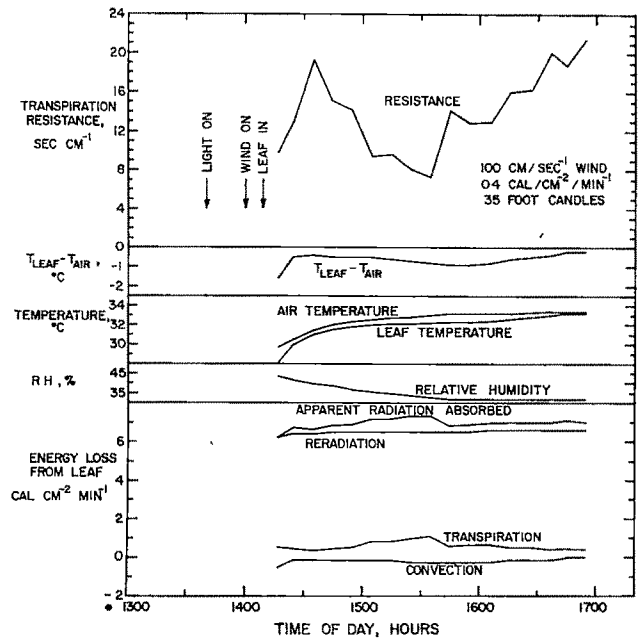


Fig. 3. Energy parameters for a cottonwood leaf under low illumination and low thermal radiation

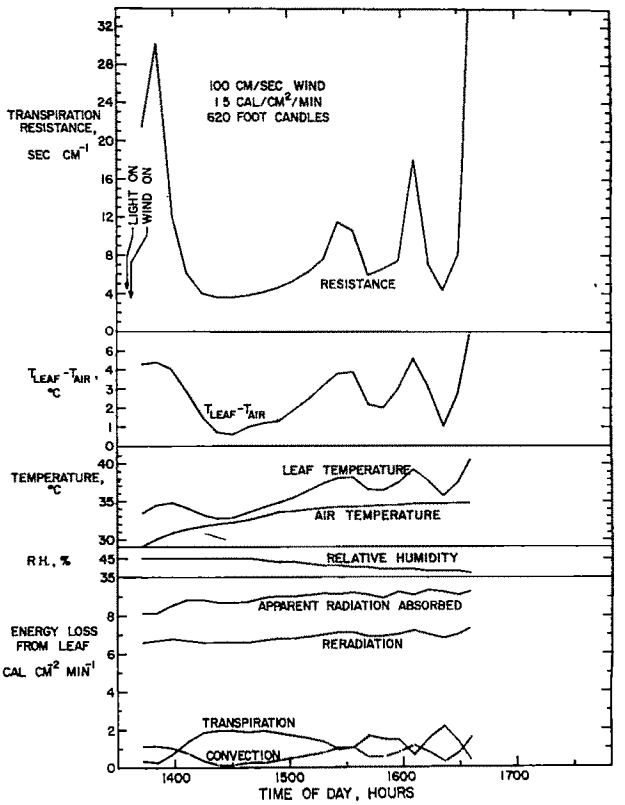


Fig. 4. Energy parameters for a cottonwood leaf under moderate illumination and high thermal radiation

The oscillatory nature of the transpiration, apparent resistance, and leaf temperature is somewhat puzzling. The fact that the leaf temperature changed simultaneously with the transpiration rate indicates that the observed transpiration was a real loss of water from the leaf. We do not profess to understand entirely what occurred here. It could have been related to the osmotic potential within the leaf and guard cells as modified by the water into which the leaf was placed for the experiment. It could have been a problem of water transport in which the leaf first dried out, the guard cells lost turgor, the stomatal opening decreased, and then water flow increased, guard cell turgor was restored, and the stomata reopened. Transpiration may next have increased, drying occurred, and the cycle repeated itself.

It is possible that the phenomenon can be explained in terms of temperature as it affects photosynthesis, respiration, and carbon dioxide concentration in the guard cells. It should be noted that the leaf temperature was not very high at first, for example 33°–35° C, at which temperature net photosynthesis was still very favourable. After the oscillation in transpiration began, however, the leaf temperature varied from 36° to 40° C, which was high enough to considerably reduce net photosynthesis and increase respiration[1]. However, purely on the basis of the processes of photosynthesis, respiration and temperature change it is difficult to postulate a feedback mechanism which could cause the oscillations.

In summary, we can say that well-lit leaves from *Populus sargentii* have an apparent transpiration resistance of about 4 sec cm^{-1}. This can be used to solve the energy budget equation for a given leaf. The resistance may be higher, and unpredictable, for a shade leaf.

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AUTORADIOGRAPHIC DEMONSTRATION OF CHOLESTEROL FILTRATION AND ACCUMULATION IN ATHEROMATOUS RABBIT AORTA

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PREVIOUS autoradiographic studies have indicated that the inner part of the rat aortic wall labels more rapidly than does the outer part after intravenous injection of tritium-labelled cholesterol[1]. This evidence was taken to show that cholesterol enters the aorta from the blood and flows from the inner to the outer part of the wall of the vessel. This view was confirmed by showing that the inner part of the rabbit aorta labelled very rapidly after the vasa vasorum (adventitial vessels) had been stripped off the vessel, but 8–16 days elapsed before the outer part labelled strongly[2]. The tritium label apparently did not dissociate from cholesterol during the experiment, as was shown by the complete recovery of plasma radioactivity from the cholesterol fraction[2].

The present investigation was undertaken to determine the fate of labelled cholesterol in rabbit atheromatous aorta. Eight young New Zealand white rabbits were fed a diet enriched with 1 per cent cholesterol[3] for 6 weeks; they were then injected intravenously with 2·0 mc. of tritium-labelled cholesterol. This amount of radioactive cholesterol was one hundred times greater than that administered in a previous investigation of the aorta in the cholesterol-fed rabbit[4]. The cholesterol for injection was prepared by dissolving 16 mc. of cholesterol–*T*(*G*) (Radiochemical Centre, Amersham) in 8 ml. of ethanol containing 0·5 per cent 'Tween 20'; this solution was evaporated under nitrogen and redissolved in 8 ml. of physiological saline[5]. The animals were killed at intervals of 1–20 days after injection; in the meantime they were maintained on the cholesterol-enriched diet. Plasma lipid radioactivity at the time of killing was estimated in an acetone–ethanol (1 : 1 v/v) extract of plasma with a liquid scintillation counter. Autoradiographs of transverse sections of the aortic arch were prepared by our previous method[2], but the exposure time was increased to 5 weeks.

Little exchange was apparent between infiltrated labelled cholesterol and sterol previously deposited in the atheromatous plaque (as can be seen by comparison of Figs. 1 and 2), yet entry of cholesterol into the atheromatous aortic wall was more rapid and resulted in heavier labelling than that found in our previous studies on the normal rat[1,2] and rabbit[2] aorta.

Granule counts were made in eight radial bands of autoradiographs of each vessel; a total area of about 0·1 sq. mm was counted in each aorta. Counts for the inner and outer half of the media are recorded as histograms (Fig. 3). These results clearly show that the atheromatous plaque labelled more rapidly than did the tunica media after injection of ^{3}H–cholesterol. An exception occurred two days after injection, when radioactivity was slightly higher in the outer media and inner plaque than in the inner media and outer plaque (Fig. 3). The greater labelling in the outer media at this stage may have been due to entry of isotope from the vasa vasorum, before ^{3}H–cholesterol had penetrated from the lumen into the middle part of the aortic wall.

This general pattern of labelling is consistent with the major entry of cholesterol into the aortic wall being from the lumen of the vessel. Nevertheless, it could be argued that this pattern is merely a reflexion of the rate of incorporation of ^{3}H–cholesterol into the different layers of the aorta. However, we have shown above that there is little dynamic exchange between radioactive cholesterol and sterol previously deposited in the atheromatous plaque (Figs. 1 and 2), so the rapid labelling of the plaque cannot be attributed to exchange with the large mass of cholesterol in the lesion. There is no reason to suppose that the rates of intracellular incorporation and intercellular deposition of cholesterol in the plaque would be greater than that in the undegenerated tunica media if both regions were equally exposed to the same 'hypercholesterolaemic' milieu. In fact it is likely in such circumstances that greater intracellular incorporation would occur in the tunica media than in the plaque, because the cell population is higher in the former than the latter. If this postulate is valid, it follows that the rate of incorporation or deposition of cholesterol in the various layers of the aorta is not primarily determined in

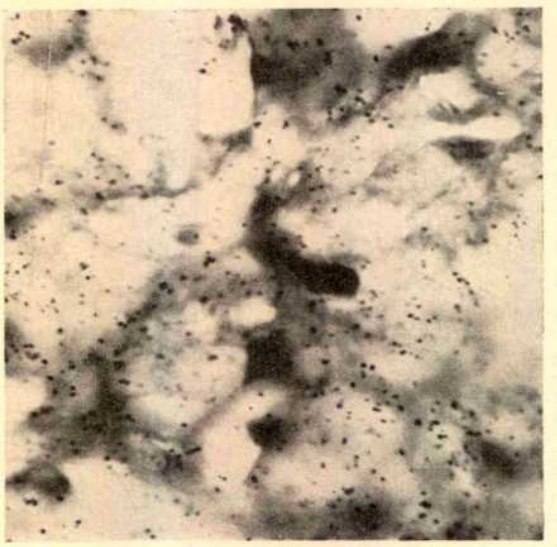


Fig. 1. Autoradiograph of atheromatous aortic intima in rabbit 20 days after injection of 2·0 mc. of ^{3}H–cholesterol (×*c.* 1,575)

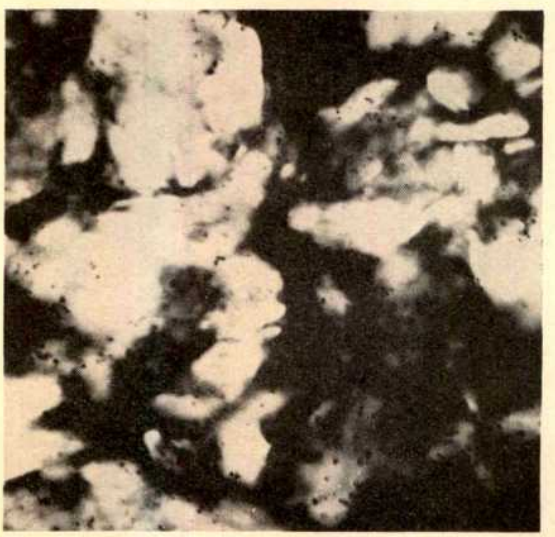


Fig. 2. Same field as Fig. 1 but photographed in polarized light, so that cholesterol crystals are birefringent (white) but autoradiograph granules remain black. Note that the cholesterol crystals previously deposited in the atheromatous intima are only trivially labelled

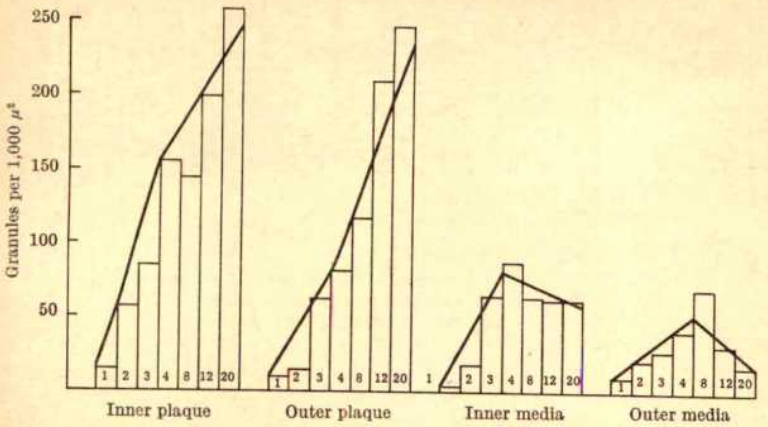


Fig. 3. Histogram of autoradiographic granule counts (less background) in atheromatous rabbit aorta after intravenous injection of 2·0 mc. of ^{3}H–cholesterol. Note the initial fast labelling of the inner plaque, followed by heavy labelling of the outer plaque and inner media, while the outer media labels slowly. The numbers at the base of each column represent days after injection. The heavy line is a guide to the overall change in radioactivity in the different aortic layers, but it has no mathematical significance

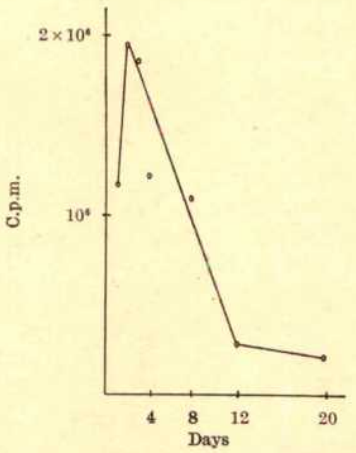


Fig. 4. Plasma lipid radioactivity (c.p.m./1·0 ml. plasma) in cholesterol-fed rabbits after intravenous injection of 2 mc. of ^{3}H–cholesterol

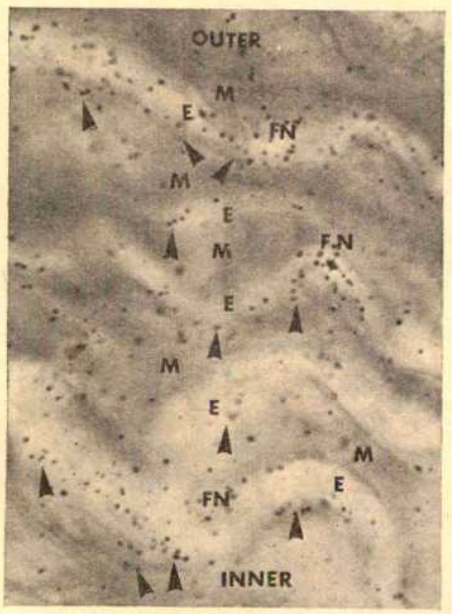


Fig. 5. Autoradiograph of inner media of atheromatous rabbit aorta 2 days after injection of 2 mc. of ^{3}H–cholesterol. Note accumulation (arrows) of label on inside of elastic laminae (*E*) and diffusion through probable fenestrations (*FN*) in the laminae; *M*, smooth muscle fibres. (×*c.* 1,575)

the young animal by metabolic differences between the cells of these layers, but is directly related to the rate of entry of cholesterol into each zone. The rate of outward transfer is exceeded when a certain rate of entry is attained, so that the excess cholesterol (overload) is deposited in that region. Thus, the quantity of cholesterol that is deposited would be directly proportional to the extent to which the rate of entry exceeds that of transfer. Studies on the fate of 4–^{14}C–cholesterol in both rabbit and cockerel aorta suggest in fact that the cholesterol overload in the inner zones may be quite small in relation to cholesterol transfer[6,7].

Plasma lipid radioactivity remained high in the initial period of the experiment but fell after 8 days (Fig. 4). The pattern of rise and fall of radioactivity in the outer media is similar to that in the blood, but is somewhat delayed; it is presumably due either to slow passage of cholesterol through the aortic wall or to relatively rapid dynamic exchange between such cholesterol in passage and steroid in the connective tissues of the tunica media. The progressive increase of radioactivity in the plaque indicates that cholesterol is both entering and being retained in the lesion. Radioactivity in the inner media maintained its level from the eighth day of the experiment onwards. This equilibrium was probably a result of deposition of cholesterol in the innermost region of the media balanced by loss of steroid by outward diffusion.

These observations are consistent with our previous hypothesis[1,2,8] that cholesterol in the blood normally filters through the arterial wall from the intima to the adventitia and with physiological evidence that metabolites and plasma constituents diffuse through arterial and capillary walls[9–13]. This view was further supported by the observation in the present investigation that labelled cholesterol occasionally accumulates on the inner side of the aortic elastic laminae (Fig. 5). If the elastic lamina can be regarded as a partial barrier to the filtration of cholesterol, a local concentration of label would be expected to accumulate on the inner side of the lamina when excess cholesterol diffuses outwards through the aortic wall.

The evidence provided by our present and previous autoradiographic investigations suggests that cholesterol diffuses through the normal and atheromatous aortic wall, but it does not indicate whether such diffusing cholesterol is bound to lipoprotein or is dissociated from this transport vehicle[14–16].

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GROWTH AND FOOD CONSUMPTION OF LABYRINTHECTOMIZED HAMSTERS DURING CHRONIC CENTRIFUGATION AT 5*g* AND 6*g*

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THROUGHOUT the past 12 years, several laboratories have reported that growth of plants and animals is possible during chronic centrifugation at survivable intensities[1]. At exposure to sufficiently intense fields, growth, though measurable, is slower, and life expectancy is shorter for experimental animals than it is for control animals. The interpretation of most of these reports rests on the assumption that the greater energy demanded to oppose the simulated high gravity diverts food materials ordinarily utilized in growth processes. An alternative explanation is that field gradients and Coriolis accelerations not associated with true gravitational fields can result in disorientation and nausea. Under these conditions, there would be a consequent reduction in growth rate due either to lower food consumption or to lower capacity for food utilization. These non-gravitational effects of centrifugation have suggested difficulties which may be encountered in generating artificial gravity in space stations, in space vehicles, or in supplying a 1*g* environment to control organisms in satellites designed for the study of the biological effects of weightlessness.

The non-gravitational effects of centrifugation are believed to develop in response to the complex stimulation of the vestibular organs. To eliminate such unnatural stimulation, female golden hamsters, *Mesocricetus auratus*, were bilaterally labyrinthectomized at the age of 22 days. The labyrinthectomy was accomplished by surgical approach into the labyrinth through the oval window, followed by aspiration of endolymphatic fluid from the cochlear labyrinth. The tympanum was completely opened. The destroyed labyrinth was filled by topical application of a few drops of streptomycin sulphate (100 mg/c.c.). This operation was performed with forty-fold magnification by means of a Zeiss operating microscope. The animals were anaesthetized with sodium pentobarbital (30 mg/kg body-weight) given intraperitoneally. Surgical details will be described more fully elsewhere. Each experimental animal had three litter-mate control animals: a sham-operated sister which accompanied it during centrifugation, a labyrinthectomized sister which remained at 1*g*, and a sham-operated sister which also remained at 1*g*.

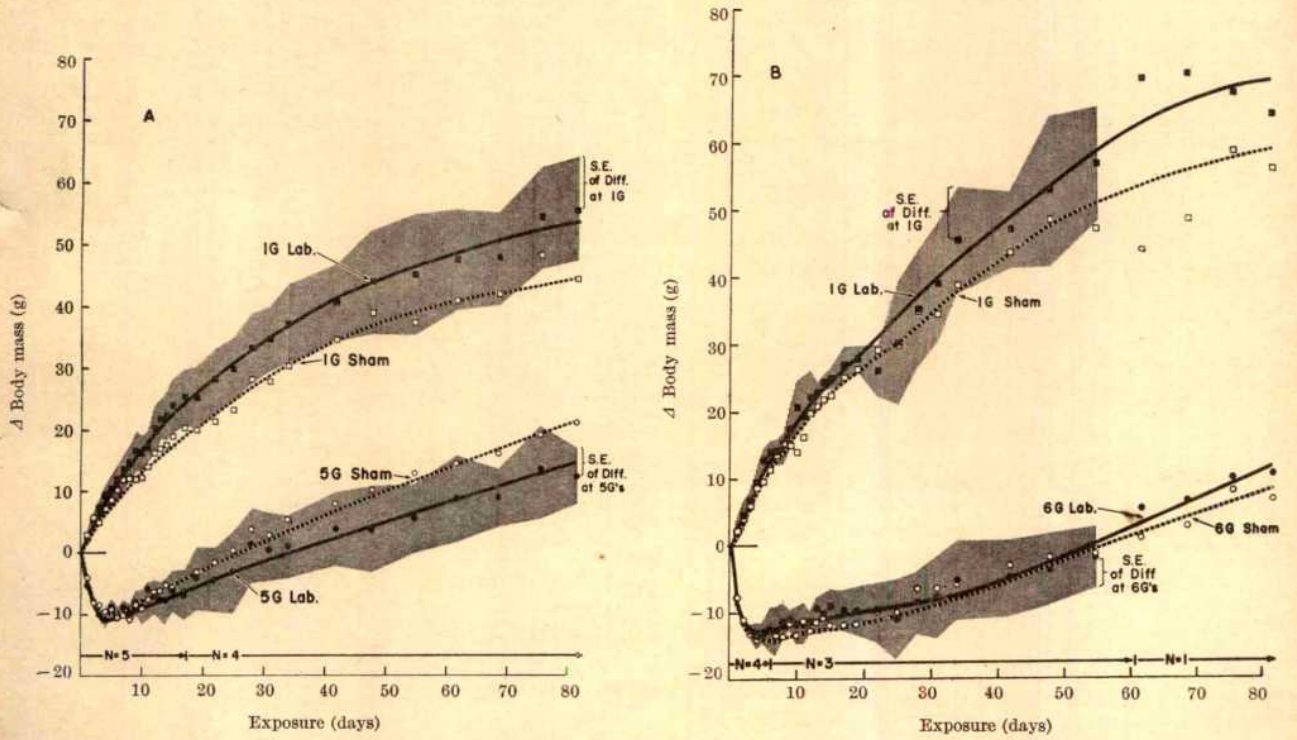


Fig. 1. Growth as measured in terms of change in body mass. *A*, Hamsters at 5*g*. Initial body-weights were 61·78 ± 2·8 g, 59·80 ± 3·59 g, 57·82 ± 1·83 g, and 59·64 ± 2·53 g for labyrinthectomized animals at 5*g*, for sham-operated animals at 5*g*, for labyrinthectomized animals at 1*g*, and for sham-operated animals at 1*g* respectively. Standard errors of differences between animals at same field are indicated. The number *N* of litters (containing one member in each of the four conditions) is indicated. Measurements were discontinued when any member of a litter died. The paired sample errors for differences between labyrinthectomized versus sham-operated animals at a given field are displayed by the height of shading above or below points for labyrinthectomized animals. The tendency for greater mass change among labyrinthectomized control animals could result from the smaller size, and thus greater growth potential, at the onset of the experiment. *B*, Growth of hamsters at 6*g* initial body-weights were 58·75 ± 3·35 g, 63·38 ± 1·46 g, 56·88 ± 3·62 g and 65·40 ± 0·39 g. The notation used is the same as that employed in *A*

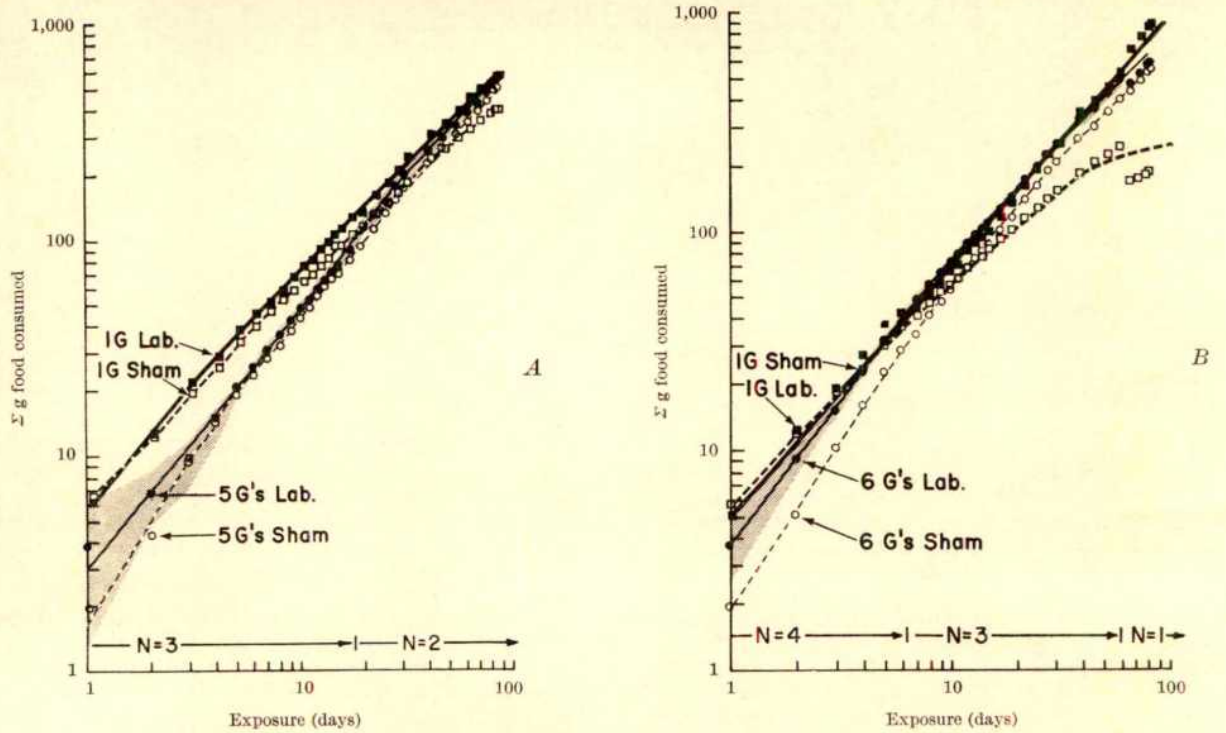


Fig. 2. *A*, Cumulative food consumption for 5*g* animals. The notation used is the same as that for Fig. 1. Standard errors are only shown for differences between animals in centrifuge. *B*, Average cumulative food consumption for 6*g* animals

Sham operations involved surgical puncture of the ear drum under sodium pentobarbital anaesthesia. Except for loss of hearing and loss of gravity reflexes associated with the inner ear in labyrinthectomized animals, all hamsters used in this investigation recovered quickly from surgical trauma and proceeded to develop at 1*g* until 36 days of age. At this time, sham-operated animals (61·79 ± 1·30 g, $N = 18$) were slightly larger than labyrinthectomized animals (58·92 ± 1·30 g, $N = 18$).

Chronic exposure at 5*g* (at a radius of 51 cm at the cage bottom) or 6*g* (radius of 62 cm) at 97 r.p.m. was initiated when animals had achieved 36 days of age. Centrifugation was performed in a modification of a previously described machine[2]. Procedures for exposure were similar to those used in earlier hamster investigations[3]. Each animal was housed in a 5 in. × 8·5 in. × 4 in. compartment where food consisted of Purina 'Laboratory Chow' with uncooked potatoes as the source of water. Exposure was interrupted for a period of 15 min each day for feeding, cage cleaning and mass measurements.

The growth pattern throughout 82 days of exposure was essentially the same as that found with normal centrifuged hamsters[3]. Experimental animals first underwent a loss in body mass, followed by some recovery of mass, with eventual growth in excess of original size but not comparable with that for the 1*g* litter-mate animals (Fig. 1). Cumulative food intakes (Purina 'Laboratory Chow' for hamsters) throughout the experiment were comparable with those of both types of centrifuged hamsters (Fig. 2). When the experiment was discontinued at the end of 89 days of exposure, the general appearance of both types of animals subjected to comparable exposure was quite similar (Fig. 3).

Throughout the first 2 or 3 days of exposure, the sham-operated animals demonstrated a post-rotatory type of nystagmus during observation at normal gravity. This behaviour did not persist throughout the remainder of the experiment. Apparently the animals adjusted by reducing the threshold for gravity and/or rotatory perception. Sham-operated animals removed from the centrifuge after 89 days of exposure did not demonstrate righting reflexes at 1*g*. Similar observations have been performed in this laboratory with kittens[4] and fish[5], which demonstrate righting reflexes only when they were exposed once more to high gravity. Centrifugation is

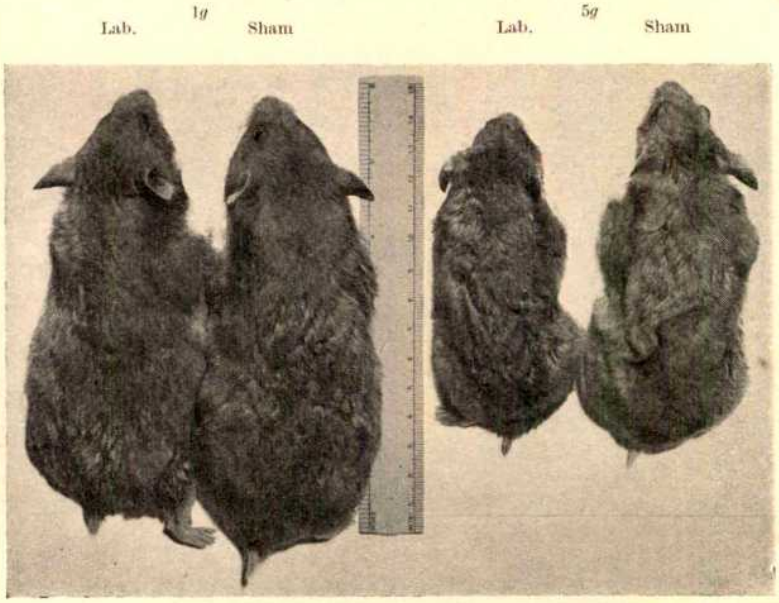


Fig. 3. Typical litter at end of experiment

Table 1. DIFFERENCES IN RESPONSE OF EXPERIMENTAL AND CONTROL ANIMALS
(5 and 6*g*'s combined)

Days of exposure	Difference of differences in change in body-weight* ± *S.E.* (g)	*N* for body-weight	Difference of differences in cumulative intake of 'Laboratory Chow'* ± *S.E.* (g)	*N* for food intake
1	0·5 ± 1·0	9	2·0 ± 1·3	7
2	0·2 ± 1·0	9	3·1 ± 1·6	7
5	−2·3 ± 1·2	9	−1·0 ± 4·6	7
6	−1·9 ± 1·7	8	0·6 ± 6·1	6
11	1·8 ± 2·0	8	0·5 ± 10·6	6
17	−3·1 ± 2·1	8	−6·8 ± 18·7	6
19	−3·5 ± 2·1	7	−4·9 ± 25·1	5
34	−9·2 ± 2·7	7	84 ± 56	5
55	−13 ± 5	7	−173 ± 98	5
62	−15 ± 5	5	−256 ± 191	3
82	−17 ± 6	5	−319 ± 247	3

* Difference in differences $= \sum_{i=1}^{i=N}$ [(Labyrinthectomized value in centrifuge − sham-operated value in centrifuge) − (Labyrinthectomized value at 1*g* − sham-operated value at 1*g*)] ÷ *N*, where *i* indicates a given litter. The number *N* is the same as indicated in Figs. 1 and 2, with the exception of data for food intake at 5*g*, where *N* is one less than measured for growth.

known to reduce the threshold for rotatory perception with chronically exposed chickens[6]. Histological observations of inner-ear structures from these hamsters are at present being carried out.

The present results support the previously unsubstantiated contention that developmental changes evoked by chronic centrifugation can be explained primarily in terms of an effectively enhanced gravity. Greater non-rotatory effects are possible with other organisms or with slightly altered centrifugation procedures. Application of labyrinthectomy to related investigations can serve as a useful tool in distinguishing the rotatory from the gravitational effects of centrifugation.

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SURFACE MICRO-STRUCTURE OF SOME VITRAINS OF BRITISH COALS

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IT has been known for many years[1] that coal has a porous nature. Laboratory investigations using such methods as adsorption, heat of wetting, and apparent density have all indicated that its ultra-fine structure consists of an extensive system of cavities interconnected by narrow capillaries. Larger micro-cracks can also be present. Such a model explains the ability of coal to act as a molecular sieve, and also accounts for its very large surface area which, it is thought, may be as high as 200 m²/g for some of the lower ranks.

Several workers have put forward theories concerning the ultra-structure of coal substance[2,3]. The work of Bangham *et al.* resulted in a micellular concept based on the existence of highly stable units of coal structure in which the size range of 1000–10 Å was considered to be the most important. It was also suggested that the size of these elemental building units, or micelles, was determined by molecular aggregation processes during coalification and that they would retain some measure of their identity in spite of the inevitable pressures of compaction.

The identification of even the coarser elements of such coal structure is of course beyond the range of even the best optical systems but it is within the range of electron microscopy. Electron micrographs by McCartney and Ergun[4] of thin sections of a vitrain band of a meta-anthracite have revealed platelet-type crystallites in the region of 800 Å in size, together with granular structure as small as 100 Å. These have been shown to be of a graphitic nature and are thought to have been formed and grown from aromatic layer clusters during metamorphic processes.

The preparation of ultra-thin sections of coal by microtomy for viewing in an electron microscope is an extremely difficult process, as indeed it is with most brittle materials. Apart from the dangers of introducing artefacts during cutting, the low degree of contrast of the image can limit the resolution obtained on the micrographs. In addition, the superimposition of the images of particles and pores lying in different planes can complicate interpretation.

An alternative is to use the replica method. Variations of this method have been used for coal and other porous materials to obtain high-contrast images of the undisturbed surface layer of the structure[5,6]. Two-stage techniques are necessary where it is found impracticable to separate carbon replica films directly from the surface which is being investigated. Careful application of the two-stage methods can enable reproducible results to be obtained from the same specimen.

Samples of freshly-fractured surfaces of vitrain bands were selected from a wide range of British coals varying in rank from the 100 group anthracites to the 900 group high volatile bituminous coals of the National Coal Board coal classification system. The vitrinite marceral group was chosen for the samples due to its relative petrological homogeneity and smoothness of fractured surfaces. The samples were crushed below 100 British Sieve Standard in order to present a variety of surfaces, embedded in softened

cellulose acetate, and then coated with a thin film of polyvinyl alcohol which is a water-soluble plastic. The film was then allowed to harden and stripped with the aid of cellulose tape. On most of the coals several films were stripped off to remove loose material before the final replica was made for further processing. In addition to those from the crushed particles, replicas were also taken from small areas of whole vitrain bands for purposes of comparison.

Selected replicas were mounted on glass slides and carbon-coated by vacuum evaporation in the standard manner, following which the plastic substrate was dissolved in successive water baths. This left the carbon replicas floating on the water surface for subsequent collection on electron microscope grids and final metal shadowing at a low angle to improve contrast and accentuate irregularities. It was found that resolutions better than 50 Å could be obtained with this procedure, particularly with fine grain of platinum shadowing. Attempts to interpret structural detail below this limit were not altogether satisfactory due to the masking effects of the deposited metal.

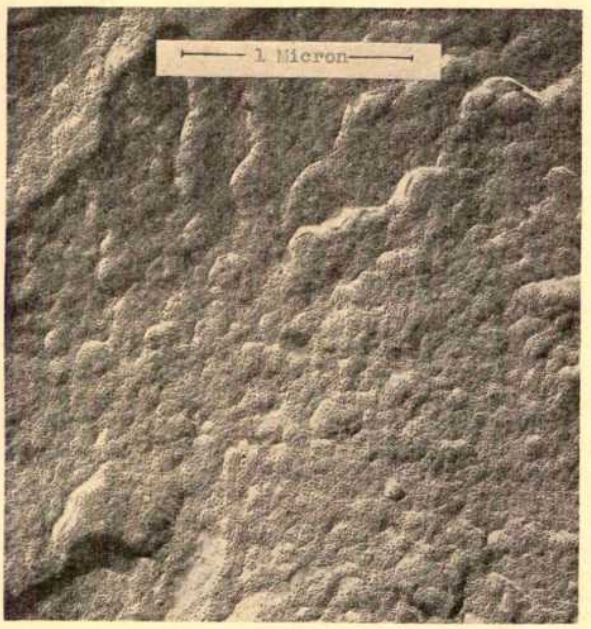


Fig. 1. 900 coa

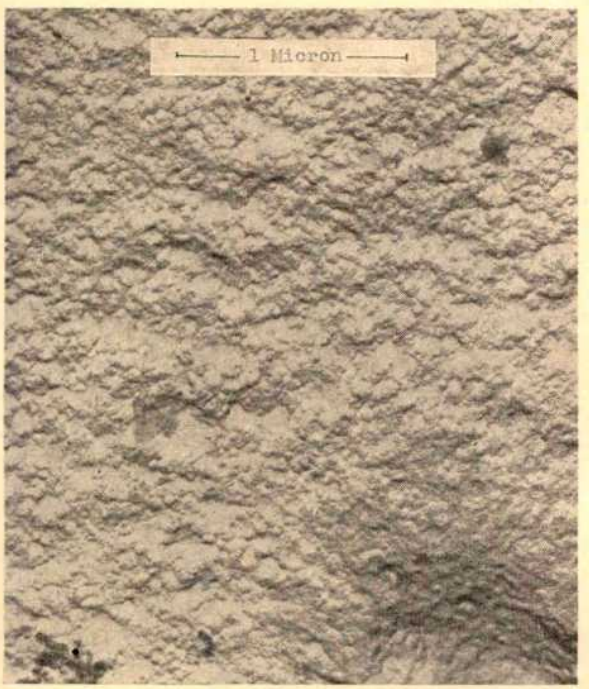


Fig. 2. 700 coal

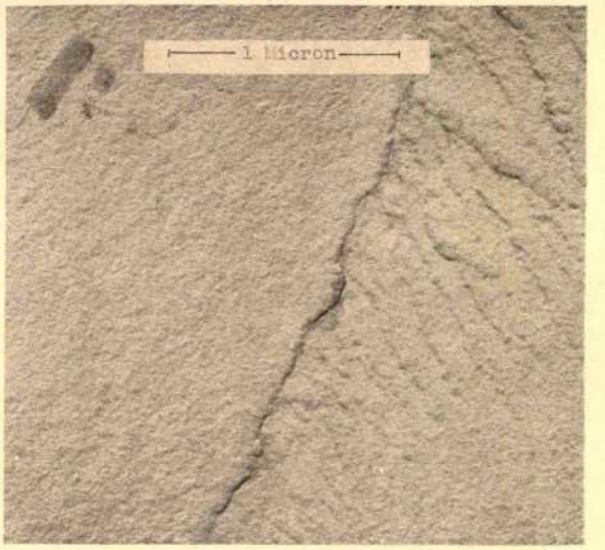


Fig. 3. 400 coal

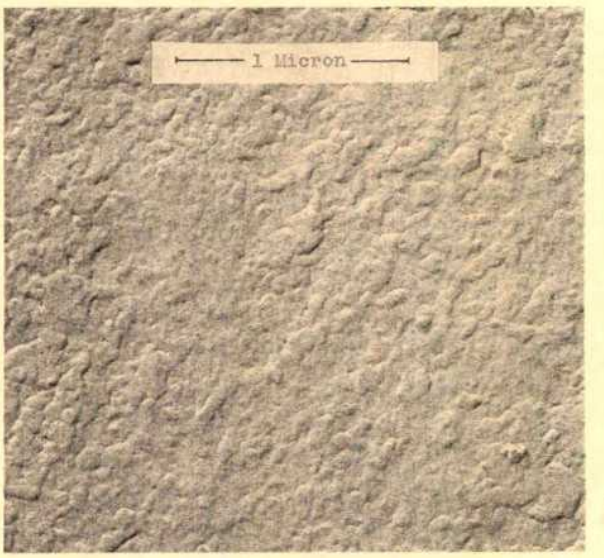


Fig. 4. 100 coal

Electron micrographs were taken of the replicas at a magnification of 10,000 using an A.E.I. *E.M.*6 instrument. Interpretation of results and measurements were assisted by projecting the negatives on to a screen to give magnifications in the region of a quarter of a million times. Photographic enlargements for record purposes were standardized at a magnification of 50,000.

The large number of electron micrographs taken revealed a marked change in surface structure with rank. The lowest rank coals exhibited a relatively coarse granular structure which generally became progressively finer and more compact towards the 400, 300 and 200 groups, increasing thereafter in coarseness in the anthracites. This trend is illustrated in Figs. 1, 2, 3 and 4, each of which is representative of the coal group mentioned. The 900 coal in Fig. 1

was shadowed with gold/palladium; platinum was used for the other three.

The surfaces appear to consist of a series of interconnected generally rounded nodules, or micelles, which give a dimpled effect. Some appear as raised areas and some as depressions. Most appear more flattened than spherical; this feature is shown in the end views along the walls of the depression in the corner of Fig. 2. Several layers are apparent on the fractured surfaces shown, as in many cases only the upper parts of the top layers of units are completely visible. A range of sizes is apparent in each of the illustrations, although close examination of some of the larger units shows them to be agglomerations of smaller ones.

Measurement of the sizes of the most clearly defined micelles for the fourteen coals examined showed them to be in the range of 300–800 Å for anthracites, generally below 200 Å for most of the 200, 300 and 400 groups and increasing to about 500–1000 Å for the lower rank groups.

The trend in micro-structure of coal substances which has been observed is further evidence for the suggested micellular structure of coal and is in keeping with its technical behaviour. The coarser, more open, structure of the lower ranks can account for their known characteristics of high porosity and large internal surface area which, in turn, lead to greater moisture retention and ease of wetting properties. This affinity also makes it much more difficult to upgrade such coals by froth flotation. The larger pore sizes, which go with a coarser structure, also make such coals more susceptible to oxidation and permit the volatile products to be more easily removed on heating so that free-burning is obtained. In addition, low-rank coals generally have high physical strength which again can be related to the relative coarseness of their structure.

Coals in which the finer, more compact structure has been observed are known to be of low porosity, difficult to wet, friable, more easily cleaned by flotation processes, and to have good carbonization properties. Such characteristics are in keeping with reduction in pore diameters. The reversion to coarser structure observed in the anthracites would also seem to be in keeping with their high strengths and other physical characteristics associated with increase in internal surface area. Optical properties of coals have also been found to follow a similar pattern[1].

Application of the electron microscopy replication techniques according to the method described makes it possible to study the micro-structure of coal, with its highly-developed transitional porosity, in much more detail than hitherto. Extension of the work to coal constituents other than vitrain would also seem to be feasible.

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RIBONUCLEIC ACID BASE COMPOSITION IN SUBCELLULAR FRACTIONS OF PINE POLLEN (*Pinus ponderosa* LAW)

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THE mature pine pollen grain contains remnants of two prothallial cells and a functional generative cell imbedded in a tube cell surrounded by a protoplasmic membrane. The pollen exine, with two air-sac-like wings, encloses the intine and protoplasmic membrane. The pollen cell and all its contents are derived from a single haploid pollen mother cell in the sporogenous tissue of the microgametophyte. The male cells derived from the generative nucleus during pollen germination contain the genetic information necessary for expression of the paternal characteristics.

Cytoplasmic organelles are transmitted to the ovule during fertilization along with the male nuclei. These subcellular protoplasmic constituents are incorporated in the developing seed. The potential role of such extranuclear protein or nucleic acid materials on subsequent genetic expressions is still not known[1]. Pollen protein, or ribonucleic acid (RNA), have been suggested as possible sources of the differences in incompatibility reactions which occur when certain pollen tubes penetrate the stylar tissue. Pollen in some cases appears to induce formation of a new protein in the female tissue; the new protein may then inhibit pollen growth[2]. The relationship of such protein to RNA in germinating pollen is assumed to be similar to that reported in other plant cells, that is, microsomal RNA serves as the template for protein synthesis.

Proteins, pigments, and probably RNA are rapidly secreted by pollen on germination[3]. One may assume that the 'incompatible protein' is formed under the influence of a messenger-like RNA diffusing from the pollen[4]; or that it represents a protein recombination diffusing in part from the pollen[5]. Protein or RNA secreted by pollen is derived from the cytoplasm within the pollen. Different proteins, and presumably RNA, are associated with the different subcellular moieties. Since some proteins or RNA do diffuse from the pollen on germination it was thought of interest to compare the composition of the template sources of these chemically reacting components.

This article compares the purine and pyrimidine base compositions of the RNA of the subcellular fractions of pine pollen. This is part of an investigation now in progress on the protein-RNA metabolic activities of germinating pollen.

6-g samples of ungerminated pollen of *Pinus ponderosa* Laws. stored 6–8 months at −15° C were used for each determination. The pollen was homogenized in a solution of 0·5 molar sucrose and 10^{-3} M magnesium chloride. Three sub-samples of 2 g were each homogenized 3 min in a plastic tube with glass beads on a dental shaker ('Wig-L-Bug', Crescent Mfg. Co., Chicago). This resulted in about 98 per cent grain breakage. All homogenizing and fractionating were done at 0° C. The homogenates

were transferred to 50-ml. plastic centrifuge tubes and centrifuged 15 min at 1,000*g* to separate large cell fragments, that is, wall, starch granules and nuclei. The precipitated cell fragments were resuspended in about 15 ml. sucrose–magnesium solution and recentrifuged. The combined supernatants (S_1) were centrifuged at 14,000*g* for 30 min to precipitate the mitochondria. The mitochondrial pellets were resuspended in 10 ml. sucrose–magnesium solution and recentrifuged. The supernatant from the mitochondrial fraction (S_2) were centrifuged at 100,000*g* for 90 min in a Spinco refrigerated preparatory centrifuge to sediment the microsomes. The microsomal particles prepared in this manner were checked for sedimentation characteristics in a Spinco analytical centrifuge and were found to consist primarily of particles sedimenting with a normal plant microsomal constant of 73 *S*. The supernatant (S_3) remaining after removal of microsomes was used for soluble-RNA analyses.

The sedimented fractions were mixed with 0·5 M trichloroacetic acid (TCA). An equal volume of solution of 1·0 M TCA was added to each supernatant fraction. The precipitates were allowed to stand overnight at 0° C and then centrifuged. The TCA supernatants were discarded and the insoluble precipitates washed in the following sequence of 5 ml. volumes of solution: twice in 2 per cent perchloric acid; three times in ethanol–ether–chloroform (2 : 2 : 1) for 15 min each at 25° C; once in 70 per cent ethanol and twice in 2 per cent perchloric acid. Except for ethanol–ether–chloroform, the washing was for approximately 5 min at 4° C. The washed precipitate was then extracted over a 12-h period at 4° C with constant slow shaking in five changes of 1·0 N perchloric acid. The perchloric acid extracts were combined and neutralized with N potassium hydroxide and the potassium perchlorate precipitate removed. The neutralized perchloric acid extracts were then concentrated by low-temperature vacuum (about 35° C) and an equal volume of 2·0 N hydrochloric acid was added; the extract was hydrolysed in a sealed tube for 1 h at 100° C. This hydrolysate was directly streaked under a stream of cold air on to Whatman No. 1 filter paper previously washed with 0·1 N hydrochloric acid and chromatographed for 18 h ascending in isopropanol–hydrochloric acid–water (68 : 17·6 : 14·4) at room temperature. Individual bands were identified under ultra-violet light, cut from the paper and eluted with 0·1 N hydrochloric acid to a constant volume of 25 ml. The absorbance of the eluate was measured with a Cary Model 11 recording spectrophotometer from 220 to 290 mμ. Yeast RNA (commercial grade, California Foundation for Biochemical Research) was used as comparison control in all extraction and hydrolytic steps; purines and pyrimidines (C_fP spectral grade, Calif. Foundation for Biochem. Res.) were used for chromatographic and ultra-violet standards.

Determinations were made in 1959, 1961 and 1964 on pollen of the same species from trees in the same locality. Efforts were made in the 1959 and 1961 analyses to compare the ultra-violet data with phosphorus and orcinol assays for total RNA and base component fractions. Results from the three methods often varied by 10 per cent. Ingle[6] showed that correlation between the three methods is generally difficult and should not be expected. Ingle[6] and Young[7] concluded that the ultra-violet values are the most reproducible and reliable. Only the ultra-violet values are used in this article. The means of three separate replicate experiments were compared. The RNA hydrolysate absorption data for all years were in good agreement. Table 1 lists the results of these experiments in terms of the mole percentage ratios of the nucleotide base components in the different subcellular RNA fractions of *Pinus ponderosa* pollen. Related values reported by other workers and control yeast determinations are included. Hydrolysis of RNA in hydrochloric acid causes a breakdown of about 5 per cent of cytosine and uridylic acid[8]. This was corrected in calculating the mole percentage values. The chromatograms were carefully scanned and eluted in search for other purines and pyrimidines which have occasionally been reported in bacteria and plant RNA hydrolysates. No other purines or pyrimidines were found.

From the results recorded in Table 1, conclusions can be drawn regarding the nature of RNA present in pollen and the significance of previous reports of nucleotide base ratios of whole plant or organ extracts. For example, values for the nucleotide components of pine pollen RNA reported by Vanyushin *et al.*[11] represent an average of the extractable RNA in all combined pollen cellular components. However, a comparison of the base composition of the RNA in the fractionated pollen cell components affords a different and more illuminating picture. The data in Tables 1 and 2 suggest that plant tissues which have high cell wall residues, such as tobacco leaf or cotton cotyledons, or the first centrifuge fraction from pollen homogenates, have a high ratio of guanine (Table 1) compared with uracil (Table 2), the usual base nucleotide with which guanine pairs.

If RNA is correctly described as a tetranucleotide with helical regions, then the molar proportions of purine nucleotides should approximate the molar proportions of pyrimidine nucleotides. Values of these proportions determined for pine pollen microsomes and yeast RNA (Table 1) agree with this concept. The values close to unity for guanidine/cytosine and adenosine/uridine ratios in pine pollen microsomes (Table 2) further indicate that the RNA of the ribosomal particles conforms to the general theory. Considerable variation from unity can be expected and was found in the soluble-RNA nucleotide fraction. The supernatant probably contains polynucleotide messenger-RNA components or hydrolysed RNA moieties. However, large deviations were also found in the purine/pyrimidine ratios of the mitochondrial fraction, and the cell wall fraction of ungerminated pine pollen. The fact

Table 1. BASE COMPOSITION OF RIBONUCLEIC ACID

	Guanine	Adenine	Cytidylic	Uridylic	Purines / Pyrimidines
			(Mole per cent)		
Pinus ponderosa pollen:					
cell wall fraction	31·1 ± 2·0	29·4 ± 1·3	24·8 ± 1·9	14·6 ± 1·9	1·53
mitochondria	29·3 ± 0·5	26·2 ± 0·2	24·0 ± 0·3	20·5 ± 0·8	1·25
microsomes	27·1 ± 0·3	22·8 ± 0·2	25·4 ± 0·5	24·7 ± 0·2	1·00
supernatant	28·9 ± 0·9	27·1 ± 2·2	24·9 ± 0·1	19·0 ± 1·2	1·28
Yeast (determination)	23·8 ± 0·6	26·5 ± 0·4	22·8 ± 0·9	26·6 ± 1·5	1·04
Yeast[9]	24·6	25·4	22·6	27·4	1·00
Pinus silvestris pollen[10]	27·6	25·3	23·4	23·7	1·12
Picea excelsa pollen[10]	28·8	25·4	22·5	23·3	1·18
Corylus avellana pollen[10]	28·2	24·8	25·0	22·0	1·13
Tobacco leaf[9]	33·1	23·5	23·8	19·4	1·29
Cotton cotyledons[11]					
mitochondria	29·6	24·6	25·6	20·2	1·01
microsomes	31·8	24·0	24·6	19·7	0·94
supernatant	29·3	21·6	28·3	20·8	1·00

Table 2. RNA–NUCLEOTIDE RATIOS OF *Pinus ponderosa* POLLEN

Fraction	C/A	G/U	G/C	A/U	$\frac{A+U}{G+C}$
Cell wall debris	0·85	2·13	1·25	2·00	0·79
Mitochondria	0·92	1·43	1·22	1·27	0·88
Microsomes	1·11	1·09	1·07	0·93	0·91
Supernatant	0·86	1·51	1·16	1·43	0·86

that our results for the pollen microsomal and yeast RNA composition (Table 1) compare well with the values in the literature suggests that these data are correct. Since the present base-paired double strand concept of RNA does not account for all the observed purine/pyrimidine ratios of pine pollen fractions, some alternative explanation is required.

In RNA the helical regions are generally considered to comprise about 50 per cent of the structure of nucleotides[12]. A disproportionate presence of one base in an RNA strand would modify the helical regions and RNA compactness. Subcellular fractions of peas, spinach, and other plant leaves have purine/pyrimidine ratios between 1·20 and 1·29 (ref. 13). The base ratios found in the RNA of supernatant and mitochondria fractions of pine pollen are similar. These modest differences from 1·0 in base ratio could be reflected in variations in the compactness of the helical RNA strands of the mitochondria and soluble-RNA components[14].

The relatively high values of the purine/pyrimidine ratio in the pine pollen cell wall fraction is probably caused by the presence of nucleotide components bound in, or on, the pollen walls. Coenzyme components of guanosine and adenosine are essential to pollen growth[15], and are presumably present in pollen. Such mononucleotides may be tightly bound in protein-cell wall complexes in ungerminated pollen. These bound nucleotides may be released with germination or ageing; or they may be released with extended acid extraction such as is used to release particulate bound RNA. The extractability and form of pine pollen protein diffusate changes substantially during storage[16]. This suggests that many enzyme–coenzyme moieties are bound, or soluble to different degrees in the mature pollen grain, and may account for the distorted base ratio values in the cell wall fraction.

The difficulties apparent in the interpretation of nucleotide base ratios of RNA from plant cellular fractions are compounded in the case of RNA of whole plant tissues. Heyes[17] has shown that the base ratio of RNA from root cells differs in cells of different ages. The soluble and particulate RNase also changes as plant cells age[18]. Thus, nucleotide base ratios of plant cells may not indicate changes in the type of RNA the plant is synthesizing, but rather changes in the concentration of a particular cell moiety: for example, it brings about more microsomes or mitochondria, or changes in the RNase activity of the cells.

Our results indicate that efforts to interpret studies of the base ratios of RNA extracted from whole organs requires greater caution and understanding of the sources of the RNA than has generally been the practice. Attempts to establish phylogenetic relationships using base composition of RNA extracted from whole organs has not met with success[10,13]. Base composition of DNA, primarily limited to cell nuclei, does provide a basis for phylogenetic comparison. A more valid comparison of base composition of RNA in different species may result when only the base components of RNA from a stable subcellular particle are compared. However, the ubiquitous nature of many enzymes in various plant cells suggests that the controlling-RNA involved in their synthesis may be very similar. Differences in RNA expression may be related to functional mechanisms rather than mere composition. Definitive knowledge of this subcellular characteristic would indeed be valuable for further studies of growth and differentiation.

We thank Dr. Paul O. P. Ts'o for the determination of the sedimentation constant of the microsomal fraction which we isolated from pine pollen. This study was initiated while the authors were visiting research workers at the Biological Laboratories, California Institute of Technology.

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LONG-TERM CULTURE OF EMBRYONIC CELLS OF *Drosophila melanogaster*

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IMPROVED methods for culturing insect tissues, particularly those of *Drosophila melanogaster*, are continually being sought due to the importance of such material for studies in genetics, molecular biology, virology and developmental biology. In a previous paper we reported the successful growth *in vitro* of embryonic cells isolated from eggs of *Drosophila melanogaster*[1]. This article describes the properties of cells which have been maintained in long-term culture. At the time of preparing this report seven separate cell lines have been maintained for periods ranging from 80 to 520 days. The latter, line *A*, is described in detail; the other lines exhibit characteristics which are essentially similar.

The techniques used for the establishment of primary cultures, the medium used (*H*-5), and the methods for determining cell number and chromosome counts have been described in the earlier paper. The initial cell suspensions were obtained from eggs collected over a 6-h period from inseminated females of the Oregon *R-EL2* stock. The cultures are grown (without shaking) at 30° C in *T*-30 flasks containing 5 ml. of medium, and are transferred every 10 days. Most of the cells in such cultures freely float in the medium, but some are attached to the glass. At the time of transfer the latter are scraped free, all the cells are randomly suspended by gentle pipetting, transferred to a tube, and centrifuged at 2,500 r.p.m.

for 5 min. The old medium is then decanted and the cells resuspended in about 1 ml. of fresh medium. If the cell count is high enough the suspension is divided into two portions, each is made up to 5 ml. by addition of medium and is transferred to a *T*-30 flask; otherwise the suspension is brought to 5 ml. and is transferred without division.

After four transfers of line *A*, crystal formation in the medium became a serious problem. The fifth transfer was made into an altered medium, *H*-6 (Table 1), and this new medium has been used in all subsequent transfers. The other lines were established by growth of the primary cultures in *H*-5 but the first and subsequent transfers were made into *H*-6. Growth rates were the same in *H*-5 and *H*-6, but crystals were not formed in the latter. Both media were supplemented with 10 per cent calf serum.

As shown in the previous paper, there are two distinctive cell types in the initial cell suspensions, small (5–10μ in diameter) and large (20–35μ). The large cells average only one division during the initial period of cultivation while the small cells enter logarithmic multiplication with a mean generation time of 25–30 h, reaching maximum number in 4–5 days, at which time they had multiplied in number almost five times.

The proportion of large cells has gradually decreased during repeated transfer. After 50 days of cultivation (five transfers) they constituted 20 per cent of the cell population, in contrast to 47 per cent in the initial suspension, and they barely maintained their number during the fifth growth period (Fig. 1*a*). After 160 days (sixteen transfers) they had decreased to 14 per cent of the population (Fig. 1*b*), and at 430 days they constituted only 4–7 per cent of the population (Fig. 1*c*). Mitotic figures are not difficult to find in the large cells of primary cultures (Fig. 2*a*), but they are only rarely observed in later transfers.

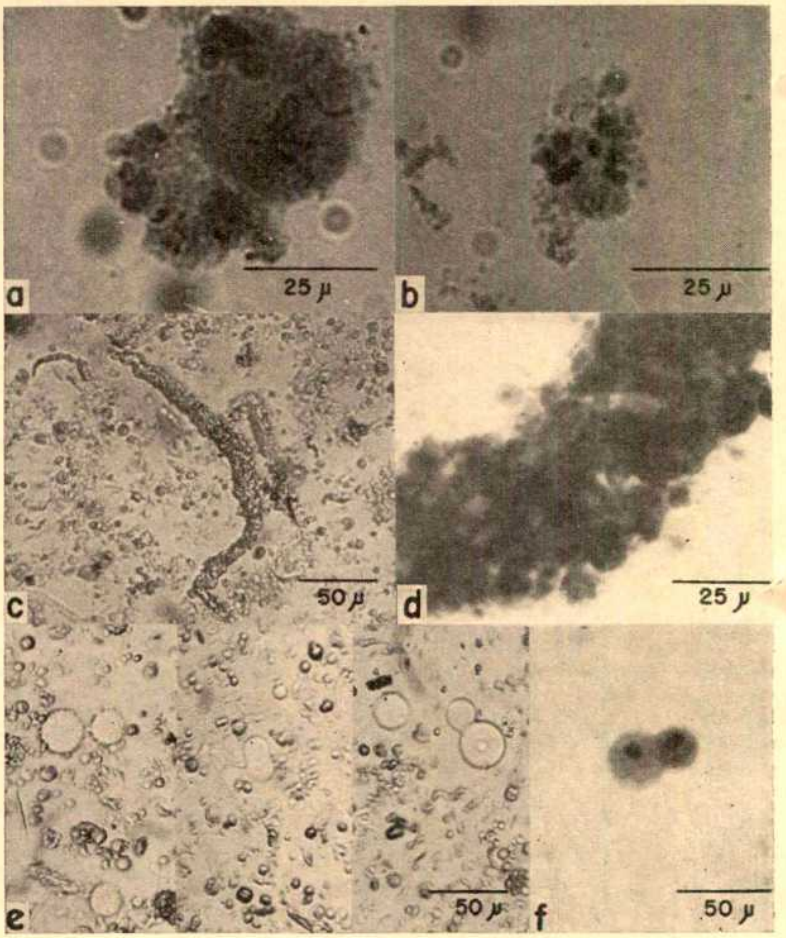


Fig. 2. Photographs of cultivated cells taken by light microscopy. *a* and *b* are cells squashed in water, stained with 1 per cent aceto-orcein; *d* is squashed in culture medium, stained with Giemsa dye; *f* is stained with Giemsa; *c* and *e* are living, unstained cells. *a*, Mitotic figure in large cell; *b*, mitotic figure in small cell; *c*, tubule-like and string-like non-random aggregates of small cells; *d*, similar aggregate showing cell nuclei; *e*, large, clear cells which first appear after 160 days of cultivation (sixteen transfers); *f*, budding large cell, showing nucleus in original cell and in bud

Small cells enter logarithmic multiplication immediately after each transfer, although their growth rate has declined with subsequent transfers. After five transfers their mean generation time was 32 h (Fig. 1*a*), while after sixteen transfers it was 48 h (Fig. 1*b*). At 430 days the mean generation time had increased to 69 h (Fig. 1*c*), but the line was still maintaining itself. The other lines have exhibited similar deceases in growth rate, and none, as yet, has undergone adaptation. Mitotic figures are readily observed in small cells both in primary cultures and in later transfers (Fig. 2*b*).

Chromosome counts are readily made in large cells but are difficult in small cells because of their size and the extreme condensation of the chromosomes. Nevertheless, we have previously shown that chromosome number remains constant at the diploid number 8 in primary cultures. The distribution of chromosome numbers after 50 days of growth did not differ significantly from that at 10 days, but some evidence of haploidy, hypoploidy and hyperploidy was evident after 160 days (Fig. 3). The modal chromosome number, however, was still diploid.

Large cells rapidly formed grape-like aggregates in primary cultures, so that the proportion of unaggregated cells of this type is small at the end of the first growth period. Small cells begin to form aggregates only after the first transfer, and continue to do so in subsequent transfers. Some of these are grape-like clusters, apparently resulting from random clumping, but others exhibit non-random structure (Fig. 2*c*). These look like tubules or strings of cells, and nuclei are apparent in many of the cells when they are squashed and stained (Fig. 2*d*). The

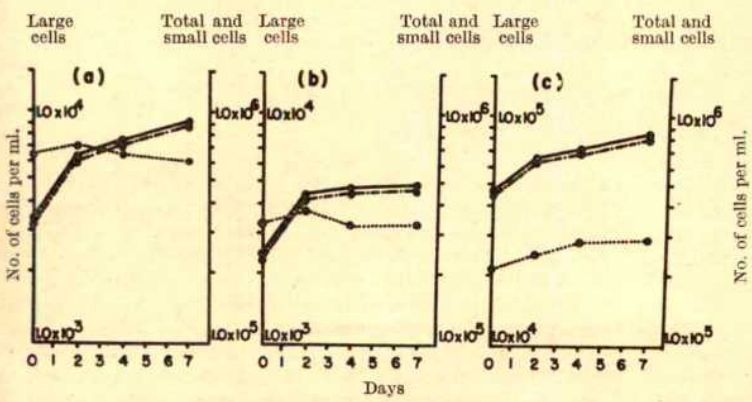


Fig. 1. Growth curves of embryonic cells *in vitro*. *a*, After 50 days of cultivation (five transfers); *b*, after 160 days of cultivation (sixteen transfers); *c*, after 430 days of cultivation (forty-three transfers). Medium H-6 supplemented with 10 per cent calf serum. Temperature, 30° C. ———, Total cells; —·—·—, small cells; · · · · · · , large cells

characteristics and consequences of aggregation are being studied.

A new cell-type, which appeared in line *A* after sixteen transfers (Fig. 2*e*), has also appeared in the other lines, and has continued to persist in all. These are large (15–30μ in diameter) and clear, and are distinct from the original large cells which possess granulated cytoplasm. Some of these cells divide unequally, giving rise to buds which may become detached from the parent cell and gradually increase in size. In favourable cases chromosomes can be seen both in the parent cell and the bud during division, and a nucleus is present in both after the completion of mitosis (Fig. 2*f*). These cells resemble embryonic neuroblasts[2] in size, appearance, and mode of division. Other cells of the same apparent type undergo equal division.

Table 1. COMPOSITION OF MEDIUM *H*-6

Constituents	Amount (mg/1,000 ml.)	Constituents	Amount (mg/1,000 ml.)
Salts		Amino-acids	
$NaH_2PO_4 \cdot 2H_2O$	200	Lactalbumin hydrolysate	7,500
$NaHCO_3$	350	L-tryptophan	80
KCl	200	L-cysteine hydrochloride	20
$CaCl_2 \cdot 2H_2O$	20		
$MgCl_2 \cdot 6H_2O$	100	Vitamins and nucleotides	
NaCl	7,000		
		Yeast extract	1,200
Sugars			
Glucose	5,500	*p*H indicator	
Sucrose	5,500	Phenol red	10
Antibiotics			
Penicillin *G* (sodium salt)	30	*p*H, 6·5. Sterilization by	
Streptomycin sulphate	100	passage through a Seitz filter	

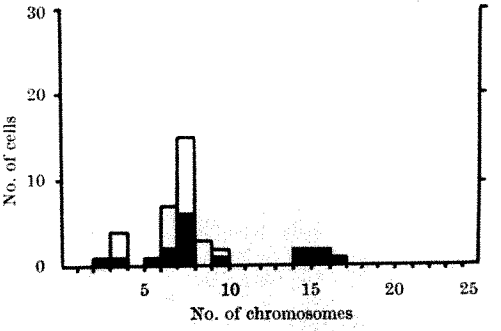


Fig. 3. Distribution of chromosome numbers in line *A* after 160 days of cultivation (sixteen transfers). ■, Large cells; □, small cells

In summary, most of the cells in long-term cultures retain their embryonic appearance, normal chromosome number, and their ability to divide. Some evidence of abnormal chromosome numbers is found, however, and the formation of non-random aggregates and of a new cell type suggests limited differentiation.

This work was supported by research grants from the National Institutes of Health and from the Wisconsin Alumni Research Foundation. One of us (M. H.) is on leave from the Department of Experimental Radiology, Faculty of Medicine, Kyoto University, Japan.

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COTYLEDONARY STIPULES IN THE MYRTACEAE

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THE presence or absence of stipules is one of the most consistent of the vegetative characters used by angiosperm taxonomists and has considerable importance in both classification and identification of flowering plants. Unfortunately, much of the information in the standard works in English is either incorrect or incomplete. For example, it is generally but erroneously believed (see, for example, ref. 1) that the members of the order Myrtales lack stipules. Weberling[2] has shown that they occur in most families of this large order. They are present on the adult leaves of 44 out of the 46 genera of Myrtaceae which he examined. He did not find stipules in *Eucalyptus* L'Hér. or *Angophora* Cav. It is true that he examined only a few species of *Eucalyptus*, but a more extensive investigation confirms his conclusion. We find that stipules are absent also from the juvenile foliage of both *Eucalyptus* and *Angophora*. However, in *Angophora* and in many species of *Eucalyptus* (as well as in many of the other genera of Myrtaceae investigated by Weberling) the cotyledons bear stipules. In the eucalypts and in *Angophora* the stipules occur on or near the margins of the petioles, two to six per cotyledon. In other genera (*Leptospermum*, *Melaleuca*, *Tristania* (Fig. 1*a*), *Syncarpia* (Fig. 1*b*), *Agonis*, *Callistemon*, *Calythrix*) there are usually six or more, forming a transverse row across the base of the petiole, as described[2] for the adult leaves of Combretaceae. In most cases, the cotyledonary stipules are differentiated into a stalk and an apical part which consists of small densely staining cells, which are apparently secretory. Although the nature of the secretion has not been adequately investigated, it is certainly not oil. The stipules are quite distinct from the emergent oil-glands which are characteristic of certain eucalypts and of *Angophora*. The occurrence in them of vascular elements in at least one species (*Eucalyptus ferruginea* Schau.) suggests that the structures, which we concur with Weberling in identifying as stipules, are not mere enations. It is fair to warn taxonomists, however, that their usual examination with a magnification of ten times or less may be inadequate to reveal the stipules.

In the aforementioned genera, other than *Eucalyptus* and *Angophora*, the cotyledonary stipules resemble those of the adult leaves. In *Angophora* each cotyledon has two three-lobed stipules; occasionally a small free lobe is associated with a two-lobed structure. Similar stipules, sometimes three-lobed, but in many cases simple (Fig. 1*c* and *d*), are present also in certain species of *Eucalyptus* L'Hér. It is convenient[3] to divide this genus into two genera, *Eucalyptus* L'Hér. emend. Carr and Carr and *Symphyomyrtus* Schau. emend. Carr and Carr. The division is based principally on the characters of the flower, inflorescence and seed, but also depends on vegetative characters. In *Eucalyptus sensu stricto* the series Eudesmieae[4] (a group which exhibits many apparently primitive characters) consists of species all of which have well-developed stipules, with the exception of *E. ebbanoensis*, *E. gamophylla* and *E. odontocarpa*. The two latter are either very closely related or conspecific[5]. These species have structures which, by analogy, can be described as stipule rudiments. Stipules also occur in series Miniatae and in most species of sections Renantheroideae and Renantherae. In *Symphyomyrtus*, on the other hand, they are lacking in most species but are especially prominent in those of the series Corymbosae

(Maiden) S. T. Blake. In the series Transverse they are well developed in some species and occasionally present in others. They are also to be found on a small percentage of seedlings of some species of series Dumosae and Leptopodae. The presence of cotyledonary stipules in *Angophora* and in the eucalypts means that all the genera of the Myrtaceae which have been examined include species which are stipulate in at least some stage of their ontogeny. It is of interest that in some genera stipules are formed only at the cotyledonary stage and that in some species of these genera they are either inconstant or completely lacking. In certain eucalypts (for example, *E. stellulata*) only about 10 per cent of the seedlings have stipules and often only one stipule appears on one of the cotyledons. Stipulate genera of a number of other families have been examined for the presence of cotyledonary stipules. They are lacking in Violaceae, Rosaceae, Salicaceae and Fabaceae. In *Viola* and in members of Rosaceae and Fabaceae the first seedling leaves are stipulate, but those of *Salix* and *Populus* are exstipulate. Other families include both stipulate and exstipulate genera. In *Clarkia* and *Godetia* (Onagraceae tribe Onagreae), which Weberling records as entirely exstipulate, there are no cotyledonary stipules, but in *Hedera* (Araliaceae), which has exstipulate leaves, the cotyledons are stipulate; in *Tropaeolum minus* and *T. speciosum* (Tropaeolaceae) all the leaves are stipulate, whereas in *T. majus* only the primary leaves[6] have stipules.

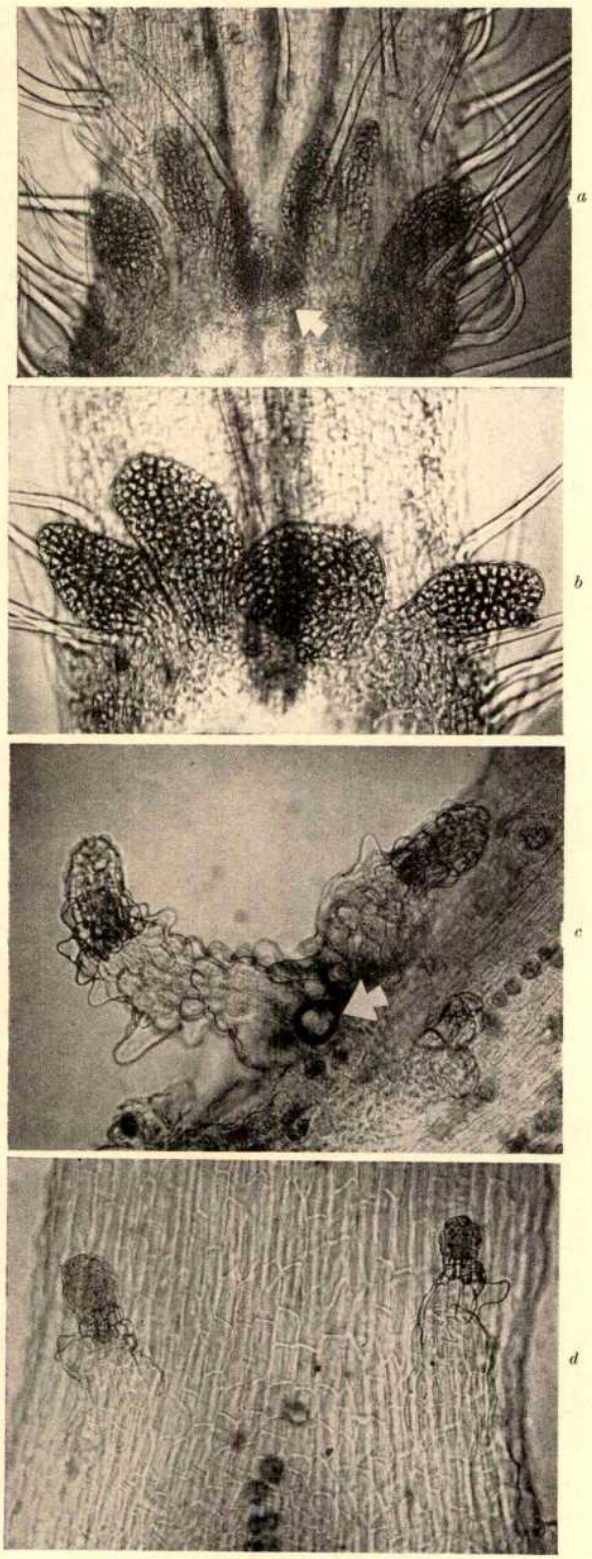


Fig. 1. Cotyledonary stipules of members of Myrtaceae. *a*, *Syncarpia glomulifera*. Arrow indicates axillary bud of cotyledon (× *c.* 68); *b*, *Tristania conferta* (× *c.* 103); *c*, *Eucalyptus tetragona*. Arrow indicates oil gland at base of stipules (× *c.* 78); *d*, *Eucalyptus rossii* (× *c.* 83)

With regard to the presence or absence of stipules one may therefore classify the flowering plants into four groups, as shown below:

Group	Cotyledons	Seedling leaves	Later leaves
1	−	±	−
2	+	+	+
3	+	±	−
4	−	±	+

+ Indicates presence of stipules.
± Indicates stipules present in some species, absent in others.

Certain genera, usually described as 'exstipulate', include species belonging to groups 1 and 3. In such a genus, the completely exstipulate condition (group 1) may have arisen by suppression of the production of stipules at successively lower nodes of the primary shoot, resulting ultimately in complete loss of ability to produce them even on the cotyledons. In the eucalypts, these observations are relevant to the evolutionary trends, evidence for which we have published elsewhere[3,5,7], many of which involve suppression of morphological structures. In *Eucalyptus* s.s., for example, there is a tendency towards suppression of the sepals. Large persistent sepals are present in some members of Eudesmieae, but sepals are completely absent from members of Renantherae; for example, *E. stellulata*. This trend parallels similar trends in other characters of the flower (for example, grouping of stamens, divergence of anther lobes) and vegetative characters (for example, stellate hairs, lignotubers). There are parallel trends in the occurrence, number, size and morphology of the cotyledonary stipules in this genus. We have described[5] the remarkable convergence of the sister genus, *Symphyomyrtus*, in which similar evolutionary trends can be distinguished. The suppression of stipules has apparently been one of the features of evolutionary change in *Symphyomyrtus*, since they are well developed in those of its series which possess larger constellations of ancestral features and are absent from species which on many grounds could be considered advanced.

We thank Mr. T. R. N. Lothian, director, Adelaide Botanic Garden, for seed of many genera, and Mrs. F. L. Ross, Waroona, Western Australia, for specimens.

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LETTERS TO THE EDITOR

ASTRONOMY

Atomic Standards of Frequency and the Second of Ephemeris Time

On the authorization of the twelfth General Conference, the International Committee of Weights and Measures assigned, in October 1964 (ref. 1), the frequency 9 192 631 770 hertz to the well-known transition of caesium-133 at zero field, for temporary use pending consideration of a new definition of the second; the second, as at present defined, is the second of ephemeris time. The number 9 192 631 770 with a quoted probable error of ±20 is that originally determined by Markowitz *et al.*[2] by the comparison, during the period 1954·0 to 1958·5, of an integrated measure of atomic time based on the frequency of the caesium transition with the measure of ephemeris time (E.T.) as determined by observations of the Moon with moon-cameras. Subsequent comparisons over longer periods have tended to confirm that this frequency lies within the uncertainty quoted.

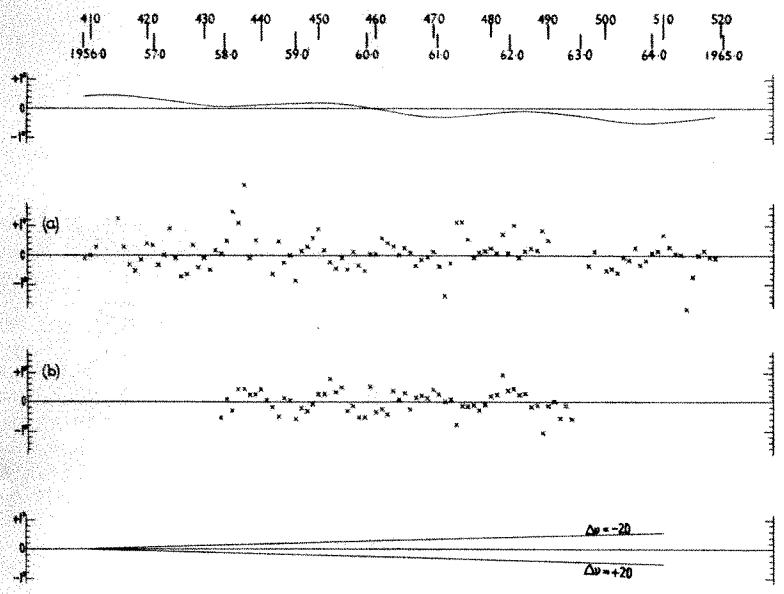


Fig. 1

However, the International Astronomical Union at its General Assembly in August 1964 recommended the use of a new IAU System of Astronomical Constants[3]; its adoption leads to modifications of a periodic nature in the lunar ephemeris which affect the determination of ephemeris time from observations of the Moon. Moreover, Eckert[4], in the course of a comprehensive recalculation of the main problem of the lunar theory, discovered a numerical error in the coefficient of one of the periodic terms of Brown's theory; the presence of this error in the lunar ephemeris has since been confirmed by analysis of both the meridian[5] and occultation (unpublished) observations.

The application of these two periodic corrections to the lunar ephemeris affects the determination of the deduced frequency in terms of the (ephemeris) second by amounts that could, over short intervals, reach as much as 40 (or even 50) hertz. The correction to the deduced frequency depends on the precise method of discussion and, for this reason, no estimates are given in respect of previous determinations. However, two new determinations, incorporating these corrections, have recently been made; the purpose of this communication is to report that they confirm that the frequency of the caesium transition is close (within its previously quoted probable error) to the adopted value of 9 192 631 770 hertz. It is emphasized that, as in all other current determinations, the measure of the (ephemeris) second is deduced from observations of the motion of the Moon around the Earth, although it is defined in terms of the motion of the Earth around the Sun.

The new determinations are based on the provisional discussion of two homogeneous series of lunation mean values of ΔT = E.T. − U.T.2 deduced from: (*a*) the Washington meridian observations[6] of the Moon from 1956·0 to 1965·0; (*b*) the observations of occultations of stars by the Moon as collected and reduced at Herstmonceux (to be published), from 1958·0 to 1963·0.

In both series the observations have been corrected for the irregularities of the limb of the Moon. The values of ΔT, amended for the effect of the corrections above, are compared with the corresponding lunation mean values of ΔA = A.T. − U.T.2, where A.T. is an atomic time scale based on the adopted frequency of 9 192 631 770 hertz, thus eliminating U.T.2. In this preliminary discussion straight lines are fitted to the differences $\Delta T - \Delta A$; the slope of the lines indicates directly the correction to the frequency (+0ˢ·01/lunation = − 36 hertz). The formal results are:

(*a*) Meridian, 1956·0–1965·0: −14 ± 5 (standard error).

(*b*) Occultations, 1958·0–1963·0: +2 ±11 (standard error).

The residuals, in seconds of time, from these fitted straight lines are plotted in Fig. 1 in which the abscissa is the lunation number. For comparison there are also given: in the upper diagram, as a continuous line, the effect on ΔT of the corrections to the lunar ephemeris; in the lower diagram, the slopes corresponding to frequency corrections of ±20 hertz.

There is some evidence, especially in the occultation values (*b*), of the presence of periodic terms; these are probably due to the effects of selection, which is inevitable in both forms of observation, on the difference between the centre of mass and the centre of figure of the Moon. The consequence is to enhance the importance of the observations at the ends of the series; for example, the omission of the last six lunations from the occultation data changes the deduced correction from +2 to −19. Pending a more elaborate discussion, all that can be said with reasonable certainty is that the frequency of the caesium transition is unlikely to lie outside the range 9 192 631 750–775 hertz. Formal estimates of error have little significance.

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On the Possible Origin of the Lunar Maria

RECENT close-up photographs of the lunar surface on the western shores of Oceanus Procellarum, televized back to Earth by the Russian soft-lander *Luna* 9 on February 4, revealed for the first time the detailed micro-structure of typical mare ground down to a cm–mm scale. While the conspicuous surface roughness manifest on this scale could have been anticipated from previous radar-echo work[1], and while most—if not all—of it may be due to micro-meteoritic etching, this new evidence of severely local nature gives rise to wider issues concerning the origin of lunar maria which have so far not been mentioned in this connexion.

As is well known, the origin of such formations has so far been sought in terms of planetesimal[2] or cometary[3] impacts, as well as of volcanic phenomena[4], with so far indecisive outcome. The aim of this communication is, however, to emphasize in this connexion another prominent and large-scale characteristic of the lunar maria which has so far not been properly faced and which—when properly understood—must bear vitally on the problem of their origin: and that is a conspicuous disparity in their distribution on the two hemispheres of the lunar globe.

Ever since the first Russian photographs of the far side of the Moon, taken by *Lunik* 3 on October 7, 1959 (and supplemented since by improved work by *Zond* 6 on July 20, 1965, of much higher resolution), became available, it has been evident that all lunar maria are very largely situated on one hemisphere facing the Earth; and with a slight rotation of the principal axes of inertia of the lunar globe this disparity could be made almost complete.

What could be the cause of this striking phenomenon? A gravitational shielding of the Moon from external impacts by the Earth having proved to be utterly ineffective[5], another type of the Earth–Moon interaction must be sought to provide the desired clue; and I should point out a possible relevance, to our problem, of certain facts which follow from retrograde extrapolation of the tidal evolution of the Earth–Moon system in the past. According to a recent fundamental work by Gerstenkorn[6], confirmed since by more extensive computations by MacDonald[7], a linear extrapolation of the present rate of tidal friction in the Earth–Moon system backwards in time shows that, about 1,800 million years ago, the Moon's centre approached that of the Earth at a distance of 2·89 Earth radii according to Gerstenkorn, and 2·72 such radii according to MacDonald—yielding a mean of 2·8 ± 0·1 Earth radii or 17,800 ± 600 km. Moreover, the angular momentum of axial rotation of the lunar globe is relatively so small that synchronism between the Moon's rotation and revolution was enforced at all stages.

It was Gerstenkorn who first pointed out that the foregoing distance of minimum approach is virtually identical with the terrestrial Roche limit of a gravitational danger zone, within which any self-gravitating body is bound to suffer mechanical damage—and eventually be torn apart—by tidal action of the Earth, regardless of whether it is fluid or solid[8].

Now—and this may be significant in our connexion—according to both Gerstenkorn and MacDonald, the Moon's dip in the terrestrial Roche limit did not engulf the entire lunar globe, but only the 'front' hemisphere facing the Earth. The mechanical damage which the lunar surface may have suffered in the hands of the Earth during their brief close embrace, and its bearing on possible origin of lunar craters, has already been considered by Alfvén[9]. Now the distribution of craters over the entire surface of the Moon appears to be reasonably uniform—if anything, they cluster on the far side. What does not seem to have previously been noted is, however, the fact that a conspicuous asymmetry in the distribution of the maria over the lunar globe appears to be consistent with an assumption that the mare plains may constitute 'scars' of mechanical damage, wrought on the lunar hemisphere which entered the Roche limit by disruptive tides of the Earth.

At the first sight, such a hypothesis would seem to require that the maria be 'lowlands' lying below the level of the continental areas, which is not found to be generally the case[10]. However, the possibility cannot be ruled out that the strain to which the lunar crust may then have been exposed could have led to its cracking, and that lava was exuded to the surface to fill up the original scars.

Could, however, an adequate reservoir of molten lava have been available in sub-surface layers, and if so, what could have been the source of heat to melt it? It could scarcely have been radiogenic heat; for no radioactive elements of sufficient cosmic abundance are known with the requisite half-lives close to 2,000 million years.

However, one gravitational process by which this could have been accomplished at the time would be through viscous dissipation of the bodily tides raised in the mass of the Moon by terrestrial attraction which, at the time of the Moon's closest approach to the Earth, should have been about 10^4 times greater than at the present time. The amount of internal heat which can be so generated[11] is at present minute, but 1,800 million years ago it could have been a different story; and it is possible that the lava-filled lunar maria represent visible reminders of that time.

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RADIOPHYSICS

Observations of Circularly Polarized OH Emission and Narrow Spectral Features

IN a recent publication we reported the detection of linearly polarized OH emission from a localized region near the radio source *W*3 (ref. 1). Although the mechanism of production of the polarized emission was not understood, we pointed out that, among several possible mechanisms, the Zeeman effect was unique because it alone would give rise to circularly polarized radiation. This communication reports observations made with a circularly polarized feed horn on the 140 ft. radio telescope of the National Radio Astronomy Observatory, Green Bank, West Virginia, between November 11 and 12, 1965. The receiver used was an autocorrelation-type radiometer with 100 channels[2]. Bandwidths of 2·5 Mc/s, 625, 250, 62·5 and 15·6 kc/s could be studied with a frequency resolution of 0·012 of the bandwidth. Therefore, on some occasions, our maximum frequency resolution was 190 c/s. The system temperature, including losses in the antenna feed line, was 250° K; the antenna beamwidth was 19 min of arc; coupling between the two senses of circular polarization in the antenna feed horn was approximately −15 db.

We have observed anomalously excited OH emission associated with the sources *W*3, Orion *A*, *W*49, *NGC* 6334, and the source near Sag *A* ($l^{II} = 0°\ 40'$, $b^{II} = 0°$), and found emission to be strongly circularly polarized in all cases.

We have not found any OH emission which is not circularly polarized. By contrast, our observations of OH absorption show it to be unpolarized. The spectra of the OH emission source associated with *W3* is shown in Fig. 1 and the polarization properties of the 1,665 Mc/s lines are given in Table 1. Note that many of the lines, at all four OH frequencies, exhibit 100 per cent circular polarization. Similar behaviour was noted in all the sources observed.

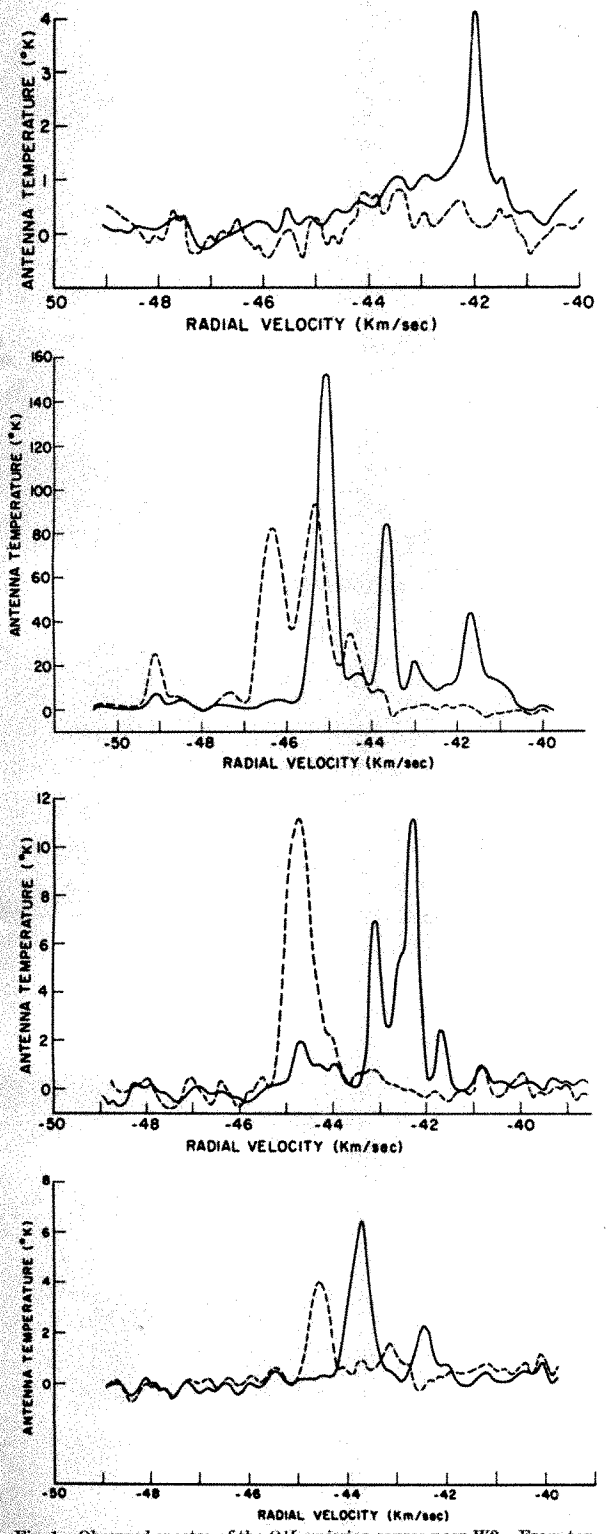


Fig. 1. Observed spectra of the OH-emission source near *W3*. From top to bottom, the frequencies are 1,612, 1,665, 1,667, and 1,720 Mc/s. The solid line shows the right-hand circularly polarized component, and the dashed line shows the left-hand. Note that the velocity scales are slightly different. The frequency resolution is 1·25 kc/s

Table 1. POLARIZATION CHARACTERISTICS OF 1,665-MC/S EMISSION FEATURES OBTAINED BY COMBINING MEASUREMENTS MADE AT HAYSTACK AND GREENBANK

Feature[1]	Velocity (OH) (km/s)	Position angle[1]	Approximate percentage polarization[5]	Comments*
1	−49·1	145° ± 10°	60	L.H. elliptical plus unpolarized radiation
2	−46·5		100	L.H. circular
3*a*	−45·5		60	L.H. elliptical plus unpolarized radiation
3*b*	−45·0		90	R.H. elliptical plus unpolarized radiation
4	−44·7	107° ± 22°	60	L.H. elliptical plus unpolarized radiation
5	−43·7	140° ± 16°	100	R.H. elliptical
6	−43·1		100	R.H. circular
7	−41·7		100	R.H. circular

* Throughout this paper, right-hand circular polarization denotes clockwise rotation of the electric vector when viewed along the direction of propagation.

Most of our observations were carried out with a frequency resolution of 1·25 kc/s and several lines were noted which appear to be inherently narrower than 1 kc/s. In particular, the −43·1 km/s line at 1,667 Mc/s in *W3* was examined with a resolution of 190 c/s and found to be approximately 600 c/s in total width, after correction for instrumental broadening. This represents the narrowest spectral line detected in radio astronomy and corresponds to a kinetic temperature of only 5° K if the width is attributed to thermal broadening. The intensity of the lines, however, argues against this interpretation.

The importance of the ability to make observations of circularly polarized emission with narrow bandwidths is clearly demonstrated by our measurements of OH near Sag *A*. McGee *et al.* have reported an emission line of 36° K intensity at +67·4 km/s when observed with 10 kc/s bandwidth at 1,665 Mc/s using a 210 ft. antenna[3]. However, when observed in left-hand circular polarization with a resolution of 1·25 kc/s using a 140 ft. antenna, this feature has an antenna temperature of 91° K, a factor of 2·5 greater than the C.S.I.R.O. observers reported even though the effective area of the antenna was a factor of 2·25 smaller. The brightness temperature of this feature must be at least 1,300° K since McGee *et al.* report an upper limit of 5 min of arc for the angular size of the OH emitting region. Other prominent emission lines detected in this source are a line of 51° K at a velocity of +73·9 km/s at 1,665 Mc/s and one line at 1,667 Mc/s with an antenna temperature of 10° K at a velocity of +69·0 km/s. The 1,665 Mc/s lines are ~100 per cent circularly polarized, the +67·4 km/s line being left-handed and the +73·9 km/s being the right-handed. The +69·0 km/s line at 1,667 Mc/s is left-hand circularly polarized but appreciably less than 100 per cent.

We find no evidence for a difference in angular size and position of the *W3 OH* source as determined by the spatial dependence of any of the emission lines at 1,665 Mc/s and 1,667 Mc/s. In our earlier communication we reported that the OH-emitting region associated with *W3* was greater than the beamwidth. This is in error. We have no evidence that any of the OH emission sources are greater than the upper limit of 5 min of arc quoted by the C.S.I.R.O. observers for *NGC* 6334 and the Sag *A* sources[3]. Also, the velocities of the 1667 Mc/s emission lines in *W3* reported earlier[1] should be increased by 0·7 km/s to read −44·8, −43·1, and −42·3 km/s in agreement with Fig. 1 of the present communication.

If it is assumed the upper limit of 5 min of arc for some of the OH-emission sources applies to *W3* also, then we must conclude from our observations that the brightness temperature of the OH at 1,665 Mc/s exceeds 2,000° K. This points out the extreme importance to be attached to an accurate determination of the angular size of these emitting regions.

Münch has reported anomalous abundances of CN molecules in the direction of the stars *BD*+66° 1675 and *BD*+66° 1674, near the HII region *W1*, and noted intensity anomalies in the CN spectra[4]. A preliminary

search revealed no OH emission in this region, but further observations should be conducted.

As noted previously, the most obvious interpretation of the existence of circularly polarized emission is the Zeeman effect. However, the presence of large intensity anomalies, the origin of which is by no means understood, makes the interpretation of our results in terms of typical Zeeman patterns exceedingly difficult, if not impossible. For example, the existence of a single line with right-hand circular polarization at 1,612 Mc/s in *W*3, without a line of the opposite sense of circular polarization being present, as shown in Fig. 1, is complotely contrary to Zeeman predictions. Even a single pair of lines at 1,612 Mc/s would be difficult to explain. Many other examples exist in our data, some of which are revealed in the *W*3 spectra of Fig. 1. We conclude that an interpretation of our observations in terms of Zeeman splittings of the lines is premature and may ultimately prove to be incorrect.

It might be possible that a maser-type amplification by the OH is occurring in the interstellar medium. This seems indicated by the intense, but very narrow, emission lines observed and by the anomalous intensity ratios between the four OH frequencies. Coherent maser amplification leads to a narrowing of the lines by an amount approximately given by $(\log_e A)^{1/2}$, where A is the power amplification factor of the medium. Therefore, amplifications of the order of 10^7 are required to narrow an emission line by a factor of 4. Furthermore, if the amplification factor is polarization sensitive, then our observations could be explained without requiring strong magnetic fields. However, it is not clear how such conditions could be produced.

We wish to express our appreciation to the entire staff of the National Radio Astronomy Observatory for their complete co-operation throughout the course of our observations. This work was supported principally by the National Aeronautics and Space Administration.

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GEOPHYSICS

A Telluric Current Experiment on the Antarctic Ice Cap

A TELLURIC current recording system was installed at the Soviet station, Vostok, on the Antarctic ice cap near the South Geomagnetic Pole in December 1964. It has generally been assumed that the high resistivity would preclude such an operation. The prime difference between an ice cap installation and a land-based operation[1] lies in the electrode installation technique and the inherent high electrode-pair resistance.

The 200-m spaced electrodes consisted of 8 m by 30 cm strips of copper screen installed in a trench 30 cm deep. Several holes 2 m deep were drilled along the trench. Next the full length of the trench, the walls of the drilled holes, and the first layer of backfill were thoroughly impregnated with 100 l. of a 5 per cent aqueous solution of sodium chloride. This technique resulted in an electrode-pair resistance of 400,000 ohms, about three orders of magnitude higher than the usual land-based values[1,2]. The 200-m spacing was sufficient to produce an adequate telluric signal level.

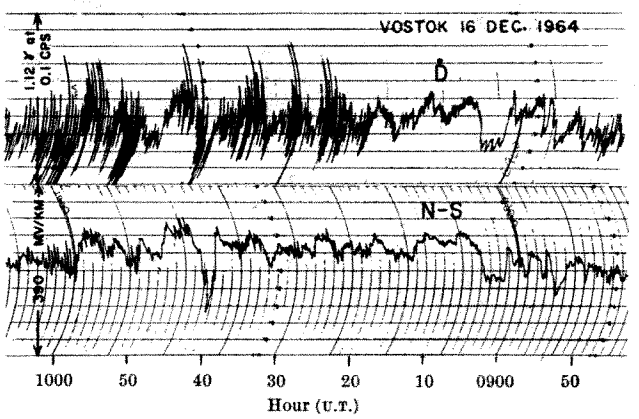


Fig. 1. North–South (in magnetic meridian) telluric current and dD/dt on the Antarctic ice cap at Vostok

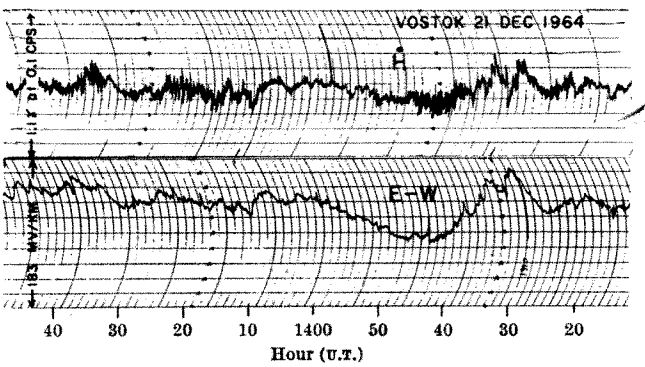


Fig. 2. East–West (perpendicular to magnetic meridian) telluric current and dH/dt on the Antarctic ice cap at Vostok

The instrumentation consisted of a 'Medistor' microvoltmeter with 1 meg input impedance and equipped with an output amplifier capable of driving the 1 mA 'Esterline Angus' recorder. The voltage gain required is indicated by the telluric signal levels shown in Figs. 1 and 2. Calibrations were made by inserting a mV signal of known value in series with one of the electrode lines, thus including the effect of the relatively high electrode-pair resistance in the calibration[1].

Figs. 1 and 2 show examples of the telluric activity and the corresponding dH/dt and dD/dt traces. The activity shown here was of more than average amplitude but characteristic of the continuing agitation observed at Vostok over a 2-week period in December 1964. This relationship between the E and H fields should be the subject of future investigation and analysis. At Vostok, on top of 12,000 ft. of a relatively good dielectric, we see that dD/dt and the North–South vector, and dH/dt and the East–West vector appear as in time phase. In contrast, over sea water on the arctic drifting stations, the same relationship holds between D and H and the corresponding electric vectors.

Telluric current recording on polar ice caps provides a useful scientific technique not hitherto available. In connexion with magnetic recordings, it permits magneto-telluric studies of the ice cap and the underlying geological structure. It provides a convenient supplementary means of studying ionospherically induced electromagnetic phenomena through a wide spectral range. It should be useful for recording the longer period P_c and P_i activity which fall in the gap which tends to exist between induction loop and normal run magnetograph records.

Readers requiring additional details of the experiment may correspond with us.

We are grateful for the Soviet invitation to work at Vostok and for the full co-operation of the station leader and staff. The magnetic data were recorded on equipment designed at the Boeing Scientific Laboratories. Dr. Pavel

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PHYSICS

Laser-induced Thermal Etching of Metal and Semi-conductor Surfaces

Chalmers *et al.*[1], Hendrickson and Machlin[2], and others[3–5] have demonstrated the possibility of thermally etching metals by evaporation. Thermal etching has been investigated most extensively in silver although it has been observed on other metals. It has been generally established that the etching is characterized by two mechanisms, namely, (*a*) the formation of grooves at the grain boundaries of polycrystalline metals, and (*b*) terrace or striation formation on the surface of single crystals. Grooving usually occurs at a lower temperature than that at which striation structure forms. The formation of a terrace structure, at least on the surfaces of copper and silver single crystals, may depend on the presence of oxygen during annealing. This communication reports a further consequence of thermal etching in air, namely, the formation of etch hillocks. This phenomenon has now been established for a number of metals and semiconductors.

Thermal etching was carried out by focusing the output of a ruby laser on to the surface of the material under investigation. The laser used was a commercial unit operating in the *Q*-switched mode. The output pulse had a width of about 80 nsec and a peak power of about 5–6 MW. The pulse was focused on to the surface with a 1·95 cm focal length lens. The surface was polished either mechanically or chemically and was subjected to only one pulse. This process usually produced a superficial crater with a diameter of about 1–2 mm. The etched area was then replicated using the selected area technique described by Fourie[6]. The replica is a shadow-cast primary replica suitable for investigation by transmission electron microscopy. In order to determine whether the laser-produced surface structures were pits or hillocks, it proved necessary to prepare a second replica of the same area. This was then sprayed with a dilute solution of polystyrene latex particles in water. The dried replica was then shadowed and coated with carbon as before. A comparison of the directions of the shadows of the latex and feature then determined the type of feature. That there were hillocks present could also be shown by making a 'through-focus' observation on the original metal surface with a high-powered optical microscope.

Typical etch patterns observed in a number of materials are shown in Figs. 1 and 2. The micrographs are of replicas of regions close to the crater periphery where it has been possible in some cases to compare surface structure as a result of deformation with the resulting etch features inside the crater.

Fig. 1*a* shows a thermally etched region on a (111) plane of a copper single crystal. The etched ridges lie along the <110> direction. This terraced structure is typical of copper crystals thermally etched in an oxygen-rich environment (compare Ponslet and Bariaux[7]). Fig. 1*d* shows a typical region on the surface of an etched α-brass single crystal (20 per cent zinc). The sample had been plastically deformed by uniaxial tension prior to the etch. The hillocks are clearly located along slip lines, a number of which are still visible. In Fig. 1*c* the crater edge in body-centred cubic iron is shown. The hillocks seem to lie along a continuation of the surface deformation markings. Note the very gradual slope into the crater. Van Wijk and Van Dijck[8] have observed similar hillocks in chemically etched iron and have associated these with dislocations in the sample[9]. Fig. 1*d* shows small etch hillocks inside a grain of polycrystalline platinum.

Figs. 1*e* and *f* are examples of laser-induced etching on the surface of polished gallium-arsenide and germanium, respectively. In Fig. 1*f* the features marked *B* are etch pits, while the fine features in the background are a mixture of etch hillocks and pits. The gallium-arsenide used in Fig. 1*e* was *p*-type arsenic-rich material with a

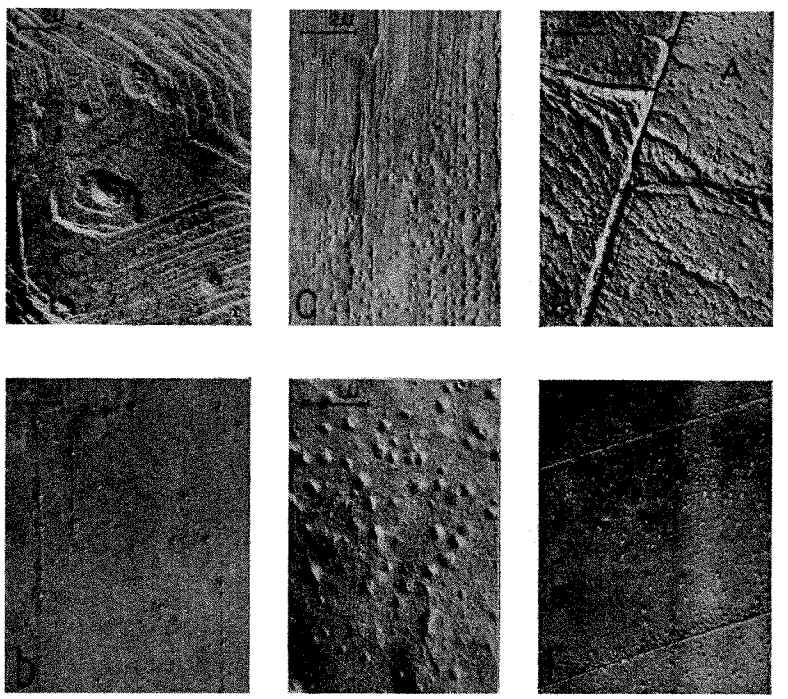


Fig. 1. Electron micrographs of thermal etch patterns. (*a*) Copper; (*b*) 20 per cent zinc α-brass; (*c*) iron; (*d*) platinum; (*e*) gallium-arsenide; (*f*) germanium

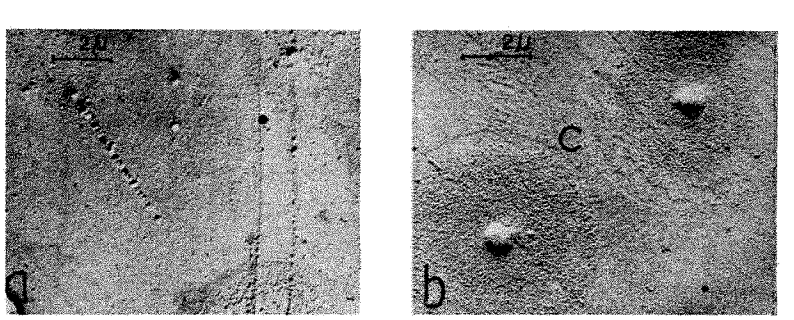


Fig. 2. Surface structure of a laser-etched α-brass crystal

specific conductivity of $2{\cdot}2 \times 10^{-3}$ ohm^{-1} cm^{-1}. This sample showed marked grooving along the grain boundaries as well as a high density of large hillocks (marked *A*). The background, in common with germanium, is a mixture of fine etch pits and hillocks.

We have further established that hillocks appear when aluminium, silver, nickel and molybdenum are thermally etched with laser radiation. Apart from the iron which has a purity of 99·98 per cent, the other materials used in this investigation are spectroscopically pure.

The correspondence of etch hillocks and crystal substructure is still being investigated. Fig. 2*a* is an example of a possible dislocation pile-up in a 20 atomic per cent zinc α-brass crystal. A number of such features have been observed in this alloy. The hillocks in these clusters are usually of the same radial size. We have also observed a number of very large hillocks ; these are usually isolated, although centred on a common slip line with small hillocks. Fig. 2*b* shows the characteristic appearance of two such hillocks. Note the rim structure at *C*. These large hillocks are similar in appearance to those observed in copper (Fig. 1*a*).

The development of etch hillocks, as described here, might be a result of the unusual method used to produce the thermal etching. The method appears to have many general applications ; for example, as laser radiation can be focused through a window, the thermal etching of a metal surface which is both under high vacuum and at a low ambient temperature is now possible.

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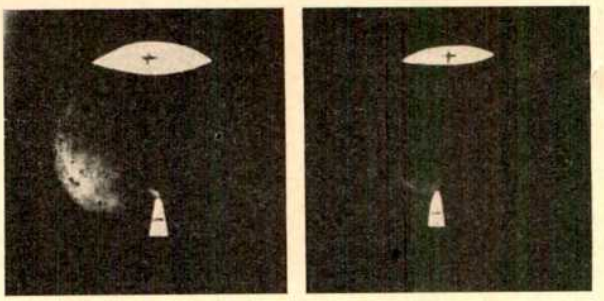
Light Emission in *n*-Hexane under High Electric Stress

LIGHT emission, resulting from the application of high d.c. fields, has been reported for insulating liquids containing fluorescent materials in solution[1,2], and for *n*-hexane by us[3]. Further, a change of refractive index has been observed between electrodes in hexane when a voltage pulse was applied across them[4].

All indications are that the phenomena commence at the cathode, and it is the purpose of this communication to report some further observations on the form of the light emission from the cathode in *n*-hexane resulting from the application of high d.c. fields.

The test cell, electrode systems and cleaning techniques were similar to those adopted by Kao and Calderwood[5] but the *n*-hexane was used as supplied without further treatment. The cathode was a steel gramophone needle having a point radius approximately 0·02 mm and the anode was a stainless steel sphere of 0·25-in. diameter. The background to the electrodes could be illuminated with a pre-focus lamp of adjustable intensity directed through the electrode gap in the test cell towards the lens which focused on the image intensifier which has been described[6]. The output screen of the image intensifier was observed visually and also photographed with a 'Polaroid' camera.

Fig. 1 shows two typical examples of weak light-emission observed at 24·5 kV and 30 kV with a gap length of 1·75 mm. The bright ring* was clearly visible on the screen although it only lasted for a short time: it originated from the tip of the point cathode. This phenomenon can be repeated experimentally. The outlines of the electrodes have been superimposed on the photographs for clarity.



a *b*

Fig. 1. Light emission in *n*-hexane under high electric stress. Gap length = 1·75 mm. (*a*) Applied voltage; 24·5 kV; (*b*) applied voltage; 30 kV

It would be reasonable to assume that a copious supply of electrons is available at the point cathode for the fields under investigation. The electrons may gain energy, excite molecules and form ions with subsequent light emission from the plasma region.

Initially the region of plasma is very small and the electric field at its front boundary is very large. As the plasma grows, this field tends to decrease since the plasma acts as an electrode of increasing effective radius: however, the field eventually begins to increase due to the decreasing effective gap length. Assuming that this explanation holds, the photographs obtained by us could be interpreted as being photographs of the plasma region. Since breakdown did not follow immediately on the event photographed, it must be assumed that the electric field at the front of the plasma had dropped to such a value that it was insufficient to propagate the plasma.

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* Clarity has been lost in Fig. 1*b*, and image intensifier background enhanced in Fig. 1*a* left.

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CHEMISTRY

Boron Carbide, a New Substrate for Fuel Cell Electrocatalysts

A NUMBER of investigators have reported high-performance, direct hydrocarbon fuel cells[1–4]. In every case, the direct hydrocarbon anode has used a large amount of high-area platinum black or Raney platinum in the range of about 10–100 mg/cm^2 of geometric electrode area. This tends to counteract the advantage of being able to operate on inexpensive hydrocarbon fuels. Furthermore, there is a tendency for performance to be proportional to

platinum content. Niedrach[5] has shown that 'Teflon'-bonded electrodes[6] containing mechanical mixtures of platinum black with tantalum or platinum deposited on tantalum give performance on propane which is just proportional to the platinum content. Thus, the advantage of reduction in platinum content is cancelled out by the reduction in performance.

In conventional catalysis, catalytic materials are often supported in order to attain high surface area, retard sintering, and achieve high activity per unit weight. In a number of instances, the catalytic activity of transition metals has been found to vary with the nature of the support material[7–10]. In supported electro-catalysis, carbon has been so widely used as a support, and diversification to other materials has been so limited that the question of whether a suitable support material might markedly enhance electrocatalytic activity remains unanswered.

This communication reports the first results[11] obtained with hydrocarbon fuel cell anodes containing boron carbide as the support for platinum electrocatalyst. The results indicate that an enhancement of platinum activity has been achieved when compared with an equal weight [illegible] an equal hydrogen adsorption surface area of platinum black. As a support boron carbide appears to be interesting [illegible] other ways as well. Platinum on boron carbide was found to be more resistant to sintering than platinum black or platinum on graphite at equal surface coverage. Also, the catalysed adsorption of hydrogen on to a graphite support[12] was found to be absent in the case of boron carbide. These results suggest interesting and probably useful features of boron carbide over and above its well-known properties of corrosion resistance and oxidative stability.

To prepare the supported platinum a commercial boron carbide powder ('Norbide' abrasive, Norton Co., Worcester, Mass.) was activated by slurrying it with a solution of platinum diammine dinitrite, driving off the solvent by heating and then reducing in hydrogen at an elevated temperature. The comparison platinum black was of commercial origin (platinum black 100 per cent, Engelhard Industries, Newark, N.J.). The materials were made into 'Teflon'-bonded electrodes of a previously described structure[6]. Surface areas of the electrocatalysts were obtained as follows.

The total surface area was determined by the Brunauer–Emmett–Teller method using the adsorption of nitrogen at −195° C. The area of the supported platinum was determined by measurements of the chemi-sorption of hydrogen at 200° C, the sample having been previously reduced and then evacuated at this temperature. The effective area of the metallic phase was calculated by assuming that each chemisorbed hydrogen atom occupied one surface metal site.

The results of typical adsorption measurements are shown in Table 1. The highest degree of metallic dispersion was obtained with low platinum loadings and low reduction temperatures. The average platinum crystallite size calculated from the adsorption measurements agreed well with estimates made from electron micrographs of the samples. For purposes of comparison a commercial platinum black has a surface area of about 20 m²/g and particle size of about 100 Å diameter.

Performance of the test anodes on propane fuel was evaluated in complete fuel cells containing similar platinum black oxygen cathodes. Phosphoric acid (85 per cent by weight) was the electrolyte, and the temperature was 150° C. The current density maintained at an *iR*-free cell potential of 0·5 V (corresponding to an anode potential of 0·42 V versus a reversible hydrogen electrode in the same electrolyte) was taken as a performance index.

Table 1. PROPERTIES OF Pt–B₄C CATALYSTS

Wt per cent platinum	Hydrogen adsorbed (ml. S.T.P./g)	Surface area of platinum (m²/g platinum)	Total metal area (m²/g sample)	Platinum crystallite size (Å)
0	0·041	—	—	—
0·5	0·197	149	0·75	16
2·0	0·310	65·3	1·31	36
4·8	0·679	63·5	3·05	38
9·1	1·007	50·7	4·61	47

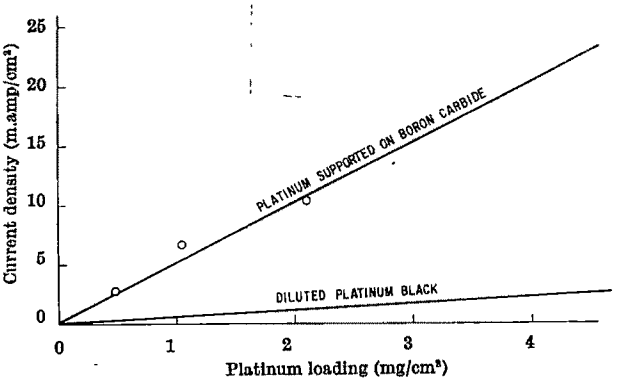


Fig. 1. Current density at cathode versus anode voltage = 0·5 (anode versus reference voltage = 0·42) V versus platinum loading for platinum supported on boron carbide and platinum black diluted with boron carbide; propane-oxygen cells, 150° C, 85 per cent phosphoric acid electrolyte

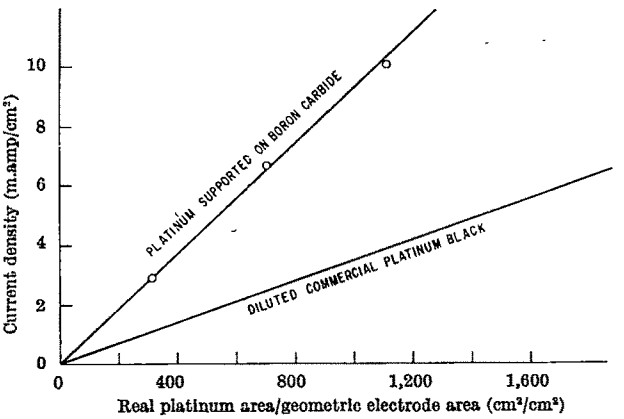


Fig. 2. Current density at cathode versus anode voltage = 0·5 (anode versus reference voltage = 0·42) V versus real platinum area per unit electrode area for platinum supported on boron carbide and platinum black diluted with boron carbide; same operating conditions as for Fig. 1

This index is plotted against platinum weight per unit electrode area in Fig. 1. The best straight line through three data points and the origin gives a slope indicating the effectiveness of the platinum in m.amp/mg platinum. The same effectiveness factor for platinum mechanically diluted with boron carbide is shown by the lower line in Fig. 1. The slopes are 5 m.amp/mg for platinum on boron carbide and 0·6 m.amp/mg for platinum black diluted with boron carbide, an improvement of a factor of 9. This is partly due to a higher platinum surface area for the supported platinum. In Fig. 2, the fuel cell performance index is plotted against the real platinum surface area per unit electrode area. These data fit a straight line better. Again the line for mechanically diluted platinum black is included for comparison. The slopes now give current density per square centimetre of real platinum area and the values are 9·3 and 3·5 μamp respectively. This factor of improvement could indicate enhancement of platinum activity by the support.

Further experimental effort is required to confirm this last conclusion. Nevertheless the results so far indicate that boron carbide is an interesting support for catalysts and electrocatalysts, and further investigation is under way.

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Frequency Assignments in Infra-red Spectrum of Water

ALTHOUGH the infra-red spectra of water and deuterium oxide vapour have been carefully mapped and satisfactorily analysed, the infra-red spectrum of water in the liquid state possesses many features that are still not clearly understood. As in the case of most liquids, the near-infra-red spectrum of the liquid is characterized by strong bands in the same spectral regions as those in which the strong vibration–rotation bands of the vapour occur. However, in the case of liquid water there are additional weaker near-infra-red bands that have no counterparts in the water-vapour spectrum. This communication offers a tentative assignment of these near-infra-red 'associational bands' in terms of monomeric vibrational modes and certain lattice modes reported recently by Draegert *et al.*[1], who have investigated the far-infra-red spectrum of liquid water between 30 cm^{-1} and 1,200 cm^{-1}.

In a report of their own recent work, Bayly *et al.*[2] have included a useful summary of numerous earlier experimental investigations of the near-infra-red spectrum of liquid water and have discussed the various attempts at making frequency assignments for the observed bands. The strong liquid-water bands in the near-infra-red and their interpretations are as follows: (1) a relatively narrow band at 1,630 ± 15 cm^{-1}, which is attributed to the ν_2 bending mode of the water monomer; (2) an extremely broad and intense band with maximum absorption at 3,430 ± 60 cm^{-1}, which is mainly attributed to the asymmetric valence vibration ν_3 of the monomer but also involves $2\nu_2$ and the normally infra-red inactive symmetric valence vibration ν_1; (3) a broad band at 5,140 ± 60 cm^{-1}, which is attributed to the combination $\nu_2 + \nu_3$. The indicated value of the frequency listed for each band is the average of the values obtained in the various investigations summarized by Bayly *et al.*[2], and the indicated uncertainties cover the range of values obtained in these independent investigations.

The best known of the associational bands of water is a broad band with frequency $\nu_x = 2{,}130 \pm 5$ cm^{-1}, which has been extensively investigated. Additional weaker associational bands $\nu_y = 3{,}950 \pm 30$ cm^{-1} and $\nu_z = 5{,}600 \pm 20$ cm^{-1} have been reported by Collins[3], Ellis[4], and others[2,5,6].

In liquid deuterium oxide the corresponding strong bands are: (1) $\nu_2 = 1{,}218 \pm 3$ cm^{-1}; (2) $\nu_3 = 2{,}514 \pm 30$ cm^{-1}; and (3) $\nu_2 + \nu_3 = 3{,}815 \pm 15$ cm^{-1}. The associational bands in liquid deuterium oxide are: $\nu_x = 1{,}555$ cm^{-1}, $\nu_y = 2{,}900$ cm^{-1}, and $\nu_z = 4{,}100$ cm^{-1}. Only Bayly's values are listed for the deuterium oxide associational bands, as work by others has not been extensive; although Bayly did not state uncertainties in frequency, it is possible that they may be comparable with those stated previously for the water associational bands. It is to be noted that the ratio of the frequencies of the water bands to the frequencies of the corresponding deuterium oxide bands is in every case approximately 1·35.

Various attempts[2] have been made to account for the near-infra-red associational bands in terms of combinations of the monomeric vibration modes with a single hypothetical infra-red-active mode producing a band in the far-infra-red. However, no experimental evidence of a band with the proposed frequency has been obtained.

The results reported by Draegert *et al.* form the basis for a more realistic assignment of the near-infra-red associational bands. For liquid water these authors report a broad major band with maximum absorption at 685 ± 15 cm^{-1} together with a second less-intense overlapping band at 170 ± 15 cm^{-1}. In liquid deuterium oxide, the corresponding bands appear at 505 ± 15 cm^{-1} and 165 ± 15 cm^{-1}, respectively. The ratio of the frequency of the major far-infra-red band in water to that of the corresponding band in deuterium oxide is 1·35—a value identical with the corresponding ratio obtained for the near-infra-red bands. Thus, it would appear that the major bands can be attributed to some molecular motion that is influenced by isotopic substitution to the same extent as the vibrations responsible for the near-infra-red bands. Since the hindered rotation of water and deuterium oxide molecules in the liquid lattice would satisfy this requirement, the major far-infra-red bands are tentatively assigned to a hindered-rotation mode ν_R.

The minor far-infra-red bands in liquid water and deuterium oxide have nearly the same frequency and therefore have an origin quite different from those of the other infra-red bands. It is probable that translational motion of entire monomeric units of water and deuterium oxide are involved. Welsh *et al.*[7] have shown that even for non-polar molecules translational absorption can occur during collisions; the collision-induced electric dipole moment provides the mechanism for interaction with the radiation field. The production of induced dipole moments during collisions between molecules is quite general except for like molecules having spherical symmetry. Ewing and Trajmar[8] have demonstrated the existence of quantized translational states of a molecule within a shell formed by its neighbours. It is therefore suggested that the minor far-infra-red bands in water and deuterium oxide be attributed to a translational mode ν_T and that they involve a transition from the lowest to the first excited translational level.

With the far-infra-red bands designated as ν_R and ν_T it is proposed that the weak associational bands in the near-infra-red involve absorption of radiation by molecules initially in the first excited translational energy state. Thus the following assignments are proposed: $\nu_x = \nu_3 + \nu_R - \nu_T$, $\nu_y = \nu_3 + \nu_R - \nu_T$, and $\nu_z = \nu_2 + \nu_3 + \nu_R - \nu_T$. Comparisons of the observed values of ν_x, ν_y and ν_z with values predicted on the basis of the proposed assignments are given in Table 1. In view of the uncertainties in the

Table 1. OBSERVED AND PREDICTED FREQUENCIES OF ASSOCIATIONAL BANDS

Band	Frequencies (cm^{-1})			
	H_2O		D_2O	
	Obs.	Pred.	Obs	Pred.
ν_x	2,130	2,145	1,555	1,558
ν_y	3,950	3,945	2,900	2,854
ν_z	5,600	5,575	4,100	4,072

frequencies of the near-infra-red bands, the agreement between observed and predicted values is quite good. For water, agreement with the data of Ellis and Sorge is nearly perfect, and for deuterium oxide agreement with Bayly *et al.* is excellent; however, in the interests of objectivity, the frequencies listed in the table are based on mean values of published results. It is interesting to note that the frequency difference $\nu_R - \nu_T$, which appears in all assignments for the associational bands, is exactly the required frequency of the hypothetical band proposed by earlier workers.

Although the suggested frequency assignments fit the existing data quite well, it is obviously necessary to establish definitely the mechanisms involved in the ν_T and ν_R modes and to consider further the processes involved in absorption by molecules in excited translational energy states. Further work on these subjects is at present in progress.

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BIOCHEMISTRY

Antigenic Properties of Soluble Elastins and their Use in Detecting Soluble Elastin in Ligament Extracts

IN this laboratory we have been interested in the existence of a possible soluble precursor of insoluble elastin (tropoelastin) analogous to tropocollagen. However, since elastin has no enzyme activity and does not possess a specific amino-acid, such as occurs in collagen, no simple method was available to detect this protein in the presence of large amounts of other proteins, which are found in tissue extracts.

We therefore turned our attention to the antigenic properties of elastin in order to use immunochemical methods to detect its soluble precursor. Previous reports[1-3] indicated that elastin was in fact non-antigenic. However, we were informed by Dr. S. M. Partridge (personal communications) that his group had succeeded in developing antibodies against α-elastin (a soluble product of partial hydrolysis of elastin), but the specificity of their anti-sera was questionable. More recently Robert *et al.*[4] have described the development of rabbit antibodies against high molecular weight peptides obtained by alcoholic potassium hydroxide digestion of bovine aortic elastin (κ-elastin)[5].

Alpha elastin was prepared in our laboratory from adult bovine ligamentum nuchae by partial hydrolysis of purified insoluble elastin with 0·25 M oxalic acid[6] followed by de-ionization on a 'Dowex' resin column[6]. A 2 per cent saline solution of the α-elastin was thoroughly mixed to an emulsified state with equal parts of a complete Freund's adjuvant. This emulsion was injected weekly into rabbits using 1 ml., subcutaneously, in four divided doses over the back. After 5 weeks, a very weak precipitin reaction was detected using the serum of all three immunized animals. A prozone phenomenon could not be documented. Primary immunization was continued for a total of 7 weeks. The animals were allowed to rest for 2 months, and a series of four secondary injections were given at weekly intervals. A weak precipitin reaction was again detected. This was insufficient to quantitate but appeared somewhat stronger than that noted during the primary response. The immune response of one of the test rabbits is reported in detail for primary as well as repeated secondary stimulation (Table 1).

This technique, including 2 months of rest between injections, was repeated two additional times. This gave distinct precipitin reactions with a maximum amount of precipitate at a 0·5 and 0·25 mg/ml. concentration of the antigen when reacted with the undiluted rabbit anti-serum. Precipitates were not apparent until the reaction mixture had been allowed to incubate in the cold overnight. Precipitins were never of a marked flocculent nature. Although areas of antigen and antibody excess were apparent, the exact zone of optimal proportions in a serial dilution of the antigen was difficult to delineate (Table 1). Precipitin bands by Ouchterlony diffusion[7] were not distinct. Only one band was seen and the sharpest band appeared to be at 0·125 mg/ml. antigen concentration when reacted with undiluted rabbit anti-serum. It was suspected that we were dealing with a system in which the antigen–antibody complex existed largely in the soluble state.

Results were more uniform with immunoelectrophoretic techniques[8]. Using large quantities of sera in repeated application, which was necessary to give visible results, one diffuse band was obtained when the original antigen was reacted with the anti-serum from any one of the three test rabbits. All control sera obtained from these rabbits prior to primary immunization were negative, as well as attempted cross-reactions with bovine serum and collagen. Alpha elastin prepared from foetal bovine ligamentum nuchae was reacted against the anti-sera from all three animals, and gave a reaction similar to that obtained with the original antigen.

Antigen–antibody interaction was also demonstrated by passive cutaneous anaphylaxis using guinea-pigs. Strongly positive tests were obtained with undiluted and 1:10 dilutions of the anti-sera.

Haemagglutination studies were carried out using formalinized, tanned, red blood cells to quantitate and

Table 1. PRECIPITATING ANTIBODY RESPONSE OF RABBIT AFTER REPEATED INJECTIONS OF 2 PER CENT α-ELASTIN WITH EQUAL PARTS OF FREUND'S ADJUVANT

Undiluted rabbit anti-sera plus	Antigen concentrations (mg/ml.)								
	1·0	0·5	0 25	0·125	0 062	0 031	0 016	Saline control	Anti-serum control
Primary response—1st and 2nd month	+/−	+/−	+/−	1+	1+	+/−	+/−	−	−
Secondary responses									
Set 1 — 5th month	+/−	+/−	+/−	1+	+/−	+/−	+/−	−	−
Set 2 — 7th month	+/−	+/−	1+	1+	+/−	+/−	+/−	−	−
Set 3 — 9th month	+/−	1+	2+	2+	1+	+/−	+/−	−	−
Set 4 — 11th month	1+	2+	2+	1+	1+	+/−	−	−	−

Precipitin tests were read after incubating at 37° for 1 h and then in cold for 18 h.

characterize the antigen–antibody reaction already demonstrated. Type 0, Rh positive human cells collected in Alsevers solution were formalinized after the method of Butler[10]. A 4 per cent solution of these cells was incubated with an equal volume of fresh 1:20,000 tannic acid solution for 30 min at room temperature. After washing three times with *p*H 7·4 phosphate-buffered normal saline, a 2 per cent saline suspension was made, and 5-ml. aliquots were quick frozen in liquid nitrogen. Aliquots were stored in this state until used for haemagglutination investigations. Immediately before testing, sufficient quantities of the cells were thawed and incubated at room temperature for 1 h with equal volumes of the serially diluted α-elastin solution. After three washes with a 1:200 normal rabbit serum in buffered saline, 0·1-ml. portions of the coated, tanned, cell suspension, in 2 per cent concentrations, were mixed with an equal volume of the serially diluted anti-serum. The presence of haemagglutination was read after incubation for 3 h at room temperature. All tubes were reshaken and read again after a total incubation time of 12-18 h. Sharper readings were obtained after 12 h, with controls more uniform.

Haemagglutination scoring was read in a fashion similar to that of Stavitsky[11]. Marked 4+ reactions were obtained with 1·0 and 0·5 mg/ml. concentrations of the antigen and a 1:50 dilution of the anti-serum. Haemagglutination was evident at dilutions down to 0·125 mg/ml. of antigen using this same concentration of anti-serum. Table 2 summarizes representative results of the haemagglutination studies. Collagen was found to interfere with haemagglutination studies. All tubes, including control antigen tagged cells without anti-sera, gave 1+ readings. However, incubation at +4° C rather than at room temperature prevented this non-specific agglutination phenomenon. Strong positive cross-reactions were obtained when the anti-serum was incubated with the κ-elastin of Robert[4] and the α-elastin from foetal tissue. The foregoing results indicated a strong antigen–antibody reaction with specificity.

Table 2. HAEMAGGLUTINATIONS OBSERVED WHEN α-ELASTIN WAS REACTED WITH ANTI-α-ELASTIN. READING MADE AFTER 12-H INCUBATION AT ROOM TEMPERATURE

Concentrations of α-elastin incubated with formalinized, tanned, red cells (mg/ml.)

Anti-alpha elastin	2·0	1·0	0·5	0·25	0·125	0·0625	0·031	Control formalinized, tanned, red cells
1·50	4+	4+	3+	2+	1+	–	–	–
1 250	2+	1+	1+	+/–	–	–	–	–
1:1250	+/1	–	–	–	–	–	–	–
Saline control	–	–	–	–	–	–	–	–

On the basis of previous work carried out in our laboratory[12], extracts were made of term foetal bovine ligamentum nuchae using a *tris*-HCl buffered normal saline solution at *p*H 8·4. Because of the interference noted earlier, collagen was precipitated from this extract by adding cold absolute ethanol to a total volume of 20 per cent. Ethanol was removed by dialysis. Haemagglutinations were then carried out at +4° C using the ligament extract as antigen and the anti-alpha elastin as antibody. Strong cross-reactions were observed. Table 3 summarizes these results.

Table 3. HAEMAGGLUTINATIONS OBSERVED IN A 20 PER CENT ETHANOL-TREATED EXTRACT OF FOETAL BOVINE LIGAMENTUM NUCHAE WHEN REACTED WITH ANTI-α-ELASTIN. READINGS MADE AFTER 3-H INCUBATION AT +4° C

Concentrations of extract incubated with formalinized, tanned, red cells (mg/ml.)

Anti-α-elastin dilutions	2·0	1·0	0·5	0·25	0·125	0 062	0·031	Saline control
1:50	4+	4+	3+	3+	2+	1+	–	–
1:100	3+	4+	3+	3+	1+	–	–	–
1.200	2+	3+	3+	2+	1+	–	–	–
Saline control	–	–	–	–	–	–	–	–

These ligament extracts were used in another study to immunize rabbits using the same régime as that described for α-elastin. The anti-sera obtained were found to cross-react with the α-elastin using the haemagglutination technique with formalinized, tanned, red cells and also the passive cutaneous anaphylaxis test. No cross-reactions were detected, however, by Ouchterlony diffusion or immunoelectrophoresis using these same anti-sera.

The availability of an anti-serum to α-elastin together with the haemagglutination technique described provides an amply sensitive method of specifically detecting small amounts of soluble elastin in the presence of other proteins. This method has been used in this laboratory to detect the presence of soluble elastin in fractions obtained by chromatography of extracts of bovine foetal ligamentum nuchae.

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Excretion of Xanthurenic Acid in the Urine of Women taking Progestogen–Oestrogen Preparations

THE purpose of this communication is to report the excretion of grossly increased amounts of xanthurenic acid in the urine of women taking a combination of a progestogen and an oestrogen for contraceptive purposes, following a tryptophan load.

Fourteen women taking various oral contraceptive preparations were investigated. An oral load of L-tryptophan (5 g) was given. All the urine passed during the subsequent 8 h was collected, the volume measured, and an aliquot kept at −20° C until the estimation was made. Xanthurenic acid was estimated by the paper chromatographic method of Coppini, Benassi and Montorsi[1], except that the solvents used were *n*-butanol/acetic acid/water (12:5:3) followed by *n*-butanol/pyridine/water (1:1:1). The results have been expressed as mg of xanthurenic acid excreted in the 8 h period following the tryptophan load. The excretions for 12 normal subjects (six males and six females) ranged from 10 to 60 mg. The authenticity of the presumed xanthurenic acid spots on the chromatograms of urine was established by comparing the R_F values and colour reactions with those of a xanthurenic acid marker (Table 1). The high excretions were dependent on the administration of a tryptophan load, and were maximal 4–5 h after giving the tryptophan.

Table 1. CHROMATOGRAPHIC CHARACTERISTICS OF XANTHURENIC ACID

	R_F values from two-dimensional chromatography				Colour reactions			
	BuA	BuP	KCl	IPrAm	UVL	SuA	Ehrlich	FAS
Authentic xanthurenic acid	0·60	0·63	0·22	Very little movement, tailing of spot	Bright blue	Red	Nil	Green
Presumed xanthurenic acid in urine	0·60	0·63	0·23	Ditto	Bright blue	Red	Nil	Green

BuA = *n*-butanol/acetic acid/water (12 : 5 : 3). BuP = *n*-butanol/pyridine/water (1 : 1 : 1). KCl = 20 per cent potassium chloride. IPrAm = isopropanol/ammonia/water (20 : 1 : 2). UVL = ultra-violet light. SuA = diazotized sulphanilic acid. Ehrlich = *p*-dimethylamino benzaldehyde 2 per cent in 1·3 N HCl. FAS = 1·7 per cent ferric ammonium sulphate followed by 5 per cent $NaHCO_3$. All chromatographs carried out on Whatman No. 1 paper.

The results obtained for the 14 women taking progestogen–oestrogen preparations are given in Table 2. The excretion of xanthurenic acid in the urine of subject 10 was re-estimated after the oral administration of pyridoxine hydrochloride (40 mg) daily for 5 days. The output had fallen from 593 mg to 80 mg in an 8 h collection of urine.

Table 2. URINARY EXCRETION OF XANTHURENIC ACID: WOMEN TAKING PROGESTOGEN–OESTROGEN PREPARATIONS

Subject No.	Preparation (daily dose)	Duration of use	Xanthurenic acid excretion (mg in 8 h)
1	*Nor*ethisterone acetate 3 mg Ethinyl oestradiol 0·05 mg	3 years	983
2	,, ,, ,,	18 months	144
3	,, ,, ,,	12 months	155
4	,, ,, ,,	11 months	407
5	,, ,, ,,	3 days	79
6	*Nor*ethisterone acetate 4 mg Ethinyl oestradiol 0·05 mg	4 months	530
7	*Nor*ethisterone 2 mg Ethinyl oestradiol-3-methyl ether 0·1 mg	2 months	211
8	,, ,, ,,	2 months	181
9	,, ,, ,,	1 month	442
10	*Nor*ethynodrel 2·5 mg Ethinyl oestradiol 0·05 mg	5 years	593
11	,, ,, ,,	2 years	187
12	*Nor*ethynodrel 2·5 mg Ethinyl oestradiol-3-methyl ether 0·1 mg	11 months	621
13	Ethynodrel diacetate 1 mg Ethinyl oestradiol-3-methyl ether 0·1 mg	10 months	135
14	,, ,, ,,	1 month	24

Increased excretions of xanthurenic acid and other tryptophan metabolites have been reported in pregnancy, and have been interpreted as indicating pyridoxine deficiency[2,3]. Brown, Thornton and Price[4] confirmed these observations, but found the output of kynurenic acid to be decreased in pregnancy, whereas in simple pyridoxine deficiency the excretion of this metabolite is also raised. They suggested that the excretion pattern of the tryptophan metabolites in pyridoxine deficiency is modified by the hormonal changes of pregnancy. This view is supported by the demonstration that sulphate esters of oestrogens inhibit the *in vitro* activity of rat kidney kynurenine transaminase, the enzyme concerned in the formation of kynurenic acid from kynurenine. The abnormal excretions of xanthurenic acid, following a tryptophan load, by women taking progestogen–oestrogen preparations may be explained by a similar mechanism. The marked fall in the excretion of xanthurenic acid by the subject treated with pyridoxine suggests that the hormonal effect may result from interference with the coenzyme function of pyridoxal-5-phosphate. Further investigations are being carried out to explore this hypothesis.

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Amino-acid Composition of the Acquired Pellicle of Human Tooth Enamel

AFTER eruption human teeth acquire a structureless film, tenaciously attached to the enamel surface, which may be liberated from clean, debris-free, healthy teeth by immersion in a solution of strong mineral acid. This acid-insoluble cuticular structure has been given various names by different authorities, and in a review of the confused nomenclature on this subject it was recommended that the term 'acquired pellicle' be used[1]. Various possible origins for this pellicle layer have been proposed, and while it has usually been assumed that it is most probably of salivary origin it has also been considered that it may be (*a*) a horny keratinous layer formed from oral epithelium[2]; (*b*) the remains of a collagenous cementum-like layer over the occlusal surface of the teeth[3]; (*c*) dental plaque which had become structureless due to lysis of its bacteria[4]. A recent suggestion is that it may be derived from denatured haemoglobin[5].

In an attempt to clarify the nature of the acquired pellicle, material was collected, hydrolysed and subjected to quantitative amino-acid analysis using a Technicon automatic amino-acid analyser. The results from analysis of three different pooled preparations of acquired pellicle are given in Table 1, where they are compared with an analysis of crude human salivary mucin.

Table 1 COMPOSITION OF ACQUIRED PELLICLE ON HUMAN TOOTH ENAMEL

Component	Acquired pellicle hydrolysates (1)	(2)	(3)	Human salivary mucin hydrolysate
Aspartic acid	76·60	70 09	67·75	83 61
Glutamic acid	141·15	136·00	132·31	126·69
Threonine	44·07	40 47	41·98	57·29
Serine	44·07	43·39	49·15	76 85
Proline	45·65	41·72	44·63	55·19
Hydroxyproline	0	0	0	0
Alanine	150 58	141·84	142·67	75·92
Glycine	82·37	81·35	82·09	96·41
Valine	50·37	63·83	45·70	51·47
Iso-leucine	33 05	30·87	28·96	38 66
Leucine	66·11	72·17	64·29	85·47
Tyrosine	17·84	20·44	20·72	26·55
Phenylalanine	27·25	28·37	26·83	32·60
Ornithine	28 35	25 45	28·16	6·75
Lysine	52 47	55·07	61·11	61·71
Hydroxylysine	0	0	0	0
Histidine	17·84	27·53	27·10	19 10
Arginine	50·85	40 47	37·73	45·18
Cysteine	0	0	0	0
Methionine	0	0	0	0
Hexosamine	41·45	40·88	48·09	21·19
Muramic acid(?)	20·45	20 86	20·49	0
Unknowns	9·44	19·19	30 31	39·36

All results are expressed as residues per 1,000 residues. Unknown components were calculated as *nor*-leucine equivalents.

The three different hydrolysates of acquired pellicle show a fairly close agreement, indicating that the structure is probably derived from material with a characteristic constant composition. Distinctive features are the relatively high concentration of glutamic acid and alanine, and the absence of both hydroxyproline and hydroxylysine, these latter features eliminating the possibility that the enamel cuticular structure under investigation is wholly or partly collagenous in nature. The apparent absence of detectable amounts of sulphur-containing amino-acids implies the absence of keratin structures. Nor do the results lend support to the concept of a haematogenous origin for the pellicle: human haemoglobin contains relatively large amounts of leucine, lysine and histidine, but no isoleucine—features not observed in the pellicle analyses. In the analyses of acquired pellicle only 43 per cent to 45 per cent of the total material was accountable for as amino-acids, and some 4 per cent to 5 per cent was shown to be hexosamine. Carbohydrate analysis using an anthrone method[6] gave total carbohydrate contents of about 15 per cent, a result representing a minimum value for the carbohydrate components. The pellicle amino-acid levels show a fair degree of similarity to those in the salivary mucin hydrolysate, the main difference being the lower levels of

alanine and ornithine in the mucin sample. Like the pellicle, only about half the mucin sample was accounted for as amino-acids, the mucin also containing large amounts of carbohydrate material, including hexosamine. From these results it can be inferred that the acquired pellicle on human tooth enamel is probably a structure derived from salivary material.

In chromatograms of the acquired pellicle there were small amounts of components not identifiable as common amino-acids. One such component, both from its position on the chromatograms and its non-separability from an added synthetic sample of muramic acid, led to its tentative identification as this substance. Since muramic acid is a component specifically associated with bacterial cell walls its presence, if confirmed, would indicate a degree of contamination of the pellicle by bacteria, a feature which is also inferred by routine bacteriological staining of individual pellicles. It is considered that the bacterial cell wall content of the pellicle could be as high as 40 per cent or more.

In summary, the acquired pellicle on human tooth enamel shows compositional similarities to human salivary mucin and it seems probable that the structure is a material of salivary origin with significant quantities of bacteria embedded or attached to it. The purchase of the Technicon automatic amino-acid analyser was made possible by a grant from the Medical Research Council.

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Structure of 'Complex' Phospholipids

COLLINS has given evidence for the presence of 'complex' phospholipids in animal tissues, and he suggested that they contained a phosphate triester group. He further suggested that they break down on silicic acid to give moieties having the currently accepted structures of the known phospholipids[1,2]. Considerations of stability led de Koning to put forward alternative structures involving pyrophosphate bonds[3].

As both suggested classes of structure have no negatively charged groups, micelles formed from a naturally occurring phospholipid mixture containing a high proportion of 'complex' phospholipids should bear a net positive charge due to the primary amine and quaternary ammonium groups. To test this possibility, measurements of electrophoretic mobility were made on emulsions of hen's egg yolk phospholipid and sheep brain phospholipid, both extracted according to the method of Wheeldon and Collins[4].

The electrophoretic mobilities were measured in the microelectrophoresis apparatus of Bangham *et al.*[5] at 25° C, and the results are presented in Fig. 1. Except for the lecithin, which exists as a zwitterion over a wide range of *p*H (ref. 6), the samples were negatively charged at *p*H values higher than the *p*K of carboxyl and primary phosphate groups. The mobility of the egg yolk phospholipid (curve *B*) is that to be expected from a mixture of 80 per cent of lecithin (curve *A*) and 20 per cent of phosphatidyl ethanolamine (curve *C*), both of which were isolated by silicic acid chromatography. This agrees with the accepted composition[7]. The high negative charge on the sheep brain phospholipid (curves *D* and *E*) is, however, inconsistent with the suggestion that 60 per cent of the phospholipids in sheep brain are in the form of tertiary esters[1].

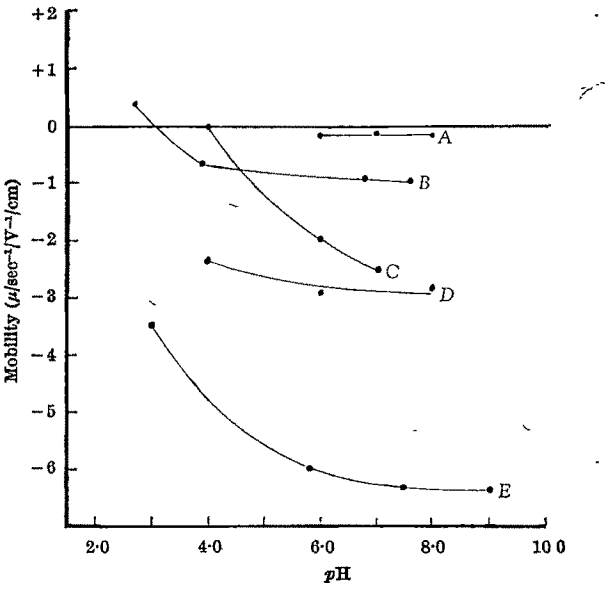


Fig. 1. The electrophoretic mobility of phospholipid micelles at various *p*Hs. The *p*H was adjusted by addition of sodium hydroxide or hydrogen chloride. *A*, Lecithin, isolated from hen egg yolk phospholipids by silicic acid chromatography, emulsified in 0 01 M sodium chloride; *B*, freshly isolated hen egg yolk total phospholipid emulsified in 0 01 M sodium chloride; *C*, phosphatidyl ethanolamine, isolated from hen egg yolk phospholipids by silicic acid chromatography, emulsified in 0·01 M sodium chloride; *D*, freshly isolated sheep brain phospholipid emulsified in 0·1 M sodium chloride; *E*, freshly isolated sheep brain phospholipid emulsified in 0 01 M sodium chloride

In general the net negative charge of naturally occurring phospholipid micelles is well documented[8] and, apart from the rather unlikely possibility that the charge on the surface of a phospholipid micelle does not reflect the ionogenic groups of the molecules forming it, the 'complex' phospholipids cannot have the tertiary ester phosphate structure suggested by Collins[1] or the pyrophosphate structure suggested by de Koning[3].

The experimental work was carried out at the Institute of Animal Physiology, Babraham, Cambridge, England. I am indebted to Dr. A. D. Bangham for the use of his microelectrophoresis apparatus.

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Dehydrogenase Isoenzymes from Legume Root Nodules

ALTHOUGH dehydrogenase isoenzymes, especially lactate dehydrogenases, have been extensively studied in vertebrate tissues[1–4], there is little information available about molecular heterogeneity among plant dehydrogenases. During enzymological studies of root nodules from three different legume species, multiple forms of lactate dehydrogenase (L-lactate NAD oxidoreductase; 1.1.1.27), malate dehydrogenase (L-malate NAD oxidoreductase; 1.1.1.37) and glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate NADP oxidoreductase; 1.1.1.49) were observed when the soluble proteins were separated by starch-gel electrophoresis[5].

The plants—*Glycine max*, *Medicago sativa* and *Trifolium repens*—were grown in quartz sand supplied with a modified Crone's nitrogen-free solution[6] and suitable strains of nodule bacteria. After 7 weeks, the nodules were removed and crushed in a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 30,000*g* for 30 min at 0° C and the clear supernatant was used for electrophoresis.

After electrophoresis[7], dehydrogenases were located on the gels by a method essentially the same as that of Markert and Ursprung[8]. For visualizing different dehydrogenases, individual gels were placed in one of the following solutions and incubated at 18° C for 16 h. The medium for locating lactate dehydrogenase[9] was prepared immediately before use from 9·0 ml. 0·5 M sodium lactate, 2·0 ml. NAD (10 mg/ml.), 5·0 ml. phenazine methosulphate (1·6 mg/ml.), 5·0 ml. nitro blue tetrazolium (1 mg/ml.), 45·0 ml. 0·2 M *tris*-HCl buffer *p*H 8·0. A similar medium was used for detecting malate dehydrogenase except that 0·2 *M* malic acid was substituted for sodium lactate. For revealing glucose-6-phosphate dehydrogenase, the substrate, coenzyme and buffer from the foregoing reaction mixture were replaced by 9·0 ml. of 0·02 M glucose-6-phosphate (disodium salt), 2·0 ml. NADP (10 mg/ml.) and 45·0 ml. 0·2 M *tris*-HCl buffer *p*H 7·4.

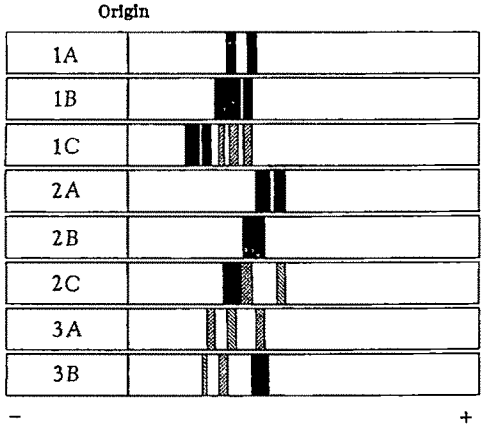


Fig. 1. Dehydrogenase zymograms from legume root nodules. Relative intensities of bands are indicated by shading. 1, *Trifolium repens*; 2, *Medicago sativa*; 3, *Glycine max*; *A*, lactate dehydrogenase; *B*, malate dehydrogenase; *C*, glucose-6-phosphate dehydrogenase

Nodules from different legume species displayed characteristic and reproducible dehydrogenase zymograms (Fig. 1). Two lactate dehydrogenase isoenzymes were visible on zymograms prepared from *T. repens* and *M. sativa*, while those from *G. max* had three isoenzymes. A similar situation was found with malate dehydrogenase, with three isoenzymes on zymograms prepared from *G. max*, two on those from *T. repens* but only one band from *M. sativa*. In contrast, however, when glucose-6-phosphate dehydrogenase zymograms were examined, only one band was detected in *G. max*, while *M. sativa* had three isoenzymes and *T. repens* five.

Further work is in progress, particularly during nodule differentiation, to determine the physiological significance of these multiple dehydrogenases.

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Induction of the Cystine-hypophosphite Reaction by Radical Initiation

THE ability of hypophosphorous acid, or its salts, to reduce the disulphide bond in cystine, peptides and proteins to the corresponding thiols has been reported earlier[1,2]. The reaction is induced either by heating or by irradiation with ultra-violet light.

In the light-induced reaction it has been shown[3] that the thiyl radicals formed by photolysis of the disulphide linkage react with the hypophosphite group (probably as the reactive enol tautomer $(H_3O_2)P\cdot$) to form a quadricovalent phosphoranyl radical of the type proposed by Walling and Rabinowitz[4]. (For the sake of simplicity the formula $(H_3O_2)P$ will be used for hypophosphorous acid as well as its salts and anions.) This radical, (H_3O_2)—$\dot{P}$SCy (where Cy = HOOC—CH(NH_2)—CH_2—), in turn reacts with intact cystine to form a phosphonium-type of double salt $[(H_3O_2)\dot{P}SCy]$-SCy which hydrolyses in water to phosphite and cysteine. Both intermediates are found[3] to be present in the reaction mixture. Neither the radical nor the double salt could be detected in the reaction products of cystine reduced by hypophosphite at elevated temperatures. This is probably due to the instability of these compounds at these temperatures[5].

Initiation of the reaction between cystine and hypophosphite by the use of a radical initiator of the chemical type has now been proved possible and, as in the case of the light-induced reaction, the products contain both the phosphoranyl radical and the double salt. To 5 ml. portions of a solution of cystine (2×10^{-4} M in 0·1 M hydrochloric acid) containing various concentrations of sodium hypophosphite, additions from solutions of hydrogen peroxide (5 μl., 1 vol.) and $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$ (20 μl., 20 per cent) were made. After about 1 min of gentle shaking, any residual activity of the radical initiator was destroyed by the addition of an equal volume of a saturated solution of the disodium salt of ethylenediamine tetra-acetic acid in water. The thiol content of the solution was then determined by Ellman's method[6]. This experiment was also repeated at *p*H 4·7 (0·2 M acetate buffer) instead of in 0·1 M hydrochloric acid medium.

Table 1. INFLUENCE OF THE HYPOPHOSPHITE CONCENTRATION ON THE EXTENT OF THE REDUCTION OF CYSTINE

Solvent	Hypophosphite concentration (M)	Reduction (per cent)
0·1 M hydrochloric acid	0·01	16·2
	0·10	61·3
	1·00	68·5
0·2 M acetate buffer (*p*H 4·7)	0·01	16 0
	0·10	64·5
	1·00	69·5

The results (see Table 1) show that the extent of the reduction increases with an increase in the hypophosphite concentration. This is in agreement with the observation that the quantum yield of cysteine in the light-induced reaction increases with an increase in the hypophosphite concentration[7]. The reason for this behaviour is that a higher hypophosphite concentration enhances the probability of a thiyl radical reacting with a hypophosphite group before being eliminated by reaction with other compounds.

Paper ionophoresis of the reaction mixture, with 10 per cent acetic acid as electrolyte, showed the presence of two compounds which gave positive reactions with both ninhydrin reagent and the perchloric acid/molybdate reagent[8] (indicating the presence of amino groups and phosphorous compounds, respectively). The ionophoretic mobility of these compounds furthermore corresponded with those of (H_3O_2)—$\dot{P}$SCy and $[(H_3O_2)\dot{P}SCy]$-SCy.

These results show that the cystine/hypophosphite reaction follows a single mechanism regardless of whether the reaction is induced by ultra-violet irradiation or by free-radical initiators. In the latter event the first stage of the reaction—that of the production of a thiyl radical

—is the result of a radical transfer mechanism while the formation of thiyl radical in the former case follows the absorption of light quanta by cystine.

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Reversible Action of Pullulanase

THE enzyme pullulanase[1] is a bacterial *R*-enzyme in that it is capable of cleaving certain α 1→6 glucosidic bonds where these occur together with α 1→4 glucosidic bonds as in pullulan, starch and dextrins. The substrate specificity of this enzyme has recently been reported[2] to embrace oligosaccharides and dextrins containing as few as two 1→4 linked α-D-glucopyranoside units in the '*A*' chain and two such units in the '*B*' chain. Thus, a tetrasaccharide linked 2 on 2 is the smallest known substrate for the hydrolytic action of this enzyme[2].

A recently developed technique[3] for examining enzyme action on micro-quantities of individual compounds of a mixture is to separate the compounds in one dimension by chromatography of a single spot on a large sheet of filter paper. The resolved components are then sprayed with an appropriately buffered enzyme, dried, and the ensemble is submitted to chromatography in a direction at right angles to the original direction. Use of this technique revealed that pullulanase acts on branched oligosaccharides (from action of malt α-amylase on amylopectin) to give the expected G_2 (maltose), G_3, and higher linear oligosaccharides. Unexpectedly, a small amount of material with the R_F of a branched tetrasaccharide was also formed from maltose. Thus, there is an apparent synthetic reaction.

We have tested this synthetic action of pullulanase on a larger scale by treating carefully purified, chromatographically pure G_2 with pullulanase, and subjecting the digest to paper chromatography. A well-defined spot appeared in the region characteristic for a tetrasaccharide containing a 1→6 linkage. In addition, there was a weak spot corresponding to a hexasaccharide containing two 1→6 links. No other compounds apart from G_2 appeared. The extent of the reaction was found to be concentration-dependent. in that little tetrasaccharide formed at a G_2 concentration of under 5 per cent, and the amount of tetrasaccharide increased with increasing concentration up to the highest concentration of G_2 we tested (about 25 per cent). Absence of other compounds, specifically D-glucose, indicates that disproportionation or transfer reactions were not involved.

Using G_3 as substrate, we obtained a spot corresponding in position to a hexasaccharide containing a 1→6 link, with no other compounds (except G_3) apparent.

These observations indicate that pullulanase action is reversible through condensation reactions, for example: 2 G_2 → tetrasaccharide + H_2O. With G_2 as substrate, there are two possible 2 on 2 structures; with G_3, there are three possible 3 on 3 structures. Studies in progress should indicate the extent to which each of these structures is produced during the reaction. The interesting possibility arises that pullulanase may condense G_2 and higher oligosaccharides with relatively long amylose chains. Such reactions could occur during the action of pullulanase on amylopectin, thus preventing complete attack by β-amylase. In a test system containing G_2 plus amylose there was definite evidence for condensation.

Similar condensation reactions should be possible with amylase; and, in fact, we were able to obtain a weak G_4 spot from G_2 by action of β-amylase. The amount produced was much less than the amount of tetrasaccharide formed by pullulanase action, however, owing to the difference in free energy of hydrolysis of 1→4 and 1→6 glycosidic bonds.

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Influence of Cortisone on the Substrate-induction of Enzymes in Rat Liver

Civen and Knox[1,2] and Feigelson and Greengard[3,4] concluded from their experiments with tryptophan-pyrrolase and tyrosine-α-ketoglutarate-transaminase that in animal tissues a distinction can be made between the two types of induction: one caused by substrate and the other caused by hormone. While substrate-induction was found to be due to stabilization of the enzyme and saturation with coenzyme, the cortisone acted by stimulating the synthesis of *m*RNA. The latter process could be inhibited by actinomycin *D*.

Previously we showed that in intact animals actinomycin *D* significantly inhibited substrate-induction of both the enzymes mentioned heretofore[5]. Substrate-induction, therefore, might also be connected with synthesis of *m*-RNA.

We used adrenalectomized rats for our investigations. Tryptophan-pyrrolase and tyrosine-α-ketoglutarate-transaminase were induced either by substrate or small amounts of cortisone or by the combination of both. The activity of tryptophan-pyrrolase was measured by the method of Knox *et al.*[6], the one of tyrosine-α-ketoglutarate-transaminase according to Rosen *et al.*[7].

On injection of 500 mg/kg L-tryptophan the enzyme activity increased 6-fold (see Table 1). This was due, of course, to the stabilization of the enzyme and to the saturation with coenzyme. Actinomycin *D* cannot change this effect. 2·5 mg/kg cortisone instead of L-tryptophan did not bring about any increase of activity. However, in the case of substrate applied together with cortisone, the hormone in an amount which cannot induce tryptophan-pyrrolase by itself, the enzyme activity even rose ten times as much. The additional stimulation is completely prevented by actinomycin *D*.

Neither the injection of 600 mg/kg L-tyrosine nor of 2·5 mg/kg cortisone led to an increase of the enzyme activity (see Table 2). But in combining the two substances one observed a 3-fold higher activity than in the control. Again, this effect does not take place in the presence of actinomycin *D*.

Table 1. INDUCTION OF TRYPTOPHAN-PYRROLASE IN ADRENALECTOMIZED RATS

	+ Cortisone	+ L-Tryptophan	+ Cortisone + L-Tryptophan	+ Cortisone + L-Tryptophan + Actinomycin *D*	+ L-Tryptophan + Actinomycin *D*
1·0 ± 0·4*	0·7 ± 0·3	6·4 ± 1·3	10·3 ± 1·3	5·9 ± 2·3	6·2 ± 2·4

* μMoles kynurenin/g liver/h.

The animals were killed 3 h after the intraperitoneal application of the substances. L-Tryptophan, 500 mg/kg: cortisone, 2·5 mg/kg; actinomycin *D*: 450 μg/kg 1 h before the other substances. Each point 6–10 animals.

Table 2. INDUCTION OF TYROSINE-α-KETOGLUTARATE-TRANSAMINASE IN ADRENALECTOMIZED RATS

+ Cortisone	+ L-Tyrosine	+ Cortisone + L-Tyrosine	+ Cortisone + L-Tyrosine + Actinomycin D
0·25 ± 0 04*	0 22 ± 0 05	0·32 ± 0 08	0·74 ± 0 12	0·36 ± 0 07

* μMoles p-hydroxyphenylpyruvic acid/mg protein/h.

The animals were killed 4 h after the intraperitoneal application of the substances. L-Tyrosine, 600 mg/kg, cortisone, 2 5 mg/kg; actinomycin D, 450 μg/kg 1 h before the other substances. Each point represents 6–10 animals

These results offer the conclusion that in animal tissues substrate-induction is dependent on small amounts of cortisone at least for the enzymes reported. As is known for other hormones, the cortisone seems to activate the synthesis of *m*RNA, which then brings forward the substrate-induction. Therefore we feel that no clear distinction can be made between substrate and hormone-induction.

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PHYSIOLOGY

Depolarization of Central Terminals of Group Ia Muscle Afferent Fibres during Desynchronized Sleep

THE stage of deep sleep is characterized by a desynchronized electroencephalogram (EEG), periodic bursts of rapid eye movements and a great reduction of the electromyographic (EMG) activity of dorsal cervical muscles. There is also a tonic depression of spontaneous EMG activity in both flexor and extensor muscles, and a further phasic reduction of residual muscular activity occurs synchronously with the periodic bursts of rapid eye movements[1]. Both the heteronymous[2,3] and the homonymous[4] monosynaptic reflexes are tonically depressed throughout the desynchronized phase of sleep. A further phasic depression frequently occurs at the time of the bursts of rapid eye movements[4]. This suggests that an abrupt but transient increase of the barrage of descending inhibitory impulses occurs phasically in relation to the rapid eye movements of deep sleep[4]. Muscular twitches have also been found to predominate during the bursts of rapid eye movements[1]. These occur, paradoxically, just when the spontaneous muscle activity[1] and the spinal reflexes[4] are most profoundly depressed.

The tonic depression of the monosynaptic reflexes during desynchronized sleep is due to hyperpolarization of the α motoneurones produced by descending supraspinal inhibitory volleys because the recurrent discharge of the α motoneurones is depressed throughout desynchronized sleep[5]. The phasic inhibition of the monosynaptic reflexes that occurs during rapid eye movement has no counterpart, however, in an enhanced depression of the recurrent discharge[5]. This finding led to the prediction[4,5] that the phasic depression of spinal reflexes during the period of rapid eye movement of desynchronized sleep is the result of presynaptic inhibition of the group Iα afferent pathway. The present experiments, in which the excitability of the spinal endings of group Iα muscle afferents was tested following Wall's method[6], have proved this prediction to be correct.

The experiments were performed on unrestrained, unanaesthetized cats. Electrodes for recording the EEG, the EMG of the dorsal cervical muscles and the ocular movements were implanted chronically under 'Nembutal' anaesthesia. The ventral roots from *L4–5* to *S2* were then severed in order to obtain complete de-efferentation. The excitability of Iα primary afferent fibres was tested by recording the antidromic responses to single shock applied unipolarly to the ventral horns through a stainless-steel microelectrode (100,000–600,000 Ω), chronically implanted and completely insulated. The tibial and the deep peroneal nerves were prepared and mounted on chronically implanted bipolar electrodes so that the antidromic response to ventral horn stimulation could be recorded in either the extensor (tibial) or flexor (deep peroneal) muscle afferents. The same electrodes could be used for stimulation.

Preliminary observations showed that under conditions of favourable population distribution of primary afferent fibres in proximity to the stimulating electrode, the number of primary afferent fibres excited was linearly related with the intensity of the stimulation. Hence, the amplitude of the antidromic spike potential could be taken as a measure of the excitability of their central segments. Rectangular pulses (0·05 msec at 1·5–2/sec) were used at a strength of 1·2–1·8 times the threshold for the antidromic response. Because the ventral roots had been cut, the spike potential so produced in the muscle nerves was due only to impulses propagating antidromically along afferent fibres. Measurements of conduction velocity indicated that group Iα afferent fibres (120–80 m/sec) were involved in the antidromic response. The size of the triphasic spike potential was taken as a measure of the number of group Iα fibres excited by the stimulus.

During relaxed wakefulness, characterized by EEG desynchronization and tonic activity in the neck muscles, the group Iα antidromic response occurred after each stimulus and was rather regular in its amplitude. Slight fluctuations of these responses corresponding to standard deviations of the order of 10–12 per cent of the mean amplitude could be detected. During a spontaneous or induced orienting reaction the Iα antidromic volley changed in an unpredictable way. In most instances, however, a short-lasting increase in the antidromic spike occurred at the beginning of an induced arousal (Fig. 1, *A*, *B*, *C*; Fig. 2, *A*, *B*, *C*).

During transition from quiet waking to drowsiness or to synchronized sleep, there was no significant change in the mean amplitude and standard deviation of the Iα antidromic volley nor was there any significant change in these values found during the EEG spindles and the interspindle lulls.

The main change in excitability of the central terminals of the Iα afferent fibres occurred during desynchronized sleep. In this stage of sleep there was an increase in the mean amplitude of the Iα antidromic spike as compared with synchronized sleep. The mean value, however, never increased above 105–110 per cent of the mean amplitude

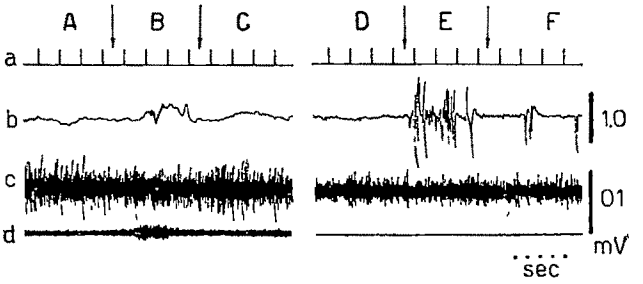


Fig. 1. Unrestrained, unanaesthetized cat Experiment made 2 days after chronic implantation of the electrodes and section of the ventral roots *L5–S2* on the left side. (*a*) Stimulus artefact by stimulating the left ventral horn at the upper level of *S1* with rectangular pulses at one every 1·7 sec, 0·05 msec duration and 1·62 times the threshold for the antidromic Iα volley recorded from left tibial nerve; (*b*) eye movements; (*c*) left parieto-occipital (EEG); (*d*) posterior cervical muscles (EMG). (*B*) Induced orienting reaction during drowsiness. (*E*) Burst of rapid eye movement during desynchronized sleep

observed during synchronized sleep. The increase in mean amplitude was also accompanied by an increase in the standard deviation (15–17 per cent of the mean amplitude). These changes cannot be considered as tonic in character because they were observed only when the episodes of desynchronized sleep were associated with large bursts of rapid eye movement. Abortive episodes of deep sleep, characterized by absence of ocular bursts, or episodes of deep sleep associated with ocular movements isolated or grouped in small bursts were not accompanied by any significant change in the mean amplitude of the antidromic volley. In the last cases only a slight increase in the standard deviation was observed.

An analysis of the relation between excitability changes of the Iα fibres and phasic events occurring during desynchronized sleep indicates that phasic increase of the antidromic volley appeared during the bursts of rapid eye movement (Fig. 1, *D*, *E*, *F*; Fig. 2, *D*, *E*, *F*), particularly when the bursts of ocular movements were rather intense. The phasic increase sometimes outlasted the period of rapid eye movement. The more intense the rapid eye movement burst, the more profound was the enhancement likely to be. The excitabilities of the Iα afferent fibres were then increased up to 110–140 per cent of the mean value observed during synchronized sleep both in tibial and deep peroneal nerves. Not every burst of rapid eye movement was associated with an increase in the antidromic Iα volley, and there were variations in antidromic Iα volley unrelated to the rapid eye movement.

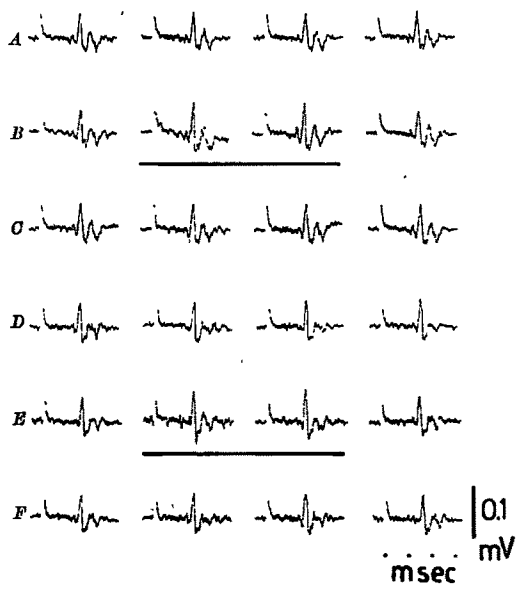


Fig. 2. Same animal as in Fig. 1, same experiment. The records show the antidromic volley of Iα afferent fibres recorded from left tibial nerve on stimulation of their central terminals. *A*, *B* and *C*, Before, during and after an orienting reaction (see *A*, *B* and *C* in Fig. 1). *D*, *E* and *F*, Before, during and after a burst of rapid eye movement (see *D*, *E* and *F* in Fig. 1). The horizontal lines indicate the enhanced responses during induced arousal (*B*) and during the burst of rapid eye movement (*E*)

Once the excitability of Iα muscle afferent fibres had been tested during physiological sleep, the animal was anaesthetized with 'Nembutal', paralysed with 'Flaxedil' and artificially respirated. By using the same stimulating and recording system utilized in the unrestrained cat, the excitability changes produced by maximal group I stimulation of the deep peroneal nerve on Iα fibres of the tibial nerve were measured. Fig. 3 shows the percentage increase in amplitude at the various intervals of the testing Iα antidromic volley in tibial nerve after conditioning by seven deep peroneal volleys, 1·6 msec apart. The time course of the excitability changes produced by the deep peroneal volleys in group Iα afferent fibres of the tibial nerve has a summit at a latency of 20–25 msec and a total duration of 300 msec, which is in agreement with previous observations[7]. The changes in excitability of the group Iα afferent fibres, produced by supraspinal volleys during the bursts of rapid eye movement, can therefore be related to similar effects produced on the same fibres by spinal afferent volleys (presynaptic inhibition)[7].

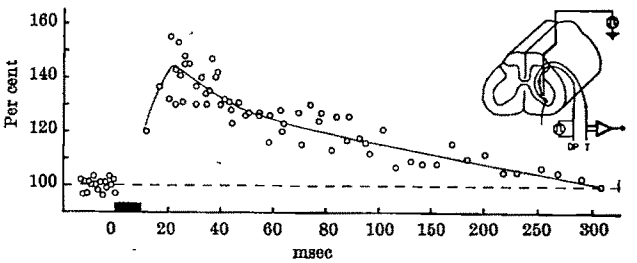


Fig. 3. Time course of excitability changes produced in group Iα afferent fibres. A stainless-steel microelectrode having a resistance of 600,000 Ω was chronically inserted into the motoneurone pool supplying the tibial nerve. After the analysis of the modulation during sleep of the Iα antidromic volley in tibial nerve was made in unrestrained cat, the animal was anaesthetized with 'Nembutal' and paralysed with 'Flaxedil'. The motoneurone pool was stimulated with single negative pulses of 0·05 msec duration, 2·4 times the threshold for the antidromic Iα volley. The testing stimulus was applied at various intervals after seven group I deep peroneal conditioning volleys, 1·6 msec apart. The size of the spike so produced at any test interval is matched against the control series in order to determine the percentage increase in excitability. Percentage increases are plotted as ordinates in the graph, against the conditioning intervals as abscissae. A diagrammatic illustration of the experimental arrangement is shown on the right side

It is postulated that the increase in excitability observed during desynchronized sleep is actually due to supraspinal influences exerting synaptic depolarizing action on terminals of group Iα primary afferents. These influences are phasic in nature and appear to be related with the large bursts of rapid eye movement. This phasic presynaptic inhibition may account for the striking depression of the homonymous monosynaptic reflex that occurs during the bursts of rapid eye movement[4].

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Organ Preservation using Ultrabaric Oxygen

ONE of the problems in grafting borrowed organs between human subjects is to obtain an adequate supply of donor material. In some cases a transplant is made on the first sign of clinical death in the donor, a technique which requires considerable organization and great care with timing. A second approach is to obtain the donor organ from the victims of violence, such as motor-car accidents. The latter are probably the most frequent potential sources of donor material but suffer the great disadvantage that the scene of the accident may be remote from the centre at which the transplant is to be made, and the problem of maintaining life in the donor organ is an acute one. One method of maintaining vitality has been

to connect the suitably treated organ to a perfusion apparatus in which it is supplied with oxygenated blood until needed for use. The perfusion and associated cooling apparatus is complicated, expensive and unlikely to be available on any large scale, and for this reason one of us (A. D. B.) suggested that, in view of successful experiments in other fields of surgery with hyperbaric oxygen at a few atm. pressure, it might be worth trying the experiment of seeing whether greater tissue permeation and maintenance of vitality could be produced by the use of 'ultrabaric' oxygen—that is, oxygen at a pressure of some thousands of pounds per in.^{2}. The experiment has now been carried out.

The right and left kidneys were removed from a 19-kgm male dog. Both kidneys were placed within a pressure vessel connected to a source of high-pressure oxygen. The pressure within the vessel was increased to 1,950 lb./in.^{2} in 5 min. During the following 120 min the pressure fell to 1,450 lb./in.^{2} due to a slight leakage. Subsequent tests indicated that under similar conditions the bulk temperature of the kidneys was lowered to 23° C during the period of subjection to the decreasing high pressure. The lowering of the pressure from 1,450 lb./in.^{2} to atmospheric pressure was accomplished in 3 min. The right kidney was immediately preserved in formalin solution. The anastomoses between the recipient's right iliac and the donor's left renal vessels were completed in 29 min, giving a total ischaemia time of 165 min. The animal voided 30 c.c. of urine immediately after recovery from anaesthesia (the donor ureter having been implanted into the bladder). One day after the operation the creatinine level was 2·7 mg/100 c.c. and on the second day the blood urea nitrogen reached 140 mg/100 c.c., both being the maximum values attained. Prior to removal of the kidneys, the measured values of these quantities were 0·9 mg/100 c.c. and 40 mg/100 c.c. respectively. Intake and urinary output daily each varied within the range 400–1,300 c.c. Clinically, the condition of the dog at the time of killing on day 39 after operation was good. The blood urea nitrogen was 47 mg/100 c.c. and the creatinine level 1·6 mg/100 c.c. Microscopically, sections of both kidneys showed no parenchymatous damage apart from superficial patchy subcapsular atrophy and fibrosis in the autotransplanted kidney, which may have been secondary to surgical trauma.

The importance of this experiment lies in the fact that the apparatus needed to preserve the kidney over a period of ischaemia of 165 min consists merely of an oxygen cylinder and a suitable pressure jar in which the organ can be placed. No basic refrigeration equipment is needed, nor any elaborate pump. Pressurized oxygen is available in almost all ambulances at the present time and the pressure vessels can be constructed quite cheaply, especially if they become commonly used.

The really surprising thing about the foregoing experiment is that, because of the rapid lowering of pressure to atmospheric, the kidney effervesced and effectively suffered such explosive decompression that it might be expected to be irreparably damaged. Since this was not the case it would seem clear that the technique is promising. Further experiments to prolong the ischaemia time with improved renal function are in progress and will be reported in due course.

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Pyrogens and Monoamine Oxidase Inhibitors

MANY nerve terminals within the hypothalamus contain either noradrenaline or 5-hydroxytryptamine (5-HT) (ref. 1). It has been suggested that these amines may play a part in the thermo-regulatory pathways[2–4].

In the cat, body temperature rises after intracranial injections of 5-hydroxytryptamine in the area of the hypothalamus[3]: in the rabbit, injections of noradrenaline have the same effect[4]. If liberation of these amines plays a part in temperature regulation, monoamine oxidase inhibitors might be expected to cause some abnormality of temperature regulation. For this reason we have examined the febrile response to intravenous injections of leucocyte pyrogen in rabbits pretreated with monoamine oxidase inhibitor (pargyline hydrochloride, Abbott, Ltd.).

Leucocyte pyrogen[5] was prepared and given, in doses of 0·25 ml. and 0·50 ml. intravenously, in two groups each of ten rabbits. The effect of an intravenous dose of 25 mg pargyline was assayed in a group of nine rabbits. The pyrogenic response to the two doses of leucocyte pyrogen was tested in the original groups of rabbits 90 min after they had received 25 mg pargyline intravenously. The responses to leucocyte pyrogen were measured again 3 weeks after the pargyline had been given.

In animals receiving the smaller dose of leucocyte pyrogen, pre-treatment with pargyline resulted in a fever initially similar to that following the control injections, but more prolonged; pargyline given without pyrogen caused a small slow temperature rise, averaging 0·3° C 4 h after injection. The time course of this effect may well account for the apparent prolongation of the fever curve in the animals given leucocyte pyrogen after pargyline.

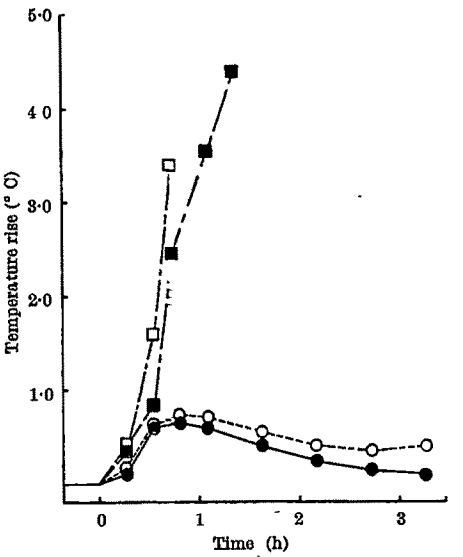


Fig. 1. Temperature responses to leucocyte pyrogen (0·5 ml.) with and without pargyline pretreatment. ● Mean response of ten rabbits receiving leucocyte pyrogen alone; ○, mean response of eight rabbits receiving leucocyte pyrogen after pretreatment with pargyline; ■ and □, responses of two rabbits which died with hyperpyrexia

With the larger dose of leucocyte pyrogen, pargyline caused a similar prolongation of fever in eight of the animals. The average temperature changes in these animals are shown in Fig. 1. In two animals there was a rapidly progressive temperature rise, and both died with rectal temperature above 44° C. This kind of response has not previously been observed by us in more than 200 animals receiving this dose of leucocyte pyrogen.

This hyperpyrexial response seemed to be related to the combined administration of pyrogen with a monoamine oxidase inhibitor, though we do not know why it should only have been manifest in two animals.

Previously the hypothesis that amines may be concerned in temperature regulation has depended on the observations that temperature changes are induced by injection of these substances into the hypothalamus. The observation that an amine oxidase inhibitor can modify a pyrogenic response in some animals is compatible with this hypothesis; though it is clearly unjustifiable to apply these findings directly to man, it seems possible that febrile illnesses might be modified in some patients receiving monoamine oxidase inhibitors.

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Lipotropic Effect of Cold on Rat Liver

Sellers and You[1,2] have shown that the total liver lipids were decreased when rats were fed a hypolipotropic diet in a cold environment. Treadwell *et al.*[3] found that a cold environment exhibits a lipotropic effect on liver triglycerides of rats fed a high fat-cholesterol diet. This observation has also been confirmed on rabbits fed an atherogenic diet[4]. The aim of our experiments was to elucidate the mechanism of the lipotropic effect of cold.

White male rats (Wistar stock, weight 180–200 g) were fed *ad libitum* a high fat-cholesterol diet[5] for a period of 7–13 weeks. One group was kept at 22° C; a second group was exposed for 8 h daily to a temperature of 2° ± 1° C. Sodium acetate labelled with carbon-14 (40 μc. per 100 g body-weight) was injected intraperitoneally into the rats 3 h before they were decapitated. After isolation of the total fatty acids of the liver[6] their specific activity was determined. In all animals the concentration of esterified fatty acids[7] and glycogen[8] in the liver and the levels of free fatty acids in the blood serum and epididymal fat tissue were determined[9]. The same analyses were also performed on a group of white rats fed normal Larsen diet which were kept at room temperature. Each group consisted of ten to fifteen animals.

In the rats kept at room temperature, the feeding of high fat-cholesterol diet caused significant increase of the concentration of esterified fatty acids in the liver ($P < 0{\cdot}01$) and a pronounced decrease in liver glycogen ($P < 0{\cdot}001$). This effect of high fat-cholesterol diet was not found in the group kept at 2° C; the concentrations of liver esterified fatty acids and glycogen in this group were nearly the same as in the rats fed control Larsen diet (Table 1). These findings are in agreement with the results of other authors[1–4] and confirm the lipotropic effect of cold environment on the liver. The concentration of free fatty acids in the blood serum and epididymal fat tissue was significantly elevated in the cold acclimatized group (Table 2); this points to an increased mobilization of fatty acids from fat depots. Biosynthesis of fatty acids from acetate-1-^{14}C in the liver was lowered in the group exposed to cold, as can be seen from the low specific activity values of fatty acids ($P < 0{\cdot}01$) in Table 1. In the group of animals exposed to cold increased mobilization of fatty acids from reserve tissues was found and a simultaneous decrease was observed in the concentration of liver fatty acids; this is in agreement with increased ability of liver tissue to oxidize fatty acids, which was observed by some authors[10,11] in animals acclimatized to cold.

Table 1. CONCENTRATION OF ESTERIFIED FATTY ACIDS, GLYCOGEN AND SPECIFIC ACTIVITY OF TOTAL FATTY ACIDS IN LIVER OF WHITE RATS FED NORMAL AND HIGH FAT-CHOLESTEROL DIET, EXPOSED TO TEMPERATURES OF 22° AND 2° C

	Normal diet 22° C	High fat-cholesterol diet 22° C	High fat-cholesterol diet 2° C
Esterified fatty acids m.equiv./100 g	17·1 ± 1·0	21·9 ± 1·1	16·4 ± 0·8
Glycogen g/100 g	2·7 ± 0·2	0·6 ± 0·1	3·2 ± 0 2
Specific activity of fatty acids/c.p.m./ m.equiv.	12·039 ± 2·468	5·442 ± 2·349	1·674 ± 325

Table 2. CONCENTRATION OF FREE FATTY ACIDS IN BLOOD SERUM AND EPIDIDYMAL FAT TISSUE OF WHITE RATS FED NORMAL AND HIGH FAT-CHOLESTEROL DIET, EXPOSED TO TEMPERATURES OF 22° C AND 2° C

	Normal diet 22° C	High fat-cholesterol diet 22° C	High fat-cholesterol diet 2° C
Blood serum m.equiv./1,000 ml.	1·41 ± 0 14	0·86 ± 0·09	1·99 ± 0·16
Epididymal fat tissue m.equiv./100 g	0·47 ± 0·04	0·52 ± 0·04	1·28 ± 0·17

On the basis of these results we assume that the lipotropic effect of cold on the liver of rats fed high fat-cholesterol diet consists in a lowered hepatic biosynthesis of fatty acids and an increased catabolism of fatty acids in the liver tissue of cold acclimatized animals.

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A Functional Caudal Photoreceptor in Blind Cavernicolous Crayfish

SEVERAL decapod crustaceans, particularly the crayfish, possess a primitive photoreceptor in their caudal ganglion[1] consisting of only two neurones[2]. An axon courses from each of the cells rostrally along the connectives in Wiersma's[3,4] area 83–84 (ref. 2). Discharge of the receptor is characterized by a relatively long latency which is inversely related to the stimulus intensity, and by a prolonged after-discharge. Stimulation of the receptor in intact animals initiates pereiopod movements and locomotion[5]. A model of the neuronal system which may account for this phenomenon has been suggested[6].

Since the crayfish possess two independent photoreceptor systems, and since some of these animals have undergone a loss of the eyes in cavernicolous environments, experiments were carried out to determine if the blind animals had also lost their central nervous system receptors. There seems to be little doubt that some of the cave crayfish are truly blind. It has been established[7,8] that the lamina ganglionaris and the retinal elements are essentially non-existent in the highly evolved troglobitic species, *Cambarus setosus*. This same blind species, however, has been shown[9] to exhibit a locomotory response on illumination of the head. The response, furthermore, was present even when the eyestalks were removed, indicating a light-sensitive dermal or anterior central nervous system receptor. Interestingly, the locomotory reflex was not produced by illumination of the caudal region of *C. setosus*[9]. The existence of these related data suggested *C. setosus* as the experimental animal.

Specimens of *Cambarus setosus* (Faxon) were obtained from Civil War Cave, Ozark, Missouri[10], and identified[11]. Three males and three females were examined. The response of the receptors of *C. setosus* were compared with those of two epigeal species, *Procambarus clarkii* and *Procambarus simulans*. Several specimens of *P. simulans* from a cave population were also used. The latter animals show sparse pigmentation but retain functional eyes.

Preparations for recording were made by isolation of the abdominal nerve cord from ganglion 1 to 6 inclusive. The cords were allowed to remain in Van Harreveld's[12] perfusion fluid at 5° C for about 8 h to suppress spontaneous discharges. Recordings were made with the 1–2 connective suspended in air on a 0·005-in. platinum electrode. The indifferent lead was a silver wire immersed in the saline. The potentials were passed to a capacity coupled pre-amplifier and into the upper vertical amplifier of a dual beam oscilloscope. The photic stimulator consisted of a 3-V tungsten lamp with a shutter and condensing lens attached. The stimulus indicator was a Texas Instruments *LS*400 light sensor. Permanent records were made on moving film or by recording the frequency of discharge directly[13].

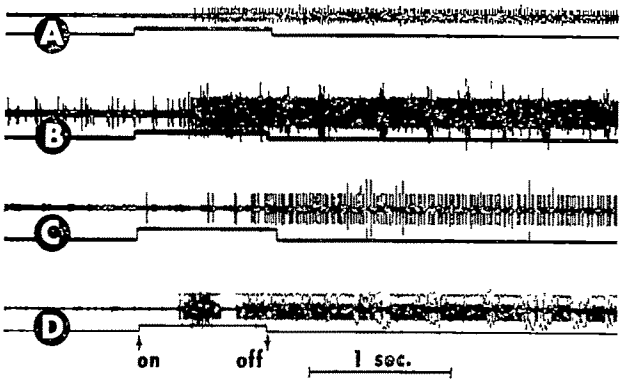


Fig. 1. Responses of sixth abdominal ganglion photoreceptors from (*A*) *Procambarus clarkii*, (*B*) *Cambarus setosus*—blind cave species, (*C*) *Procambarus simulans*, and (*D*) *P. simulans*—cave population. Each preparation received equal intensity 1-sec stimuli. Gain in (*A*) is lower than in the others. The magnitude of the responses more nearly reflects the length of time the cords were isolated than their thresholds

The responses from the caudal photoreceptors of the various species are shown in Fig. 1. Each preparation received a 1-sec flash of equal intensity. Every individual, including *C. setosus*, showed active photoreceptors in its caudal ganglion. Latencies in response to the standard stimulus were approximately equal for the receptors of all three species. This is not apparent from the recordings shown, since each preparation had been isolated for different periods before testing. The after-discharge appears to be more prolonged, however, for the receptors of *P. clarkii*, but approximately equal for *P. simulans* and *C. setosus*.

The light-activated response of the photoreceptor may be modified by appropriate synaptic input[2]. Analysis of this phenomenon led Kennedy to the conclusion that the neurones of *P. clarkii* not only possess properties of primary photoreceptors, but function as interneurones by receiving inputs from the tactile hairs of the telson and uropods. Stimulation of the contralateral roots of the sixth ganglion inhibits light-induced activity, while stimulation of the ipsilateral roots facilitates[2].

The cephalically-blind cavernicolous crayfish rely on tactile and proprioceptive information for locomotion, orientation and position sense. They may, therefore, have retained the important interneurone functions of the caudal receptors for their positive adaptive value, without loss of the photosensitive properties.

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PHARMACOLOGY

Testing Chemicals for Mutagenicity in a Mammal

WHILE admitting that mammals would be preferable to diptera for testing newly introduced drugs for possible genetic (that is, mutagenic) effects, Prof. Lüning[1] argues that tests with mice would be expensive and time consuming and therefore advocates the use of *Drosophila* larvae grown on medium containing the drug, which are then tested for chromosome loss and non-disjunction.

The mouse is, however, not so impracticable as test material for mutagenicity as Prof. Lüning implies. The most readily detectable mutation in the mouse is the dominant lethal, which is probably due to chromosome loss. It manifests itself as lethality *in utero* of embryos arising from gametes in which the mutation has arisen.

The most convenient technique is to inject males with the test compound at a dose determined from $L.D._{50}$ tests, which we can assume to have been carried out in any drug of pharmacological interest. The treated males, together with untreated controls, are then mated to untreated females. The females are dissected at mid-term (we have found the most convenient interval to be 12 days after the formation of a mating plug) and the number of implants and their condition recorded. There are two main classes of lethal embryo among the implantations. The first is the deciduoma or 'mole', an outgrowth of the uterus at the site of the implanting blastocyst which itself fails to develop much after implantation. The second type of lethal embryo has developed to a relatively advanced stage before death so that placenta and foetal membranes can be seen. The frequency of this latter kind of lethal is unaffected by mutagens and seems, therefore, to be non-genetic in this test system. The most sensitive test for mutagenicity is the proportion of all implants which are deciduomata. In control matings this runs at about 4 per cent[2], so that the doubling dose, which would yield 8 per cent, is readily detectable.

If the implantation rate (the ratio between number of implants and number of corpora lutea) is much depressed, this may indicate that too high a dose-level is being used, as a low implantation rate can result from a high mutation rate[3,4].

The sensitivity to mutagens varies greatly between the stages of spermatogenesis. To survey all the stages of spermatogenesis from spermatogonia through spermatocytes to mature sperm it would be necessary to make test matings continuously for 8 weeks after treatment. Experience with a wide range of mutagens has demonstrated, however, that dominant lethals are most readily induced in post-meiotic stages which are sampled during the first 3 weeks after treatment. The whole test would then take

about 5 weeks, using, say, thirty males (ten controls and ten at each of two dose-levels) and 360 females (four females per male per week).

Rats may also be used as test animals but, being larger, more accommodation is required to obtain the same information. The vaginal smear has to be used to detect inseminations instead of the convenient mating plug. Moreover, we have found rats unable to mate regularly with more than two females per week; so that more males are needed to produce the same number of pregnancies.

This technique has now been used to study the mutagenic action of X-rays[2], neutrons[5], radioisotopes[6], triethylenemelamine[4] and alkane sulphonic esters[7,8]. Following reports that the radio-opaque medium diodone might be mutagenic we have tested this compound with negative results[9]. Negative results have also been obtained for caffeine[10] by other workers using a similar test.

It is obviously preferable to use a mammal rather than an insect as the test animal for drug assays. The technical simplicity and the few weeks required for the dominant lethal test in mice make it a very suitable system for testing the mutagenicity of any new drug.

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Reactivation of Paraoxon-inactivated Cholinesterase in the Rat Cerebral Cortex by Pralidoxime Chloride

IT has been demonstrated by Firemark, Barlow and Roth[1] that the concentration of pralidoxime (PAM) in the rat brain cortex after a single intravenous injection of PAM-^{14}C is significantly higher than in other brain regions. The present communication reports an attempt to see whether such a relatively small dose of PAM as used by these authors (20 mg/kg) is able to reactivate satisfactorily the phosphorylated cholinesterase in the brain cortex of rats poisoned with lethal doses of paraoxon.

Female albino rats weighing 200–250 g were given 10 mg/kg of atropine sulphate followed 20 min later by a subcutaneous injection of paraoxon. Ten minutes after injecting paraoxon, 20 mg/kg of PAM (chloride) was given intravenously. One hour later the animals were killed by decapitation. Brains were quickly removed and immediately chilled in ice. The cerebral cortex (parietal region) was placed on a frosted glass slide and sliced with a razor blade. Cholinesterase activity was measured manometrically at 37° C with the following reaction mixture: 0·15 M sodium bicarbonate, 0·164 M sodium chloride and 0·01 M acetylcholine chloride in a total volume of 2·0 ml.; *p*H 7·4, gas phase nitrogen + carbon dioxide (95 : 5). Enzyme activity was expressed in μl. carbon dioxide liberated per mg of tissue (dry weight) during the first 30 min (Q value). All results were corrected for non-enzymatic hydrolysis.

The results presented in Table 1 show that 1 h after the injection of PAM the mean enzymatic activity in the cerebral cortex of rats poisoned with paraoxon was considerably higher than the activity in the cortex of rats injected with paraoxon only. The differences at each dosage group are statistically significant ($P < 0·05$). With a dose of paraoxon of 0·25 mg/kg the reactivation was on average 54 per cent. With higher doses of paraoxon (0·5 and 1·0 mg/kg) the mean reactivation was 45 per cent and 25 per cent, respectively. In the last group all animals injected only with paraoxon died 10 min after poisoning, and had a cholinesterase activity which was significantly lower than that in PAM-treated animals which survived and were killed 1 h after injection of oxime.

Table 1. CHOLINESTERASE ACTIVITY IN THE BRAIN CORTEX OF RATS INJECTED WITH PARAOXON FOLLOWED BY PAM*

Paraoxon (mg/kg)	Cholinesterase activity Without PAM Q	Without PAM Per cent	With PAM Q	With PAM Per cent	Reactivation per cent
0	12·25 ± 0·17	100	12·54 ± 0·70	100	—
0·25	5·26 ± 1·04	42	9·13† ± 1·32	73	54
0·5	2·51 ± 0·71	20	6·60† ± 1·20	53	45
1·0	3·76‡ ± 0·45	30	5·95† ± 0·30	48	25

* Each value represents the mean and standard error of the activity measured at least in five brains.
† Mean values differ significantly ($P < 0·05$) as determined by the t-test.
‡ Dead 10 min after poisoning.

These data are in contrast to the general belief that PAM, owing to its quaternary structure, does not penetrate the blood–brain barrier in a concentration which is sufficient to reactivate phosphorylated cholinesterase in the brain (reviewed in ref. 2). However, this conclusion is based mainly on results obtained with homogenates of whole brain and makes no allowance for the uneven distribution of PAM in the brain. The use of cortical slices instead of homogenates of whole brain has the advantage that the tissue slices contain most cells in an intact form. This may result in a greater effectiveness of PAM in reactivating the so-called functional cholinesterase on the cell surface than the reserve cholinesterase within the cells. The latter accounts for more than 95 per cent of the enzymatic activity in the isolated frog rectus abdominis muscle[3]. The situation in the brain might be similar and the ability of PAM to reactivate paraoxon-inhibited functional cholinesterase in the rat cerebral cortex would provide an explanation for some of its central actions which have been observed both in animals and human beings poisoned with various organophosphorus anticholinesterase agents[4–6].

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PATHOLOGY

Effect of Environmental Temperature on the Metabolic Response to Injury

THE pattern of the metabolic response by man and the rat to injury (for example, fracture of a long bone) has been under investigation for some time. The reaction is characterized by a marked rise in the urinary excretions of nitrogen, sulphur, phosphorus, potassium and creatine, which reach a peak in the rat at the third day after the injury and then slowly decline[1–3]. It was early noted that in man once shock had subsided there was also a rise in

the 'basal' consumption of oxygen and a slight rise in body temperature paralleling the increase in the catabolites[1]. It was suggested that following injury there is an increased demand for energy from endogenous sources[2,4,5]. At that time it seemed reasonable to assume that in some way by releasing energy from endogenous sources metabolism is raised following moderate to severe injury and this facilitates the healing process.

Using a direct gradient-layer calorimeter constructed by one of our colleagues, Dr. J. D. Pullar, it was shown that the average total metabolism of rats maintained on a moderately high protein diet also rises to a maximum on the third day following such injury which is also the time of the maximum excretion of nitrogen[6]. The rise was essentially due to a rise in the minimum metabolic rate which was found to be increased by 7 per cent by the third day. This could be explained in terms of a greater restlessness during the hours when the metabolic rate is normally low. From the metabolic data it was also inferred that both in man and the rat the increased heat output must arise mainly from oxidation of protein. Earlier observations had indicated that when rats were fed a diet deficient in total protein they did not respond to trauma by increasing their protein catabolism[7], for no increase in metabolic rate nor in urinary excretion of nitrogen appeared[6]. Wilkinson[8] believed that these disturbances of bodily composition in the normally nourished organism might be held to assist the patient in recovery from a major operation.

It is therefore interesting to note that Caldwell[9] recently showed in the rat that at the higher environmental temperature of 30° C the normal metabolic response to injury (burning) no longer occurred if the animal's intake of food was fixed at the pre-burn level, while animals with similar wounds but maintained at 20° C, also with their diets fixed at the pre-burn level, lost weight, had a high mortality and exhibited a negative nitrogen balance. When both environmental groups were allowed free access to food in the post-burn period these striking differences between the groups were not seen and the apparent adverse effect of restriction at the lower temperature disappeared. The rate of wound healing was found to be significantly faster at an ambient temperature of 30° C than at 20° C with both groups having free access to food. Mortality was then low in both groups and the differences not statistically significant. Caldwell's hypothesis is that when rats with burn wounds are kept at an environmental temperature below that of the surface temperature of the wound they must then compensate for the heat they lose as heat of vaporization from extra-renal water loss which occurs through the wound. This hypothesis seemed to us to demand an investigation on wounds such as fracture of the femur where surface phenomena would not be involved—or not to such an extent. According to Caldwell's theory, an obligatory heat loss requires a corresponding increase in total heat production to maintain body temperature.

In our preliminary experiments, 3-month-old male hooded Lister rats of the Rowett Institute strain were used and the animals were kept in individual cages and fed the Institute's stock diet No. 86 once daily at 9 a.m., the food being left with them. The average intake for each rat for the 3 days, that is, −5, −4, −3 days, prior to the beginning of the urinary collections was the basis of the food fed throughout the experiments. Body-weight, food intake and urinary nitrogen were measured in a direct gradient-layer calorimeter by the technique previously described[6]. All operations were performed under ether anaesthesia. The basal urinary nitrogen was the average for the 3 days (that is, −3, −2, −1 days) selected before treatment and was expressed as mg nitrogen/100 g body-weight above or below basal level (the average of the 3 days prior to treatment being taken as zero).

In the first experiment, twenty-four rats were kept in an ambient temperature of 30° C: twelve rats were fractured and twelve littermates received the same manipulation but without fracture and were termed the incision group. The food intake for the fracture group was 19·4 g/day for the pre-injury period and 18·6 g/day for the 10-day post-injury period. For the incision-only group the value remained at 19·9 g/day throughout and probably accounts for the higher urinary nitrogen excretion. The fractured animals gained 17 g in weight over the post-operation days as against 22 g gained by the incised group.

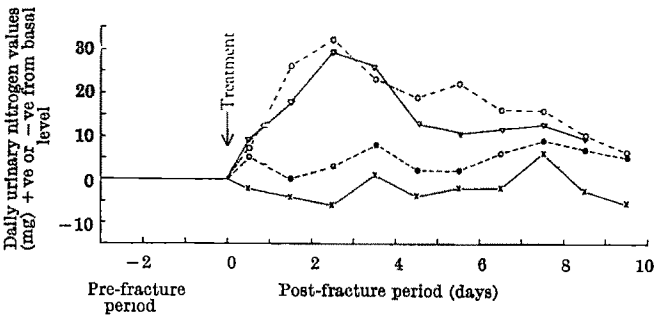


Fig. 1. Variation of daily urinary nitrogen values from basal level in mg nitrogen/100 g body-weight. ×——×, Fracture, 30° C (average of 12 rats in each group), ●- - -●, incision, 30° C (average of 12 rats in each group); O—·—·—O, fracture, 22° C (see ref. 3); ▽———▽, fracture, *c.* 20° C (see ref. 2)

The fractured group did not show the characteristic protein catabolic effect seen in similar apparently well-nourished animals held under normal environmental temperature conditions (compare with Cuthbertson *et al.*[2] and Campbell *et al.*[3]); indeed, 22 mg less nitrogen (that is, negative from base-line) was excreted in the urine/100 g body-weight over the 10-day post-operative period. The rats were apparently in positive nitrogen balance (see Fig. 1). In the incised group, 47 mg nitrogen in excess over the base-line were excreted/100 g body-weight over the 10-day period. In one of the earlier fracture experiments[3] conducted under normal laboratory temperature conditions, the urinary nitrogen excreted over the 10-day period was 177 mg/100 g body-weight in excess over the base-line; in a still earlier experiment[2] it was 146 mg in 9 days.

The disappearance of the characteristic increase in urinary nitrogen excretion following injury at 30° C was rather unexpected, but agreed with the findings of Caldwell[9]. The question arose whether the increase in heat production following injury would likewise disappear.

Our second experiment, using the gradient-layer calorimeter, was designed to answer this question. Nine male rats were placed in individual cages in the calorimeter at 30° C. After stabilization they were fractured and held in the chamber. They maintained their body-weight throughout the experimental period, but the food intake fell by 1·7 g/day/rat during the 10 days of the post-injury period (that is, from 16·0 g pre-injury to 14·3 g post-injury). There was no evidence of the increased heat output previously found to be a characteristic response to injury induced in animals at normal room temperature. A pair-fed fractured group also at 30° C, but maintained outside the calorimeter in metabolism cages, also showed a decreased urinary nitrogen excretion throughout the 10-day post-injury period, as was noted in Exp. 1—the figure being 63 mg (negative from base-line) over the 10 days, again tending towards a positive balance.

Further experiments are now in progress involving injury on animals on *ad libitum* feeding in ambient temperatures at 30° C and 20° C and the rates of healing of skin wounds are also being followed, but in view of present interest in this problem we report the present stage of our experiments for Caldwell's hypothesis would not seem to cover these observations on the disappearance of the normal protein catabolic response to fracture when the animals are held at a high environmental temperature.

We thank Dr. J. D. Pullar for his assistance with the calorimetry.

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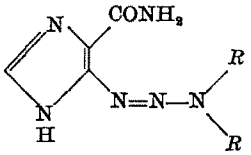
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Complete Inhibition of Mouse Leukaemia *L*1210 by 5(or 4)-[3,3-*bis*(2-Chloroethyl)-1-triazeno]imidazole-4(or 5)-carboxamide (*NSC*-82196)

IT has been shown that 5(or 4)-(3,3-dimethyl-1-triazeno)-imidazole-4(or 5)-carboxamide (*NSC*-45388) and related triazenoimidazoles inhibit the growth of experimental neoplasma[1]. The triazenoimidazoles were prepared by reaction of various amines with 5-diazoimidazole-4-carboxamide[2,3]. This investigation has been extended to embrace a variety of substituted triazene derivatives. We now report the synthesis of one of these, 5(or 4)-[3,3-*bis*(2-chloroethyl)-1-triazeno]imidazole-4(or 5)-carboxamide (*NSC*-82196), and its inhibitory action in preventing the development of mouse leukaemia *L*1210.



NSC-45388: $R = CH_3$
NSC-82196: $R = CH_2CH_2Cl$

NSC-82196 was prepared by treating 5-diazoimidazole-4-carboxamide with *bis*(2-chloroethyl)amine free base in a mixture of methanol and ethyl acetate. The atomic analysis was found to be carbon, 34·54 per cent; hydrogen, 4·27 per cent; nitrogen, 30·25 per cent; chlorine, 25·5 per cent. The atomic analysis calculated for $C_8H_{12}N_6OCl_2$ was: carbon, 34·42 per cent; hydrogen, 4·32 per cent; nitrogen, 30·10 per cent; chlorine, 25·4 per cent. *NSC*-82196 is sparingly soluble in water and in methanol, but stirring a suspension of the compound in either solvent transforms it into a water-soluble ionic compound having the same composition. Analysis gave the result: carbon, 34·35 per cent; hydrogen, 4·18 per cent; nitrogen, 29·85 per cent; total chlorine, 25·2 per cent; ionic chlorine, 12·7 per cent. Calculated values for $[C_8H_{12}N_6OCl]^+Cl^-$ were: carbon, 34·42 per cent; hydrogen, 4·32 per cent; nitrogen, 30·10 per cent; total chlorine, 25·4 per cent; ionic chlorine, 12·7 per cent. Infra-red absorption bands in the spectrum of *NSC*-82196 at 3,390, 1,655 and 740 cm^{-1} are replaced by bands at 3,460, 1,685 and 770 cm^{-1} in the spectrum of the ionic transformation product; but the appearance of a strong band at 1,515 cm^{-1} in the spectrum of the ionic transformation product and the absence of this band in the spectrum of *NSC*-82196 is of greater diagnostic value. The infra-red spectrum of a specimen of *NSC*-82196 stored at −15° C was unchanged after 7·5 months; on the other hand, an earlier specimen that had been kept during most, but not all, of a corresponding length of time at approximately 5° C was partially transformed into the ionic product. The structure of the ionic transformation product has not been established; it may be an aziridinium, a *v*-triazolinium, or a piperazinium salt. Owing to the sparing solubility of *NSC*-82196 in water, specimens containing small quantities of the ionic transformation product may be rendered essentially free of this impurity by rapid washing with water and rapid drying.

Because of the instability of *NSC*-82196, it was injected into test animals as a suspension in carboxymethylcellulose solution within 5 min of the preparation of the suspension. Results of daily treatment of leukaemic mice beginning 2 days after intraperitoneal inoculation of the animals with 10^5 *L*1210 cells and continuing to death or to day 30 are shown in Table 1. In two tests at 75 mg/kg/day the average life-span of the treated mice was more than three times that of the control groups. In initial tests, *NSC*-82196 has also demonstrated activity against sarcoma 180 and adenocarcinoma 755 in mice and against Walker carcinoma 256 in rats. The ionic transformation product was inactive at doses of 50 and 75 mg/kg/day in initial trials against *L*1210.

Table 1. DAILY TREATMENT OF LEUKAEMIC (*L*1210) MICE UNTIL DAY 30 OR DEATH*

Dose (mg/kg/day)	Average weight change, *T*/*C*†	No. of 30-day survivors	Average life span *T*/*C* in days	Average life span Percentage of controls
100	−2·0/+0·2	0	21·6/8·7	248
100	−1·9/+0·4	0	15·6/8·7	179
75	−1·1/+0·4	3	29·3/8·7	336
75	−1·9/+0·8	6	34·3/8·8	389
50	−1·8/+0·4	0	27·2/8·7	312
50	−1·3/+0·8	5	31·3/8·8	355
25	−0·3/+0·8	0	15·3/8·8	173

* Intraperitoneal administration of suspensions of *NSC*-82196 in carboxymethylcellulose; six BDF_1 mice per test. *T*, treated group; *C*, control group.
† Average weight-change (in g) of treated and control mice during first 5 days of treatment.

Table 2. SINGLE-DOSE TREATMENT OF LEUKAEMIC (*L*1210) MICE*

Dose (mg/kg)	Average weight change, *T*/*C*	Average life span of non-survivors *T*/*C* in days	Percentage of controls	Survivors No.	Survivors Time in days†
Group 1					
1,000	−4·5/+0·1	13·2/8·9	148	0	
750	−3·5/+0·1	23·8/8·9	267	1	>240‡
500	−2·8/+0·1			6	>240‡
250	−1·0/+0·1	19·2/8·9	215	1	>240‡
Group 2					
625	−2·8/+1·3	11·0/8·7	126	5	>160§
375	−2·2/+1·3	17·0/8·7	195	2	>165‡
187	−0·6/+1·3	17·2/8·7	197	1	>165‡
93	−0·3/+1·3	14·7/8·7	168	0	
Group 3					
500	−2·9/+1·2	20·5/8·8	233	4	>120‖

* See footnotes for Table 1.
† Survivors beyond 30 days maintained on oxytetracycline therapy to minimize the danger of bacterial infection.
‡ Still alive at this writing.
§ One death after 162 days; four alive after 165 days.
‖ Experiment terminated after 120 days.

Skipper, Schabel and Wilcox[4] have demonstrated the necessity for complete eradication of leukaemic cells *in vivo* and the possibility of achieving this goal through massive, single-dose treatment with active compounds. As shown in Table 2, some of the leukaemic animals treated in this way with *NSC*-82196 have survived for periods of 5·5 and 8 months. At 500 mg/kg/day, for example, all the treated animals in one experiment have survived for 8 months, and four of six survived until a second experiment was terminated after 4 months.

In summary, *NSC*-82196 administered daily exerts a strong inhibitory action against mouse leukaemia *L*1210 and also shows inhibitory activity against certain other tumours. The massive, single-dose therapy of Skipper *et al.*[4] has brought about complete inhibition of *L*1210 in a high proportion of the treated mice. *NSC*-82196 is rather easily transformed into an ionic compound that was inactive in preliminary trials.

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ANATOMY

Role of the Lens in the Morphogenesis of the Iris and Cornea

WITH the view of analysing the part played by the lens during the morphogenesis of the eye, the following experiment was performed: lens primordia of chicken embryos between stages 16 and 22 of Hamburger and Hamilton[1] were implanted into the optic cup of host embryos at the same stage of development. The lens primordium was placed through a section in the wall of the optic cup (at the level of the posterior chamber) by means of a rounded glass needle. The pupil of the host embryo thus came to possess two lenses—its own and an implanted one.

The host embryos were killed with a 10 per cent formalin solution, between stages 17 and 32. After embedding them in paraffin–'Celoidin', 8μ serial sections were made and stained with haematoxylin–eosin. In five of the twenty-five embryos that we investigated, plastic reconstructions were prepared according to Born's method but with slight modifications. The left eyes of the host embryos were considered as controls.

The most pronounced effect of implanting the new lens was that it evoked very intensive structural changes at the wall of the optic cup and in the cephalic mesoderm at the level of the pupil. At the latter, a new pupillary border was reconstituted and the pupillary became greatly increased in size. In front of this newly-formed pupillary area, the cephalic mesoderm was re-organized in such a way that an anterior chamber and a corneal membrane was formed.

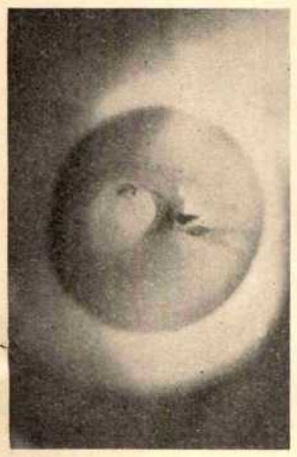


Fig. 1



Fig. 2

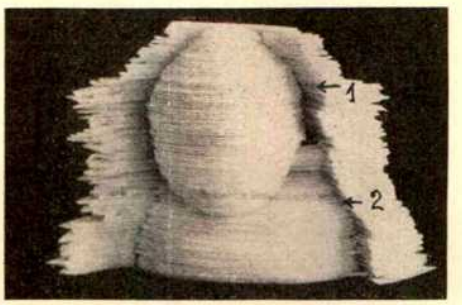


Fig. 3



Fig. 4



Fig. 5

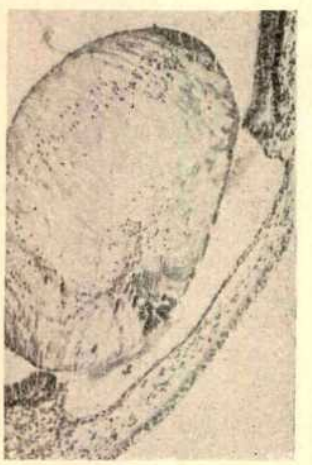


Fig. 6

Compared with the controls, the pupil of those eyes receiving implanted lens were noticeably larger: in front of the latter some pigmented material was present from the primitive iridian border (Figs. 1 and 2).

The plastic reconstructions have enabled us to demonstrate the general aspect of the pupil with the two lenses. In embryo 1252 the cranial equator of the host's crystalline demonstrated the adherence of the implanted lens (Fig. 3). A partial aspect of the same reconstruction (Fig. 4) shows the very clear and definite limits of the anterior chamber and cornea.

Fig. 5 corresponds to a histological section taken through the region marked by arrow 2 in Fig. 3. Both the lens of the host and the implanted lens are sectioned, but only a small section of the latter can be seen. The pupillary border, anterior chamber and cornea all show the same structure as that observed in the control eyes.

Fig. 6 represents a more cranial section through the region marked by arrow 1 in Fig. 3, and a section of the implanted lens can be seen. Although some structural differences exist when a comparison is made with the

control eyes, a pupil is present and the mesenchyme is organized to form an anterior chamber and a cornea.

Our results confirm the suggestion that the lens plays an important part in corneal morphogenesis[3–5]. At the same time they agree with the views put forward by Giroud[6] and Stroeva[7], who emphasize a strong organogenetic connexion between the iris and the lens.

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RADIOBIOLOGY

Manganese Free Radicals in Cobalt-60 Gamma-irradiated Strawberries

THE formation of free radicals in biological systems exposed to ionizing radiation is well established[1–7]. Some of the induced radicals, as well as some of those occurring naturally[8], have long lives[9–11]. The surprising stability of the induced radicals may have far-reaching biological implications. As an example of stability, cystine, on irradiation *in vacuo* at room temperature, gave electron paramagnetic resonance signals even after 35 days[12]. Numerous investigations in connexion with irradiation damage of proteins and amino-acids confirm the stability of these radicals[13–18]. This stability must be considered carefully in assessing the wholesomeness of foods preserved by ionizing radiation. Such assessment involves the concepts of stored chemical energy and consequent radiomimetic hazard[19–20]. Chromosomal aberrations have been shown to occur in germinating barley and onion seeds following treatment with orange or apple juice, which had been irradiated with 200 Krad of γ-rays[21]. Although the radiomimetic precursors generated by irradiation of fruit juices have not been ascertained, preliminary studies indicate that peroxides may be implicated.

From research in our laboratory, strawberries appear to be one of the most promising fruits for application of irradiation to extend shelf life[22]. This communication reports on an investigation to determine whether long-lived organic free radicals were present in irradiated fruits. Greenhouse-grown 'Shasta' strawberries were irradiated at 200 Krad at 0° C, and maintained at this temperature for 64 h. Samples taken at regular intervals, starting immediately after irradiation, were held in evacuated quartz tubes submersed in liquid nitrogen, until electron paramagnetic resonance measurements. The spectra were determined with a Varian electron paramagnetic resonance spectrometer having a 9-in. magnet system, over the temperature range of $-196°$–0° C. The scan was varied from 500 to 1,000 G at roughly g 2. The samples were evacuated continuously during the measurements.

We found no organic free radicals and were only able to detect Mn^{2+} in the samples. Similar results were recorded from commercially available fruit irradiated over a dose range of 200–600 Krad. Atomic absorption spectroscopy showed 19 p.p.m. Mn^{2+} (dry weight basis) in the greenhouse strawberries.

Preservation of foods by radiation, as a novel concept, led to extensive investigations concerning the wholesomeness of irradiated foods. Among the various general principles used to evaluate the biological safety of such foods, sub-threshold toxicity and delayed response are particularly significant when considering wholesomeness in relation to long-lived free radicals.

Due to the absence of any long-lived organic free radicals, the foregoing concept is of no significance in irradiated strawberries. Comprehensive feeding trials of the irradiated foods have shown no adverse effects in test animals. Likewise, in trials conducted by the United States Army Quartermaster Corps, troops fed sterilized rations showed no ill effects. Manganese resonance also has been detected in various non-irradiated plant materials, such as fallen oak leaves and pine needles and naturally dried ivy stems.

On the basis of the evidence available, it is logical to assume that wholesomeness of irradiated strawberries is not affected by the presence of Mn^{2+} free radicals. We have been unable to locate any references in the literature as to the fate of ingested transition metal free radicals. More definitive evidence regarding the biological safety of Mn^{2+} free radicals will come from such an investigation.

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Strontium-90 Content of First Bicuspids

THE tooth crown, once formed, is a stable calcified structure removed from direct equilibrium with body fluids and therefore undergoes minimal remodelling, exchange or turnover of mineral elements. During development and calcification, the mineral elements of the crown are in equilibrium with body fluids and, indirectly, with the diet. The concentration of strontium-90 in the tooth crown is, therefore, a measure of the equilibrium between the tooth and dietary strontium-90, and the tooth crown represents a permanent record of the equilibrium existing at the time of tooth formation.

We have recently reported[1,2] that the accumulation of fall-out strontium-90 in deciduous teeth of children is adequately described by a linear equation of the form $C_T = K\,C_D$, where C_T and C_D are the strontium-90 concentrations expressed as pc. strontium-90/g calcium in the tooth crown (C_T) and the diet (C_D). The slope, K, was

derived from considerations of the percentage of the tooth crown formed *in utero* and *post partum*, known discrimination factors between the foetus and its mother, and discrimination factors between the baby and its diet. The slope, K, varies with the type of tooth and time of formation and has been found to be 0·59, 0·76, 0·69 and 0·77 for healthy incisors, healthy cuspids, carious first molars and carious second molars, respectively. The strontium-90 content of the deciduous teeth represents a function of the dietary strontium-90 content some 5–8 years before natural exfoliation.

For permanent teeth that are formed after birth and are predominantly formed after breast feeding is discontinued, the factors of placental discrimination and the influence of the maternal nutrition are effectively absent. Thus, the slope (K) of the equation is simplified and is primarily equivalent to the discrimination factor between permanent tooth crown strontium-90 and the dietary strontium-90. The slope (K) must be experimentally determined. Sound first bicuspids, often extracted for orthodontic purposes, are readily available for estimation of strontium-90 content[3]. Furthermore, the rate of growth of the first bicuspid crown is well characterized[4], and data concerning the age of the patient, geographical area of and later development and other pertinent informa- may be readily obtained.

This report describes the strontium-90 content of the crowns of first bicuspids and the relationship to dietary strontium-90 from fall-out.

Healthy first bicuspids, extracted between July 1, 1964, and May 1965, from children born within a 150-mile radius of St. Louis, Missouri, and ranging in age from 9 years to 17 years were analysed for strontium-90 by methods previously described[1,2,5]. The crowns and roots were separated at the crown-enamel junction and the crowns were pooled to make a sample containing four crowns and weighing 2–4 g. Of forty-six samples analysed, twenty-four samples (52 per cent) contained four crowns from one child, eighteen samples (40 per cent) were pooled from two or three children, and four samples (8 per cent) were pools of teeth from four children. When crowns were pooled, care was taken to check that they came from children born within 6 months of each other. Because strontium-90 analysis of milk in the St. Louis area was not carried out before 1959, milk strontium-90 values were estimated as previously described[1]. For American children of this age group, previous estimates have shown that the daily dietary strontium-90 intake is equal to the milk strontium-90/g calcium multiplied by 1·20 (ref. 6).

The first bicuspid forms between the ages of 1·5 years and 6 years[4], and an average value of 4 years after birth was taken as the mid-point for time of development.

As shown in Fig. 1, the strontium-90 concentration in the crowns of permanent first bicuspids increases with time for children born between 1948 and 1955, corresponding to a tooth development time between 1952 and 1959, a period associated with increasing fall-out. When the strontium-90 content of the tooth crown is plotted against the strontium-90 content of the diet (Fig. 2), the data are adequately described by a linear equation, $C_T = K\,C_D$ (calculated by the method of average points), with a high correlation coefficient of 0·98. The slope of the line (K), equivalent to the discrimination factor between tooth crown and diet, is calculated from the data to be 0·31.

Discrimination against strontium relative to that of calcium is assumed to be absent in new-born infants[7] and a factor of 0·25 has been accepted for calcified tissues of adult subjects between 20 years and old age[8]. These and other known discrimination factors have been plotted (Fig. 3) to indicate the rapid decrease of the discrimination factor from the time of birth until about 4 years of age, thereafter decreasing slowly to adult values. We estimate, therefore, that the discrimination factor approaches the adult value at about 10–12 years of age.

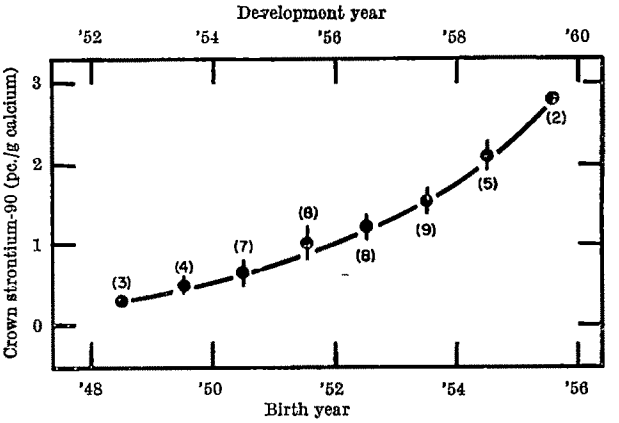


Fig. 1. Strontium-90 content of first bicuspid crown versus year of birth and year of average development. Vertical lines are standard error of the mean for the number of samples in parenthesis

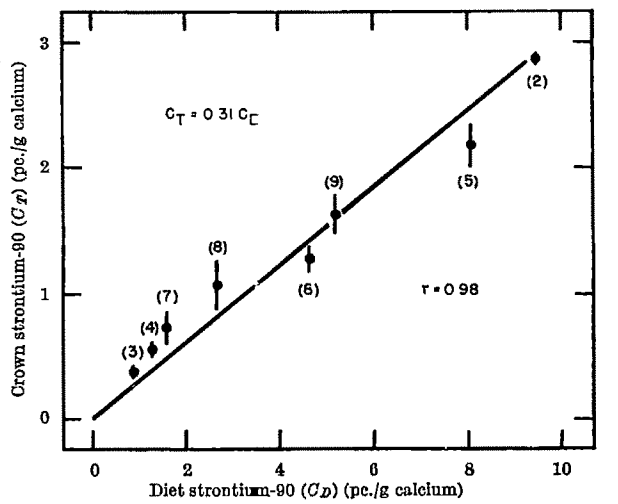


Fig. 2. Strontium-90 content of first bicuspid crown (C_T) versus diet strontium-90 (C_D). Vertical lines are standard error of the mean for the number of samples in parenthesis

The data described in the present report for first bicuspids and our previous reports for deciduous teeth[1,2,5] indicate the usefulness of the teeth as a permanent record of strontium-90 deposition at the time of tooth formation. Furthermore, the stability of the formed crown with respect to minimal turnover time, exchange and remodelling and the known time for tooth development[4] makes it possible to estimate the changing discrimination factors in the growing child—estimates that are difficult to determine by other means.

We recognize the influence of the time lag of about 4 years for development of the bicuspid crown and the

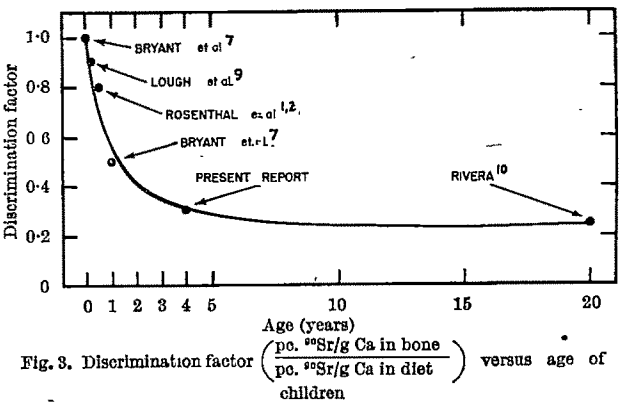


Fig. 3. Discrimination factor $\left(\frac{\text{pc. } ^{90}\text{Sr/g Ca in bone}}{\text{pc. } ^{90}\text{Sr/g Ca in diet}}\right)$ versus age of children

fact that the crown consists of enamel and dentine that calcify at different times. These and other factors need to be clarified, but they would not seem to influence seriously the results and are presumed to be much smaller than the analytical error of strontium-90 analysis. Although Starkey, Bryant and Henderson[3] have criticized the usefulness of deciduous teeth and have preferred to analyse permanent first bicuspid and third molars, we believe that the tooth crown reflects the equilibrium between calcified tissues and diet strontium-90 concentrations at the time of tooth formation.

Deciduous teeth are useful because they are readily available in large numbers by natural exfoliation, reflecting the strontium-90 incorporation during a short calcification period of less than 1 year. The various factors influencing the strontium-90 content of deciduous teeth such as discrimination by the placental barrier during *in utero* development, or discrimination between the baby and its diet, have already been evaluated[1]. Permanent teeth, although not so readily available as deciduous teeth, are larger, require fewer teeth for adequate sampling and represent the strontium-90 equilibrium with the diet in the absence of minimal maternal influence. The longer development time of permanent teeth represents an average incorporation of strontium-90 during the period of calcification, thus obviating the influence of sharp and rapid changes in dietary strontium-90 levels.

It appears, therefore, that all kinds of teeth may be useful as an index of dietary strontium-90 when due consideration is given to the many factors involved in tooth and bone formation and metabolism.

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Unexpected Dose-rate Effect in the Killing of Mice by Radiation

IT has been well established for many years that the effectiveness of ionizing radiation may be correlated with dose-rate[1–4]. The relative ineffectiveness of radiation at low dose-rates is generally regarded as resulting from the operation of intracellular repair or recovery mechanisms, which may act between successive damaging events, when an accumulation of these is required to give the effect under investigation. In most radiobiological investigations on mammals, or with mammalian cells, experimenters have used dose-rates of up to a few hundred rads per min, so that the duration of 'acute' doses is usually to be measured in minutes or tens of minutes.

In mammalian work, only Lindop and Rotblat[5], so far as we know, have examined the relative effectiveness of a series of dose-rates greater than 1,000 rads/min, their measurements being concerned with the mean dose required to kill mice within 30 days ($L.D.$ 50/30), and with the life-shortening effect of radiation. They used an electron beam to deliver dose-rates varying between 80 and 160,000 rads/min, and found very little change in the $L.D._{50/30}$ in this range. Ainsworth *et al.*[6] likewise found no difference in the $L.D._{50/30}$ for mice with γ-ray dose-rates of 100 and 10^6 rads/min. These results justify the assumption implicit in much mammalian cell-survival work that it is a matter of indifference whether dose-rates of, say, 100 rads/min or 1,000 rads/min are used.

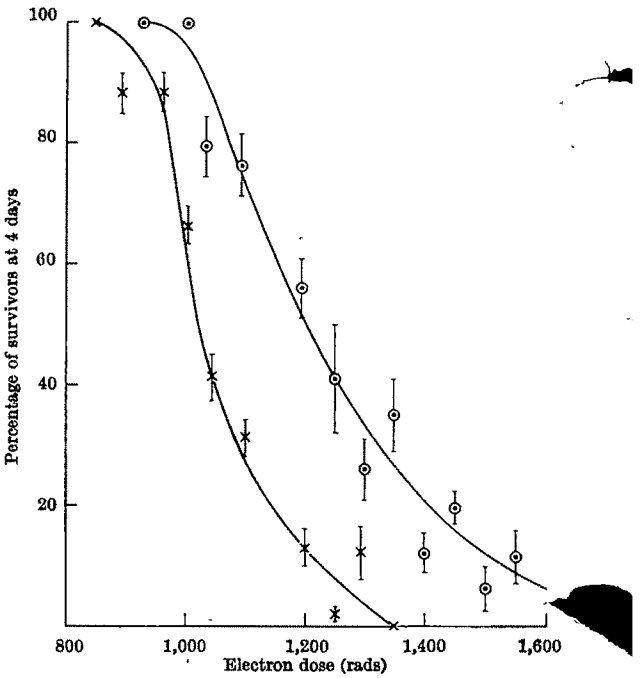


Fig. 1. 4-day survival curves for mice, strain *TO*, exposed to electron beams at 6,000 rads/min (×) and 100 rads/min (⊙)

It was therefore unexpected to find that the mean dose required to kill mice within four days ($L.D._{50/4}$) was strongly dependent on dose-rate for rates up to 6,000 rads/min. This mode of death is attributable to depletion of the stem cells in the crypts of Lieberkühn, which give rise to the epithelial cells covering the villi in the small intestine[7]. Fig. 1 shows survival curves for female mice 6–8 weeks old, strain *TO*, breathing oxygen during exposure to an electron beam (energy about 8 MeV) at dose-rates of 100 and 6,000 rads/min. (Exposure time about 10 min and 10 sec.) The data have been collected from several experiments, in some of which the mice were anaesthetized with 'Nembutal' before irradiation. The results for these and for unanaesthetized animals were the same, provided the mice were breathing oxygen.

We have determined the $L.D._{50/4}$ for electron beams or 250 kV X-rays at dose-rates between 20 and 50,000 rads/min, with results shown in Fig. 2. We do not think that this strong dependence of $L.D._{50/4}$ on dose-rate, observable up to about 6,000 rads/min, can be due to experimental artefact. Dosimetry for the electron beam was based on measurements with a Baldwin/Farmer substandard ionization chamber, used at a low dose-rate; these measurements were used to calibrate the monitor present in the electron beam[8]. The monitor chamber is not fully saturated at dose-rates greater than 2,000 rads/min, but the correction factors we apply have been verified by dosimetry with lithium fluoride[9]. At 6,000 rads/min, the dose-rate at which many of the observations were made, the correction factor was only 1·1 per cent. An additional internal check was provided by using dose-rates of 100, 250 and 6,000 rads/min for determining

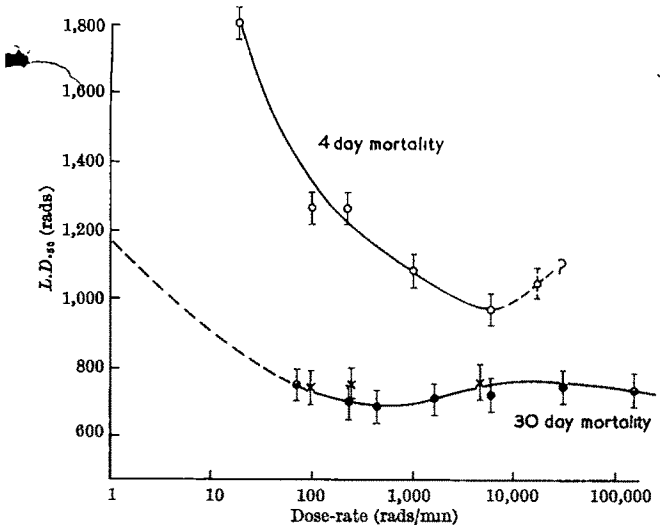


Fig. 2. (○), 4-day mortality with *TO* strain mice; (×), 30 day mortality with *TO* strain mice; (●), 30-day mortality results taken from Lindop and Rotblat (ref. 5)

values of the $L.D._{50}$ at 30 days. These were the same at the three dose-rates, and agreed with the results of Lindop and Rotblat[5] (Fig. 2). The dose-rate effect we have observed is not peculiar to the strain of mice (*TO*) used for most of these experiments. We have carried out preliminary experiments on *C3H* mice inbred in these laboratories. These are not yet available in numbers sufficient to obtain full survival curves; but experiments have been carried out with small homogeneous groups. When a group irradiated at 100 rads/min was given a dose 200 rads more than a similar group irradiated at 6,000 rads/min, the patterns of death up to 9 days were closely similar.

The 10–15 min exposure time required for delivery of lethal doses at 100 rads/min might be long enough to permit of some "recovery from sub-lethal damage", in the sense used by Elkind[10]. We tested this possible explanation for the dose-rate effect by giving a series of doses at 6,000 rads/min, each split into two fractions separated by 10 min. As shown in Table 1, this procedure resulted in survivals equal to those seen after low dose-rate irradiation, when the total doses were 1,050 rads or less. With the higher doses, however, the doses given at 100 rads/min were less effective than doses given at a higher dose-rate but split by 10-min intervals. This may perhaps be accounted for by the relatively small amount of recovery which can occur during the few seconds' irradiation time required for delivery of the second fraction, at the higher dose-rate. No increased sparing effect was observed when the interval was extended to make the overall time always equal to the time required for the delivery of the same doses at 100 rads/min; for example, when a total dose of 1,300 rads was split into two fractions separated by 13 min.

Since data dealing with dose-rate effects at greater than 100 rads/min are sparse, it is not possible to know whether gut-death in mice is exceptional in demonstrating dependence on dose-rate up to 6,000 rads/min. In these laboratories Miss B. Cullen has found no difference in the effectiveness of the electron beam in killing mouse ascites tumour cells (Landschütz strain) for dose-rates varying from 100 to 12,000 rads/min. Thus there is one *in vitro* system, at least, for which the results are like those for haemopoietic death in mice, in that maximum effectiveness is reached at a dose-rate less than 100 rads/min.

Table 1. COMPARISON BETWEEN EFFECTS OF DOSES GIVEN AT 100 RADS/MIN AND AT 6,000 RADS/MIN IN TWO FRACTIONS 10–14 MIN APART

Dose (rads)	Survival at 4 days							
	6,000 rads/min						100 rads/min	
	Single shot		Two fractions				Single shot	
			Interval 10 min		Interval shown in brackets			
	%	S.E.	%	S.E.	%	S.E.	%	S.E.
1,000	66	± 3	96	± 7			100	
1,040	42	± 3	81	± 4			80	± 5
1,000	31	± 3	46	± 5			77	± 5
1,200	18	± 3	22	± 4	8	± 6 (12′)	56	± 5
1,250	2	± 1			23	± 9 (12·5′)	41	± 9
1,300	12	± 4	21	± 5	4	± 4 (13′)	35	± 6
1,400	0		8	± 6	8	± 6 (14′)	13	± 3

From two reviews on dose-rate effects in mammals and mammalian cells[11,12] the impression might be gained that the effectiveness of radiation increases indefinitely with dose-rate, perhaps because data on dose-rates above 100 rads/min were not available when those reviews were written. The three sets of data discussed here show that effectiveness approaches a maximum level, and indeed the results of Lindop and Rotblat[5], on life-shortening in mice, show that effectiveness decreases with increasing dose-rate, above 500 rads/min. In any event, it could be expected that at very high dose-rates local oxygen depletion would start to play a part in reducing the effectiveness of radiation.

In the range of dose-rates we have used, namely 20–50,000 rads/min, our results do not fit the conclusion reached by Bateman *et al.*[11] that the effectiveness of radiation varies with the cube root of the dose-rate. The relationship between dose-rate and effectiveness must clearly depend on the kinetics of intra-cellular repair mechanisms, and, for the epithelial cells of the small intestine, a particularly rapid form of repair would seem to operate.

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Use of Multiple Biopsies for the Study of the Cell Cycle of the Mouse Hair Follicle

IN investigating the cell proliferation of normal tissue in rodents individual animals have to be used for each experimental time interval and thus the problem of inter-animal variation is always present. This presents a particular problem when the cell cycle is investigated by the method in which the fraction of mitoses labelled is plotted against time[1].

This communication describes the use of multiple biopsies for the study of the cell cycle of the hair follicle in mice. It also describes a method of obtaining sections of skin for investigation prepared from autoradiographs using a cold microtome technique.

Adult Carworth Farms No. 1 female mice of the Argonne strain, about 90 days old, had their hair plucked from their back in order to induce the anagen phase of the hair cycle. Experiments were carried out 5 days and 10 days after this plucking. Although there is no diurnal cycle in the hair follicle[2], intraperitoneal injections of 20 μc. of tritiated thymidine were made at 10 p.m. in all animals.

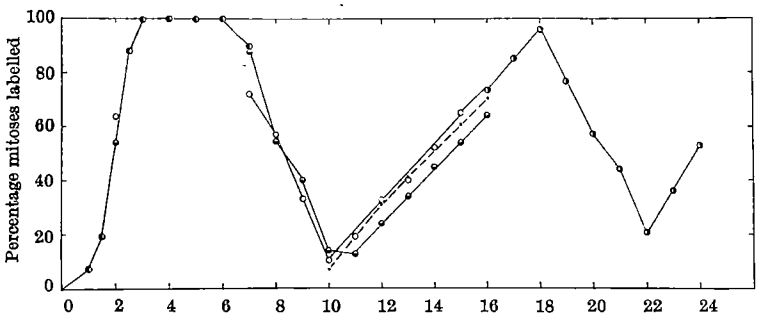


Fig. 1. Percentage of mitoses labelled as a function of time interval between injection of label and biopsy. O - - - O, Data taken from a mouse 5 days after plucking; O——O, data taken from several mice 10 days after plucking. The following symbols are used for individual mice. O, ◒, ◐, ◑

Because of the size of the mouse the largest number of biopsies carried out on one mouse was eleven. (In the rat it is possible to obtain thirty or more biopsies of the skin. Additional experiments on rats are in progress.) Ten days after plucking, one mouse was biopsied at 1, 1·5, 2, 2·5 and hourly intervals between 3 and 7 h after injection. Two mice were biopsied at 2 h and at hourly intervals between 7 and 16 h. Two mice were biopsied hourly between 16 and 24 h. One animal was biopsied from 10 to 16 h after injection of tritiated thymidine and 5 days after plucking.

The 4-mm punch biopsies were dipped in mounting fluid, frozen with a carbon dioxide quick freeze unit and sectioned on a cold microtome at −30° C. Sections 4μ thick were made, fixed in 4 : 1 methyl alcohol–acetic acid fixative and then taken through graded solutions of ethyl alcohol to water. The slides were dipped in Kodak *NTB* 3 liquid emulsion, dried and stored for 1 or 2 weeks in light-tight boxes, developed in *D*-19 developer for 1·5 min at 20° C, washed, fixed, and then stained with Mayer's haematoxylin and eosin. Labelled mitoses were counted in the region of the matrix in sections cut perpendicular to the skin, and longitudinal to the hair shaft. Proliferative cells in the dermal papilla and external root sheath were not counted.

Following the punch biopsy the skin healed well without appreciable scarring. The cold microtome sections proved to be an advantage from the point of view of convenience and speed of preparation, and yielded undistorted sections of the skin and subcutaneous structures with good nuclear detail. The autoradiographs were exposed for 1 and 2 weeks in order to establish whether there were false negative results following 1 week of exposure to the label. No difference was observed.

Fig. 1 is a composite curve constructed from the data obtained from the several animals biopsied at various time intervals after injection of tritiated thymidine. The presence or absence of label was scored for between 100 and 300 metaphases observed in the follicles available in each sample. In some follicles it was noted that there were clusters of cells which appeared to be in the same phase of the cell cycle. The composite curve so obtained showed that the percentage of mitotic figures labelled reached 100 per cent at 3 h and remained at this level for 3 h. Following this there was a rapid decrease in the percentage of mitoses labelled to a minimum between 10 and 11 h. Afterwards the curve of the percentage of mitoses labelled rises to a second maximum of 96 per cent at 18 h. A third minimum point of 20 per cent is reached at 22 h. From this curve an estimate of the *S* phase can be made from the time elapsed between the 50 per cent points (at 2 h and at 8 h) resulting in an *S* period of 6 h. A similar estimate of *S* is obtained between the 50 per cent points on the second cycle (at 14 and 20 h). If the duration of $G2 + M$ phase is considered to be the time from 0 h to the 50 per cent point, a figure of 2 h is obtained from these data. The duration of the cell cycle may be obtained as the time elapsed between homologous points on the curve; for example, between successive 50 per cent levels. The time elapsed between the 50 per cent point on the first ascending limb to the 50 per cent point on the second ascending limb gives a cell cycle of about 12·5 h. If the time elapsed from the origin of the first ascending limb to the beginning of the second ascending limb is taken, a cell cycle of about 9·5 h is obtained. If the duration from the second minimum at 10·5 h to the third minimum at 22 h is measured, this yields a cell cycle of 11·5 h.

It is of interest that there is a gross correlation between the data from various animals so that similar results were obtained at the same time-interval irrespective of the number of biopsies that had previously been carried out. The percentage of mitoses labelled at intervals between 10 and 16 h was similar for the anagen hair follicle at both 5 and 10 days after plucking. This suggests that there is little or no change in the cell cycle during this period of anagen.

The data obtained at 5 days and 10 days differ from previously published data in that there is a plateau on the first curve and there is less clear-cut dampening of the curve in the first 24 h than was previously reported by Cattaneo[3].

From these results it would seem that there is little inter-animal variation; however, it remains to be seen whether this will be so under other conditions. This method is being extended to study the whole period from 0 to 30 h in the hair follicle of rats. This technique may be useful in investigations in which comparisons are made of the curve of the percentage of mitoses labelled under various conditions such as radiation, changes in temperature, and the like.

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Malonaldehyde from the Autoxidation of Methyl Linolenate

THE pink colour obtained when 2-thiobarbituric acid (TBA) reagent is heated with foods or tissues has often been used as an index of lipid oxidation. It has been assumed that malonaldehyde (MA) is responsible because the colour obtained with oxidized food or fat has the same absorption spectra as that from MA[1,2]. Recently, however, Saslaw *et al.*[3] and Saslaw and Waravdekar[4] irradiated fatty acids in air, separated by thin-layer chromatography several different substances which reacted positively with TBA, and suggested that none of these was MA, thus casting some doubt on the previous assumption. The present experiments show that the principal TBA-reactive substance produced during the air oxidation of methyl linolenate is indeed MA.

A column (2 cm × 75 cm) was prepared from 'Sephadex *G*-10' with 0·05 M *tris*-HCl buffer containing 0·1 M sodium chloride, *p*H 7·4. 1 ml. of 2×10^{-4} M MA[5] in buffer was added to the column and 250 fractions (1 ml.) were collected at a flow rate of 1 ml./1·5 min (Spinco 'Accu-flo' pump). 1 ml. of aqueous 0·02 M TBA had been added to every other test-tube in the fraction collector. The void volume (*Vo*) of the column was determined with blue dextran 2000. The fractions with TBA reagent were maintained at room temperature for 22 h in order to develop the colour, and then the absorbance was measured at 532 mμ. Fractions which had not had TBA reagent added were examined for their ultra-violet absorption spectra: the results are shown in Fig. 1. The elution volume (*Ve*) of MA was 148 ml. with *Vo*, 80 ml. The ratio $A_{532\ m\mu}/A_{267\ m\mu}$ was 2·4 (ref. 5) for the MA peak.

5 g of methyl linolenate (Mann Research Laboratories) in a 10 ml. beaker were stirred continuously in air at 40° for 16 h. 2 ml. of distilled water were added to the rancid ester and the whole was mixed for 5 min. The water extract ($A_{532\ m\mu} = 52$) was separated by centrifugation and subjected to fractionation as already outlined. Three TBA-positive peaks were detected as shown in Fig. 2. Peak No. 1 was not further characterized. Peak No. 2 was identified as MA by the following criteria: (1) The elution volume ($Ve = 148$ ml.) was the same as that of authentic MA. (2) The ratio $A_{532\ m\mu}/A_{267\ m\mu}$ was 2·4. (3) On acidification to *p*H 3, the absorption maximum was shifted from 267 to 245 mμ; the ratio ($A_{267\ m\mu}/A_{245\ m\mu}$) was 2·3. A similar change occurs with MA, and this can be attributed to the transformation of enolate anion to the *s-cis* chelated form[5]. Although MA accounted for about 90 per cent of the total TBA-reactive substances in this experiment, the concentration in the peak fraction was too low to give a positive ferric chloride test[4]. Saslaw *et al.*[3] had used this criterion as one argument against the presence of MA in their extracts. Peak No. 3, which represented about 8 per cent of the total TBA-reactive substances, had an absorption spectrum with maximum at 230 mμ independent of *p*H. The material in the three peaks gave identical visible absorption spectra with TBA.

Saslaw and Waravdekar[4] demonstrated the presence of two TBA-reactive substances in fatty acids that had been irradiated with ultra-violet light, but only one from substrates irradiated with cobalt-60. Fränz and Cole[6] isolated four TBA-reactive substance peaks on gas chromatograms of methyl linolenate that had been irradiated with ultra-violet light; however, they were unable to identify the substances concerned. According to Thiele and Huff[7], TBA-reactive substances may result from both enzymatic and non-enzymatic oxidations of liver fractions.

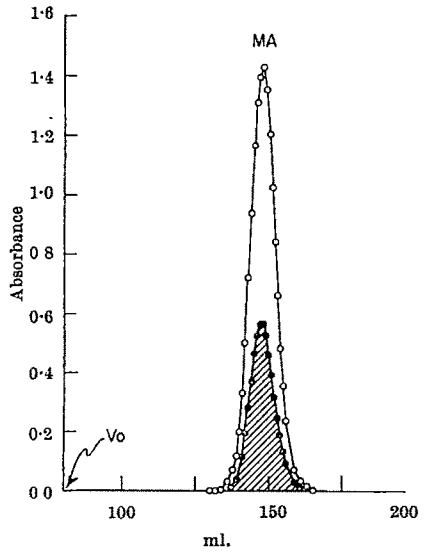


Fig. 1. Elution of malonaldehyde (MA) (1 ml. of 2×10^{-4} M) from 'Sephadex *G*-10' column (2 × 75 cm) with 0·05 M *tris*-HCl buffer containing 0·1 M sodium chloride, *p*H 7·4. ○——○, 532 mμ; ●——●, 267 mμ

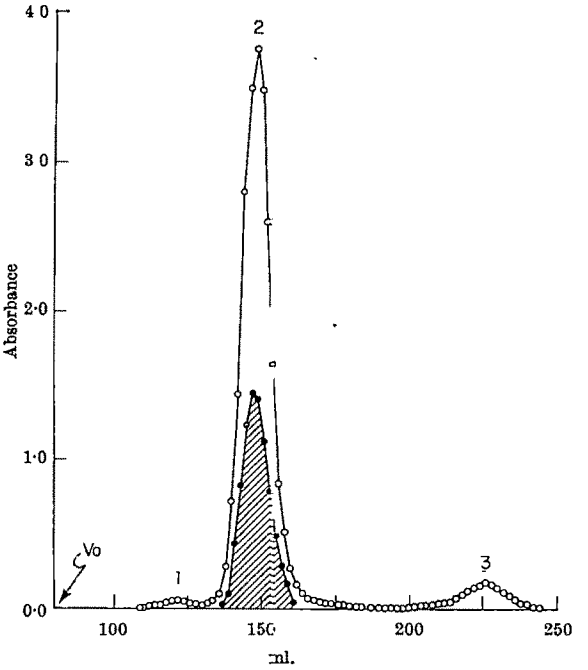


Fig. 2. Elution of water extract of oxidized methyl linolenate from 'Sephadex *G*-10' column. ○——○, 532 mμ; ●——●, 267 mμ

Saslaw *et al.*[3] reported that MA, obtained by distillation from the bisulphate addition product, migrated only slightly and with tailing in thin-layer chromatography. The solvent used was *n*-butanol–dichloromethane–water, 20:4:1. These results were confirmed in this experiment.

However, when MA which had been prepared by acid hydrolysis[5] of malonaldehyde *bis*(-diethyl acetal) was used two TBA-positive spots could be separated by the same thin-layer chromatography technique with silica-gel *G* (E. Merck *AG*, Darmstadt, Germany). The main spot (R_F, 0·77) clearly separated from the other which migrated slowly (R_F, 0·16) with tailing. When the MA solution (10^{-3} M) was heated at 90° for 15 min the original principal spot disappeared and the pink colour of the second spot became more intense. These observations suggest that either polymerization or some other as yet unidentified change may occur during the application of MA to the thin-layer chromatography plate even at room temperature or during heating of MA solution, or during distillation of the bisulphite addition product.

In any event, MA seems to be the single most important TBA-reactive substance of air-oxidized methyl linolenate, The identity of the other TBA-reactive substances is under investigation.

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BIOLOGY

Deviant Index : a New Tool for Numerical Taxonomy

A MEASURE of the extent to which an individual differs from the norm of a population, taking many attributes into account, would often be useful in numerical taxonomy. With metrical attributes, the distance in an attribute space between the point representing the individual and the centroid of the population would serve such a purpose[1]. Similar measures have been proposed for binary attributes, but no fully appropriate way of combining binary and metrical attributes has been proposed, and no suggestion has been made for the inclusion of ordered non-metrical attributes except by allotting arbitrary metrical equivalents to the class values. Significance tests for these distance measures have proved elusive except in the multivariate normal case; but a significance test is often required as a guide to taxonomic action. The index proposed here not only combines information from all types of attributes, but also provides a direct test of significance.

When dealing with a metrical attribute, the natural choice for the norm is the arithmetic mean (unless the distribution is very skew, in which case one might, for example, prefer the geometric mean), and the deviant index for a particular value of such an attribute (as we will call the proposed index of deviation from the norm) is simply the proportion of the population further from the mean than the individual in question.

For ordered but non-metrical attributes, the appropriate choice for a norm seems to be the median value. Here, distance from the median has no meaning, but one may fit classes above and below the median together in accordance with the proportion of the population they contain. Thus, a class is considered to be further removed from the norm than a class on the other side of the median if, together with the tail of the distribution beyond it, it contains a smaller proportion of the population than the class in question with its tail. If the proportions in the successive classes are p_i $(1 \leqslant i \leqslant n)$, with m as the median class, the deviant index for this attribute for an individual with a value x $(< m)$ is thus:

$$D_x = \sum_{i=1}^{x} p_i + \sum_{i=y}^{n} p_i$$

where y is defined by:

$$\sum_{i=y}^{n} p_i \leqslant \sum_{i=1}^{x} p_i < \sum_{i=y-1}^{n} p_i$$

For a purely qualitative attribute, the only possible choice of norm seems to be the most frequent value. No natural ordering of classes around this norm suggests itself, but, as in the similarity index already proposed[4], it seems reasonable to use the proportion of the population in the class for this purpose, the rarer class values being considered to represent wider deviations from the norm than the commoner values. Thus, in this case:

$$D_x = \sum_{i \in S} p_i$$

where:

$$S = \{i : (p_i \leqslant p_x) \wedge (1 \leqslant i \leqslant n)\}$$

When all the D_x for all m attributes have been calculated, they need to be combined to give an overall value $\mathfrak{D}_x$ for the deviation of individual x from the population norm. This can be done by Fisher's[2] method:

$$\chi^2_{2m} = -2 \sum_{i=1}^{m} \ln D_i$$

or the modification with a correction for continuity introduced by Lancaster[3]. $\mathfrak{D}_x$ is then defined as the probability of the χ^2 value obtained, or a greater one.

The analogy between this deviant index and the similarity index described earlier[4] is clear. The latter is the complement of the probability that a pair of individuals with a random selection of attributes would differ as greatly as the pair in question. This amounts to the complement of a deviant index calculated not as above for a single individual but for the deviations from the norm common to a pair. Generalization of this concept to larger sub-sets can be envisaged.

The deviant index proposed here could clearly be used for deciding whether, at a given significance level, a certain individual should or should not be included in a proposed taxon, or for a decision between two possible taxa to which an individual could be ascribed.

Programmes for computing $\mathfrak{D}_i$ for all individuals in a set have been written in *Fortran II* for the IBM 1620_3 computer, and are available on request.

I thank Mr. D. W. G. Moore for facilities in the Computing Centre of the University of Western Australia.

Note. While this communication was under editorial consideration, Hall's note[5] about a proposed 'peculiarity index' was published. He, too, was concerned to express the extent to which different individuals deviated from the norm of a set, and the purposes of the two proposed indices are very similar. The peculiarity index, however, covers binary attributes only, and is unstandardized, so that its magnitude depends on the number of individual (taxa) in the set under consideration. It may be defined as:

$$P = \frac{Nm}{2} - N \sum_{i=1}^{m} q_i$$

where N is the number of individuals (taxa), m the number of attributes. and:

$$q_i = \min \{\tfrac{1}{2}, p_i\}$$

p_i being the proportional frequency of the observed value of the ith attribute.

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Present Status of Known Populations of the Vendace, *Coregonus vandesius* Richardson, in Great Britain

AT least thirteen different populations of *Coregonus* have been recorded in Great Britain and Ireland; four of these were of the Vendace, *Coregonus vandesius* Richardson. The status of these four populations was discussed by Regan[1], who divided them into two sub-species: the Lochmaben Vendace, *C. vandesius vandesius*, from two lochs near Lochmaben in Dumfriesshire; and the Cumberland Vendace, *C. vandesius gracilior*, from two lakes in Cumberland. The most recent revisions of the genus in Europe[2-4] agree that *C. vandesius* in Great Britain is equivalent to *C. albula* (L.) in Europe, though little evidence is brought forward to prove this. Practically nothing original has been published on the species in Great Britain since 1908 (ref. 1), and, in fact. other than very occasional specimens found accidentally at the water's edge, the species has not been taken in any numbers since the beginning of the century. Because of this, and the fact that several unsuccessful attempts have been made in recent years to capture specimens, many workers have assumed that this fish is extinct in Great Britain, or almost

so. Thus it was stated in 1958 of the Lochmaben vendace, "Cette forme doit être considérée comme éteinte"[4].

There has been no recent revision including all the populations of *Coregonus* in this country, and in 1963 I started making collections of material for this purpose. In April 1964, netting was carried out in the Castle Loch and in the Mill Loch near Lochmaben, from both of which *C. vandesius* had been previously recorded[5]. No fish was caught on that occasion. A second attempt, however, was made in August 1965, and this time thirty-nine specimens were taken in the Mill Loch. None was taken in the Castle Loch, which is now extremely eutrophic owing to the discharge into it of the Lochmaben sewage effluent; it may well be that the species is extinct there now. (The possibility of specimens occurring in it cannot be ruled out, however, because the outflow from the Mill Loch eventually runs into the Castle Loch, and an occasional fish may pass down by this route.) In September 1965 similar netting was carried out in the recorded localities for the species in Cumberland, at Bassenthwaite and Derwentwater. Both of these lakes were found to have thriving populations of *C. vandesius*, 225 specimens being taken in Bassenthwaite and eighty-four in Derwentwater. Unfortunately many of the latter fish were badly damaged in the nets by eels *Anguilla anguilla* (L.).

This material is being retained for future study and comparison with other British and Irish *Coregonus*. It is of great interest, however, to note immediately that the species is still present in at least three localities in Britain, and in two of them at least in considerable numbers—sufficient to enable a very useful and exciting study of their ecology to be made there. The fact that thriving populations such as these can remain undetected over long periods in bodies of water where they are known to have occurred makes it seem quite feasible that there may well be unknown populations of this and other forms of *Coregonus* in some other British lakes. It is hoped that several of the most likely of these may be investigated in the next few years.

I am grateful to Mr. J. F. Flannagan and to my wife, Kathleen, for help with netting in Dumfriesshire, and also to Mr. H. C. Gilson and his staff at the Windermere Laboratory of the Freshwater Biological Association for assistance in Cumberland.

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Photoperiodism and Fecundity in *Plutella maculipennis* (Curt.)

INVESTIGATIONS on the population dynamics of the diamondback moth, *Plutella maculipennis* (Curt.), at Ottawa[1,2] revealed that its numbers were regulated by factors affecting the adult stage. One of the more important of these was fecundity of the insect, which declined during the season. More recent findings, communicated here, indicate that fecundity is influenced by photoperiod.

The diamondback moth is a cosmopolitan species, occurring almost everywhere where cruciferous crops are grown. In temperate climates it has two to seven generations per year and overwinters most often in the adult stage[3,4]; in the warmer parts of the world it breeds without interruption[5,6]. There is neither true nor facultative diapause, and in the northern parts of its range the moth succumbs to the frost annually. At Ottawa, infestations arise each year from adults that overwinter in the central and southern United States and migrate north in the spring as the weather moderates[7]. There are usually four generations per year but sometimes as many as six.

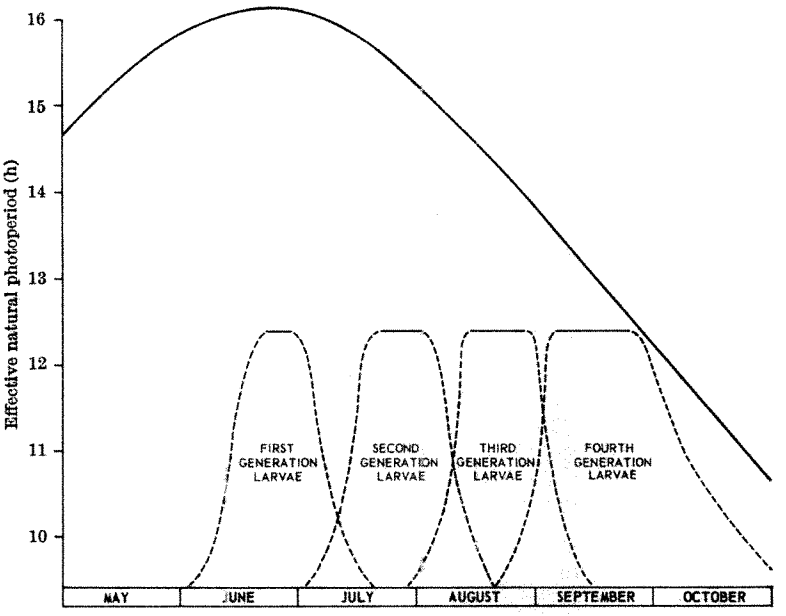


Fig. 1. Effective natural photoperiod at Ottawa (latitude, 45° 34′) in relation to seasonal activity of *P. maculipennis*, 1960–64

In the population studies, life tables have been compiled for thirty-two generations since 1958, and fecundity in each generation has been measured since 1960. Table 1 shows that the reproductive rate declined gradually in successive generations each year. To shed light on this phenomenon, we plotted photoperiod in relation to seasonal activity of the insect at Ottawa for the past 5 years. Fig. 1 shows that larvae in each generation were exposed to different lengths of day, the effective natural photoperiod (length of day as measured from sunrise to end of civil twilight—solar elevation, −6·00°) diminishing gradually from 16·1 to 13·0 h or less. These data suggest a causal relationship between photoperiod and fecundity of the insect. To test this hypothesis, progeny of generation 5, 1964 (photoperiod 11 h, fecundity 32), were reared on cabbage leaves in the laboratory at 20° C. Batches of larvae were placed in a divided rearing chamber designed to provide both a 12-h and 16-h period of light. Sixteen adults (eight males, eight females) from each light regime were liberated in 2 × 2 × 2 ft. screened oviposition cages containing young cabbage plants. The moths were fed on a solution of sugar-water, given a 16-h photoperiod, and held at a temperature of 25° C, relative humidity 70 per cent. The test was replicated twice. Differences in egg production (Table 2) provide experimental evidence of a photoperiodic effect.

Table 1. NUMBERS OF EGGS LAID PER FEMALE IN SUCCESSIVE GENERATIONS OF *P. maculipennis*, OTTAWA, ONTARIO, 1960–64

Year	Generation 1	2	3	4	5
1960	187	119	97	14	—
1961	192	108	129	20	—
1962	189	198	151	80	—
1963	144	115	98	60	30
1964	164	164	125	59	32
Mean	175	141	120	47	31

Table 2. NUMBERS OF EGGS LAID BY *P. maculipennis* AFTER LARVAL EXPOSURE TO PHOTOPERIODS OF 12 AND 16 H

Photoperiod	Replicate *A*	Replicate *B*	Mean per female
12 hours	260	331	37
16 hours	544	639	74

The relationship between photoperiod and fecundity in *P. maculipennis* appears to be analogous to that between photoperiod and diapause in other insects, and the decline in its fecundity may, in fact, represent incipient reproductive diapause. Short photoperiods frequently promote development of the insect fat body instead of the ovaries[8,9]. This process is accompanied by other body changes that result in cold-hardiness. Thus, decreasing day length may enable the moth to survive moderate frosts such as occur at the northern limits of its overwintering distribution.

Experiments are now in progress to determine the seat of photoperiodic induction and possible temperature-photoperiod interactions.

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Migration of Epithelio-muscular Cells in *Hydra*

RECENT reports have directed attention to the part played by connective tissue in the histogenesis and morphogenesis of epithelial tissue[1]. In *Hydra* the possibility that the acellular mesoglea influenced the differentiation of cells almost suggests itself. It is widely held that epithelio-muscular cells in the epidermis and digestive cells in the gastrodermis undergo a net movement down the body[2] and change in certain cytological and physiological details as a function of the region in which they temporarily reside[3].

Among the possible ways connective tissue could be influencing adjoining tissues are two that appear to be mutually exclusive, if defined in a strict sense: (1) connective tissue could act as a substratum for cell migration or (2) as an adhesive, a 'cement', holding the cells in one place. The object of our experiment was simply to determine whether the epithelial cells were capable of disassociating themselves from given positions on the mesoglea and migrating to other positions.

Hydra viridis of both green and white (bleached) varieties were used. The experiment consisted of placing a ring of both cell layers and mesoglea, cut from a normal white animal, over the isolated gastrodermis and accompanying mesoglea, taken from a green animal stripped of its epidermis. The green colour derives from the presence of an algal symbiont found exclusively in the gastrodermal piece. Such algae allowed us to distinguish between the two sorts of gastrodermal cells in histological sections. The composite animals were fixed about 2 h after grafting. Our object was, ultimately, to determine if epithelio-muscular cells could be found overlying gastrodermal cells containing the algal symbiont. Such epidermal cells would have to have broken whatever contacts they originally had with the mesoglea in the ring and migrated to their new positions.

A mildly acidic solution[4] (*p*H 2·5) was used to loosen the attachment of the epidermis to the mesoglea in order to obtain the green gastrodermal piece. Animals were placed in the solution for about 1 min during which the tentacle ring and adhesive foot were cut off. The gastrodermis, surrounded by the mesoglea, thereupon stretched into a cigar-shaped piece while the epidermis contracted into a thick annulus. The two cell layers could then be separated with the aid of forceps. The gastrodermis was placed in a small petri dish containing a solution known to support the life of isolated gastrodermal cells[5].

The petri dish had been prepared with a slant of paraffin over half the bottom and a piece of filter paper over the other half. A normal animal of the white variety was placed into the dish and a ring cut from its mid-gastric region. This ring was stretched over a pair of forceps until it could accommodate the isolated gastrodermal piece. The former was slipped over the latter and set into place at about its midpoint. The paraffin slant provided a working surface; the isolated gastrodermal pieces adhered to the paraffin sufficiently well not to slide around while the rings were slipped over them. The mesogleas were, however, damaged by prolonged contact with the paraffin. The composites were therefore transferred to the half of the bottom of the dish covered by filter paper; the paper proved to be a more suitable substratum on which the composites could rest since the gastrodermal pieces did not adhere to it. Neither glass nor agar was as suitable as either paraffin or paper in their respective roles.

Following grafting, the rings seemed to expand and encompass the isolated gastrodermal pieces. The grafted animals were then fixed in Bouin's fluid, and later dehydrated in alcohol and embedded in paraffin. Sections were cut at 6μ and stained with the periodic acid-Schiff technique followed by toluidine blue, or by aqueous light green and carmine[6].

In all, eighteen grafts were made and gave unambiguous results; a continuous epidermis could be seen overlying both algae-less gastrodermal cells and algae-rich gastrodermal cells. Such a free edge of migrating epidermis (*E*) is seen in Fig. 1. Interstitial cells accompanied the migrating epithelio-muscular cells. Mitotic figures were rarely seen in the epidermis or in any other part of these preparations.

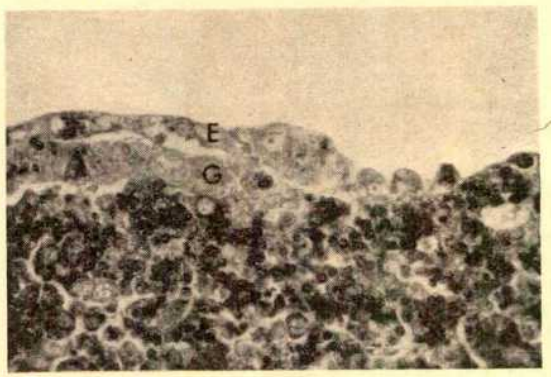


Fig. 1. Longitudinal section through part of grafted white ring and host green gastrodermis. The algal symbionts in the latter stain intensely with periodic acid-Schiff and are seen here as black granules. The gastrodermal cells derived from the ring (*G*) lack these granules. The epidermis derived from the ring (*E*), likewise lacking these granules, covers both the gastrodermal cells of the ring and some of those of the isolated gastrodermis. (×300)

Often, extracellular spaces were seen between the epidermal cells and the mesoglea in the original ring. These spaces can be seen in Fig. 1, and suggest that the cells are undergoing a sort of mobilization for migration. In addition, the epidermis, particularly near the edge of the ring, seems to be thinner than normal, suggesting that the cells are stretched.

It is not possible, in most of our sections, to identify the mesoglea originally accompanying the isolated gastrodermal piece even though the mesoglea brought along with the ring was often conspicuously stained.

However, the latter never extended beyond the edge of the gastrodermis of the ring. Possibly the difficulty was due to our having dissolved a carbohydrate moiety of the mesoglea in either the solutions used for isolating or maintaining the gastrodermal piece, or in that used for fixation. The outer surface of the gastrodermal piece remained smooth throughout the experiment, suggesting that the mesoglea, or at least its structural component, was still present. Moreover, what appeared to be a mesoglea could be dissected away from completely isolated gastrodermal pieces even 12 h after removing the epidermis.

Clearly, the question of whether the epithelio-muscular cells can migrate has been answered positively. These cells, originally adjacent to digestive cells lacking algae, evidently migrated and came to overlie other digestive cells containing algae. At least under the experimental conditions described here the mesoglea of *Hydra* permits cells to migrate over it, and does not act as a 'cement' prohibiting cell migration.

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Fig. 1. Shows a group of melanocytes in the skin of the pinna of the ear of an anaesthetized mouse. (×*c.* 196.) (Taken from ciné film)

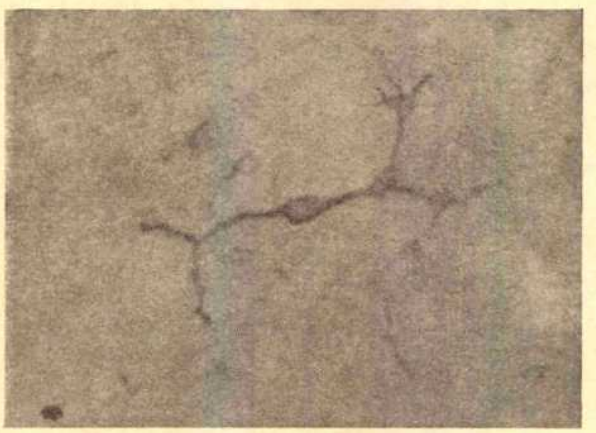


Fig. 2. A single melanocyte in the skin of the pinna of the ear of an anaesthetized mouse. The detailed morphology of the cell body and its dendritic processes can be clearly seen. (×*c.* 244.) (Taken from ciné film)

New Method for studying Mammalian Melanocytes *in vivo*

MOST reports on the morphological changes of mammalian melanocytes associated with alterations in melanogenesis have been based on observations made either with a split skin preparation or by the use of tissue culture. The former method involves the use of trypsin[1,2] or other chemical agents[3] or the use of a freezing microtome[4] to bring about separation of the epidermis from the dermis. Tissue culture of melanocytes using the technique described by Cruickshank[5] requires that the epidermis be broken down into individual cell units in the early stages, and this results in a dissolution of the melanocyte keratinocyte relationship. Furthermore, the continued growth of the culture is dependent on the presence of an artificial medium[6]. The method which is described here allows the observer to study for hours, days, months or even years the activity of a single melanocyte or groups of melanocytes in their natural undisturbed environment. The method is simple and is particularly suited to time-lapse photography.

Black *C57* mice, 3–4 weeks old, are commonly used. The animals are anaesthetized with 'Nembutal' given by the intraperitoneal route (80 mg/kg body-weight). The hair of the pinna of the ear is removed with a commercial depilating agent ('Neet'). The cream is applied for about 5 min and the hairs are washed off. If the cream is allowed to act for too long it will produce an inflammatory reaction. The ear is then moistened with glycerine which is gently massaged into the epidermis. The glycerine improves the optical properties of the epidermis so that the melanocytes can be more clearly seen. The pinna is placed dorsal surface downwards on to a glass slide and the pinna edges are glued in position with rubber cement. (Rubber cement has the advantage that the ear can be separated from the slide at the end of the period of study without damage to the epidermis.) The rest of the animal is then loosely tied down to the microscope stage leaving the proximal end of the pinna slack. The latter precaution is important since it reduces the possibility of the respiratory movements and heart beat being conducted to the slide holding the pinna. The microscope objectives (× 10, × 25 and × 40) can then be lowered on to the pinna and further glycerine is added to immerse the lens. Having selected a particular field this may now be observed continuously for a number of hours. Continuous anaesthesia is facilitated by leaving the 'Nembutal' syringe needle in the peritoneal cavity. This allows the drug to be administered as required without disturbing the animal. We have found that open ether plus 'Nembutal' is a satisfactory combination when the melanocytes are to be observed for a long period of time. If repeated studies of the same melanocyte are to be made at long intervals, then either the ear skin must be marked at the site or the general topography of the blood vessels and hair follicles in that region carefully noted.

This method is at present being used by one of us (R. S. S.) in the study of the influence of hormones and other agents on the morphology of mammalian melanocytes. It is also being used to observe the circulatory and follicular changes which accompany the hair cycle (A. F. S. and H. B. C.)[7,8]. The wing and interfemoral membrane

of the bat can similarly be used for the direct observation of melanocytes by transmitted light[9].

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MICROBIOLOGY

Transduction in *Proteus morganii*

PHAGES capable of generalized transduction among strains of *Proteus mirabilis* have been described[1–3]. *Proteus morganii* differs in many respects from *P. mirabilis*[4] including a growth requirement for pantothenate and *l*-cystine in addition to the nicotinic acid requirement of the latter organism[5]. Several gene loci of these two species are being investigated in this laboratory. With *P. mirabilis* biochemical work is complemented with genetic (transductional) studies. In order to do the same with *P. morganii* it was decided to search for transducing phages active on this species.

The seventeen temperate *P. morganii* phages previously isolated[4] from an equal number of *P. morganii* strains[6] were investigated. The screening test used was the ability of phage to transduce a one-step marker (*str-r*) controlling resistance to 1 mg streptomycin/ml. from a mutant to the wild-type host. The materials and methods used for this part of the investigation were those previously used[1]. The minimal medium used was that of Porter and Meyers[5] solidified when necessary by the addition of 1·5 per cent agar. Under these conditions the growth factors *l*-cystine, calcium pantothenate and nicotinic acid were added to the agar at 47° C just before the plates were poured. Auxotrophic mutants of *P. morganii* strain N.C.T.C. 2815 were selected by the penicillin method after ultra-violet irradiation of washed suspensions of the organism to about 1 per cent survival. With transductions of prototrophy to auxotrophic mutants 0·05 ml. of the adsorption mixture (constituted as for streptomycin transductions) was plated on minimal medium 15 min after mixing. Colonies were scored after 4 days at 37° C.

One of the seventeen phages was capable of intra-strain transduction of the *str-r* marker. This phage (named *M*) was derived from *P. morganii* strain N.C.T.C. 10041 by ultra-violet induction of an overnight broth culture. Phage *M* is strictly inducible. No phage was detected in supernatants of an unirradiated broth culture tested at intervals for 10 days. The host of the phage is *P. morganii* strain N.C.T.C. 2815 on which it forms markedly turbid plaques. The transduction rate of the *str-r* marker from mutant 2815 *str-r* to wild-type 2815 is 6–9 × 10^{-6}/phage particle adsorbed. The transducing ability of phage *M* is completely abolished by prior addition of 0·1 ml. pure phage antiserum which reduces the phage titre from 7 × 10^{9} plaque forming units/ml. to 8 × 10^{4} P.F.U./ml., but it is unaffected by treatment of the lysate with deoxyribonuclease. A large number of strains of all the genera of the family Enterobacteriaceae were tested for susceptibility to the phage with negative results. The infectivity of phage *M* is Ca^{++} independent and undergoes 90 per cent inactivation at 60° C for 15 min and 99·9 per cent at 70° C for the same period of time. The structure of the phage was examined[7] before its transducing potential was recognized. It resembles the *Salmonella* transducing phage *P22* (ref. 8), but its overall dimensions are slightly smaller and it has a collar around the short neck.

Mutants of strain N.C.T.C. 2815 with individual growth requirements for histidine, tryptophan, isoleucine plus valine, ornithine and adenine were isolated. Phage lysates of the wild-strain transduced all the foregoing auxotrophs to prototrophy at rates similar to the *str-r* transductions. Phage *M* is thus capable of generalized transduction. Particular attention was given to the detection of abortive transductants, but none of the systems yielded any[2]. All prototrophic transductant clones examined so far are lysogenic and yield no *str-r* transductants when used as recipients for phage prepared on 2815 *str-r*.

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Thermophilic Micro-organisms from Rotting Maize

TEMPERATURES of up to 58° C were observed within a 20-ton stack of maize which accidentally became wet in Ado-Ekiti, Western Nigeria.

Samples collected deep in the stack were plated out on Difco corn meal agar (for fungi) and on glucose-yeast extract agar[1] (for bacteria) and incubated at 55° C.

Three fungi, *Thermomyces lanuginosus* Tsiklinsky (I.M.I. 110803), *Mucor pussilus* Lindt (I.M.I. 110805) and a possibly new species of *Rhizomucor* (I.M.I. 110804), were regularly isolated. The last-mentioned isolate is at present being studied.

Three strains of spore-forming bacteria and one of an actinomycete were also obtained. The morphological and physiological properties of the bacteria (N.C.I.B. 9667–9669) suggest that they are strains of *Bacillus licheniformis* (Weighman) Chester *emend. Gibson*, while the actinomycete (N.C.I.B. 9670) belongs to the genus *Thermoactinomyces* and is probably *T. thermophilus* Krassilnikov.

Table 1 illustrates the temperature requirements of the isolates on the aforementioned media.

Table 1

	Minimum (° C)	Optimum (° C)	Maximum (° C)
Thermomyces lanuginosus	28–32	45–50	58–60
Mucor pussilus	21–23	40–45	50–53
Rhizomucor sp.	25–30	45–53	60–61
Bacillus licheniformis	27–30	38–45	58–59
Thermoactinomyces sp.	30–35	45–47	59–61

All the isolates hydrolysed starch; none 'cleared' chitin or cellulose agar[1]. The bacteria and the actinomycete visibly dissolved finely ground particles of maize incorporated with mineral salts into agar; the fungi did not.

These isolates have been recorded in other parts of the world in various habitats, and especially in decomposing vegetable matter[2,3]. A new species of *Thermomyces* was recently isolated from self-heating oil-palm produce in Nigeria[4].

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'Polyspheroids' from American Soils

A RECENT electron microscopic examination of a soil sample from Texas revealed the presence of unusual forms of micro-organisms. As a result, a more extensive survey of North American soils was undertaken, to find whether the so-called 'polyspheroids' were more widely distributed, or restricted to limited areas.

Soil samples from New Jersey, Louisiana, Florida and New York yielded, on inoculation into selective culture media, mixed microbial cultures that were examined by electron microscopy. Among several unusual forms observed, some closely resembled the 'helicoidal polyspheroids' described by Nemec and Bystricky[1,2] and Bystricky and Nemec[3] and seemed to be identical to those encountered from Texas. These unusual forms (Fig. 1) were of the general size of bacteria, about 0·5μ by 0·6μ–1·5μ. Electron micrographs revealed several strongly contoured, longitudinal rows of roughly spherical sub-units, with diameters of about 0·1μ, in a maize cob-like arrangement (Fig. 2). The sub-units were not detected by light microscopy. A helicoidal arrangement of the sub-units was suggested in some instances, but was less pronounced than in the forms described from Europe[1–3].

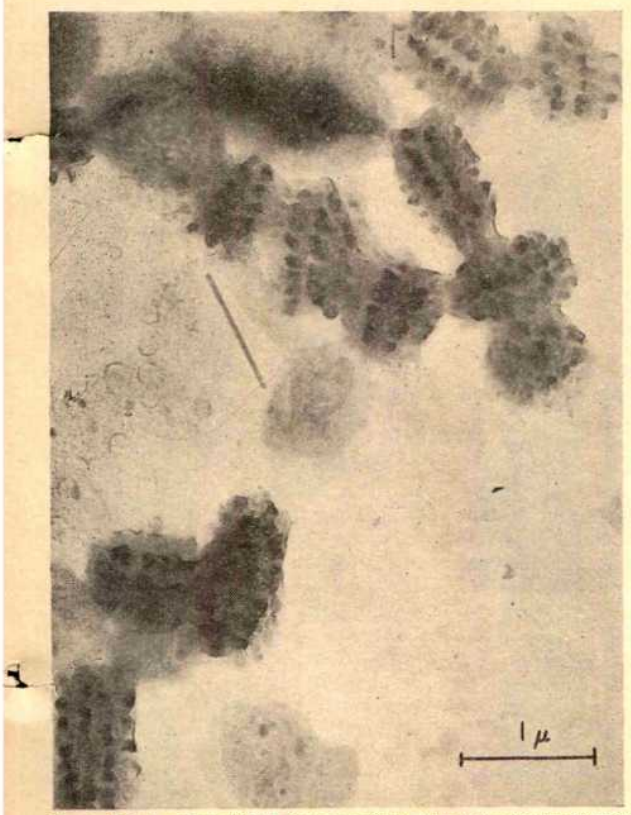


Fig. 1. Mixed culture of soil micro-organisms, with several polyspheroids

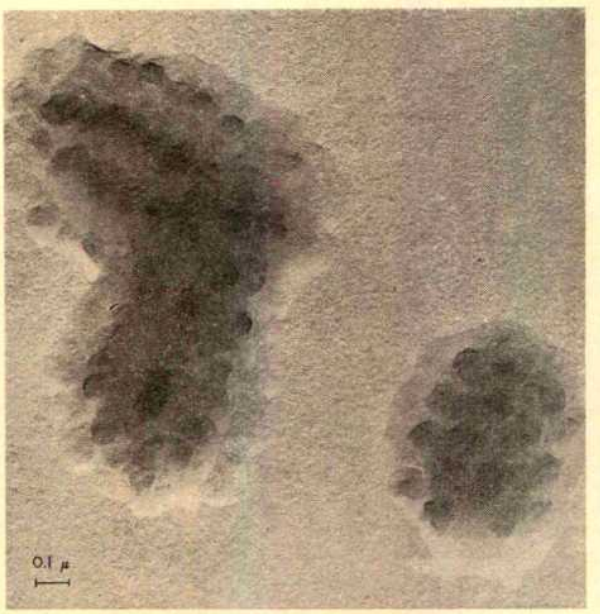


Fig. 2. Polyspheroids, at higher magnification

As to the nature of the observed forms, the following interpretations are suggested: (*a*) they might represent a stage in the normal or abnormal life cycle of a bacterium, or of some other soil micro-organism; (*b*) they could be a hitherto undescribed soil bacterium, of unusual morphology; (*c*) they might belong to a new, hitherto undescribed group of micro-organisms. The latter interpretation is the most challenging, and seems very likely at present.

Attempts are at present being made to obtain enriched mixed cultures with a higher percentage of these forms, and it is hoped eventually to gain pure cultures of the micro-organisms. Their tentative designation as polyspheroids will be maintained.

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GENETICS

Genetic Control of Lipid Metabolism in *Tribolium*

GENE blockages of protein synthesis in micro-organisms are now classical to the study of gene action. While a few metabolic defects are well known to be under genetic control in higher organisms, including man, experimental evidence of genetic control of lipid metabolism is not known. Therefore, discovery of a gene in *Tribolium* which apparently influences the metabolism of fatty acids may be of general interest.

The mutant gene arose in a quantitative genetic study of selection response of 13-day larval weight in *Tribolium*

castaneum when cultured under two levels of nutrition[1]. Initially, the base population's 13-day larval weight on the 'good' diet (percentage composition: vitamin premix 10, soybean meal 17, corn (maize) meal 58, dried brewers' yeast 10, and corn oil 5) was about twice that on the 'poor' diet (percentage composition: vitamin premix 3, soybean meal 11, and corn meal 86). However, after twelve generations of selection for small 13-day larval weight on the 'good' diet, the average weight in one experimental population (GS_2) was considerably greater on the 'poor' diet than on the 'good' diet. Typical growth of the GS_2 population on the two diets in comparison with the base population is illustrated in Table 1. It should be noted here that the quantitative trait 13-day larval weight has been studied using biometrical techniques and has also been investigated with the intention of specifically identifying the genetic basis of selection. The importance of the latter approach has been emphasized by Thoday[2].

An analysis of the influence of the components of the 'good' diet on larval growth revealed corn oil to be the inhibiting factor for the GS_2 population. The population grown in the absence of corn oil showed normal growth. Inheritance investigations revealed the observed sensitivity to corn oil to be inherited as a single autosomal recessive gene and is referred to as *cos* for corn *oil sensitive*.

The action of *cos* suggests that the system (or systems) of corn oil metabolism is altered in homozygous *cos*/*cos* individuals. To investigate experimentally the possibility of differential utilization of fatty acids in the sensitive (*cos*/*cos*) and normal (+/+) genotypes, larvae of these two types were grown on 'good' and 'standard' (95 per cent wheat flour, 5 per cent yeast) media for 13 days, then washed free of media and analysed by gas-liquid chromatography. The relative percentages of C_{16}—C_{18} fatty acids in a representative analysis are reported in Table 2. Fatty acids with less than 16 or greater than 18 carbons occurred only in trace amounts and no trend as to diet or genotypes was apparent.

Table 1. GROWTH OF THE GS_2 AND BASE POPULATIONS OF *Tribolium* ON 'GOOD' AND 'POOR' DIETS AS MEASURED BY 13-DAY LARVAL WEIGHT

	Mean ± standard error of 13-day larval wt. (μg)	
Population	'Good' diet	'Poor' diet
Base	2,303 ± 25	1,215 ± 37
GS_2	350 ± 15	906 ± 36

Number of larvae weighed in each treatment group = 100.

Table 2. RELATIVE PERCENTAGES OF THE FATTY ACIDS OF THE SENSITIVE AND NORMAL GENOTYPES REARED ON 'GOOD' AND 'STANDARD' DIETS

Medium	Genotype	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic
'Standard'	*cos*/*cos*	32·0	2·8	10·2	37·4	17·6
,,	+/+	31·0	3·5	12·7	34·6	18·2
'Good'	*cos*/*cos*	18·5	2·1	7·4	31·8	40·2
,,	+/+	26·0	2·1	12·1	33·9	25·9

Dietary corn oil produced an increase in the linoleic acid content of the tissues in both the corn oil sensitive and normal genotypes. However, the marked difference in the distribution of fatty acids in +/+ and *cos*/*cos* genotypes on the 'good' diet suggests an alteration in lipid metabolism in the corn oil sensitive population.

Evidence for a fatty acid oxidizing system in insects has been reported[3]. The present investigations suggest a genetic block in the oxidation of linoleic acid which results in a build-up of this fatty acid. It appears reasonable that the accumulation of linoleate may interfere with other systems (for example, membrane permeability) and thus account for the observed growth reduction. Whether or not the genetic block is specific for linoleate, or represents a general block in the oxidation of all fatty acids, is not known as, in either case, an accumulation of linoleate would represent the predominant change resulting from dietary corn oil because of the high concentration of linoleate in corn oil (58 per cent). Further biochemical analyses of the *cos* gene are now being undertaken.

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VETERINARY SCIENCE

Effect of Plane of Nutrition on Changes in the Rate of Wool Growth in Sheep induced by Hexoestrol

TREATMENT with exogenous oestrogen is known to influence growth in ruminants, for example the retention of nitrogen, the rate of weight gain and ossification of bone are increased. The effect is considered to occur by way of the anterior pituitary gland, oestrogens facilitating the synthesis of growth hormone[1]. It has been claimed, however, that, while causing a general increase in somatic growth, treatment with oestrogens decreases the rate of wool synthesis in sheep, possibly by means of a lowered thyroid activity or a pituitary or adrenal effect[2]. In view of this apparent anomaly, and the fact that most of the evidence to support it was obtained using gross methods of measurement such as fleece weight, the following work was undertaken.

The experiment was designed to use an absolute method of assessing the rate of wool growth in order to determine more accurately how this is influenced by hormone implantation. An autoradiographic technique involving incorporation of sulphur-35 cystine into wool which enables growth in single fibres to be accurately measured has been described by Downes and Lyne[3] and this was adopted without modification.

The experiments were carried out using four cross-bred wethers maintained in metabolism cages in a controlled temperature room (65° F) under a controlled light régime equivalent to that of the time of year at the commencement of the experiments (11 h daylight). They were acclimatized to a standard ration before taking measurements of wool growth. At the beginning of the experimental period wool was removed by shaving areas 4-in. square on each sheep, one on each shoulder, flank and rump. After 10 days, sulphur-35 cystine was injected intravenously twice with a 3-day interval between injections. One week later, the squares were reshaved and the rate of wool growth for each sheep was measured on a number of fibres selected at random from the six patches. After the determination of wool growth each animal was implanted with a 15-mg pellet of hexoestrol subcutaneously in the ear. After a further 2 weeks the animals were reinjected with sulphur-35 cystine, the squares reshaved as before and the rate of wool growth again measured. Investigations of nitrogen retention were undertaken throughout the experiment in order to establish that the hormone implant was having the expected effect. All animals showed a significant increase in nitrogen retention following hormone treatment (3·748 g nitrogen/day for the control period; 6·066 g nitrogen/day after treatment ($S.E. = 0{\cdot}477$; $P < 0{\cdot}01$)).

In order to determine nitrogen retention accurately it was necessary to control the weight of food consumed by each animal. Observations were made on the appetite of each animal during a preliminary period and, after examining the data, each sheep was given a maximum of 5,675 g of food per week and uneaten food was weighed back. Weekly food consumption is given in Table 1.

Table 1. FOOD CONSUMPTION OF EXPERIMENTAL SHEEP
Food consumption per sheep (g./week) (Max. given = 5,675 g)*

Week	A	B	C	D
1	5,664	4,342	4,196	5,675
2	5,675	5,269	3,958	5,217
3	5,675	5,568	3,690	5,512
4	5,675	5,675	4,047	5,675
		Implantation		
5	5,668	5,675	4,391	5,600
6	5,675	5,675	5,078	5,579
7	5,675	5,675	5,606	5,675
8	5,675	5,675	5,643	5,675
9	10,050	10,125	5,650	9,985

* The results for week 9 are for the sheep fed *ad libitum*.

Table 2. EFFECTS OF HEXOESTROL IMPLANTATION ON THE RATE OF WOOL GROWTH OF SHEEP UNDER CONTROLLED FEEDING

Animal	Rate of wool growth (mm/3 days) Control period	Rate of wool growth (mm/3 days) Treatment period	Effect of treatment	Significance (P)	S.D.
A	1·306 (43)*	0 962 (40)	Decrease	< 0 001	± 0·1723
B	1·375 (64)	1 322 (39)	Decrease	N.S.	± 0·2771
C	1·121 (52)	1·444 (45)	Increase	< 0 001	± 0 2811
D	1·297 (34)	1·260 (41)	Decrease	N.S.	± 0·2066

* Numbers of observations are given in parentheses.

Table 3. EFFECTS OF HEXOESTROL IMPLANTATION ON THE RATE OF WOOL GROWTH OF SHEEP UNDER *ad lib.* FEEDING COMPARED WITH WOOL GROWTH UNDER CONTROLLED FEEDING

Animal	Rate of wool growth (mm/3 days) Control	Rate of wool growth (mm/3 days) Treatment	Effect of treatment	Significance (P)	S.D.
A	1·3057 (43)*	1·365 (41)	Increase	N.S.	± 0·1673
B	1·375 (64)	1·552 (34)	Increase	< 0 01	± 0·2793
C (a)*	1·121 (52)	1·769 (31)	Increase	< 0·01	± 0·2702
C (b)†	1·444 (45)		(over b)		
D	1·297 (34)	1·622 (25)	Increase	< 0 001	± 0·2324

* Numbers of observations in parentheses.
† b, Result after first treatment.

The results of observations on the rate of wool growth are shown in Table 2.

Three sheep, namely *A*, *B* and *D*, showed decreases in wool growth rate although only *A* was significant ($P < 0{\cdot}001$). Sheep *C* showed a significant increase in the rate of wool growth ($P < 0{\cdot}001$). This difference in response appeared to be related to the level of feed intake of the sheep. Sheep *A*, *B* and *D* consumed virtually all the food offered at the commencement of the experiment whereas sheep *C* did not. After implantation its appetite increased, but on no occasion did the animal consume all the food offered. The appetites of sheep *A*, *B* and *D* were re-established at the end of the experiment by feeding the animals *ad libitum* for a further week (week 9, Table 2). This demonstrated that sheep *A*, *B* and *D* were capable of consuming considerably more than they were actually being fed and in consequence possessed a potential to retain more nitrogen which was not satisfied by food intake. It was possible that, in order to satisfy this potential, the animals utilized nitrogen from sources which, under normal conditions would have been available for wool synthesis, leading to the observed effect of a decrease in the rate of wool growth. Sheep *C*, however, was fed to appetite throughout and therefore the increasing potential to retain nitrogen could be satisfied without recourse to nitrogen utilized for wool synthesis.

To test this theory, all four animals were fed *ad libitum* for 6 weeks, re-implanted, and the experiment was repeated. The results are shown in Table 3.

There was an increased rate of wool growth in sheep *A*, *B* and *D* when compared with the original control values (non-significant for sheep *A*). The rate of wool growth in sheep *C* also increased, due possibly to variations in the rate of release of hexoestrol. The amount of nitrogen retained daily was also increased in all sheep.

The results show that the mechanism of action of oestrogens on the rate of wool growth must be more complex than was previously realized, and that the nutritional status of the animal has a direct effect on the changes in wool growth induced by hexoestrol.

It has been established that there is an order of priority of tissue growth which becomes more apparent under restricted dietary régimes[1]. These experiments indicate indirectly that, in the sheep, wool follicles have a low priority, since the rate of wool growth was depressed in animals which were not fed *ad libitum*. Thus, under conditions of restricted feeding, hexoestrol treatment, which increases the demands for nutrients for systems which have a high priority, would be expected to decrease the growth rate of wool. It is possible that the availability of amino-acids might be the factor limiting wool growth under these conditions. On the other hand, hexoestrol treatment coupled with *ad libitum* feeding would result in an increase of nitrogen retention with no restriction in the use of this nitrogen for wool growth. This would be expected to give the result observed in the second experiment, that is, an increase in the rate of wool growth. The foregoing results emphasize the need for careful definition of the conditions used in work of this nature.

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Prenatal Loss of Foetuses and Abortion in Guinea-pigs

DURING experiments designed to determine the effects of stresses on pregnancy in guinea-pigs, it was noted that a large proportion of sows aborted within 4–7 days when their diet was restricted. The effect of a hot environment on foetal resorption and abortion in pregnant rats has already been reported[1], but only one brief reference[2] has been found describing prenatal losses caused by dietary restriction in guinea-pigs. This communication describes two experiments illustrating the effects of severe sudden dietary restriction during pregnancy.

In the first experiment, eighteen multiparous guinea-pigs were arranged in pairs of similar weights and allotted at random into one of two groups. From the 30th to 39th day of gestation they were caged individually and offered 5 g lucerne hay and 400 g freshly picked clover and ryegrass daily. From 40 days' gestation, the control group continued on this diet and 25 mg ascorbic acid (Parke, Davis and Co.) daily in tablet form *per os*. The daily intake of the second group was restricted to 5 g lucerne hay, 20 g fresh clover and grass and 25 mg ascorbic acid. All animals were weighed daily and observed twice daily until abortion or normal parturition occurred.

The results are summarized in Table 1.

Table 1

	n	Mean weight at mating (g) ± S.D.	Litter size Living: Mean	Litter size Living	Litter size Dead	Litter size Total	Mean gestation length (days)
Restricted diet	9	747·7 ± 100·0	2 9 ± 0 82	0	26	26	48·1 ± 5·57
Unrestricted diet	9	780 0 ± 95·7	2·8 ± 0·65	22	3	25	67·1 ± 1·67

There was a highly significant difference between the restricted and unrestricted groups in foetal survival ($\chi^2 = 37{\cdot}7$, $P < 0{\cdot}001$) and gestation length ($t = 9{\cdot}8$, $P < 0{\cdot}001$). Six of the nine animals the diet of which was restricted aborted fresh foetuses between 44 and 46 days' gestation. The others aborted degenerating foetuses after 12 days (two sows) or mummified foetuses after 20 days (one sow).

In the second experiment, thirteen multiparous sows were allotted into one of two groups as before, were caged at 42 days of gestation, and to 51 days' gestation were fed a diet of 45 g freshly picked clover, 10 g lucerne hay leaf and proprietary guinea-pig pellets *ad lib*. From the 52nd to the 62nd day the control group received 90 g,

and the restricted diet group received 8 g, of lucerne leaf hay daily. All experimental animals received 50 mg ascorbic acid in tablet form daily *per os* during this period. After the 62nd day they were offered fresh clover, hay and pellets as before 52 days.

The results are presented in Table 2.

Table 2

	n	Mean weight at mating (g) ± *S.D.*	Litter size Mean	Living	Dead	Total	Mean gestation length (days)
Restricted diet	7	773·6 ± 84·6	3 3 ± 0·74	1	22	23	61·3 ± 2·21
Unrestricted diet	6	763·2 ± 74 6	3 2 ± 0·73	19	0	19	68·3 ± 2 89

There was a highly significant difference between the restricted and unrestricted groups in foetal survival ($\chi^2 = 34{\cdot}4$, $P < 0{\cdot}001$) and maintenance of gestation after the onset of treatment ($t = 6{\cdot}26$, $P < 0{\cdot}001$).

Two sows in the restricted diet group developed a condition resembling pregnancy toxaemia at the 56th and 58th days of gestation and were destroyed. In each, the foetuses were dry and had been dead for some time as estimated by opacity and wrinkling of the cornea and haemoglobin staining of subcutaneous tissues. The placentae contained brown or grey necrotic areas and fell away from the endometrium with very light traction. One sow was destroyed on the 65th day of gestation following opening of the vaginal membrane on the 63rd day, and passage of a profuse vaginal discharge for 2 days. The four foetuses were degenerating and emphysematous. It may be assumed that normal pregnancy was interrupted in each of these three instances, but they might not have been able to pass the foetuses through the narrow unrelaxed pelvis. They have not been included in the data referring to gestation length and maintenance of gestation.

The litters of the other four female guinea-pigs were aborted from the 58th to the 63rd day and included three mummified, two degenerating, six freshly dead, and one living foetus. One litter, delivered at 63 days, consisted of a mummified foetus, a degenerating foetus, a freshly dead foetus and a small living foetus. Bacteriological examination of eighteen uterine swabs and foetal stomach contents failed to reveal the presence of any significant pathogens.

The severe sudden stress imposed in these experiments usually resulted in the loss of the whole litter. This was commonly accompanied by the abortion of fresh foetuses, and less frequently by retention, resorption and delayed delivery of foetuses. Mummification occurred if foetuses were retained for a sufficient period, and when this happened the foetal fluids and products resulting from embryonic degeneration appeared to be largely absorbed by the uterus, as the vaginal membrane may remain intact until delivery is imminent. Brambell[3] found no evidence that products of embryonic disintegration were absorbed by the maternal tissues although the possibility had not been excluded.

The factors which determined whether abortion or foetal degeneration and mummification would occur have not been elucidated, but did not appear to be related to size of dam at mating or at the beginning of dietary restriction, or to the amount or the rate of weight loss during restriction. The loss in weight between the beginning of dietary restriction and abortion ranged in a linear fashion between 13 per cent when abortion occurred on the 4th day, and 30 per cent when abortion occurred on the 20th day.

It has been suggested by Csapo *et al.*[4] that foetal death *in utero* results in a reduction of both uterine volume (v) and placental progesterone (p) and, if the rate of decrease is such that the ratio v/p stabilizes as in normal gestation, pregnancy is maintained. This could account for the retention of litters resulting in their mummification. The prompt delivery of freshly dead, or occasionally of living premature foetuses, might have been the result of sudden placental damage with diminished production of progesterone.

I thank Prof. D. McFarlane for advice and Mr. K. L. Hughes for help with the bacteriological examinations.

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ANTHROPOLOGY

Cusp Number, Occlusal Groove Pattern and Human Taxonomy

EVER since Gregory and Hellman, the number of cusps on the molar teeth and the pattern of the occlusal grooves have been used as taxonomic criteria in the assessment of hominid and hominoid remains[1,2]. Both number of cusps (4, 5 or 6) and the groove pattern (+ or *Y*) have been used in the comparison of many recent human populations[3–11]. The addition of the *X* groove pattern by Jørgensen in 1955 (ref. 8) completed the series of polymorphisms, giving three groove patterns (+, *Y* and *X*) and three or more cusp numbers (4, 5 and 6), or at least nine possible combinations in all. However, it has not been known for certain whether cusp number and groove pattern represent a single pleomorphism or two independent dental polymorphisms[8].

In our studies on 258 White subjects with unworn dentition, we have found side-to-side concordance to exceed 96 per cent both for cusp number and groove pattern. Further, extreme discordances have not been found; individuals did not differ by more than one cusp or by one step in the (+, *Y*, *X*) groove series. However, the two polymorphisms on M_1 proved to be essentially unrelated. For males taken separately, for females taken separately, and for both sexes considered together, the association between groove pattern and cusp number was not significant ($\chi^2 = 1{\cdot}99$).

Despite the independence of these two dental polymorphisms within individuals, they do appear to be correlated among recent human populations. Ranking twenty different groups, sixteen taken from the literature[3–11], with due attention to problems of grouping cusp numbers in excess of five, and groove patterns other than *Y*, there was a clear-cut relationship between the percentage of individuals exhibiting the *Y* pattern and the percentage of individuals with five or more cusps on the

Table 1. MANDIBULAR FIRST MOLARS WITH *Y* GROOVE PATTERN AND FIVE OR MORE CUSPS

Group	*Y* pattern per cent	5 cusps or more per cent
Texas Indians (ref. 6)	69 3	99·3
Recent Dutch (ref. 8)	76 5	89·8
Old Harbor Koniag†	76·7	97·6
Lublin Jews (ref. 11)	78·0	78·0
Fels Institute subjects*†	82·2	85·6
East Africans (ref 9)	86·9	95·1
Pecos Indians (ref. 10)	88·6	99·4
Alaska Eskimos (ref. 5)	91·1	95·6
Chicago Whites (ref. 4)	92·0	86·0
Ohio orthodontic patients*†	93·5	84·7
European male Whites (ref. 5)	94·0	89 0
Ninth-century Hungarians (ref. 5)	94·0	83·0
East Greenland Eskimos (ref. 7)	95·7	98·5
New Britain Melanesians (ref. 3)	96·8	96·3
Neolithic Mesopotamians (Kish)†	97·2	91·7
American Negroes (ref. 5)	99·0	99 0
Pima Indians (ref. 10)	99·4	100 0
West African Negroes (ref. 5)	100·0	99·0
Buriats (ref. 5)	100·0	100 0
Amerinds (ref. 5)	100·0	100·0

Samples below 30 excluded
* Observations by Kinai Tomita.
† New or unpublished observations.

mandibular first molar (Table 1). Although sampling effects, inter-observer differences in techniques of morphological assessment, and the failure of earlier workers to recognize the *X* pattern may be expected to minimize such association, the rank order correlation (ρ) was 0·54 on a population basis.

Since our data show high monozygotic twin concordance with respect to both cusp number and groove pattern, and excellent bilateral symmetry for both sets of dental traits, they appear to be genetic polymorphisms of considerable potential value in the analysis of recent human populations. Because cusp number and groove morphology are unrelated within individuals, it would seem expedient to separate the two, describing a particular subject as "*Y* and 5", or "*Y* and 4", rather than as *Y*5 or *Y*4 as has been the custom. Finally, the degree of association within recent populations suggests that common selective factors have brought about a simultaneous reduction in cusp number and deviation from the *Y* pattern, even though the two are independent dental polymorphisms.

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PSYCHOLOGY

Attentional Factor in Delayed Response

THE loss of an animal's ability to perform delay tasks following bilateral prefrontal lobe ablations is well established and has been attributed to diverse causes, notable among which is the factor of attention. Equally established are techniques which, by altering the animal's physiological state or the problem situation, are effective in restoring the animal's task performance to the preoperative level. Typically, the techniques reduce the animal's distractability or emphasize the cueing procedure thereby presumably enhancing the animal's ability to attend[1,2]. While this evidence implicates attention as an important factor in delayed response, it does so indirectly, and thus far attention has eluded direct study as a dependent variable.

Delayed response deficits comparable with those seen in ablated animals have been reported in the essentially intact animal as the result of electrical stimulation of certain brain foci through chronically implanted electrodes[3,4]. The technique of electrical excitation has also been used to elicit alerting or 'attentional' responses. This behavioural 'attention' is characterized by: (i) arrest of the on-going behaviour of the animal; (ii) arousal, that is, the animal seems to be more alert and often undergoes a change in facial expression and attitude; (iii) orientation, the animal looks about usually toward the side contralateral to the stimulation. On the basis of these behavioural observations and electroencephalographic evidence, it has been concluded that the frontal lobes have their own attentional system unrelated to any known sensory system[5,6].

The purpose of this investigation was to examine more directly the part that attention may play in delayed response performance. After mastering a 5-sec *L–R* delayed response task by the direct method, five macaque monkeys were implanted with stainless steel electrodes insulated except for the cross-sectional area at the tip. Two weeks after surgery each brain point was stimulated to see if an 'attentional' response could be elicited. A 60 c/s sine wave pulse of 1–2 sec duration was delivered monopolarly in these determinations; current values ranged from 50 to 400 μamp. The animals were seated in restraining chairs modified to include components of the Wisconsin general test apparatus. A brain point was judged to be 'attentional' in nature if on stimulation the animal manifested alerting behaviour according to the stipulated criteria. In so far as attention may be a critical element in the performance of the delay task, one would expect a high degree of overlap between brain points eliciting 'attention' and those causing delayed response deficits on stimulation.

The effect of electrical excitation on delayed response was then investigated. Each brain point was stimulated monopolarly during the associative and retentive phases of the delay task. A current intensity below the threshold for eliciting the attentional response was used during this phase of the investigation to preclude direct interference with behavioural performance. The electrical stimulation lasting for 5 sec, 0·5 sec on and 0·5 sec off during each phase. Details of this aspect of the study have been reported elsewhere[4].

Table 1 summarizes the results. Twenty-nine of the 55 brain points were judged to elicit an alerting response while 34 of the 55 resulted in a statistically significant deficit in the delayed response score when stimulated. A χ^2 test revealed no significant relationship between the two classes of points, indicating that the delay deficits seen were not due to disruption of processes in neural tissue judged to be part of an attentional system.

Table 1. EFFECTS OF ELECTRICAL STIMULATION ON DELAYED RESPONSE PERFORMANCE AND 'ATTENTION'

		'Attention' Yes	No	Total
Delay deficit	Yes	18	16	34
	No	11	10	21
	Total	29	26	55

Table 2. RELATIONSHIP BETWEEN 'ATTENTION' AND PHASE OF DELAY PROBLEM WHERE DEFICIT WAS SEEN

	Phase related delayed response effect: Assoc. +	−	Retent. +	−	Both +	−	No effect +	−	Total +	−
Cortex and basal ganglia	3	5	3	1	0	0	7	8	13	13
Limbic and deep extrapyramidal	0	3	2	0	6	6	0	0	8	7
Thalamic and brain stem	1	3	1	1	2	0	4	2	8	6
Total	4 (12)	8	6 (8)	2	8 (14)	6	11 (21)	10	29 (55)	26

\+ Symbolizes an 'attentional' point.
− Symbolizes a 'non-attentional' point.

Table 2 shows the problem phase during which the stimulation-caused deficit was seen, its relationship to attention and the gross neuroanatomical locus of the electrode. With regard to attention, the locus does not appear to be important since attentional and non-attentional points are distributed about equally within the three classes of tissue. While the number of points in each cell is too small for meaningful statistical analysis, it would appear that the deficits seen were neither specific to the animal's ability to attend, that is, form an associa-

tion, nor to retain, that is, be distracted from an already formed association. This suggests that the delay deficits seen were more likely due to interference with associative and retentive processes directly rather than due to interference with an attentional mechanism. While this investigation demonstrates that attention is not the critical factor in accounting for delayed response deficits it does not suggest that the task can be performed without attending. Indeed, the validity of results in delayed response experiments is contingent on the experimenter's judgment that the animal attended to the cueing procedure on each and every trial. Thus it seems more likely that the delay deficits are not due to an inability to attend, but rather may be due to an inability to process the information.

I thank Prof. J. Olds, University of Michigan, for providing facilities for this work.

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Effects of Loudness and Presentation-rate on Auditory Short-term Memory

MACKWORTH[1] has presented evidence suggesting that if lists of, for example, letters or digits are presented auditorially for immediate recall, fast presentation is more advantageous than slow. The converse has generally been found true for visual presentation[2–4]. It has been hypothesized by Mackworth that subjects might use a short-lasting auditory memory after-image for recall purposes, this image probably persisting for longer than the visual memory after-image. It might then be that fast presentation-rates of auditory material facilitate recall because more of the list is still available in image-form after a fast presentation than after a slow presentation.

At the same time we[5] have found that when seen lists were voiced aloud by the subject at presentation, such vocalization also gave relatively more advantage for recall after fast than after slow presentation-rates. If it be argued that, when voicing, the subject is presenting himself with an auditory message (the sound of his own voice) in addition to the visual message, then our results would be consistent with Mackworth's hypothesis. In other investigations, however, we have found only slight, insignificant advantage for loud-voiced lists over soft-voiced lists for immediate recall. The question is therefore raised whether loud presentation in itself will give rise to a 'stronger', more persistent after-image which ought to aid immediate recall of loud material: we might also ask whether, if lists are presented rapidly, less of the image will have disappeared when the list comes to be recalled. To examine this, immediate recall has been obtained for lists of eight letters presented either quickly (120 letters/min) or slowly (60 letters/min), the lists also being presented at three intensities.

Thirty-six subjects, mainly from introductory psychology courses at Queen's University, came in groups of six to the University Language Laboratory. In this laboratory, each subject had a private booth and a set of earphones through which messages from a central tape-deck could be relayed. After initial practice on six lists, they heard a total of twenty-four lists of eight consonants each (for example, LVMKTNCS), having four lists at each of the six combinations of rate and loudness. Variations in rate were obtained by recording the sequences of lists in either fast or slow versions, using a metronome; loudness was varied by adjusting a dial on the central tape-deck. With each booth having been previously set to be equal in general sound-output to the other booths, the three levels of loudness as measured at the earphone were: 50 db (soft), 80 db (medium), and 90 db (loud). At these levels, each list was clearly audible, but the differences between the soft, medium and loud levels were plainly discernible to subjects. The design was balanced so that each group of subjects worked through the six presentation-conditions in a different order; and subjects were always warned of the rate and loudness to expect for each list. As soon as each list ended, subjects wrote down their recall on prepared answer-sheets: they were told they could omit or guess if they could not recall a letter, and were allowed to write down their recall in any order provided they put each letter into its correct original position. The main index of recall was the number of letters in their correct positions.

The percentages of letters correct out of the total possible are shown in Table 1.

Table 1. PERCENTAGE OF LETTERS CORRECT AS A FUNCTION OF LOUDNESS AND PRESENTATION-RATE

	Soft	Medium	Loud
Slow (60/min)	59·11	64·32	63·37
Fast (120/min)	60·24	57·03	55·96

Analysis of variance showed that the effects of loudness and rate were not significant; there was a significant subject-variance ($F = 7{\cdot}707$, $P < 0{\cdot}005$); and there was a significant interaction between loudness and rate ($F = 5{\cdot}08$, $P < 0{\cdot}01$). Other interactions were not significant. Increasing the loudness appeared to improve recall of slow lists except at the loudest level, but to depress the recall of fast lists.

From these results it would seem that we shall have to qualify our original suggestions. Rather than aid recall of fast lists, increasing loudness seems to hinder it. We suggest that the results might most simply be explained if we assume that loud lists did leave stronger after-images but that these images themselves interfered with the rapid intra-presentation rehearsal required on fast lists. Furthermore the images probably interfered with simple grouping of letters, a strategy which Mackworth[6] has found to facilitate the recall of fast lists in particular. Posner[7] has also found that if recall is forced (that is, if the results must be given in the order in which they are presented) the advantage of fast lists increases. This type of recall, also used by Mackworth[1], was not used in the present situation.

It can therefore be concluded that it is not appropriate to generalize that fast auditory lists will necessarily be better recalled than slow auditory lists. Apart from grouping and recall-strategies, we have found that increasing the loudness of fast lists is detrimental to their recall, probably because of a greater difficulty of rehearsal due to interference. With regard to our own work on vocalization, these findings suggest that what advantage might have accrued to the recall of loud-voiced lists by virtue of a more persistent after-image was probably diminished due to various kinds of interference being simultaneously produced by loud voicing.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Tuesday, April 12

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

INSTITUTION OF THE RUBBER INDUSTRY, LONDON SECTION (at the Royal Overseas League, Park Place, London, S.W.1), at 6.30 p.m.—Annual General Meeting.

Wednesday, April 13

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, 26 Portland Place, London, W.1), at 3.30 p.m.—Symposium on "Automation in Coal Mining".

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP—NUTRITION PANEL (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Annual General Meeting, followed by Dr. R. J. L. Allen: Chairman's Address.

INSTITUTE OF INFORMATION SCIENTISTS (at the Whitehall Hotel, Bloomsbury Square, London, W.C.1), at 6.15 p.m.—Mr. H. Schur: "The Sheffield University Postgraduate School's Course on Scientific and Industrial Librarianship and Information Work".

CROYDON NATURAL HISTORY AND SCIENTIFIC SOCIETY (in the Maple Room, Fairfield Halls, Croydon), at 7.30 p.m.—Dr. K. Fea: "Some Aspects of Lunar Geology".

Thursday, April 14

INSTITUTION OF CIVIL ENGINEERS, ENGINEERING MANAGEMENT GROUP (at Great George Street, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Difficulties of Communication in Management", introduced by Mr. R. F. Heynes.

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion Meeting on "Non-Optical Transmissions".

Friday, April 15

INSTITUTE OF NAVIGATION (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, S.W.1), at 5.30 p.m.—Colonel J. Kelsey and Mr. D. N. Bisgood: "Land Navigation".

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. A. H. Reeves, O.B.E.: "Equilibrium Coding for Pulse Code Modulation".

TELEVISION SOCIETY (in the Conference Suite, I.T.A., 70 Brompton Road, London, S.W.3), at 7 p.m.—Mr. W. Silvie: "Domestic Video Recording".

Monday, April 18

OPERATIONAL RESEARCH SOCIETY (at the Royal Aeronautical Society, 4 Hamilton Place, London, W.1), at 4.30 p.m.—Forum on "O.R. and Accountancy".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. E. C. Claydon: "Electricity for Grain Drying".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.E.–I.E.R.E. COMPUTER GROUPS (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Computer Bulk Storage".

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Annual General Meeting followed by Ordinary Meeting—The Chairman's Address.

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"The Days of Whiskey Gap" and "City of Gold" (films).

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER (with experience in general experimental psychology and an interest in laboratory teaching) IN PSYCHOLOGY—The Secretary, The Queen's University, Belfast, Northern Ireland (April 13).

LECTURER and an ASSISTANT LECTURER (with special interests in North America and/or economic geography) IN THE DEPARTMENT OF GEOGRAPHY—The Registrar, The University, Leicester (April 15).

LECTURER IN INORGANIC CHEMISTRY in St. Salvator's College—The Secretary, University of St. Andrews, College Gate, St. Andrews, Fife, Scotland (April 16).

LECTURER (preferably with a medical qualification) IN MEDICAL VIROLOGY—The Registrar, University Senate House, Tyndall Avenue, Bristol, 2 (April 16).

LECTURER (with appropriate academic qualifications, good teaching experience with recent developments concerning education in chemistry) IN EDUCATION—The Deputy Secretary, The University, Southampton (April [illegible]).

READER or SENIOR LECTURER IN APPLIED MECHANICS, and a LECTURER or ASSISTANT LECTURER IN MECHANICAL ENGINEERING IN THE SCHOOL OF APPLIED SCIENCES—The Assistant Registrar (Establishment), The University of Sussex, Stanmer House, Stanmer, Brighton, Sussex (April 16).

CHAIR OF ELECTRONICS—The Registrar and Secretary, The University of Sussex, Stanmer House, Stanmer, Brighton, Sussex, marking envelope "Confidential" (April 17).

LECTURER (man or woman graduate with experience of research in education and an interest in philosophy) IN THE SCHOOL OF EDUCATION—The Registrar, The University, Liverpool, quoting Ref CV/3 (April 18).

ASSISTANT LECTURER or LECTURER (with a medical qualification and preferably an additional dental qualification) IN ORAL PATHOLOGY—The Registrar, The University, Sheffield (April 23).

LECTURER IN HYDRODYNAMICS—The Registrar, The University, Manchester 13, quoting Ref. 73/66 (April 23).

LECTURER IN MEDICINE—The Registrar, The University, Sheffield (April 23).

LECTURER (with a good honours degree in mechanical engineering together with industrial and/or research experience and a special interest in applied thermodynamics) IN THE DEPARTMENT OF ENGINEERING—The Secretary, The University, Aberdeen (April 25).

UNIVERSITY RADIATION PROTECTION OFFICER (with experience in the use of nuclear radiations)—The Assistant Registrar (Science and Engineering), University of Birmingham, Birmingham, 15, quoting Ref. PO/N (April 23).

ASSISTANT LECTURER IN STATISTICS, preferably in mathematical statistics or probability theory—The Registrar, The University, Hull (April 25).

DEMONSTRATOR (graduate) IN INORGANIC CHEMISTRY; and a DEMONSTRATOR (graduate) IN ORGANIC CHEMISTRY—The Secretary, Trinity College (University of Dublin), Dublin 2, Republic of Ireland (April 25).

RESEARCH ASSISTANT (preferably with a higher degree and research experience in biological productivity and/or methods of plant and soil analysis) IN ECOLOGY IN THE DEPARTMENT OF BOTANY for work on the role of bryophytes in the productivity and mineral cycle of woodland ecosystems—Prof. P. W. Richards, Department of Botany, University College of North Wales, Bangor, North Wales (April 25).

VETERINARY OFFICERS (men or women aged at least 23, M.R.C.V.S. and at least one year's appropriate postgraduate experience) WITH THE MINISTRY OF AGRICULTURE, FISHERIES AND FOOD—Civil Service Commission, Savile Row, London, W.1, quoting Ref. 30/66/1 (April 26).

JUNIOR LECTURERS (2) (with an honours degree in physiology with zoology, chemistry or physics, or a medical qualification, and preferably some postgraduate experience in teaching and research) IN PHYSIOLOGY—The Registrar, King's College (University of London), Strand, London, W.C.2 (April 29).

LECTURER IN MATHEMATICS at Makerere University College, University of East Africa, Uganda—The Inter-University Council, 33 Bedford Place, London, W.C.1 (April 29).

ASSISTANT LECTURER IN GEOGRAPHY—The Registrar, The University, Manchester, 13, quoting Ref. 76/66 (April 30).

ASSISTANT LECTURER or LECTURER IN CHEMISTRY—The Registrar, The University, Sheffield (April 30).

ASSISTANT LECTURER (with an honours degree in physiology or prepared to attend an honours course, and preferably with a veterinary qualification) IN THE DEPARTMENT OF VETERINARY PHYSIOLOGY—The Secretary, The University, Edinburgh (April 30).

ASSISTANT LECTURER (with research interests in comparative physiology, preferably neurophysiology) IN ZOOLOGY—The Registrar, The University, Sheffield (April 30).

DEMONSTRATOR (graduate or about to graduate) IN BIOLOGY—The Registrar, The University, Keele, Staffs (April 30).

KEEPER (with a good university degree and relevant experience in an academic or scientific library, and preferably professional qualifications and experience or interest in the field of scientific librarianship) OF SCIENCE BOOKS—The Librarian, University Library, Palace Green, Durham (April 30).

LECTURER or ASSISTANT LECTURER IN BIOLOGICAL CHEMISTRY—The Registrar, The University, Manchester, 13, quoting Ref. 71/66/Na (April 30).

LECTURER or ASSISTANT LECTURER IN MATHEMATICS at the University of the West Indies, St. Augustine, Trinidad—The Inter-University Council, 33 Bedford Place, London, W.C.1 (April 30).

SENIOR LECTURER (Grade I) or LECTURER (Grade II) IN APPLIED MATHEMATICS—The Registrar, University Senate House, Tyndall Avenue, Bristol, 2 (April 30).

THIRD CHAIR OF CHEMISTRY—The Registrar, The University, Hull (April 30).

TUTOR (with a good honours degree in either social anthropology or sociology) IN THE DEPARTMENT OF SOCIAL ANTHROPOLOGY—The Secretary, The University, Edinburgh (April 30).

LECTURER or ASSISTANT LECTURER (with special qualifications in organic chemistry) IN ORGANIC CHEMISTRY—The Secretary, The University, Edinburgh (May 2).

CHAIR OF BACTERIOLOGY—The Secretary, The University, Edinburgh (May 3).

LECTURER IN THE DEPARTMENT OF PSYCHOLOGY—The Secretary, The University, Edinburgh (May 4).

AGRICULTURAL SCIENCE or SCIENCE GRADUATE (with an honours degree in agriculture or science (majoring in botany) or equivalent, and suitable postgraduate research experience) for research on the manuring, ecology and related management aspects of grassland—The Director, An Foras Taluntais (The Agricultural Institute), 33 Merrion Road, Dublin 4, Republic of Ireland (May 6).

LECTURER or ASSISTANT LECTURER IN PHILOSOPHY—The Registrar, The University, Sheffield (May 7).

LECTURERS or ASSISTANT LECTURERS (for one post preference will be given to candidates with a special interest or experience in X-ray crystallography) IN THE DEPARTMENT OF INORGANIC AND STRUCTURAL CHEMISTRY—The Registrar, The University, Leeds, 2 (May 7).

TEACHING FELLOWS (with a Ph.D. degree) IN THE DEPARTMENT OF INORGANIC AND STRUCTURAL CHEMISTRY, to give laboratory and tutorial instruction and to engage in research—The Registrar, The University, Leeds, 2 (May 7).

LECTURER (with research interests and industrial experience in the fields of automatic control, power systems analysis or electronic circuit design) IN ELECTRICAL ENGINEERING—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (May 13).

POSTDOCTORAL RESEARCH ASSISTANT IN THE DEPARTMENT OF PHYSICAL CHEMISTRY, for the investigation of ion molecule reactions, using a differentially pumped mass spectrometer—Dr. M. J. Henchman, Department of Physical Chemistry, The University, Leeds, 2 (May 31).

LECTURER (with a postgraduate degree and preferably some experience of teaching biochemistry at University level) IN BIOCHEMISTRY, at Lincoln College, University of Canterbury, New Zealand—The Association of Commonwealth Universities, Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, June 30).

GRADUATE MICROBIOLOGIST or BACTERIOLOGIST (male, between 20 and 30, and industrial experience of research or quality control, preferably in the

food industry) to work on problems associated with the microbiological spoilage of baked products and the introduction of new methods of prevention—The Secretary, British Baking Industries Research Association, Chorleywood, Rickmansworth, Hertfordshire.

LECTURER IN STATISTICS to teach statistics and some mathematics to the standard of the B.Sc. General Degree of the University of London—Clerk to the Governing Body, Northern Polytechnic, Holloway, London, N.7.

LECTURER or SENIOR LECTURER (with a first- or second-class honours degree or equivalent in civil or mechanical engineering) IN THE MATERIALS BRANCH OF THE DEPARTMENT OF CIVIL ENGINEERING, primarily to lecture on the properties of materials and stress analysis at degree level and to supervise the relevant laboratory work—The Registrar, Royal Military College of Science, Shrivenham, Swindon, Wiltshire.

LECTURER (with a good honours degree in psychology) IN PSYCHOLOGY (Experimental Psychology/General Psychology) for work mainly with honours and general degree courses—The Registrar, Goldsmith's College (University of London), London, S.E.14.

LECTURERS or ASSISTANT LECTURERS (or exceptionally Senior Lecturers) IN MATHEMATICS—The Assistant Registrar (Establishment), University of Sussex, Stanmer House, Stanmer, Brighton, Sussex

MASTER TO TEACH MATHEMATICS (this is largely a Sixth Form appointment)—The Headmaster, St. Albans School, Abbey Gateway, St. Albans, Herts.

PHYSIOLOGIST to join a small team of scientists studying the human environment—D. C. Page, Head of Personnel Services, The Electricity Council Research Centre, Capenhurst, Cheshire, quoting Ref. N/19.

POSTDOCTORAL FELLOW IN ORGANIC CHEMISTRY to join a team working on a major synthetic project—The Registrar, The University, Manchester, 13.

PRINCIPAL LECTURER IN MATHEMATICS; a PRINCIPAL LECTURER IN CHEMICAL ENGINEERING; a LECTURER IN BIOCHEMISTRY; a LECTURER IN MICROBIOLOGY; a LECTURER IN PRODUCTION PROCESSES (Machine Tools or Metal Forming); an ASSISTANT LECTURER IN PRODUCTION ENGINEERING, an ASSISTANT LECTURER IN ELECTRICAL ENGINEERING; and RESEARCH ASSISTANTS IN ELECTRICAL AND IN MECHANICAL ENGINEERING—Clerk to the Governing Body, Borough Polytechnic, Borough Road, London, S.E.1.

SENIOR LECTURER (with a good honours degree and preferably with a further degree or research experience in animal physiology or genetics/cytology) IN ZOOLOGY; and a LECTURER (with a good honours degree and preferably a special interest in plant physiology) IN BOTANY—The Registrar, Regional College of Technology, Leicester.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Transactions of the Royal Society of Edinburgh. Vol. LXVI, No. 6 (1963–64): On the Cupule Structure of *Eurystoma angulare*. By Albert G. Long Pp. 111–128+1 plate. 8*s*. 6*d*. Vol. LXVI, No. 7 (1964–65): Nuclear Phenomena in *Marasmius androsaceis* (L. ex Fr.) Fr. and M. *Rotula* (Scop. ex Fr.) Fr. By E. Julian Duncan and J. A. Macdonald. Pp. 129–141+4 plates. 10*s*. 6*d*. Vol. LXVI, No. 8 (1964–65): Raised Shorelines Associated with the Perth Readvance in the Forth Valley and their Relation to Glacial Isostasy. By J. B. Sissons and D. E. Smith. Pp. 143–168. 10*s*. 6*d*. (Edinburgh: The Royal Society of Edinburgh, 1965.) [281

Institute of Personnel Management. The Labour Market. By John Corina. (Part 1 of Incomes Policy—Problems and Prospects.) Pp. 35. 7*s*. 6*d*. The Development of Incomes Policy. By John Corina. (Part 2 of Incomes Policy—Problems and Prospects.). Pp. 82. 17*s*. 6*d*. (London Institute of Personnel Management, 1966.) [281

Bulletin of the British Museum (Natural History). Geology. Vol. 11, No. 8: On Certain Triassic and Liassic Representatives of the Family Pholidophoridae S. Str. By Prof. O. Nybelin. Pp. 351–432+15 plates. (London: British Museum (Natural History), 1966) 60*s*. [281

Forestry Commission. Booklet No. 15: Know Your Conifers By Herbert L. Edlin. Pp. 56 (London: H.M. Stationery Office, 1965.) 5*s*. net. [281

Fabian Society. Fabian Research Series, No. 252: The Mental Health Services. By Peter Mittler. Pp 29. 3*s*. Fabian Research Series, No. 253. The Remoter Rural Areas. By Keith Openshaw. Pp. 24. 2*s*. 6*d*. (London. Fabian Society, 1966.) [12

Metallurgical Abstracts, Series III, Vol. 1, No. 1 (January, 1966). Pp. xvi+1–80. Subscription for Non-Members £20 p.a. (56.50 dollars). Booksellers, Public and University Libraries £15 10*s*. (49.50 dollars). (London: Institute of Metals, in association with the Iron and Steel Institute and the Institution of Metallurgists, 1966.) [12

Titles and Dissertations Approved for the Ph.D., M.Sc., and M.Litt. Degrees in the University of Cambridge during the Academical Year 1964–1965. Pp. iii+30. (Cambridge: Board of Research Studies, The University, 1966.) [12

Ministry of Technology. Problems of Progress in Industry, No. 18: Work Flow in Batch Production. By R. H. Hollier and E. N. Corlett. Pp. 32. (London: H.M. Stationery Office, 1966) 3*s*. net. [12

The New Mercantilism By Prof. Joan Robinson. (Inaugural Lecture delivered in the University of Cambridge on October 15, 1965.) Pp. 26. (London: Cambridge University Press, 1966.) 3*s*. 6*d*; 75 cents. [12

Ministry of Agriculture, Fisheries and Food. Agricultural Chemical Approval Scheme (Insecticides, Fungicides and Herbicides). List of Approved Products 1966 for Farmers and Growers. Pp. 144. (Pinner, Middx.: Ministry of Agriculture, Fisheries and Food, 1966.) [12

Ministry of Agriculture, Fisheries and Food. Bulletin No. 154: Grass and Grassland. Pp. iv+114. (London: H.M. Stationery Office, 1966.) 8*s*. net. [12

Bulletin of the British Museum (Natural History) Geology. Vol. 11, No. 9: Some British Jurassic and Cretaceous Ostracoda. 1: New Genera of Purbeck and Wealden Ostracoda. By Dr. Frederick William Anderson. 2: Ostracods from the Portland Beds of Dorset. By Dr. Dennis Barker. 3: Ostracods from the Portland and Purbeck Beds of the Aylesbury District. By Dr. Dennis Barker. Pp. 433–487+9 plates. 44*s*. Zoology. Vol. 14, No. 2: The Elopoid and Clupeoid Fishes in Richardson's "Ichthyology of the Seas of China and Japan" 1846. By P. J. P. Whitehead. Pp. 15–54+7 plates. 26*s*. Vol. 14, No. 1. The *Oedura tryoni* Complex: East Australian Rock-Dwelling Geckos. (Reptilia: Gekkonidae). By H. Robert Bustard. Pp. 1–14+3 plates 10*s*. (London: British Museum (Natural History), 1966.) [12

Other Countries

Annuaire pour l'An 1966 publié par Le Bureau des Longitudes. Pp. viii+882. (Paris: Gauthier-Villars et Cie., 1966.) 55 francs. [12

The Annual of Czechoslovak Medical Literature 1963. Edited by Dr. Josef Navrátil. Pp. xv+672. (Praha: Czechoslovak Medical Press, 1965.) [12

Organization for Economic Co-operation and Development. Ministerial Meeting on Science. Government and Allocation of Resources to Science. Pp. 66. 4 francs; 6*s*.; 1 dollar. Government and Technical Innovation. Pp. 60. 4 francs; 6*s*.; 1 dollar. Fundamental Research and the Policies of Government. Pp. 72. 6 francs; 9*s*.; 1.50 dollars. The Social Sciences and the Policies of Governments. Pp. 102. 6 francs; 9*s*.; 1.50 dollars. (Paris: Organization for Economic Co-operation and Development; London: H.M. Stationery Office, 1966.) [12

Consejo Superior de Investigaciones Cientificas: Diputacion Provincial de León. Trabajos de la Estacion Agricola Experimental de Leon, Vol. 1, Año 1964. Pp. 166. (Leon, España: Facultad de Veterinaria, Paseo de la Facultad, 25, 1965.) [12

Unesco Scientific Institutions and Scientists in Latin America. Argentina, (Instituciones), Fasciculo 1. Pp. 396. Mexico (Cientificos) (S-Z), Fasciculo 3. Pp. iv+687–889. (Montevideo: Centrao de Co-operacion Cientifica para America Latina en colaboracion con la Organizacion de los Estados Americanos, 1965.) [12

Svenska Linné-Sallskapets Årsskrift, Årgang XLVII, 1964. Pp. 98. (Uppsala: Svenska Linné-Sallskapet, 1965.) [12

New Zealand. National Roads Board: Road Research Unit. R.R.U. Bulletin No. 2: The California Bearing Ratio Test on Loess By J. P. Blakeley. Pp. 18. (Wellington, N.Z.: National Roads Board, 1965.) [12

Commonwealth of Australia. Department of National Development: Bureau of Mineral Resources, Geology and Geophysics. Petroleum Search Subsidy Acts. Publication No. 12: Summary of Data and Results—Drilling Operations in the Sydney Basin, New South Wales, 1958-1962, of Australian Oil and Gas Corporation Limited; Farmout Drillers No Liability; and Exoil (N.S.W.) Pty. Limited. Pp. 29+4 plates. Publication No. 31: Tambo-Augathella Aeromagnetic and Gravity Surveys, Queensland, 1959-1960, by Magellan Petroleum Corporation. Pp. 25+2 plates. Publication No. 49: Summary of Data and Results—Carnarvon Basin, Western Australia—Exmouth Gulf Marine Seismic Survey; Whaleback Seismic Survey of West Australian Petroleum Pty. Limited. Pp. 12+5 plates. Publication No. 52: Summary of Data and Results—Drilling Operations in the Murray Basin, New South Wales and South Australia, 1961-1962, of Australian Oil and Gas Corporation Limited; Woodside (Lakes Entrance) Oil Company N.L.; and Australian Oil Corporation. Pp. 34+5 plates. Publication No. 53: Summary of Data and Results—Surat Basin, Queensland, U-K-A. Wandoan No. 1, U-K-A Burunga No. 1, of Union Oil Development Corporation, Kern County Land Company; and Australian Oil and Gas Corporation Limited. Pp. 19+3 plates (Canberra: Bureau of Mineral Resources, Geology and Geophysics, 1963, 1964 and 1965) [12

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Museum of Comparative Zoology, Harvard University. Breviora. No. 231 (October 29, 1965): A New Anole (Sauria, Iguanidae) from Puerto Rico. By Ernest E. Williams, Juan A. Rivero and Richard Thomas. Part 1: Description. By Ernest E. Williams and Juan A. Rivero. Pp. 18. No. 232 (October 29, 1965): Hispaniolan Giant Anoles (Sauria, Iguanidae)—New Data and a New Subspecies. By Ernest E. Williams. Pp. 7. No. 233 (October 29, 1965): South American *Anolis* (Sauria, Iguanidae)—Two New Species of the *Punctatus* Group. By Ernest E. Williams. Pp. 15. No. 234 (October 29, 1965): Studies on Neotropical Pompilidae (Hymenoptera). By Howard E. Evans. 1: The Genus *Agenioideus* Ashmead in South America. Pp. 7. No 235 (November 19, 1965): A New Salamander of the Genus *Chiropterotriton* (Caudata: Plenthodontidae) from Mexico. By George B. Rabb. Pp. 8. No. 236 (December 10, 1965): Variation in the Number of Marginal Tooth Positions in Three Species of Iguanid Lizards. By Clayton E. Ray. Pp. 15. No. 237 (December 15, 1965): A New Species of the Ant Genus *Dacetinops* from Sarawak. By Robert W. Taylor. Pp. 4. Bulletin of the Museum of Comparative Zoology, Harvard University. Vol. 133, No. 5: Comments on some Recent Changes in the Classification of the Clidae (Coleoptera) By John F. Lawrence. Pp. 273–293. Vol. 133, No. 6: The Fossil Elephant Shrews (Family Macroscelididae). By Bryan Patterson. Pp. 295–335+1 plate. Vol. 133, No. 7: Panamanian Spiders of the Genus *Tmarus*. By Arthur M. Chickering. Pp 337–368. (Cambridge, Mass.: Museum of Comparative Zoology, Harvard University, 1965.) [12

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DEAD HEAT ON THE MOON

IT is beginning to look as if the race to the Moon could be decided by what happens in 1966. The United States has already carried out a successful rehearsal of an encounter between two rockets in orbit about the Earth, and the first flight of a version of the *Apollo* spacecraft is imminent. The U.S.S.R. has landed instruments on the Moon in working order, and has now put *Luna* 10 into an orbit about the Moon. These are all steps in the process of sending a man to the Moon, and of bringing him back as well. It is only a matter of time before they are integrated into sequence. In a few months, those who are so inclined will be able to guess with some show of plausibility which of the two competitors is likely to win. Bookmakers, no doubt, will start taking bets.

Deciding what the race has accomplished and will accomplish is unfortunately more difficult. The undoubted benefits and the undoubted penalties are incommensurable. Whether to cheer or to hang the head in gloom is therefore a question for subjective judgment, not mere logic.

The benefits are easier to catalogue. Exploration of the Moon and of its geology will be extremely valuable, but a good start should have been made with automatic devices long before the first men arrive. So close is the development, indeed, that a lot of fun has already gone out of the Earth-bound controversy about the nature of the surface of the Moon; only a few more months, and this will be known once and for all. Beyond the Moon itself lies its potential usefulness as a site for telescopes and other instruments, but not even the most vigorous supporters of the competition for the Moon would claim that the technical benefits of victory can justify more than a tiny fraction of the cost.

The disparity between the cost of sending a man to the Moon and the costs of the most ambitious projects on which Earth-bound scientists set their hearts is by itself sufficient proof that space travelling is not mere science. The cost of the large reflecting telescope in the southern hemisphere, the lack of which has been a painful frustration in the past decade, is perhaps a thousandth of the cost of Project *Apollo* in the United States. The same programme of space development might alternatively provide the cost of a hundred proton synchrotrons to succeed the machines at Brookhaven and Geneva. It would also support scientific research in British universities for the best part of a century or provide every university in the world with a really up-to-date computing installation. Obviously such a distribution of the efforts of skilled people would not have been settled in the light of strictly scientific considerations. Even the most ardent space-travellers would have sought a more balanced effort.

"But we only send the rocket to the Moon—not the money spent on developing it." This is the crypto-Keynesian justification. It is in this spirit that officials in the United States have from time to time extolled the virtues of what is called 'spin-off', and it is true, at least in the United States, that a large space programme does introduce industry to new techniques. The trouble is that many of the new techniques have no application outside rocketry. The educational consequences of the space programme—again in the United States—have been mixed. Plentiful funds have provided the United States Government with a convenient means of channelling supporting scientific research into universities and other institutions. Inevitably, however, the result has been lop-sided. Already there are some who claim that much greater benefits would have been forthcoming, in industry and in education, if a part of the money spent on rocketry had been spent, instead, on the development of fast surface transport (on the Earth) or on the advancement of computer techniques. The defect in this analogy with Keynes's argument that a nation with a slump on its hands can usefully employ men to dig holes in the ground and then to fill them in again is plain. Any research and development is better than none, but for advanced nations such as the United States and the U.S.S.R., where research and development are limited by skilled manpower and not by money, it is meaningful and certainly prudent to seek an optimum strategy for the effective deployment of resources. To aim to get to the Moon by such and such a date introduces a needless constraint on this operation.

But must we not go to the Moon because it is there? President Kennedy made good use of the analogy of the Moon and Everest. There are also on record the unforgettable words of the distinguished American who told Congress that going to the Moon is unavoidable "because that's the kind of person Man is". And, of course, it is exciting. Which of us will not wish, at least in part, for a place in an early expedition? Next best, we shall sit glued to our television sets—not every day is there a chance to see a kind of Colombus go about his business.

But why the hurry? Nothing vital would be lost if the first landing were delayed a decade. Even a few years of grace would help to lend moderation to the present course of events. The ideal would be some kind of co-ordination of the programmes of the United States and the U.S.S.R., tacit or otherwise, for there is an urgent need to see that the race to the Moon does not become an empty but symbolic trial of strength between two great nations.

The dangers are already great. Both nations can probably afford the cost. They can also afford some substantial departure from the ideal pattern of research and development. But because so much prestige has now been attached to reaching the Moon, neither nation can afford to lose. Unfortunately, however, success will not be an accurate yardstick of prowess, technological or otherwise. Luck will matter. So will the somewhat arbitrary decision the two nations have already made about the commitment of resources to this project. The best man may not be the winner—and the loser will be bound to save face by nominating some more distant celestial body than the Moon as the next finishing post.

The reputations of nations are chiefly at stake, but the reputation of science and technology is also involved. Even if—again in the United States—scientists have been able to establish that the cost of going to the Moon should not be thought of as a charge against scientific research, popular and not-so-popular opinion still holds

that the competition for the Moon is a comparative assessment of science and technology in the two nations. In reality, it would be as sensible to assess the merits of two chess-players by pitting them one against the other at *Karate*. It will do no good, and much harm, if the competition for the Moon should lead to another spate of invidious and pointless comparisons of the conditions of science in the U.S.S.R. and the United States. It will be damaging if science and technology are found to have lent themselves, without protest but even with a sneaking sense of delight, to a hollow competition. Obviously it is too late now to stop the race but, in the circumstances, it may be best to hope for a draw.

BRITISH GAS

THE discovery of natural gas in substantial quantities under the North Sea will still further sharpen the need that the British Government should have a consistent policy on fuel. A quarter of a century ago the British industrial economy was virtually dependent on a single fuel—coal. Since then, and particularly in the past decade, there has come almost bewildering diversity. First came a rapid growth of the import of petroleum. The prospect of nuclear power competitive in price with electricity from coal has finally become reality. Now there is natural gas—or the promise of it—and nobody will blame the British Government for hoping that the exploration of the North Sea will do for the British industry what the natural gas at Laq did for French industry a decade ago, and what the more recent finds of gas in Holland have done for the Dutch. There will even be some who will point to the way in which cheap coal sustained the Industrial Revolution in Britain.

Unfortunately, however, exploiting natural gas from the North Sea will not simply be a matter of laying pipes and turning on the taps. It will also somehow be necessary for the British Government to arrange for this new fuel to fit easily into the existing fuel economy. Negotiating prices will be relatively easy, although there is likely to be hard bargaining between the nationalized gas industry and the oil companies which have found the gas. The real difficulty will be to cushion the changes which there are bound to be elsewhere in the British economy. How, for example, is the coal industry to be persuaded to contract even faster than at present? And how are the communities thus robbed of livelihood to be recompensed? Hitherto, successive British Governments have vacillated between the opposing beliefs that social considerations should determine—and limit—the pace of change, and that prices should be settled in the market and that supply and demand should then determine the balance with the fuel industries. If the natural gas finds in the North Sea turn out to be as plentiful as everybody hopes, the pressure on the Government to find a consistent compromise may even be great enough to prompt a satisfactory solution. Briefly, it needs to be recognized that marked changes in the pattern of the fuel economy have been and will be inevitable, and that the full costs of these are met by the prices charged for fuels of different kinds. Only in such a way can the most economic pattern be evolved with the greatest speed, but without grave social injustice to those with a vested interest in the preservation of an existing pattern—communities of coal-miners in Britain, for example.

NATIONALIZATION AND POLITICS

Politics and the Airlines

By Prof. David Corbett. Pp. 350. (London: George Allen and Unwin, Ltd., 1965.) 35*s*. net.

ONE of the most surprising things about Prof. Corbett's study of the policies and the politics governing airlines in Australia, Britain and Canada—to which the first six chapters of *Politics and the Airlines* are devoted—and, to a lesser extent, in the United States and India, is that in so technical a matter he never discusses the question of the technical or scientific adviser and his position in the formation of policy. Not that he is unaware of the problem. He remarks, for example, that studies of public enterprise tend to be sterile when they ignore the technology and the social environment of the industry they purport to analyse. He recognizes that the vital managerial decision for an airline is the choice of aircraft and that such choice must lie with the technical staff of the airline. Further, he maintains that airline managements make sound decisions when they can stand up to their Ministers. Airlines need strong boards and strong managements if they are to continue their tradition of excellence, and Prof. Corbett's study endorses the verdict of Prof. W. A. Robson in *Nationalized Industry and Public Ownership* as to the importance of resisting Ministerial pressure.

Again, he is well aware of the difficulties which, in Britain at least, arise out of the relations between civil aviation and the aircraft manufacturing industry. The interests of the two industries inevitably conflict at some points, and the Ministry tends to resolve differences at the expense of the airlines. For this reason, *Politics and the Airlines*, particularly the chapter in which Prof. Corbett discusses the means of Government control, is of some interest in connexion with the report of the Plowden Committee of Inquiry into the Aircraft Industry (see *Nature*, 209, 1053; 1966). He does not, however, pursue the implications of the view expressed by the Chairman of British Overseas Airways Corporation that the expense attendant on introducing an aircraft into service is part of its development and should be regarded as a proper charge against research and development funds, which are at the disposal of the Government. He is, in fact, concerned with the issue of public enterprise in general, and it is as a contribution to the discussion on the future of nationalization and a comparison of experience in different countries that his book derives its main value.

As such it is an admirably objective study though, as already indicated, of less interest to the scientist and technologist because scientific and technical issues are so largely ignored. Prof. Corbett first describes civil aviation before nationalization, and in his next four chapters examines the issue of public ownership, the great debate on nationalization, public versus private airlines and the issue of the public corporation. The chapter on Government control, already noted, is followed by two chapters on competition and regulation of airlines and on the International Airline of India—an example of enterprising bureaucracy; the conclusions of the study are set forth in a final chapter. These chapters reiterate the need for strong boards of directors in publicly owned airlines and the inherent inconsistency of placing related industries, such as aircraft manufacture and air transport, under the regulatory power of a single department. The tensions and political pressures thus generated can prevent either activity from being performed well. Prof. Corbett's own leaning is towards a mixed economy of private and public ownership, but he suggests that the system may have its own virtues and requires deliberate creation rather than that even the theory of such a system already exists. When regarded from an economic point of view, private and public ownership differ only marginally, and Prof. Corbett insists that party politics are increasingly irrelevant to the questi[illegible] though the rise and fall of

public enterprises depends as much on the political mood of the times and on the country in which they operate as on the formal relations linking them to the executive and legislative branches of Government. Although the book has copious references, a bibliography would have increased its usefulness; there appear to be important omissions among the British works cited.

R. BRIGHTMAN

ARCHAEOLOGICAL IDENTIFICATION

The Inorganic Raw Materials of Antiquity

By Dr. Andrée Rosenfeld. Pp. xiii+245+26 plates. (London: Weidenfeld and Nicolson, 1965.) 45*s*. net.

THE intention of Dr. Rosenfeld in writing *The Inorganic Raw Materials of Antiquity* was to provide the archaeologist with an introduction to the principles of mineral and rock identification, emphasis being given particularly to those materials most commonly used in antiquity. The need for such a book could only be doubted by those who have never had to cope with objects directly from archaeological excavations, for the identification of the materials from which antiquities were made can often only be described as frightening. Not only may wrong identifications find their way into final excavation reports, but also a failure at the time to appreciate the nature of the materials excavated may even result in inadequate excavation.

Allowing that most archaeologists have scant scientific training, in preparing this book Dr. Rosenfeld was clearly faced with one of two possible approaches. On the one hand, she could have provided a kind of 'cookery-book' for the identification of materials; on the other, she could begin by explaining the structure and composition of minerals, and go on from there. Wisely she chose the latter and, from the point of view of a writer, more difficult course. Molecular structure and crystal systems may seem remote when all the archaeologist wants to do is to ascertain whether a neolithic battle-axe was made of granite or syenite, but it is difficult to see how a really reliable method of mineral identification could be presented without these basic ideas. As it is, Dr. Rosenfeld has compacted the essence of mineral classification into a mere twenty pages of text; and this alone is no mean feat.

The contents of this book fall into two main sections. The first four chapters deal with the properties and identification of minerals and rocks. These chapters are of necessity brief, and the author has clearly taken pains to eliminate from the text all but the really vital materials. So far the book could have been written as an introduction to the subject for students of petrology, and one has little doubt that many such students will find it useful.

In the second half of the book are discussed the ores and metals used in antiquity; the manufacture of implements and ornaments; building materials; and the dating—by physical means—of rocks and minerals. Finally, a four-page appendix suggests how the archaeologist should set about identifying the materials that confront him. These final chapters are particularly valuable for their discussion of the merits and shortcomings of the various materials used by early man for tool-making and construction.

The book is clearly written and well illustrated, although many of the line drawings would have been better reproduced at a greater reduction. It is to be hoped that all archaeologists—professional and amateur alike—will not only read this book, but also continue to use it as a work of reference. It will be a pleasure indeed to open one of those neatly docketed little bags and to find that the description bears some resemblance to the contents: that the 'corroded bronze object' really is what it says, rather than a fragment of chlorite schist.

H. W. M. HODGES

NIGGLI METHODS FOR THE MANIPULATION OF GEOCHEMICAL DATA

Petrochemical Calculations

By Conrad Burri. Translated from the German by A. Katz. Pp. viii+303. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1965.) 108*s*.

A BASIC problem in geochemistry is the relation between the bulk chemical composition of the rock and the individual compositions of the component minerals. Prof. Niggli was interested in this problem throughout his life, and gradually evolved a series of procedures of which no connected account appeared during his lifetime. *Petrochemical Calculations* is a translation of a work which had been contemplated as a joint publication but which, in the event, was compiled by Prof. Burri after the death of Prof. Niggli in 1953.

An introductory section on some general problems of rock chemistry and on graphical methods of displaying analytical data leads to an exposition of the calculation, and application, of 'Niggli values'. These values are obtained by recalculation from the weight-percentage data of a chemical analysis, and their relation to other representative data such as cation percentages and molecular equivalent percentages is discussed. Their utility in geochemical calculations is fully demonstrated in the main section of the book, on the 'equivalent norm'. In contrast with the earlier CIPW norm, the equivalent norm permits rapid recalculation to different heteromorphic assemblages, and enables one to use the actual chemical compositions of modal minerals as easily as the standardized normative compositions. Thus the methods of calculation are equally applicable to metamorphic rocks, as the author demonstrates by examples of the determination of standard katanorms and epinorms and some modal variants. Brief reference is made to more recent proposals by T. F. W. Barth and P. Eskola concerning calculations in terms of anions and of a 'standard cell' based on 160 (O + OH).

The appendix provides an alphabetical index, with name, formula unit and equivalent weight, of the symbols of all the equivalent normative components used; a compilation of equations showing important reaction relations; and tables of equivalent numbers allowing for interpolation of 0·01 wt. per cent. A short section on "The Concept of a Magma-type" is followed by the tabulation, first published in 1945, of more than 180 supposed magma-types. The reappearance of this long list of recalculated rock-analyses under this head is regrettable, although it is stated in partial justification "in the context used here . . . a magma-type is defined merely as an abbreviated designation for the characterization of the rock's chemism". Many of the types listed are represented by rocks, such as manifest accumulates, for which the existence of a magma of the same composition is out of the question or has never been demonstrated.

The translation is adequate for a work of this kind, though phrases such as "the modus is not unequivocally determined by the chemism" or "the CO_2 evolved during the assimilation of the calc" do jar on the reader. The CIPW norm is misprinted as 'CPIW' in many places, including the index. The nonsensical term 'mesocratic' should read 'mesotype'.

The German edition, first published in 1959, referred to the "completely new development in rock analysis" which was beginning to take shape, in the form of rapid methods, at the hands of Shapiro and Brannock and other analysts. Because a much greater number of determinations could be carried out in a given time, it was becoming feasible to apply statistical methods to their interpretation. The intervening period has seen further developments which make this statement still more applicable to-day;

the advent of X-ray fluorescence procedures has so increased the rate of output of analytical data that the 'small calculating machine' recommended by the author must now give place to the computer. The large number of fully worked examples is a specially valuable feature of the book, the same three selected igneous rock-types being used wherever appropriate. Now that this first comprehensive account of the calculations is available to English readers it is likely that there will be greatly increased interest in, and use of, the elegant Niggli methods.

F. C. PHILLIPS

THEORY AND PRACTICE OF ELECTRON OPTICS

Electron Optics

By Prof. P. Grivet. Revised by A. Septier. Translated by P. W. Hawkes. Pp. x+781. (London and New York: Pergamon Press, Ltd., 1965.) 200*s*. net.

AN author setting out these days to write a book on electron optics has to stand comparison with the established texts: on the theoretical side with Glaser's *Grundlagen der Elektronenoptik* (1952), on the experimental side with Klemperer's equally comprehensive account of practical devices, *Electron Optics* (1953), which almost burst the bounds of a Cambridge Monograph on Physics. Yet it has long been time for a more up-to-date treatment. A great deal has happened in the past ten years or so, in both the theory and the practice of electron optics. For example, quadrupole lenses are barely mentioned by Glaser, nor electron probe microanalysis by Klemperer. Prof. Grivet has now set out to fill the gap, not without some feeling that a French contribution was needed, since he speaks in his preface of "his delight at seeing that this book is going to present the ideas of the French school of electron optics to an English-speaking public".

The task of preparing an account of electron optics in all its major aspects is formidable. I (and, I suspect, Dr. Klemperer also) have quailed before the task of revising my own book, fifteen or twenty years after. With Glaser's untimely death, the only theoretical magnum opus has remained untouched. The subject is now so ramified that only a team of specialists in its different departments could do justice to it, and this is the solution adopted by Grivet.

The origin of *Electron Optics* goes back over ten years, in fact, for it is based on a French text which came out in two parts, in 1955 and 1958, under the general title of *Optique Electronique*. Part 1, subtitled *Lentilles Electroniques*, was written in collaboration with M. Y. Bernard and A. Septier, and Part 2, *Microscopes, Diffractographes, Spectrographes de Masse, Oscillographes Cathodiques*, with F. Bertein, R. Castaing and M. Gauzit, as well as Bernard and Septier. There was also a third volume, dealing with particle accelerators and β-spectrographs. The present work follows the same general scheme as Parts 1 and 2, with some material from Part 3, but it is much more than a straight translation of them. The treatment has been thoroughly revised and brought up to date by Dr. Septier, with help (so the preface hints) from the translator. The extent of the revision can be judged from the length of the English text, longer by almost a third than the original.

In the first part, dealing with electron lens theory, the chief changes have been the inclusion in Chapter 8 of a full treatment of the triode electron gun, as used in electron microscopes, and of an even fuller description of the properties of quadrupole ('strong-focusing') lenses in Chapter 10. These latter have become increasingly important, apart from their uses in particle accelerators, with the realization that they offer a means of correcting the spherical aberration which afflicts the conventional form of electron lens, whether magnetic or electrostatic. The amount of research now being done in this field, mainly in Britain, France and Russia, is reflected in an increase in the space given to it from 7 to 26 pages; this concise review is very timely.

Two completely new chapters have been added to Part 2, on electron probe devices (including a section on electron-machining) and on β-spectrographs, the latter taken from Part 3 of the original work. In addition, the chapter dealing with the effect of an electron beam on the specimen has been considerably extended with an exposition of the uses of velocity analysers and velocity filters in electron microscopy. The book is rounded off by four tables of useful data, including one of Legendre functions of fractional order, and a bibliography of twenty-five pages. A great improvement on the French edition, the latter is arranged by chapter topics, making reference much easier.

Taken as a whole, this English edition is the most complete account of electron optics available in any language. It will be invaluable as a text for introductory courses in the theory of the subject, previous treatments being either too cursory or too detailed. The coverage of applications, however, is likely to find more critics, depending on their particular specialism. The electron microscope gets very full space, in all its aspects, possibly because of the early interests of the senior author. Those concerned with mass- or β-spectrometers, on the other hand, may well feel that they have been neglected, though it must be recognized that these instruments have been adequately covered in other works, such as K. Siegbahn's α-, β-, *and* γ-*Ray Spectrography*. Similarly, specimen preparation for electron microscopy, the brief survey of which is indeed rather out of place here, is now fully covered in a number of manuals.

Technically the book is very well produced, the text being much easier to read than in the French edition. The diagrams have been re-drawn and have also gained in the process. As for the translation, it is rarely possible to detect that it is such. Dr. Hawkes started with the advantage of having spent some time in the laboratory of the École Normale from which the project originated, and so was familiar with the outlook of the French school of electron optics as well as with the language. He is to be congratulated on the easy flow and correctness of his English rendering of a long and, in parts, mathematically intricate text.

Two criticisms may nevertheless be made of this edition, in regard to its subject index and its price. The former is too small, the latter too large for those most likely to benefit from reading the book—postgraduate students and other beginners in research. It would have been better to produce the book in two separate volumes in paper-back, as was the original, one on the theory and the other on the applications of electron optics, which might have been priced at about £2 each. Such a course would have multiplied the usefulness, and also perhaps the total income from sales, of a work which has a large prospective readership. Having supplied the needs of libraries and electron optical laboratories with the present edition, the publishers might care to consider adopting this course for the next.

V. E. COSSLETT

PROBLEMS OF STRUCTURAL DESIGN

The Design of Structures of Least Weight

(International Series of Monographs in Aeronautics and Astronautics. Division 1: Solid and Structural Mechanics, Vol. 8.) By H. L. Cox. Pp. vii+134. (London and New York: Pergamon Press, Ltd., 1965.) 40*s*. net.

THE design of structures, as opposed to their analysis, has two principal facets. One is concerned with the optimum disposition of material in a member, once its

loading and a characteristic dimension are known; the other concerns the layout of the members—clearly much more difficult, as evidenced by the smaller amount reported in *The Design of Structures of Least Weight* compared with the former aspect.

In his introduction, the author relates how Fairbairn attempted a rational design for the Menai Bridge some 120 years ago, and refers back repeatedly in succeeding chapters as to how the bridge might have been designed a century of knowledge and thirty years of metal aircraft building later. Mr. Cox himself has pursued both aspects of the structural design problem for more than twenty years, so that his exposition of the underlying principles is quite sound.

In successive chapters, the search for the optimum configuration in which local and overall buckling coincide is treated exhaustively for struts, wide columns and panels in compression, beams under pure bending, cantilevers under tip load and very simple braced frames. In each case there is a ruling structure-loading coefficient (elsewhere called the structural index) which determines how high the nominal stresses can be before instability occurs, and therefore the lowest possible weight achieved. An interesting conclusion is that the weight of end fittings is more significant for heavily rather than lightly loaded structures.

In the chapter on braced frames, wide geometrical variations are considered and this, in effect, leads to the question of layout dealt with next. The procedure explained enables families of plane framework to be constructed which yield the minimum weight. However, this is only possible in a few restricted cases. Recent work along these lines with the aid of a computer reportedly takes us little further. A chapter devoted to layout in practical design indicates the amount of effort required to design a simple frame for only two loading cases. Where optimum configurations are determinable, they may not be practical. The author likens these optima to the Carnot efficiency for heat engines. The conclusion summarizes the gaps in knowledge and is coloured by the poem on the "one-hoss shay" illustrating the ultimate object in engineering design.

The book is well produced, but marred by a few misprints and odd wordings. A further blemish is the author's penchant for developing analysis and arguments in parallel. One has to search back to find the definition of a symbol, and sometimes it has not been defined at all.

N. K. BENSON

PHYSIOLOGY AND FERTILITY

The Control of Fertility

By Gregory Pincus. Pp. xvii + 360. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 72*s*.

IT is now widely accepted that there is a pressing need for controlling human fertility. As yet, however, relatively few laboratories are devoted to the investigation of reproductive physiology or to developing new methods of fertility control in man, and many major grant-giving agencies remain aloof for various reasons. All the more praise, then, to Pincus and his collaborators for discovering and developing oral contraception. Their work stands out as the one major successful application of scientific knowledge to the control of fertility, most of the other methods in use having devious origins in history.

It would be difficult to find another author who has contributed so much to pure and applied knowledge in the field. Pincus and his collaborators provide the major sources of data, yet a glance at the reference list shows the breadth of coverage. *The Control of Fertility* has three major subdivisions: a brief introduction to reproductive physiology of two chapters is followed by six chapters on animal studies and then five on clinical studies. In each chapter a wealth of detail is discussed in a clear concise manner, and supported by data in tables and figures. Brevity is the rule throughout, so that the actual text occupies only three hundred pages, and the book is written with considerable authority.

Since many approaches to the control of fertility have not yet reached the level of clinical trials, the animal studies cover the widest scientific disciplines. Here the author is clearly at his best in dealing with the endocrinological side of reproductive physiology, and not surprisingly this approach provides the constantly recurring major theme of the book. Massive lists of compounds, mostly steroids, with their relative potency for the inhibition of ovulation or with their activity as anti-gonadotrophins testify to the effort put into this field. Pincus emphasizes the lack of relationship between the ovulation-inhibiting potency of synthetic steroids and their structural and functional features, and the differing potency of many steroids when given orally or by injection. Successive chapters deal with various aspects of fertilization and free ovum development, including the effect of agents on embryos in the fallopian tubes, a potentially valuable approach to controlling fertility. This section of the book is completed by detailed chapters on the suppression of implantation, especially by steroids and anti-steroids, and the biological action of compounds affecting fertility, mostly describing effects on the reproductive system. More detail on several topics, for example, the metabolism of spermatozoa, the structure of antibodies and their migration through the body, or the ultrastructure of gametes and embryos, would have widened the scope of this section of the book.

Most readers will perhaps turn to the clinical chapters first, and notably to Chapters 11 and 12, for the fundamental data on fertility control in man. New approaches to the control of male fertility, all so far unsuccessful, by hormones and other agents are described first, including the *bis*(dichloroacetyl)amines as inhibitors of spermatogenesis which proved unacceptable because of side-effects in combination with alcohol. After a chapter describing what is known of ovogenesis and ovulation in man, Chapter 11 describes how the inhibition of ovulation is assayed, and the remarkable effectiveness of progestin–oestrogen combinations as contraceptives. The effects of the pill on reproductive organs, blood clotting, somatic tissues, etc., and the interplay between different endocrine systems are described in detail in Chapter 12, the longest in the book. Many of the medical objections raised against the pill are dealt with, and quite fairly; no doubt controversy will continue, but the evidence certainly suggests that the pill is safe. Notice, too, how the pill can be beneficial, for example, in the correction of various uterine malfunctions. The final chapter describes the effectiveness and acceptability of contraception; some women readers might have strong feelings when they learn that their 'reactions' (vomiting, dizziness, etc.) have a psychogenic basis. The intrauterine contraceptive device is also dealt with here, although insufficiently, for more detail on this second major new method of controlling fertility would have been welcome, for example, on cost, acceptability in different countries or by different people, chance of mass application. This omission is perhaps explained by the recent advances in this technique, especially in the past 2–3 years.

The main story in this book is of the successful application of the inhibition of ovulation to the control of human fertility. Data from other scientific areas also make abundantly clear the effort now being put into the field. Scientists, clinicians and laymen will find a wealth of reference and detail, an awareness of the breadth of the field, and a clear linkage between biological data and clinical achievement. And many facts in the text confirm

Pincus's thesis that the methods of Nature can be improved on by the intelligent application of biological discovery.

R. G. EDWARDS

COMPARATIVE INVERTEBRATE NEUROLOGY

Structure and Function in the Nervous Systems of Invertebrates

By Prof. Theodore Holmes Bullock and Dr. G. Adrian Horridge. With chapters by H. A. Bern, I. R. Hagadorn and J. E. Smith. Vol. 1: Pp. xx+1–798. Vol. 2: Pp. viii+799–1719. (San Francisco and London: W. H. Freeman and Company, 1965.) 540*s*. per set.

INVERTEBRATE neurophysiologists have been compelled to work with a large, scattered, and unwieldy literature; Hanstrom's *Vergleichende Anatomie des Nervensystems der wirbellosen Tiere* appeared in 1928, and there has been no comprehensive account of invertebrate neurophysiology. The authors of *Structure and Function in the Nervous Systems of Invertebrates* have attempted to survey the present state of both these fields of endeavour. While I am still concussed by the magnitude of the enterprise, its scope may be indicated by a brief summary of the contents.

Part 1 (429 pp.) establishes the general mechanisms and principles governing the operation of nervous systems, and includes sections on micro-anatomy, excitability and conduction, transmission and integration, and a contribution on neurosecretion by H. A. Bern and I. R. Hagadorn. Parts 2–5 describe the special features of the groups and deal respectively with the lower Phyla (365 pp.), the Arthropoda (469 pp.), the Mollusca (242 pp.), and the Deuterostomes (70 pp.).

It must be said at the outset that the bibliography alone makes these books indispensable. Throughout, they are lavishly illustrated with photomicrographs, electron micrographs and excellent line-drawings. In addition to a careful use of headings and summaries, bold type is deployed in the text in two ways: to emphasize key words which indicate the topic under discussion, and to underline phrases which the authors feel to be of particular importance. This device, in practice, works well enough, although I find it visually rather irritating; occasionally, a phrase stands out from its surroundings with merely eccentric effect: **"lower phyla are on an equal plane with the nervously more advanced animals"**, for example (Volume 1, p. 271). However, the ease with which a particular topic can be run down more than compensates for these disadvantages. In general, the text is lucid, although there are some startling lapses, which sometimes seem to result from too strenuous an attempt at concision. Thus, on p. 16 of Volume 1 a paragraph, clearly intended to stand by itself, reads: "Correlated with cell-types is the **histological architecture of nervous tissue.** Whenever it occupies the superficial, basepithelial position characteristic of the simplest systems (coelenterate, echinoderm, enteropneust, the epidermal plexus of nemerteans, even the brain and cord of some simple acoel flatworms, terebellids and limicolous oligochaetes), the diagnostic tissue, a stratum of nearly cell-free nerve-fibres, lies deep in the epithelium, just external to the basement membrane, if present, and to muscle-fibres. It is visible even in sections stained by common unselective dyes as a **layer of punctate texture** but little structure, unless locally there are too few fibres to form a distinct layer." The paragraph which precedes this discusses the classification of neurones in terms of the differentiation of their processes, and it is followed by a paragraph on the organization of ganglia. It is not immediately apparent that the section I have quoted is intended to define the characteristics of a basepithelial nervous system, for the subject is neither mentioned by name, nor given the benefit of bold type.

In fact, the merits of these volumes are distributed in a way which clearly reflects the interests of the two authors; both are neurophysiologists of distinction, and the sections which display our present understanding of neural function are admirably comprehensive and well organized. They can be used as a quick source of information and read for pleasure, and to combine both roles in one text is a remarkable achievement. Nevertheless, only a small proportion of invertebrate nervous systems has been investigated physiologically, and many chapters are, in the nature of things, almost solely concerned with structure. Whenever an equivocal anatomical concept or finding affects a physiological hypothesis (for instance, the neurone doctrine, or controversies opposing continuity versus contiguity at synapses), we are given a scholarly and historical summary of the several points of view. But it does not help the neuroanatomist to be told, somewhat baldly, that dendrites can be defined as "those processes of neurones specialised as though to act as receptor regions for the soma", for he is more concerned to find labels which will enable him to categorize the units which he sees in his preparations, and the physiological function of which is probably quite unknown. He should, perhaps, be warned against the rising dangers of applying rigid terminologies with functional implications to structures which, in invertebrates, are often physiologically ambiguous.

But the pitfalls of neuroanatomical interpretation go far beyond matters of definition: they are implicit in the very nature of the histological methods which are available. Where so much depends on the limitations of individual methods of metallic impregnation, and on the difficulties of combining the different kinds of information which they provide, it is disappointing to find only a brief discussion of these techniques and of their application in practice. This is particularly serious in the case of such structures as the optic lobes of insects, for the amount of reliance that can be placed on the early accounts by Cajal and Sanchez and by Zawarzin requires some knowledge of the vagaries of impregnations of the Golgi type and of methylene blue staining respectively. The authors do not always tell us how the information which they present was obtained.

Obviously, a single reviewer is not competent to assess all the chapters. Those dealing with the Insecta manage to cover a vast range of material, but it is not always well organized. There appear to be some inconsistencies in the nomenclature of the optic tracts, and here a table giving the usages of the various sources would have helped. There is one curious omission: despite the importance of the corpora pedunculata, and a good account of recent work on their physiology and fine anatomy, the beautiful and accurate figures from *Apis* which Kenyon published in 1896 are nowhere represented. The exact topography of the intrinsic neurones of these structures, and of the afferent and efferent endings which lie within them, are crucial to hypotheses about the ways in which they may work, and only in this early study have they been displayed by selective silver methods.

These comments may seem ungenerous. Against them may be set the difficulties inherent in this monstrous labour of love, and the improbability of anyone attempting it again. Few comparative physiologists could have achieved the very real merits of these volumes, or have scoured the literature with the diligence and thoroughness which are everywhere evident. A physiologist who works in any one of the fields covered by this treatise is naturally inclined to look for the kind of critical evaluation of the literature which he would hope to find in a specialized review. Such hopes are extravagant; we should be grateful for what the authors have given us.

A. D. BLEST

THE SOIL MAP

Soil Survey

A Guide to Field Investigations and Mapping of Soils. Edited by I. V. Tyurin, I. P. Gerasimov, E. N. Ivanova, and V. A. Nosin. Translated from the Russian by N. Kaner. Edited by Mary Singer. Pp. 356. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1965.) 90*s*.

THE scientific examination of soil profiles, as distinct from the surface layers of soil, has been in progress since the end of the nineteenth century, and, commonly enough, by dedicated investigators intent on the identification and distribution of dominant types of soil over a particular territory. When Governments appreciated that the soil was a national resource worthy of systematic recording the pedologist acquired respectable standing, at least in some of the larger and thinly populated continental countries where land was still being developed. It is perhaps of interest to mention that soil maps were carefully consulted by bank managers in the Middle West of the United States in the early 1920's.

International co-operation became highly important in reconciling differences in nomenclature and classification. Nomenclature has, naturally, always presented trouble between different countries, and this has led to the fairly recent production of a dictionary of pedological terms in five languages by the International Society of Soil Science. A complete re-appraisal of soil language has also been made in the *U.S. Seventh Approximation*.

The first soil map of Europe was printed some 40 years ago and presented a broad picture of the main soil groups throughout the Continent; we are now awaiting a new map arising from the combined labours of European pedologists, who have thrashed out the differences and similarities in the various regions in order to produce an acceptable classification for soils derived from widely differing parent materials and climatic influences.

Britain was a slow starter in soil mapping, for there was a genuine feeling that British farmers knew all that was necessary about the suitability of their fields for the normal crops. Successes in plant breeding and machinery development, and economic pressures, seemed to outweigh all else in any change in agricultural practice. Nevertheless, the soil survey in Britain has accomplished much, for example, in dealing with apparently sterile areas or planning afforestation programmes or producing large-scale soil maps around expanding urban areas in the hope of preserving the potentially most useful soils for agriculture.

There are several manuals written for the soil surveyor. *Soil Survey* is a most comprehensive treatment of principles, techniques and applications. It goes into details that might seem to be superfluous to anyone who has not taken part in an expedition to an inaccessible desert or mountainous region that is remote from base. In this book, from the Dokuchaev Soil Institute of the U.S.S.R. Academy of Science, soil cartography is regarded as the basis of the quantitative and qualitative description of the terrain, having in mind the complexity of the problems involved in production. It discusses the principles of soil classification based on genetic characteristics, and cartography based on the relationship between soils and soil-forming factors. It is intended to remove misapprehensions in classification and to improve techniques.

The volume is divided into two parts, the first covering such topics as mapping scales, programmes of work and equipment required, the actual techniques of field work, including descriptions of territorial features and soil sections, the special conditions of mountainous areas, the processing of field samples and the drawing of maps, and the interpretation of aerial photographs. In Part 2, the utilization of the maps is discussed with respect to land management and planning, to special studies for soil amelioration, irrigation, reclamation, and for afforestation for such purposes as protective strips in arid zones or along ravines. The appendixes describe procedures for measuring the physical properties of soils.

The text is based on conditions and experiences in the U.S.S.R. and the bibliographies seem to be comprised exclusively of Russian papers. The translation, editing and production, apart from a few photographs, are excellent.

A. M. SMITH

REVIEW OF MODERN DIVING TECHNIQUES

The Principles of Diving

By Mark Terrell. Pp. 240+27 photographs. (London: Stanley Paul and Co., Ltd., 1965.) 50s. net.

DIVING equipment and techniques have changed radically during the past few years. *The Principles of Diving*, in addition to covering standard diving procedure, describes many recent developments, and provides a welcome and very useful source of information for anyone wishing to study advanced diving methods.

The early chapters deal with aqualung and sporting diving equipment, but the major proportion of this book is devoted to industrial and deep diving problems, even going so far as underwater blasting and wreck recovery.

The modern diver is no longer limited to the copper-helmeted equipment used thirty years ago; he now varies the clothing and breathing apparatus to match the conditions he expects to encounter. Terrell describes various forms of breathing apparatus which use high and low pressure air supplies from the surface, and follows this with details of various forms of portable self-contained breathing sets of both open- and closed-circuit patterns. At depths below 240 ft., both oxygen and nitrogen exert certain toxic effects on the diver, and the author describes the techniques which are used to substitute oxy-helium or oxy-hydrogen mixtures for air at these depths, and so allow the diver to descend as deep as 1,000 ft.

At great depths the water temperature is almost stable at 39° F. This poses quite a problem on how to keep the diver warm and capable of working, without excessive clothing to restrict his movements. In addition to the cooling effect of the water, the oxy-helium mixture he uses conducts heat away from the body almost seven times as rapidly as air. As diving from offshore oil drilling rigs becomes more commonplace, I can envisage the various points raised by Terrell being considered in great detail.

After reading the chapter on underwater medicine, one is left with the feeling that the only sensible thing to do is to stay on terra-firma. The symptoms associated with pressure injury vary from hallucinations, epileptic fits to blue skin and paralysis. The following chapter on underwater fishing is a welcome relief, until you read that the conger-eel can bend an underwater harpoon like a hairpin.

The views expressed by Terrell on future developments are already in part coming true. The recent work by Commandant Cousteau and the American *Sea Lab II* are the early stages of what can be described as the underwater industrial revolution. His scheme for an underwater tent to cover oil drilling rigs is already within the bounds of possibility. Multi-cell recompression chambers and submersible chambers are already being made by Siebe, Gorman and Co. Ltd. for prolonged deep diving projects in the North Sea and elsewhere.

In conclusion, I can recommend this book to anyone who has an interest in the problems and techniques of diving. Some of the sections on respiration and medical effects are necessarily complex, but the range of subjects covered gives an insight into the problems and achievements of the diving world, and it provides a fascinating story.

L. R. PHILLIPS

The Invisible Government

By David Wise and Thomas B. Ross. Pp. 375. (London: Jonathan Cape, 1965.) 30*s*. net.

THIS book might well have been entitled, perhaps more appropriately, *Invisible Power*. This suggestion is not made to emphasize any features the book has in common with Mr. Cater's *Power in Washington* or Prof. R. E. Newstadt's earlier *Presidential Power*, but because it is concerned essentially with the theme of power and influence within the working of a reputedly democratic system of government and not directly with the Government itself. It is concerned, as are both those books, with the situation in the United States and with what can happen under the cloak of secrecy or security, especially if these two terms are not clearly distinguished, when institutions created for valid purposes become their own custodians or judges. The book describes in some detail the organization and functioning of the intelligence service of the United States, including Army Intelligence, the oldest service, the Office of Naval Intelligence, the smallest, Air Force Intelligence, mechanically the most sophisticated, the Atomic Energy Commission, the Federal Bureau of Investigation, the Bureau of Intelligence and Research, and finally the major institutions—the National Security Agency, the Defence Intelligence Agency and the Central Intelligence Agency.

For the British reader, the real interest of the book does not lie in these descriptions, nor in the account of various episodes of the past fifteen years which indicate their weakness and the acute difficulties of control which have resulted in them appearing on occasion to determine policy rather than to be ruled by it. It lies in the way the book raises the real questions and problems of control which confront a democracy in this age of rapid technological development. There is displayed, for example, even more insistently than in the recent report from the Estimates Committee on Electrical and Electronics Equipment for the Services, the problem which secrecy presents and the way in which it can react to endanger the security which secrecy itself purports to serve. There is the issue of advice, and insistently, naggingly, all the time the question of who shall control the controller. Nor are the issues confined to defence. The implications for the Fulton Commission are plain, and if the authors offer little in the way of constructive suggestions, their warnings are unmistakable. This sombre book should not be overlooked by anyone seriously concerned with the relations of Government and science, though it will challenge his thought rather than contribute to his peace of mind.

R. BRIGHTMAN

Fluorine Chemistry

Vol. 4. By Harold C. Hodge and Frank A. Smith. Edited by J. H. Simons. Pp. xviii+786. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 224*s*.

VOLUME 4 of *Fluorine Chemistry* is not a book which will be of primary interest to chemists who are engaged in the very broad field known as fluorine chemistry in the same sense that the first three volumes in this series were. It is a condensation of some 3,500 references on clinical information pertinent to the physiological effects of fluoride ion.

The first chapter deals with the biological properties of inorganic fluorine compounds which provide fluoride ion in solution, and *in vivo* may cause acute or chronic poisoning. The mechanism of fluoride poisoning, its treatment, and the nature of fluoride toxicity in organs, glands, blood and other parts of the animal and human anatomy are considered in detail. The purpose of the book "has been to collect information about the biological effects of fluorides in compact form . . . has not attempted to formulate and state critical opinion of the knowledge about every biological effect of fluorides".

The second chapter is concerned with the effect of fluorides on the bones and the teeth. The various factors effecting fluorosis, osteosclerosis and osteofluorosis are critically correlated. The relationship between fluoride ion intake, mottled tooth enamel and dental caries is covered extensively. In view of the many debates which have arisen in many communities concerning the topic of fluoridation of drinking waters, part of this chapter is most helpful in providing scientific evidence which can be used to point out the benefits and possible damage to tooth structure which may be associated with the adjustment and control of fluoride ion concentration in drinking water. The authors do not take a position on this controversial subject. The terminal portion of the book concerns itself with hard tissue metabolism of fluoride ion in bony structures and teeth.

This volume of *Fluorine Chemistry* is directed not to chemists but to medical and dental scientists. It contains a vast quantity of information on the causes of, possible preventive procedures against and possible cures for disabling physiological effects caused by various fluorochemicals as well as the harmful effects of fluoride ion deficiency. Nevertheless, it will be of more than passing interest to the fluorine chemist and of special interest to those concerned with water treatment and to practitioners of industrial medicine and dentistry.

It is most fitting that the authors and the editor of Volume 4 of *Fluorine Chemistry* have seen fit to dedicate their efforts to Prof. Kaj Roholm of Copenhagen.

RICHARD DRESDNER

Fair Isle and Its Birds

By Kenneth Williamson. With a List of the Birds of Fair Isle by Peter Davis. Pp. xvi+311+27 photographs. (Edinburgh and London: Oliver and Boyd, Ltd., 1965.) 30*s*. net.

FAIR Isle and Its Birds is a book for the general reader whose interests include remote places, wild birds and the scientific riddles of migration; it brings together, in a pleasant form, material that has appeared before in scattered articles and papers. The first part deals with the island, its people, its history, its general natural history, and the habits of some of its breeding seabirds—skuas of two species and the fulmar. The second part gives an account of the tremendous phenomena of bird migration that can be observed from this remarkable vantage point. In the third part Peter Davis provides an annotated systematic list of the birds of Fair Isle. John Peterson contributes excellent photographs of scenery, human activities and birds.

Fair Isle was made ornithologically famous by the visits of Eagle Clarke in the period 1905–11 to observe the spring and autumn passages. It provided part of the material on which his pioneer studies of migration were based, but there remained much more to be extracted from this rich source. In 1948 a regular observatory, manned except in mid-winter, was established by the efforts of George Waterston and others. Kenneth Williamson was appointed as the first warden, serving for eight years, and Peter Davis was his immediate successor.

In the middle part of the book the author describes the observatory and its work and gives an account of movements to be observed in spring and in autumn. He lays emphasis on the influence of weather on both the actual and the observable performance of the migrants. He attaches special importance to the effect of wind direction in causing lateral displacement of lines of flight; some of his views, he says, are personal. This theme is notably exemplified in a chapter called "Wheatears and Redwings", illustrated with weather maps relating to certain strongly marked autumn movements in which the origin of the birds was indicated by recognizable subspecific characters. A final chapter deals with some of the astonishing number of rarities that have been recorded from Fair Isle.

LANDSBOROUGH THOMSON

THE PLACE OF THE ENGINEER IN SOCIETY*

By LORD SNOW†

THE status of engineers in Great Britain is lower than in any country that I know of. This condition of things reflects a good deal that is wrong with our society. Right at the heart of this discussion there is the need which all sensible people increasingly feel for engineers all over the world to be close to all the decisions which are going to affect our human society.

To an extent, however, engineers get the rough end of the stick even in countries where they are more esteemed than in ours. There is the bitter American crack—invented by an engineer. You fire off a rocket and a satellite moves successfully around the world. That is a scientific triumph. On the other hand, if it flops on the launching pad, that is an engineering failure. The only country I know where they really do have maximum prestige is the U.S.S.R. It is hard to imagine an English or even an American engineer being given a State funeral—but Korovolov got one in Moscow this winter. The present President of the Soviet Academy of Sciences is an engineer. So, curiously enough, is the present Soviet Prime Minister. It is still difficult to imagine similar appointments to the Royal Society or to No. 10 Downing Street.

The position in Britain is much less favourable. Somehow, and soon, this position has to be altered. But the first thing I want to tell you is that engineers themselves cannot do much about it. The protest and change have almost certainly got to come from others. Unless we raise it to an extent commensurate with the importance of engineers to our society, then our society cannot possibly exist in economic health or in intellectual and moral health either.

There is not much that engineers can do for themselves, and I suspect that the less engineers talk about the problem, the better for all that we want to attain. I am entirely in favour of the new Council of Engineering Institutions. But formal organizations of this kind usually do not affect the status of an activity by 1 per cent. With some reserve, I am in favour of the new designation of Chartered Engineer. But again experience suggests that professions riding the crest of the wave tend to take this kind of designation rather lightly. Scientists in Great Britain, for example, enjoy a very high status, probably as high as in any country in the world. Our pure science continues to be remarkably successful. After the United States, we come a clear second in the Nobel Prize race.

It is no accident that among our scientists there is still a cheerful and relaxed attitude to qualifications. Cockcroft is a great physicist, but he has never taken a physics course in his life. Einstein and Dirac, two of the most profound theoretical scientists, not only of this century but of all time, started life as engineers. Crick has revolutionized modern biology; but he has had as much formal instruction in biology as he has in Hebrew. The point I wish to stress is that the scientific climate is easy-going and encouraging. It would be a mistake and to my mind a suicidal mistake if engineers, feeling, and rightly feeling, that they were undervalued, went on the defensive and, being on the defensive, became not less rigid but more rigid.

It seems to me probable that engineering has already suffered through the rigidity of its training. This is one factor, but an important one, in the lack of appeal of the profession to men of original and unorthodox minds. At all costs, we need to attract such unorthodox minds. I believe that constantly we ought to look over our shoulders and ask why pure science is so good at attracting them—and what is wrong both with the education and the career of engineers that acts as a negative inducement.

Pure scientists have been taken, although with some difficulty, into the main stream of British society. They can be depicted, with some hope of establishing communication, in films and plays and novels and in the mass media. With engineers the barriers are much more formidable. Why has this happened? And how are we going to put it right?

Why it has happened seems mainly a matter of our social history, but a good many of the reasons we still do not begin to understand. It is at first sight puzzling that the British, who began the first industrial revolution, one which was closely linked with the work of engineers, should during the nineteenth century come to think less of engineers and care less about their education: whereas in the United States and Germany, industrializing themselves about 20 or 30 years later, exactly the reverse was true. This is one of the oddest phenomena in English history. From it has followed many of our existing discontents. Relative to the United States and Germany, Britain was on the technological decline very early. One can even put a date to it. It was somewhere between 1851 and 1867. If Britain had put one-tenth of the effort into engineering as into the Indian Empire it would now be a very prosperous country, individually more prosperous than Sweden and able to look American technology in the face.

It is no use repining about where our ancestors went wrong. The important thing is not to continue to go wrong.

It is, however, going to take a long pull to get rid of the lack-of-insight-syndrome—the various symptoms which prevent us recognizing what is necessary for us in terms of rudimentary social health. Features of this syndrome are lack of awareness of who makes the social wealth; absence of interest in, or even active hostility to, the technological basis of our society; nonsensical patterns in education. These malaises are very deep. Even with good fortune it is going to take 10 to 20 years before we breathe a freer air and walk about with a more robust common sense.

Above all, we need a powerful educational effort, an educational effort which reaches to the people who have to make the decisions, and also produces a new generation of those who will make the decisions in 10 or 20 years. We need to produce new generations of

* Substance of the Graham Clark Lecture delivered at the Institution of Electrical Engineers on March 24.

† Lord Snow was Parliamentary Secretary to the Ministry of Technology in the British Government from October 1964 until April 1966.

engineers who have been taught engineering as a humane education.

Not that I want all our bright boys and girls educated as engineers—any kind of talent is worth training to the full. Our society would be immensely impoverished without full scope for its artists and linguistic scholars. But I am more than ever convinced: (1) that our secondary education is too specialized; (2) that we must search for and train—and probably specially train—every kind of really high ability; (3) that we have to make special efforts at the undergraduate level to make engineering education something which it is not at present or at least has scarcely begun to be.

It is not enough to think that someone who is a natural engineer will find his way into the profession. Very largely that is true of engineering, as it is true of pure science or music or painting. But the number of people with a deep and passionate vocation for any walk of life is quite small, a tiny fraction of 1 per cent. What is needed is to attract, to valuable social functions such as engineering, people who could do many other things. Most of us respond to the pressures of our society. Where the esteem and the rewards appear to be, there able people will go. Most of us, if we had been born 800 years ago, would not have studied engineering or science, for the very good reason there was no engineering or science to study. We should probably have become canon lawyers, however, just because that was the road to all the administrative jobs there were. In the same way the high-class professional English administrators in the nineteenth century studied Greek and Latin. Not because they could gain an intellectual training, which indeed they did, certainly not because of profound literary interests, but because that was the accepted training for anyone who aspired to run this country. Our task is to make engineering just as natural, just as accepted educationally—not the only way, but an important way—for young people of high ability who want to test themselves to the full.

It is surprising that engineering has not in fact become more of a humane education. The training is rigorous, and that is a good thing. No subject is really worth years of time for a good mind unless it is, in the old-fashioned academic sense, 'hard'. Engineering is certainly that. It is, or certainly ought to be, deeply concerned with human beings. That is, an engineer does not exist in a vacuum; his working life is going to be intimately concerned with people; the results of his activities are going to affect much larger numbers of people. And yet, somehow, engineering, like medicine, with which it has much in common, has up to now been something of a failure as an educational discipline in its own right.

I am not being abusive. I have spent much of my life among engineers and count some of them among my most intimate friends. By and large engineers turn out to be very good citizens, honourable, responsible and upright. But I think that most of us would agree that they are not by and large as fully educated as we should like. It is partly for that reason that they do not play the prominent part in British society that we should also like. There ought to be far more engineers where the decisions are really made—in Government—in Parliament and at the top levels of the Civil Service. This does not happen. In the United States it happens a good deal more, in the U.S.S.R. incomparably more. When we have good engineering education I believe it will begin to happen in Britain.

I believe one can identify two defects—there are probably many more—in contemporary engineering education. For one thing, it does not encourage enough the speculative and rebellious intelligence. This is its great handicap, and its great lack of appeal, as opposed to pure science. Engineers are good citizens. It is rare for engineering students to question everything under heaven or earth in the way that good scientific students will. Medical education also seems to repress the speculative intelligence. Engineering students and medical students are nearly always the most conservative-minded of any large student groups. This tells its own story. I am not asking the engineering profession or the medical profession to move as one man sharply to the left. What I am saying is that a uniform approach and a conformist approach to all the major problems of society suggests either that we are getting too homogeneous an entry in these professions or, alternatively, that the training itself has a rigidifying effect. I should like to see as part of all engineering education what one can already see at the Massachusetts Institute of Technology and the California Institute of Technology and some colleges of technology in Great Britain—a serious study of social history or economics or any subject which of its nature is bound to arouse the speculative mind.

The second of these two defects seems to me to be the lack of verbalism in engineering training. Engineers, as engineers can get on very comfortably without words. Engineering students can make do with a very small vocabulary. But engineers when they are not being engineers, when they are moving into management, boardrooms and government, are often handicapped just because they have neglected or despised the use of words. Language, verbal language, is a great invention. It is different from symbolic language, and a man is lucky if he is equally at home with both. But without some feeling for verbal language there is much of human wisdom shut off from one for ever. If engineering students are going to hold their own in 10 years' time with people of comparable intelligence but different training, they will have to be as good on paper and as good speaking on their feet.

I believe this widening of engineering training would of itself attract a great many students of high ability who at the moment are being drawn into pure science or economics. It is precisely such students that we need. Some of them will be among the decision-makers of the 'eighties.

If we get our education right, then in the long run the place of the engineer in our society will become right. But what a society recognizes is ultimately expressed in concrete terms. These terms are not only financial. To most sane persons, esteem is more important than pay. If we had a choice most of us, I hope, would prefer to be President of the Royal Society than the most successful pop singer in the world. Yet the most successful pop singer in the world has been out of proportion better rewarded financially than any of the Presidents of the Royal Society. Nevertheless, pay, in a qualified sense, does matter and is likely to continue to matter. Modest differentiations are well understood by people who do not expect to bring to the community the special contribution that a creative engineer can bring. If that is agreed, at present we certainly are not lavish with our awards to these creative engineers. Take one of my theoretical young people of high general ability who could become reasonably accomplished as a pure scientist or economist or engineer. At the moment, if he is thinking only of money, then engineering is not the first profession he would choose. His starting salary as an engineer would be as good as, or better than, the others. His terminal salary is likely to be appreciably lower. This is another surprising fact, but the evidence is overwhelming.

I am sure this cannot be right. Once again, it will not be adjusted overnight. The proper value placed on engineers will only happen when we who are interested contrive to get our side of the house right. And when we have persuaded the rest of society what engineers are like, what they can do, and how on them depends a large part of whether Britain flourishes or dwindles, not rapidly but imperceptibly, into decline.

LONG-TERM (GEOTECTONIC) STRESS GENERATION, PROPAGATION AND ACCUMULATION IN THE EARTH

By JEROME B. CARR*

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A GREAT deal of effort in the past has gone into the development of theories and hypotheses for explaining particular geotectonic expressions of stress relief in the Earth. For example, efforts have been made to explain where the stress for earthquakes originates and how continents maintain their above sea-level expression against the high erosion rates that are known to exist. Looking at the results of all this labour, one is faced with a geotectonic mechanism for each expression of stress relief in the Earth and quite often these mechanisms have little or nothing in common.

To reduce this multiplicity of mechanisms, one must be aware of the time spectrum of geotectonic stress relief (see Fig. 1). Given the existence of this spectrum, one would suspect that all the expressions of stress relief are due to the same source of stress generation. Thus it can be presumed that there is a single source of stress generation in the Earth and that the mechanisms giving evidence of this stress are a function not only of the origin of the stress, but also of the methods and limitations of stress propagation and accumulation within the Earth.

Among the elements of stress relief it is known that earthquakes are associated with plateau uplift, island arc formation, mountain building and continental maintenance. (The last-mentioned is defined as "the ability of a continent to maintain itself above sea level".) However, even though we know that interrelationships exist between geotectonic elements, the exact nature of these relationships cannot be fully understood by studying the extreme ends of the geotectonic spectrum because the relationships are masked by intermediate scale geotectonic components. Thus the logical place to start studying stress generation is with the meso-scale geotectonic structures which are direct indications of recently applied stress. If a mechanism can be uncovered which would explain the meso-scale geotectonic structures, then this same mechanism should also be capable of explaining some properties of the extreme ends of the geotectonic spectrum. At the same time, the finding of relationships between geotectonic elements should throw light on stress propagation and accumulation once the generating mechanism is understood.

Let us first direct our attention to plateau uplift. There are two types of plateau uplift—the first type being of moderate area but of very rapid and high to very high uplift. The Colorado Plateau and the Tibetan Plateau are two prime examples. The second type of plateau uplift covers a much greater area but is slower in rising and tends to be lower in elevation. Areas representative of this type of uplift are the Brazilian Highlands, the western half of Australia, and Africa south of the Congo Basin.

To produce these regions of plateau uplift, material must be volumetrically added to the sub-crustal areas where the plateau uplift mechanism occurs. The two causes of volumetric increase postulated at present are convection currents in the mantle and phase changes at the Moho. The three main objections to the concept of convection currents are: (1) the volume increases required by plateau uplift are too great to be produced by slow moving convection currents and rapid moving convection currents are precluded by the physical properties of the upper mantle[1]; (2) mantle-wide convection has been shown to be impossible due to the changes in the Earth's physical properties between 400 and 1,000 km (ref. 2); (3) studies of seismic surface waves show that continental and oceanic structural differences extend to 400 km depth[3], thus requiring mantle-wide convection to produce continental drift (see 2 above) and preventing convection cells from passing through continental borders to reach regions of plateau uplift.

For the reasons just listed, it is concluded that the eclogite-basalt phase change at the Moho offers the best mechanism for volumetric increase to produce plateau uplift. However, there is one major objection to interpreting the Moho as a basalt-eclogite phase change; that is, the Moho appears to be too shallow in oceanic regions for this explanation.

Recent work[4,5], which validates the concept of the world-wide shear pattern proposed by Vening Meinesz, clearly implies that the ocean bottom is much older and more stable than continental regions. This extreme stability of oceanic structure raises an important question. That is, "What is the effect of pressure on the eclogite-basalt phase change over very long time periods?". Put in other words, "What is the effect of a pressure of $3{\cdot}3 \times 10^9$ dynes/cm^2 over a time period of 2,800 million years or more?". Obviously the phase change experimentation that occurs in to-day's high pressure laboratories is completely incapable of evaluating the effects of such long periods of time. Thus, the Moho under the ocean could be a basalt-eclogite phase change that is in complete

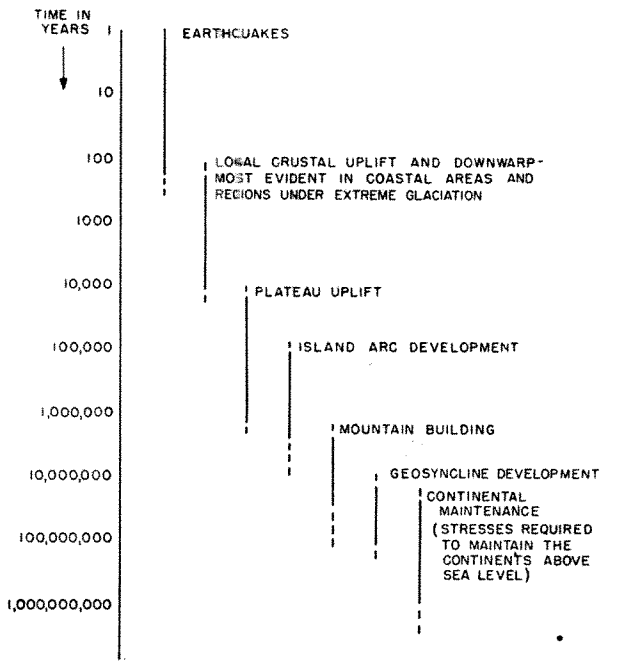


Fig. 1. Time spectrum of geotectonic elements

* The author wishes to acknowledge that all but the final preparation of this publication was made while he was at Sperry Rand Research Center, Sudbury, Massachusetts.

hydrostatic stability due to lack of vertical motions that are so prominent in continental areas.

Given that the foregoing statement is true, let us take the Colorado Plateau as a case study region. The Colorado Plateau is delineated in Fig. 2 and the major faults in the adjacent Basin and Range Province are also shown. From Cambrian time to middle and late Mesozoic time, sediments were deposited (though not continuously) on both the Colorado Plateau and the Basin and Range Province. The sediments, being poor thermal conductors, acted as an insulator and caused trapping of heat generated by the decay of the radioactive elements in the underlying sial. This heat expressed itself as vulcanism and plutonism in the Basin and Range Province due to the presence of faults and other zones of weakness which allowed the magmas to migrate towards the surface. The Colorado Plateau, however, is lacking these faults and zones of weakness and therefore the thermal energy at depth was used primarily to convert eclogite to basalt and uplift the region as a whole. The depth to the Moho under the Colorado Plateau averages 40 km (ref. 6) and this is some 10 km deeper than the location of the Moho underneath the Basin and Range Province. Since the phase change generates a 10 per cent increase in volume, the uplift due to volume increase alone would be 1·15 kilometres or some 3,600 ft. In addition to the volume increase, however, the phase change also causes a local reduction of g. The amount of uplift required to satisfy once again the geopotential conditions which existed before the phase change occurred is an additional 4 km. Thus there is easily enough energy generated by phase change to allow for the present 1·5 km (5,000 ft.) elevation of the Colorado Plateau and also enough energy left over to account for uplift and erosion in the past, uplift in the future, and propagation of energy into other locations at the present time.

Now that the case study region is found to satisfy the physical requirements of the uplift region, let us consider the geography of island arc regions as these regions also reflect stresses which are, or were, recently active. The most complicated island arc regions are the Indonesian and Caribbean regions. Indonesia is found between the Tibetan Plateau and the continental mass of Australia, while the Caribbean area lies between the Colorado Plateau and the continent of South America. Since there is an apparent geographical relationship between these two geotectonic elements, let us infer that this relationship includes a common stress source. Thus, at first glance, the development of complex island arcs appears to be due to: (1) stress generated in a region of plateau uplift; (2) this stress being propagated through the Earth until it strikes an immovable continent; (3) since the mass of the continent is too large for this stress to move, the oceanic crust close to the continent breaks; (4) it breaks along pre-existing zones of weakness which were generated as a part of the world-wide crustal shear pattern[4,5]. This is the initial picture which arises when the Earth is considered in Mercator's projection. Let us now switch our attention to a spherical Earth. First it is known that the initial phase change causing plateau uplift originates at the Moho at its initial depth of about 30 km. The change in volume at this depth will produce stresses which may be propagated rectilinearly in all directions. Looking at the two complex island arc regions it is seen that the distance from the closest border of the Colorado Plateau to the extreme limit of the Caribbean arc is 47° of arc and the corresponding distance to the Indonesian arc region from the edges of the Tibetan Plateau is 53° of arc. Plotting this larger figure (see Fig. 3) it is seen that the maximum depth of stress propagation is 690 km. The known limit for depth of earthquakes is 720 km and thus it is seen that the limit imposed by seismic data does not rule out the proposed new mechanism for island arc formation.

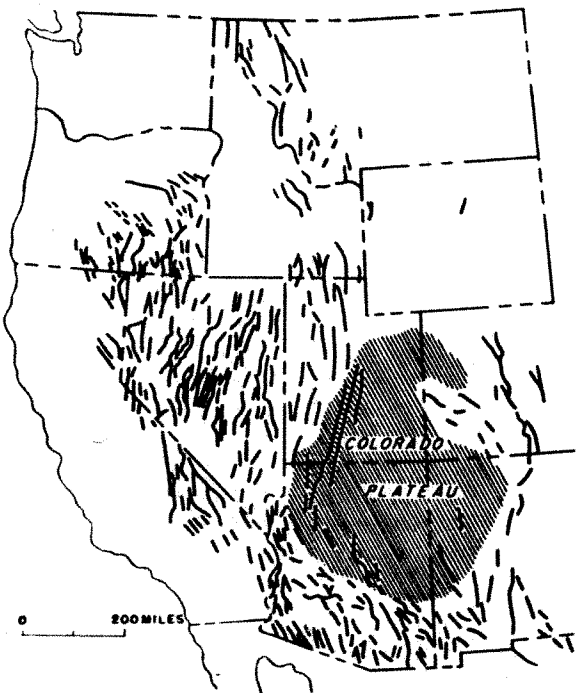


Fig. 2. Delineation of the Colorado Plateau (shaded area) and the faults of the Basin and Range Province (heavy lines). Figure taken from ref. 12

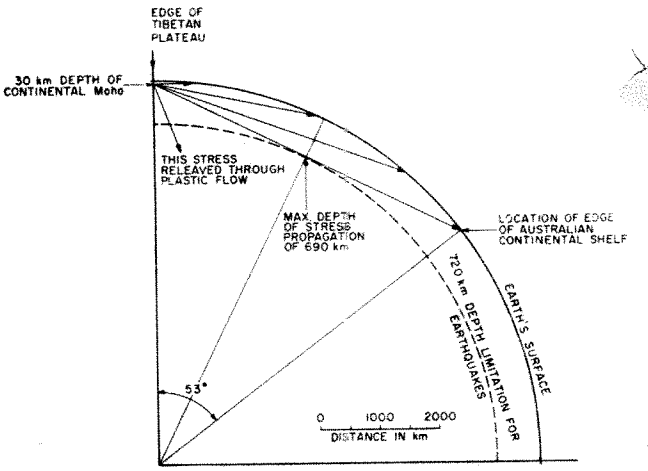


Fig. 3. Rectilinear stress propagation in a spherical Earth. This figure shows a spherical Earth with stresses propagating from a region of plateau uplift to the extreme limit of a complex island arc located 53° of arc from the edge of the plateau uplift

Looking again at Fig. 3, it is to be noticed that the stress propagated 53° just touches the continental shelf of Australia and that no stress is directly acting on Australia. Thus it is suspected that no continental backstop is needed for island arc generation, but that the backstop merely generates a more complicated set of arcs when the backstop is available. Checking the globe, it is seen that all the island arcs from Indonesia to the Kamchatka Peninsula are within 53° of the edge of the Tibetan Plateau. It is also seen that the island arc region located off the southern tip of South America is within 53° of the Brazilian Highlands and the Argentine Plateau. Lastly, the Aleutian Islands Arc, with the exception of the westernmost few islands, is within 53° of the Colorado Plateau. When the flattening of the poles is taken into account, the entire Aleutian Arc may be accounted for without exceeding the stress depth limitation. Thus, by examination of the globe, it is found that every island arc region in the world can be accounted for by stress generation from a region of plateau uplift.

Before checking this concept of island arc formation against the geological evidence, it should be noted that the arcuate structures observed in the Earth's crust are amazingly similar to the arcuate structures observed as crystal dislocations. In Fig. 4, some linear shears are shown with the arcuate structure rounding off the intersection of these linear shears. The same type of rounding off is observed when one superimposes the Mariana Islands Arc on Vening Meinesz's crustal shear pattern. The correlation coefficient between island arcs and the crustal shear pattern is 0·90, thus showing that island arcs fit the linear zones of weakness proposed by Vening Meinesz's crustal shear pattern[6]. Since the physics of shear can account for the arcuate nature of the island arcs, we need not try to propose one mechanism for arcuate structures and a second mechanism for linear structures.

The geological evidence concerning the island arcs is of two types. The first type of geological evidence is that the structures of the Earth are generally found to be due to compression. Thus, if the concept of island arc formation outlined here has any basis of reality, the directions of compression should point to regions of plateau uplift. With respect to the Indonesian Island Arc, it is known that the stress required to generate this structure came from the north-west[7]. Thus it is seen that the proposed mechanism of island arc formation agrees with the required direction of stress in at least one area that is structurally well explored.

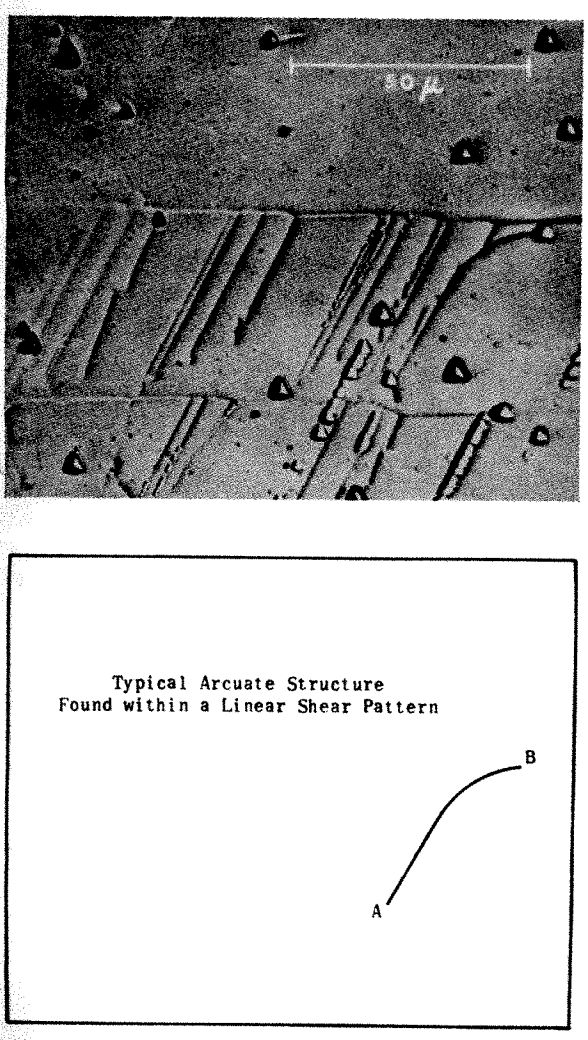


Fig. 4. Typical arcuate structure found in a linear shear pattern. The upper figure is a photograph of a shear pattern in a silicon crystal. The lower figure shows the location of the arcuate structure (line *A–B*). The relationship between this arcuate structure and the linear shear pattern is analogous to island arcs when compared with Vening Meinesz's crustal shear pattern

The second type of geological evidence which offers a check on the new concept of island arc formation is the relative ages of the structures involved. That is, if the island arcs developed before the plateau uplift areas began their ascent, then obviously the new concept is invalid. However, on examination of the data available, it is seen that the arcuate structures of Indonesia, the Mariana Islands and the Kamchatka Peninsula are all younger than the first uplift motions in Tibet and that the Japanese Islands Arc is younger or at least the same age (Upper Carboniferous) as this first uplift. Thus the independent evidence from geology offers no contradiction to the proposed mechanism for island arc formation and, in fact, the compression evidence from Indonesia substantiates the proposed mechanism.

To sum up the development of island arcs it appears that: (1) the stress generated in a region of plateau uplift is rectilinearly propagated into previously stable ocean regions over a distance not greater than 53° of arc; (2) this stress expresses itself in arcuate or linear structures controlled by a shear pattern produced in the Pre-Cambrian; (3) after initial stress relief has occurred, the Moho, which was previously in hydrostatic equilibrium, now acts as a local stress source in seeking to re-establish the lost equilibrium; (4) normal geological processes will be triggered by the initial disturbance, thus producing volcanoes, etc.; (5) should continental backstops be present, complex island arc regions develop—otherwise simpler structures are produced.

Now let us pay detailed attention to earthquakes—the high-frequency end-member of the geotectonic spectrum. With respect to earthquakes it is well known that shallow earthquakes (focal depth less than 70 km) dominate the seismic scene and that a large number of shallow focus earthquakes occur at the depth of the continental Moho. This latter fact strongly reinforces the concept of the Moho being a phase change because, if the Moho were only a change in material, it would be very difficult to explain the high number of earthquakes which occur near this discontinuity.

Also, since the continents are known to be continuously undergoing complex vertical movements (which we now assume are due to phase change at the Moho) and since we have also commented on the extreme stability of oceanic regions, it should be expected that earthquakes will occur anywhere on a continental mass and be severely restricted (numerically) in ocean regions. This is indeed what is seen in a plot of earthquakes. Fig. 5 shows a plot of earthquakes for a one-month period and it is seen that only 2·5 per cent of these earthquakes occur in open ocean regions; that is independent of island arcs and continental structure.

As stated earlier, there is an obvious relationship between individual earthquakes and meso-scale geotectonic elements. However, it remains to be seen if there is any gross relationship between the world-wide earthquake distribution and the phase change which occurs under plateau uplift regions. As it turns out, if the oceanic structure is indeed as stable as earlier work indicates[5], and if it is true that stress cannot be propagated more than 53° from its source, then there should be one or more regions of no earthquakes on the oceanic structure of the Earth. Fig. 6 shows the locations of the major regions of plateau uplift; and the area delineated in the centre of the Pacific Ocean is the only area on the surface of the Earth that lies outside of the 53° range of stress propagation. On investigation[8–11], it is found that within historical time there has never been an earthquake within the area delineated. At the present time, the probability of generating such a region by a random process is less than 0·02. Thus the concepts of phase change stress generation, rectilinear stress propagation and a stable ocean structure are strongly reinforced by the mapping

and satisfactory explanation of the "zone of no earthquakes". Since the ocean structure reflects the assumed stress propagation mechanism so nicely, perhaps some similar evidence can be found with respect to continental areas. Mapping the European earthquake distribution one would expect (based on area) about 25 per cent of all European earthquakes to occur at distances greater than 53° from the Tibetan Plateau. However, on plotting all European earthquakes during 1956 (ref. 9) and for the first half of 1963 (ref. 10), it was found that less than 1 per cent of them occurred at distances greater than 53° from the edge of the Tibetan Plateau (see Fig. 7). Thus major plateau uplifts are seen to be tied to earthquake distributions on continents as well as on oceanic structure.

Now that we have considered the two-dimensional distribution of earthquakes, let us extend our outlook to three dimensions. Figs. 8 and 9 show the distributions of intermediate (70–300 km) and deep (300–720 km) focus earthquake regions.

The results of this extension of the previous investigation of earthquakes lead to a preliminary classification of earthquake regions (see Table 1). Special notice should be paid to the 'Special Ocean Area', that is, the Fiji Islands. In the Fiji Islands Area the distribution of earthquakes with respect to depth is completely abnormal and unique when compared with all other areas of the world which possess deep focus earthquakes. Table 2 shows that the Fiji Islands are unique when comparing percentages of deep focus earthquakes to total numbers of earthquakes. Besides that, this region suffers approximately 70 per cent of all deep focus earthquakes in the world, and the deep focus earthquakes appear to be independent of shallow earthquakes because there is a singular lack of intermediate depth earthquakes. This latter fact implies that there is a unique source of stress in the Earth that is operational at depth in this region. The exact nature of this stress source may be surmised as one of two possible mechanisms, namely: (1) phase change is actively occurring in the region below 400 km; (2) that there is a convection cell operating in the lower mantle.

To compare the aforementioned two suspected causes, let us examine the areal extent of the high-frequency

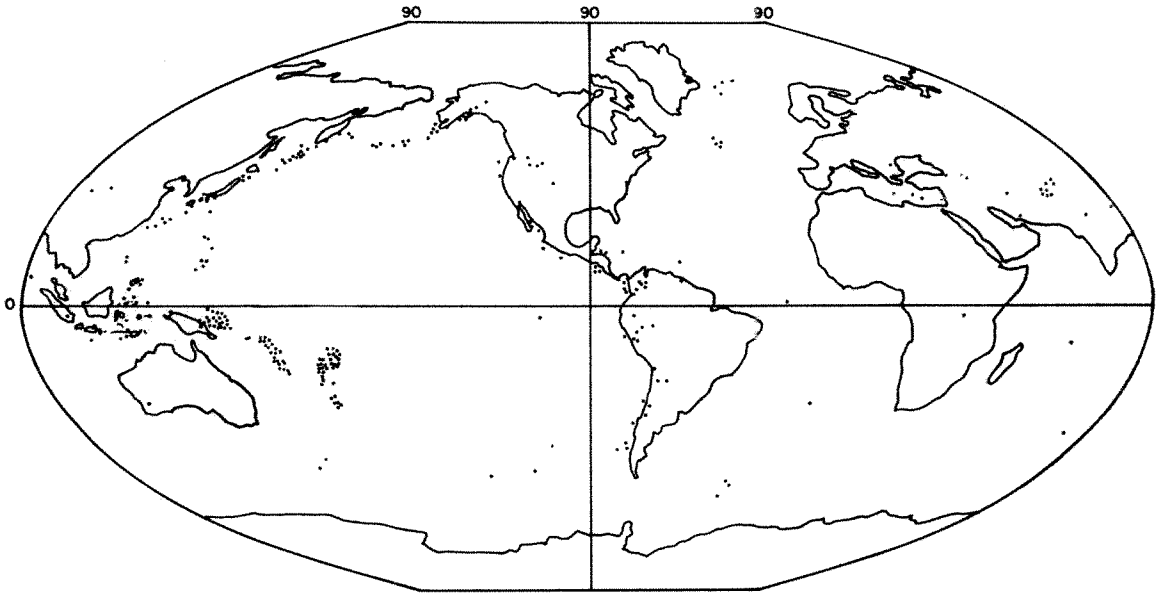


Fig. 5. Earthquake locations for a one month period (January 1963). Data from ref. 10

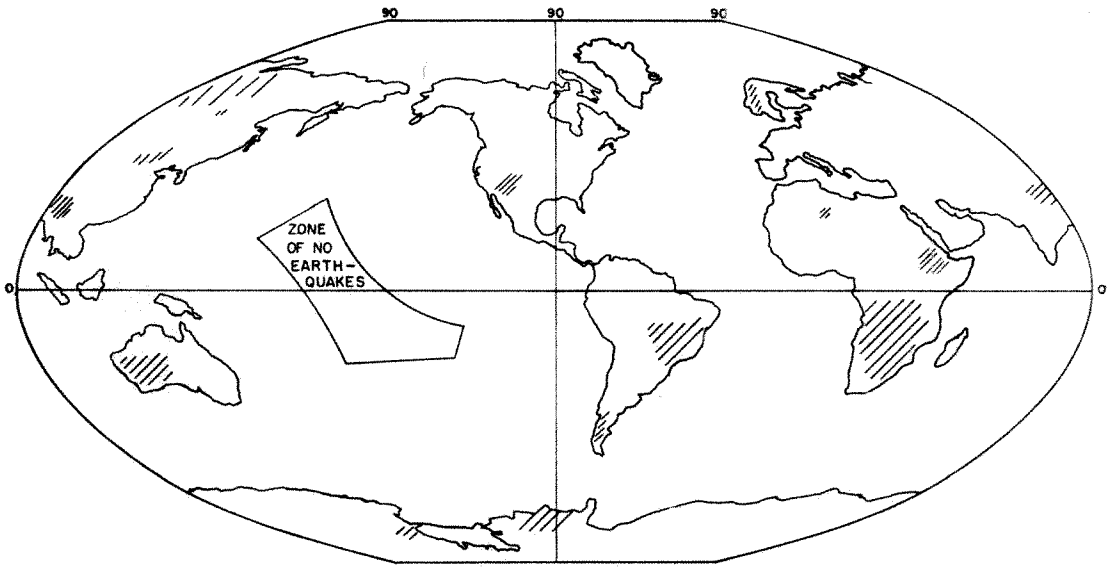


Fig. 6. Major areas of plateau uplift (diagonally lined areas) and the 'zone of no earthquakes'. This 'zone of no earthquakes' is delineated by striking off arcs of 53° from the borders of the plateau uplift regions

Table 1. GEOTECTONIC CLASSIFICATION OF EARTHQUAKE REGIONS

Region designation	Region description	Type locations	Stress mechanism
Very active mountains	Possesses shallow, intermediate and deep earthquakes; continental structure	Andes, Japan, Indonesia, Philippines	Mountain building
Active mountains	Possesses shallow and intermediate earthquakes; continental structure	Himalayas, mountains in the Mediterranean, area Aleutians (continental portion)	Mountain building
Less active mountains	Shallow earthquakes only, continental structure	Rockies, Appalachians, Atlas, Alps, Urals	Mountain building
Continents in general	Shallow earthquakes not associated directly with major mountain chains	Continental shields, old mountain roots, etc.	Local stress accumulations are due to general continental activity
Normal oceanic regions	Ocean areas in general with preference for stress release along mid-ocean ridges and less active island chains	Hawaii, Iceland, mid-ocean ridges	Stresses generated in regions of plateau uplift and propagated generally less than 53°. Majority of these earthquakes occur along mid-ocean ridges, as these ridges represent pre-existing zones of weakness generated as part of the world-wide shear pattern
Active oceanic areas	Possesses shallow and intermediate earthquakes; few deep earthquakes; oceanic structure often with associated deep ocean trench	Mariana Islands, New Hebrides, Aleutians (oceanic portion), Kermadec Islands, Philippine and Indonesian Oceanic structures	Island arc generation
Special ocean area	Possesses a very high number of deep earthquakes relative to shallow earthquakes; 70 per cent of all deep earthquakes that occurred in the first six months of 1963 occurred here	Fiji Islands	Stress mechanism located in mantle; suspected cause of stress generation is phase change in the 400–1,000 km region (*C* region)
Completely inactive oceanic region	No earthquakes, region completely inactive	N.W.–S.E. area in Pacific area. See Fig. 5	Lack of stress accumulation because this area is located more than 53° of arc from all plateau uplift regions

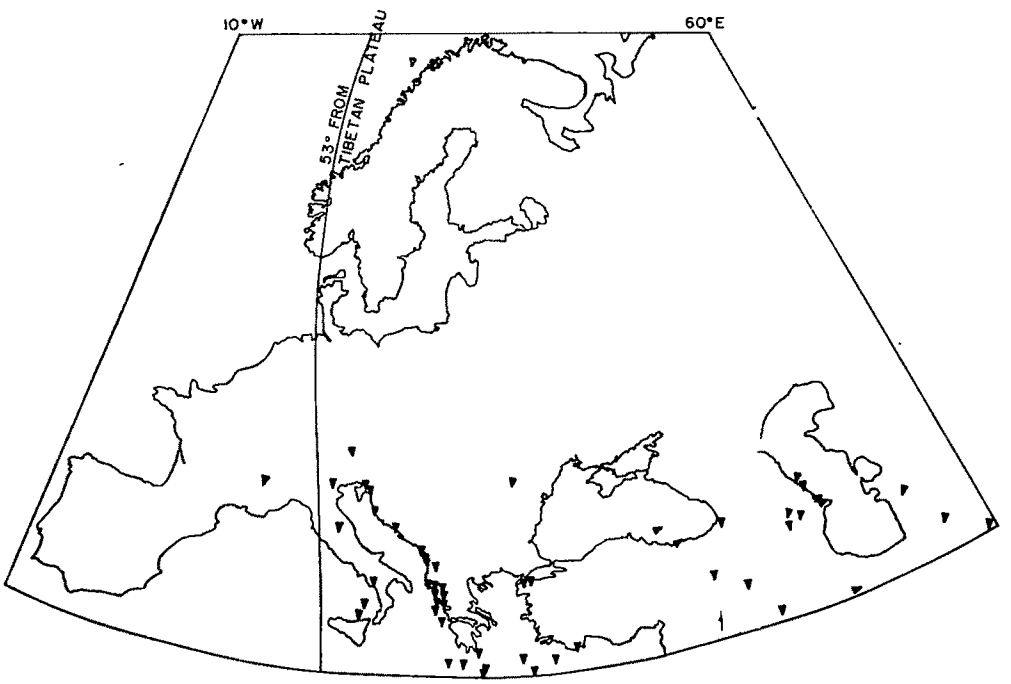


Fig. 7. European earthquakes for the first half of 1963. Data from ref. 10

deep-focus earthquake region. On examination, it is found that the area of deep focus earthquakes is small and has a distinct N.N.E.–S.S.W. trend. If large-scale convection was operational in the lower mantle, then one would expect to find the area of high-frequency deep-focus earthquakes to be very large. However, the surrounding island groups—the New Hebrides, the Tonga Islands, the Kermadec Islands and the Samoa Islands—all have normal earthquake distributions while the Ellice Islands are comparatively earthquake-free.

Table 2. EARTHQUAKES OF THE SPECIAL OCEAN AREA VERSUS THE NORMAL EARTHQUAKE DISTRIBUTION

Earthquake range (km)	Normal earthquake distribution (per cent)	Fiji island earthquake distribution (per cent)
0–100	63	17
100–300	31	6
300–720	6	77

The figures for the normal earthquake distribution are the average percentage of earthquakes from all other regions containing deep focus earthquakes. (Data from ref. 10.)

This consideration of the areal extent of the high-frequency deep-focus earthquake region indicates that the stress generation mechanism is indeed a phase change located below 400 km and is expressed along a zone of weakness which penetrates the entire crust. The presence of a phase change below 400 km—which should be capable of propagating a stress into very deep regions—and the fact that the 720-km depth limitation on earthquakes is not exceeded, strongly implies that geotectonic stress cannot be accumulated or propagated below this depth. Any geotectonic stresses arriving below this depth limit must therefore be released, not through normal geotectonic expression, but by plastic flow or some other related phenomenon.

Finally, it is to be stated that the phase change at the Moho in continental areas is somewhat sensitive to changes in temperature and pressure. Thus, phase change can most likely explain how many kilometres of sedimentary rock can be deposited in a shallow water environment and how mountain ranges can maintain themselves as sediment sources for tens of millions of years in excess of their expected erosional lifetimes. This pressure and temperature sensitivity should also yield a basis for developing a detailed explanation of continental maintenance.

The conclusions that can be drawn from this investigation of stress in the Earth are:

(1) Geotectonic stress generation is due to phase change at two levels in the Earth—(i) the Moho and (ii) the '*C*' Region, that is, 400–1,000 km depth.

(2) Geotectonic stress propagation is limited to 53° of arc and is probably rectilinear in nature.

(3) Stress accumulation required for geotectonic expression is limited to regions above 720 kilometres depth and that below 720 kilometres stress is dissipated in some form of plastic flow.

Finally, it is concluded that the entire geotectonic spectrum of stress relief may be explained by the stress generation, propagation and accumulation as described here, and that convection currents are not needed to explain any geotectonic phenomenon.

The verification of the foregoing conclusions may be based on resolution of the following predictions:

(1) That earthquakes will eventually be observed: (*a*) in continental Antarctica; (*b*) between Hawaii and the Aleutian Islands; and (*c*) between Hawaii and California and between Hawaii and Mexico further west than has been observed so far.

(2) That earthquakes will not be observed in the 'zone of no earthquakes' between now and the time required to satisfy predictions 1*b* and 1*c*.

(3) That project Mohole, if successful, will find that the Moho is indeed due to a basalt-eclogite phase change.

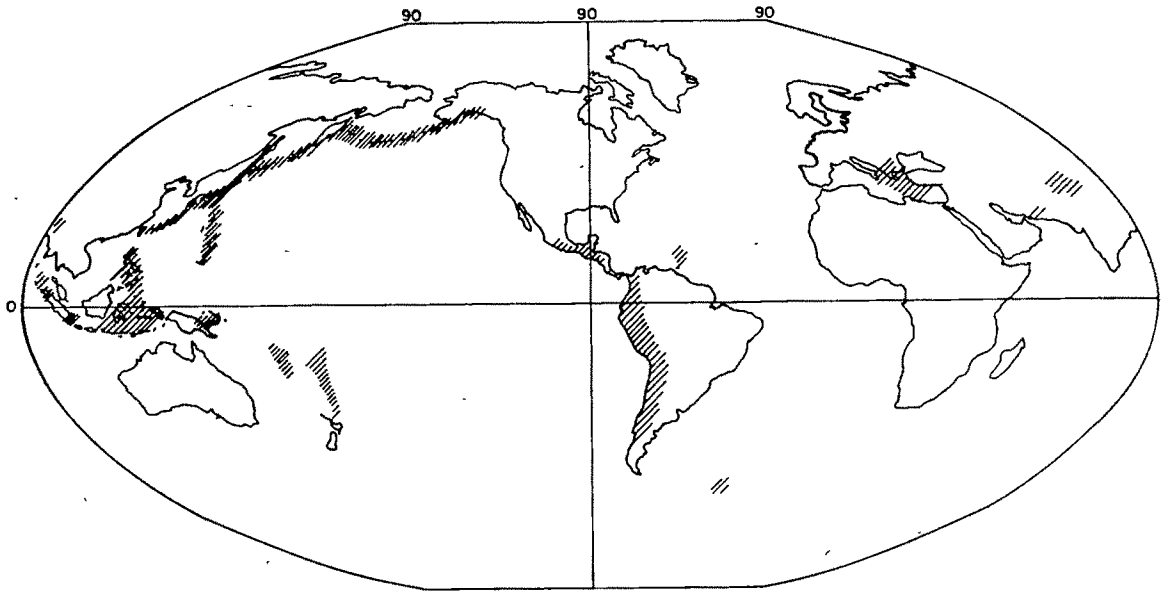
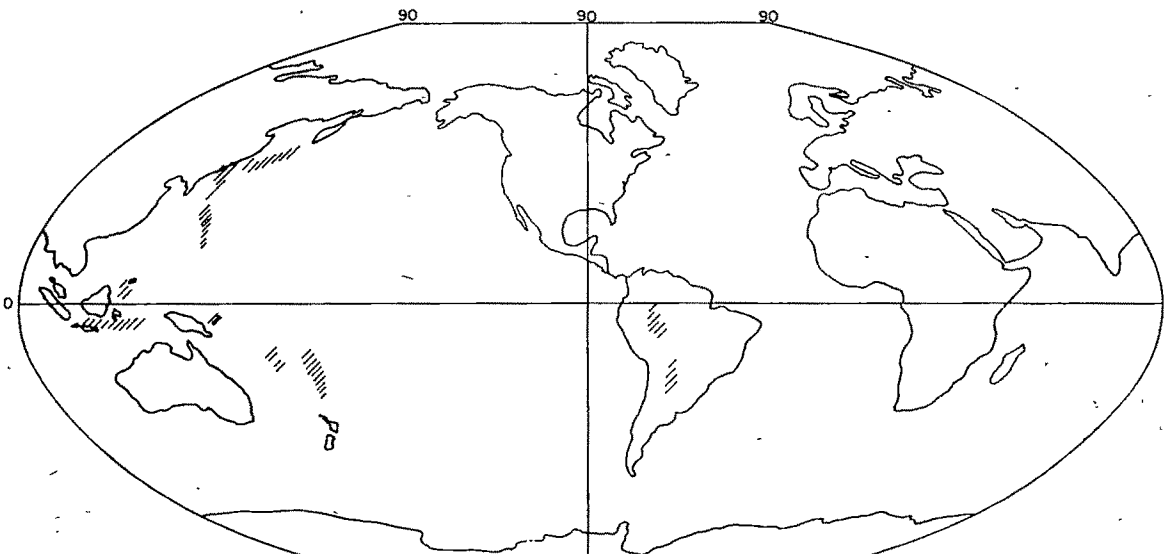


Fig. 8. Distribution of intermediate depth earthquakes



(4) That a Moho will be found on the Moon at a depth that is, from the point of view of pressure, comparable to the depth of the Moho under the stable ocean regions on Earth. Preliminary calculations (which assume a surface density on the Moon of 3·0) give the approximate depth of the lunar Moho as 50 km.

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DATING OF POTTERY BY THERMOLUMINESCENCE

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University Museum, University of Pennsylvania, Philadelphia

EXPERIMENTS with thermoluminescence as a possible technique for the dating of pottery have been pursued actively for the past 3 years as one of the projects of the Applied Science Center for Archaeology.

The suggestion that thermoluminescence might provide a means of dating pottery was made by Farrington Daniels[1] and also by F. G. Houtermans[2] some years ago. The technique was investigated further by George Kennedy[3] and is now being engaged in actively at the Research Laboratory for Archaeology and the History of Art, University of Oxford[4], as well as in the Applied Science Center for Archaeology and other laboratories.

Thermoluminescence in pottery is due to the fact that radiations from the traces of radioactive elements in pottery bombard the other constituents of the clays and raise electrons to metastable levels. When the pottery is heated, such as in firing, each electron falls back to its stable position and emits a photon of light. On being reheated, the amount of thermoluminescence observed is, therefore, representative of the accumulated radiation damage and hence of the time elapsed since the original firing of the pottery.

Once an object has been heated to a temperature of 400°–500° C and its electrons have emitted their thermoluminescent light, no further light may be obtained by reheating after a relatively short time. Consequently, recently fired ceramic ware or freshly cooled lava, all of which have all electrons in stable sites, should show no thermoluminescence.

The basic principle is, therefore, straightforward, but there are a number of uncertainties which must be investigated or circumvented before it can definitely be stated that thermoluminescence will provde a means of dating pottery. Some of the major uncertainties are as follows: (1) Is the radiation damage caused primarily by α-bombardment, or is it a combination of α-, γ- and β-particles ? (2) How many metastable electrons are lost, especially near the surface, during the passage of time ? (3) How, and how much, do the susceptibilities of clay to radiation damage vary ? (4) How do the differing transparencies of clays affect the detection of the photons emitted on heating ? (5) Does the bombardment from radioactivities in soils cause significant radiation damage to potsherds from external sources ? (6) What is the effect of the grinding of the pieces of pottery, a necessary preliminary to thermoluminescent detection ?

Our first concern has been to assemble apparatus sufficiently sensitive and reliable to make a start on measurements. We have proceeded, therefore, in an over-simplified way at first, and have succeeded in making quantitative measurements with adequate sensitivity.

First of all, we have assumed that the major damage is caused by α-bombardment or is proportional to it, and have, therefore, constructed special low-background zinc sulphide screens and associated components for this low-level detection of α-particles. The α-emissions are counted in 'infinitely' thick samples with the result that only comparative values are obtained.

For the detection of the thermoluminescent photons emitted on heating, our numerous preliminary experiments indicated that very rapid heating rates of thin layers of powdered potsherds would allow detection of the maximum light output. Since the accumulation of metastable electrons is very small, very high sensitivity is required to detect the light output in the visible range. However, this high sensitivity enables the apparatus to detect also the longer wave-lengths of the heat radiation even at temperatures considered usually well below the emission of visible light. These are the reasons why rapid heating (now 13° C/sec) is essential—namely, to drive out all the photons before the surrounding materials get too 'hot'.

The apparatus for detection of thermoluminescence is shown in Fig. 1. Samples are heated in a nitrogen atmosphere to prevent possible combustion of organic particles and also to prevent spurious changes in intensity due to oxygen. To obtain very thin uniform layers of powdered pottery, the pottery is ground in a ball mill to less than 200 mesh. Separate pre-baking experiments have shown that the grinding induces negligible thermoluminescence. Then the powder is mixed with silicone oil and applied to aluminium foil on a $\frac{3}{4}$-in. diameter 'spot' by means of a No. 10 silk screen. This produces a uniform, thin and stable coating of pottery on the foil. The size of the 'spot' of powdered pottery was dictated by the area that could be heated uniformly by the furnace.

One of the important unknown factors is the quantitative relationship between the bombardment and the resultant metastable electron accumulation in ancient pottery. It may be determined empirically, however, with series of pottery of known ages. The first series run consisted of fourteen samples, representative of five different ages, from the Solduz Valley, Iran. The archaeological layers from which these samples were excavated were dated by carbon-14 techniques from associated charcoal samples[5]. Even though carbon-14 dating is not so precise as tree-ring or historical dating, this series affords a long range in time, from 5500 to 900 B.C. The obvious advantage of this is that the older samples give more light output, but, additionally, a long range should afford more information about the possible loss or decay of metastable electrons with time, one of the basic uncer-

tainties. The results of the fourteen thermoluminescent measurements when plotted against age showed poor correspondence and inconsistencies in replicate runs of the same sample.

The determination of potassium contents affords an indirect measure of the relative bombardment rate from β-radiations (due to potassium-40) within the pottery. The results of eight measurements (percentage potassium range was 2·14–3·29) indicate that this factor is not the cause of the discrepancies in age correspondence for the series of pottery from Iran.

The lack of consistency was thought to imply different susceptibilities. The obvious way to obtain a measure of the susceptibility of a potsherd to radiation damage is by the application of an external dose, from a source with much greater intensity than the natural bombardment in order to duplicate the original radiation damage in a short time. On the basis of the low rates of α-bombardment in this series of pottery, we estimate that the natural thermoluminescence is caused primarily by the filling of lattice imperfections with metastable electrons rather than by the creation of new 'holes'. Therefore, with a relatively small artificial dose, it should be possible to repopulate the lattice vacancies. If the dose is increased, then it might be possible to note when the dose becomes sufficient to create new radiation damage.

Our initial susceptibility experiments were carried out with low-voltage X-rays. Duplicate samples of pottery were pre-baked for 1 h at 600° C, and then mounted on aluminium foil in the usual manner for irradiation with X-rays. After several experiments made at various energies and intensities, the effects of these variables appeared to be linear. Subsequent tests were made at 30 kV and 12·7 m.amp (G.E. model *XRD*-5 X-ray unit) and the times of bombardment were varied. From these measurements, the amount of irradiation required (measured in seconds) to produce the same amount of light output as that obtained naturally was determined. If these times of irradiation are then used as correction factors for varying susceptibilities, the results obtained, if anything, are slightly worse.

Experiments with longer times of irradiation and applications of these results in various ways as correction factors for susceptibilities did not significantly improve the age correspondence. Then, during the past year, two important observations were made. First, it was found that even though replicate samples were taken from a single piece of pottery that had been ground and mixed thoroughly, there were large variations in susceptibilities among the replicate samples from the one piece. This indicated that the correction by artificial bombardment should be applied to the identical samples that were measured for natural thermoluminescence. Fortunately, our mounting technique with silicone oil on aluminium foil allows this to be easily done. Therefore, when each sample was heated and measured for natural thermoluminescence, bombarded with X-rays (for 60 sec at 30 kV and 12·7 m.amp), and then reheated, the area of the artificial glow curve, applied as a correction factor, gave some improvement toward consistent results. The reduction of the overall uncertainty in a series of replicate runs is shown in Table 1. For this particular sample, the statistical deviation of the ratio of natural divided by artificial thermoluminescence is only ± 5 per cent whereas the individual spreads were 20 and 17 per cent.

Table 1. EXAMPLE OF REPLICATE RUNS AND CALCULATION OF DEVIATIONS FOR ONE SAMPLE

The uncertainties for each column represent $\sqrt{\frac{\Sigma(n_i-\bar{n})^2}{N-1}}$

Sample No 6. From. Plain of Sybaris, Italy
Age: 550 B.C. α Activity· 32 5 ± 1·2 counts/h ± (3·7%)

Run No.	Area of natural glow in cm² Nat. Th-L $\pm\sigma_s$	Area of artificial glow in cm² Art Th-L $\pm\sigma_i$	Area ratio $\frac{\text{Nat. Th-L}}{\text{Art. Th-L}} \pm\sigma_s$
1	17·3	46·5	0·372
2	14·0	37·5	0 373
3	14·9	43·5	0·343
4	17·8	51·0	0·349
5	17·0	48 0	0·354
6	20·3	57·0	0 356
7	21·5	54·0	0·398
8	13·0	39·0	0·333
9	18·8	49·5	0·380
10	22·0	57·0	0·386
11	16·0	44·4	0·360
12	26·0	69·0	0·377
13	16·1	46 5	0 346
14	20·3	51 0	0·398
15	25·0	63·0	0·397
16	23·5	61 5	0·382
17	23·0	60 0	0·383
18	16·5	45·0	0·367
Average	19·1 ± 3·8 ± (20%)	51·3 ± 8 6 ± (17%)	0·370 ± 0·019 ± (5·1%)

Calculation:
Specific thermoluminescence = 0·370/32·5 = 0 0114.
Percentage deviation of the specific thermoluminescence = $\sqrt{(5·1)^2+(3·7)^2}$ = 6·3 per cent. 6 3 per cent of 0·0114 = ± 0·0007.
Result: specific thermoluminescence = 0 0114 ± 0·0007.
Abbreviations: Nat., natural, Art., artificial; Th-L, thermoluminescence.

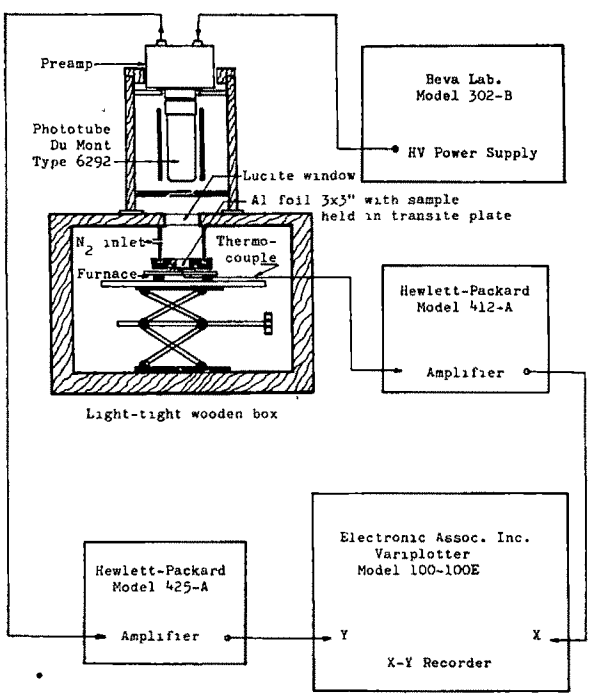


Fig 1. Block diagram of the glow curve apparatus

More important, however, is the second observation made within the past 6 months, namely, that the low-temperature peak or peaks in the glow curves induced by X-ray bombardment are unstable and decay within a period of 1–2 weeks. In the natural glow curves these unstable low peaks have already disappeared due to thermal environment, and possibly decay. Therefore, one must wait for these to decay after the artificial bombardment in order to obtain a reliable correction factor. Alternatively, ideally one could measure only the area under the high-temperature peaks, but with the rapid heating rates which we have found necessary to use for this weak thermoluminescence, the peaks become integrated together into one single peak. A week or two after X-ray bombardment, however, the low temperature part of the integrated peak does decay and a small discernible second peak appears (see Fig. 2). When the area of the latter is used as the correction factor, significant improvement in age correspondence is achieved. This is shown in Table 2 and Fig. 3, where measurements of samples (Nos. 6, 7, 12 and 13) from Italy are included as well as those from Iran. The samples from Italy, representative of classical periods, are more firmly dated.

Recently several examples of thermoluminescent decay have been reported. Zeller and Ronca[6] mentioned that workers engaged in thermoluminescence research have had the impression that inconsistent results were obtained if glow curves were run immediately after samples were irradiated. They suggested that this was the result of the thermal decay of the lowest temperature peak. This effect was reported also by J. M. McNellis[7]. B. E. Sabels[8], in studies of basaltic lava flows, has estimated a hypothetical half-life of thermoluminescent decay for these lavas

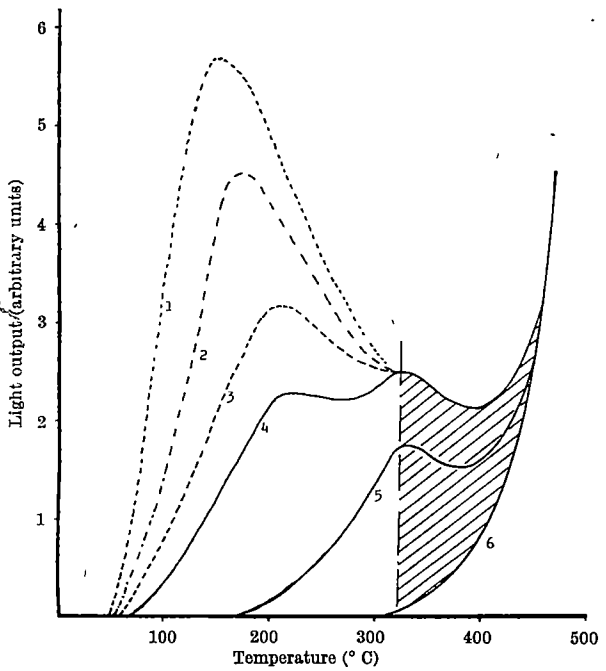


Fig. 2. Typical glow curves. (1) Artificial glow immediately after irradiation with X-rays; (2) artificial glow 24 h after irradiation with X-rays; (3) artificial glow one week after irradiation with X-rays; (4) artificial glow two weeks after irradiation with X-rays; (5) natural glow curve; (6) background (heat radiation only) curve. The hatched regions are the significant areas for both the natural and artificial thermoluminescence

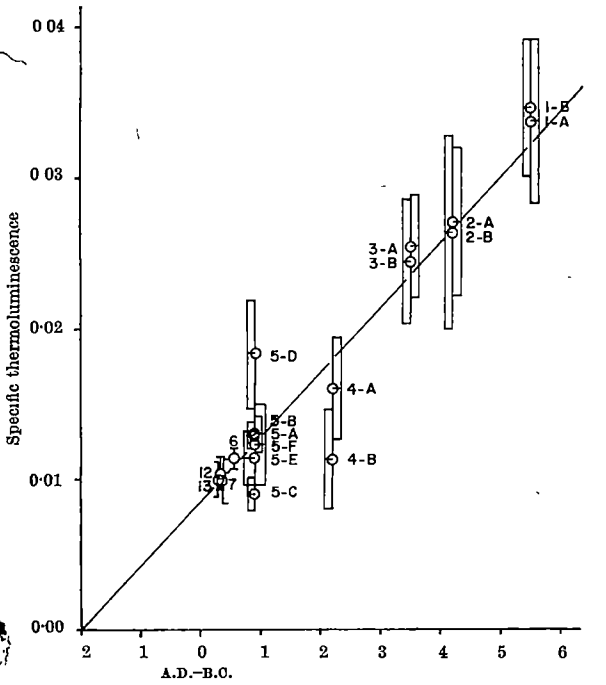


Fig. 3. Plot of specific thermoluminescence versus known ages for samples listed in Table 2

Table 2. α RATES, AREA RATIOS, AND SPECIFIC THERMOLUMINESCENCE FOR SAMPLES FROM SOLDUZ VALLEY, IRAN (NOS. 1 TO 5), AND PLAIN OF SYBARIS, ITALY (NOS. 6, 7, 12 AND 13)

Sample No.	Age B.C.	Relative α activity in counts/h $\alpha \pm \sigma_1$	Average area ratios Nat. Th-L / Art. Th-L $\pm \sigma_s$	Specific thermoluminescence Area ratio / α counts/h $\pm \sigma_s$
1–*A*	5500	11·7 ± 0·3	0·393 ± 0·063	0·0336 ± 0·0054
1–*B*		11·3 ± 0·4	0·390 ± 0·050	0 0345 ± 0·0045
2–*A*	4200	11·4 ± 0·6	0·308 ± 0·053	0·0270 ± 0·0049
2–*B*		11·0 ± 0·4	0·289 ± 0·070	0·0263 ± 0·0064
3–*A*	3500	16 9 ± 0·8	0·429 ± 0·053	0·0254 ± 0 0034
3–*B*		13·1 ± 0·4	0·320 ± 0·053	0·0244 ± 0·0041
4–*A*	2200	17 1 ± 0·5	0·274 ± 0·057	0 0160 ± 0 0034
4–*B*		18·0 ± 0 5	0·204 ± 0·060	0·0113 ± 0·0033
5–*A*	900	17·6 ± 0·5	0·227 ± 0·015	0·0129 ± 0·0009
5–*B*		8·8 ± 0·5	0·114 ± 0·009	0·0130 ± 0·0012
5–*C*		9·1 ± 0·3	0·0823 ± 0 0101	0·0090 ± 0 0011
5–*D*		16 1 ± 0·4	0 294 ± 0·058	0·0183 ± 0·0036
5–*E*		10·2 ± 0·5	0·116 ± 0·017	0·0114 ± 0·0018
5–*F*		11·2 ± 0·3	0·138 ± 0·030	0·0123 ± 0 0027
6	550	32·5 ± 1·2	0·370 ± 0·019	0·0114 ± 0 0007
7	360	23·0 ± 0·6	0 227 ± 0·035	0·0099 ± 0·0015
12	330	22·0 ± 0·4	0·228 ± 0·025	0·0104 ± 0 0011
13	300	24·2 ± 0·3	0 243 ± 0·030	0·0100 ± 0·0012

The postscripts *A*, *B*, etc. (column 1), represent different sherds of the same age for each number listed.

Abbreviations as for Table 1.

to be 20,000 years. After various experiments, Sabels concluded that the loss in thermoluminescent output was due to natural decay rather than limitations of storage capacity for excited electrons. Although this limits the dating range for basaltic lavas, it should not be a serious problem for pottery, most of which was fired much more recently than the lavas studied. However, some evidence of decay in pottery has been observed by Ichikawa[9]. Decay of the high-temperature peaks in pottery has not been observed in our laboratory as evidenced by the linear correspondence of age with time (Fig. 3), and it has been reported to be insignificant in a recent article by Aitken[10].

With the application of the X-ray induced correction factor after time allowance for decay, the improvement in age correspondence is encouraging. This is especially true for the pottery from Italy, which is representative of classical periods, and presumably made of finer and more homogeneous clay. For the four samples from Italy (Nos. 6, 7, 12 and 13), the average uncertainty is ± 0·0006 or ± 300 years.

If, after the completion of several different series of pottery, it is found that the time-scale derived from the series now completed (based on sherds from Iran and Italy) is applicable for all, there will be no question that thermoluminescence will provide a reliable means of dating pottery. Additionally, if the consistency of the measurements is improved, the precision may be comparable with carbon-14 dating. It affords the extra advantage that the pottery itself (abundant and primary) may be dated rather than something (such as charcoal) associated with it.

We thank Prof. Froelich Rainey, director of the Applied Science Center for Archaeology, and Prof. William E. Stephens, chairman of the Department of Physics, for their guidance and encouragement. We also thank Dr. Martin Aitken, associate director of the Research Laboratory for Archaeology and the History of Art, University of Oxford, for his many helpful suggestions and advice. This work was supported by the National Science Foundation.

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NEWS AND VIEWS

New Fellows of the Royal Society of Edinburgh

THE following have been elected Fellows of the Royal Society of Edinburgh: PROF. G. H. BEALE, Institute of Animal Genetics, Edinburgh; PROF. F. F. BONSALL, Department of Mathematics in the University of Edinburgh; DR. G. S. BOYD, senior lecturer in biochemistry in the University of Edinburgh; PROF. A. BUXTON, Royal (Dick) School of Veterinary Studies, Edinburgh; DR. J. A. CRANSTON, formerly principal lecturer in physical chemistry in the Royal College of Science and Technology (University of Strathclyde), Glasgow; PROF. E. C. ELLWOOD, Department of Metallurgy, University of Strathclyde; PROF. W. N. EVERITT, Department of Pure Mathematics, Queen's College, Dundee; MR. H. FERGUSON, chief of the Agricultural Research Division, Sudan Ministry of Agriculture; DR. G. A. GARTON, Rowett Research Institute, Aberdeenshire; DR. J. A. K. HAMILTON, a partner in Messrs. Mott, Hay and Anderson, civil engineers, Edinburgh; MR. D. M. HENDERSON, principal scientific officer, Royal Botanic Garden, Edinburgh; PROF. L. G. JAEGER, head of the Department of Mechanical Engineering in the University of Edinburgh; MR. B. R. S. MEGAW, director of the School of Scottish Studies in the University of Edinburgh; DR. B. MELTZER, reader in electrical engineering in the University of Edinburgh; DR. C. F. MILLS, Rowett Research Institute, Aberdeenshire; PROF. J. M. MITCHISON, Department of Zoology, University of Edinburgh; PROF. E. O. MORRIS, Department of Applied Microbiology and Biology, University of Strathclyde; PROF. W. MULLIGAN, Department of Veterinary Physiology, University of Glasgow; PROF. D. R. NEWTH, Department of Zoology, University of Glasgow; DR. A. S. PAINTAL, director of the Valhabhai Chest Institute in the University of Delhi; PROF. M. R. POLLACK, Department of Molecular Biology, University of Edinburgh; LORD POLWARTH, chairman of the Executive Committee, Scottish Council (Development and Industry); DR. A. ROBERTSON, Unit of Animal Genetics of the Agricultural Research Council, Edinburgh; PROF. A. P. ROBERTSON, Department of Mathematics, University of Keele; PROF. H. F. W. TAYLOR, Department of Chemistry, University of Aberdeen; DR. P. L. WILLMORE, senior seismologist, Royal Observatory, Edinburgh; SIR CHARLES WILSON, principal and vice-chancellor, University of Glasgow; MR. R. B. WILSON, principal geologist, Geological Survey and Museum.

Deputy Directors at the National Physical Laboratory

THE Minister of Technology, Mr. Frank Cousins, has appointed Dr. N. P. Allen and Dr. O. Simpson to be deputy directors at the National Physical Laboratory with effect from April 1, 1966. Dr. N. P. Allen will become deputy director (*A*) and Dr. O. Simpson deputy director (*B*). The deputy directors will have a collective responsibility for advising the director on the work of the Laboratory as a whole, but will also have functional responsibilities for particular areas of the work. Thus, initially Dr. Allen will be concerned with materials science, and Dr. Simpson with measurement science.

Dr. N. P. Allen

DR. ALLEN graduated in metallurgy at the University of Sheffield in 1923, and after a period at University College, Swansea, became a member of staff in the University of Birmingham. He obtained the D.Sc. degree in 1934 for his work on gases in metals. Dr. Allen transferred to the Mond Nickel Company in 1935 to take charge of various research projects connected with alloy steels and creep-resisting nickel–chromium alloys, and remained there until 1944, when he joined the National Physical Laboratory as superintendent of the Metallurgy Division. Dr. Allen's interests at the National Physical Laboratory have ranged over a wide field, but particular mention may be made of his work on pure iron, and on fatigue, creep and fracture of metals and alloys. He was awarded the Grand Médaille F. Osmond by the Société Française de Métallurgie in 1963, and the Bessemer Gold Medal by the Iron and Steel Institute in 1965. The honorary degree of Doctor of Technical Sciences was conferred on him in 1964 by the Technical University of Prague. Dr. Allen was elected a Fellow of the Royal Society in 1956, and was president of the Institution of Metallurgists in the period 1961–62.

Dr. O. Simpson

DR. SIMPSON graduated at Trinity College, Cambridge, and joined the Admiralty Research Laboratory during the Second World War to work on lead sulphide infra-red detectors. He returned to Cambridge after the War to continue research on photoconduction in semiconductors, and went on to become a Fellow of Trinity College. In 1949 Dr. Simpson was appointed assistant professor of physics at the University of Michigan, and returned to England in 1952 as Imperial Chemical Industries research fellow in the Department of Theoretical Chemistry at the University of Cambridge. He joined the Services Electronics Research Laboratory of the Admiralty in 1953 to initiate research on organic semiconductors, later becoming head of the solid state physics group. Dr. Simpson transferred to the National Physical Laboratory in 1963 as superintendent of the Basic Physics Division, and in 1965, following the merger of the former National Chemical Laboratory with the National Physical Laboratory, became superintendent of the newly formed Division of Molecular Science.

National Physical Laboratory

PROF. B. H. FLOWERS, Langworthy professor of physics in the University of Manchester, has been appointed chairman of the National Physical Laboratory Steering Committee. Eight members of the Steering Committee have now been appointed: R. G. Clayton, General Electric Co., Ltd.; H. G. Conway, Bristol Siddeley Engines, Ltd.; Dr. H. N. Finniston, International Research and Development Co., Ltd.; Prof. G. Gee, University of Manchester; Dr. H. Kronberger, U.K. Atomic Energy Authority; Vice-Admiral Sir Frank Mason; Sir James Taylor; and the director of the National Physical Laboratory. Two further members will be appointed in due course. The Steering Committee will submit annually to the Minister of Technology a research programme for the following year, together with proposals for budgets and staff to carry out that programme. It will be responsible to the Minister of Technology for advising on the selection of projects and for the allocation of effort to them within the financial and other resources allocated to the laboratory.

Sir John Leslie (1766–1832)

SIR JOHN LESLIE, the noted physicist and mathematician, was born in Largo, Fifeshire, two hundred years ago on April 10. He entered the University of St. Andrews at the tender age of thirteen, to study for the Church, and later went on to the University of Edinburgh. Here, Leslie began the scientific investigations which he was to follow for the rest of his life. His first paper concerned electrical theory, but his most notable contributions

were in the field of heat radiation. He invented a differential thermometer, and 'Leslie's Cube' appears in every school physics textbook. He wrote a number of papers and books including *Experimental Inquiry into the Nature and Properties of Heat* and *Radiation of Heat* and also published on geometry, analysis and trigonometry. In 1805, Playfair resigned his chair of mathematics in Leslie's favour, and in 1819, Leslie became professor of natural philosophy at Edinburgh. Leslie was a first-class experimentalist and designer of apparatus, but a poor lecturer. He travelled widely and took a considerable interest in European, especially French, science. He died on November 3, 1832.

Science and Parliament

UNDER the title *Science and Parliament*, the Organization for Economic Co-operation and Development has published an admirable final report, presented by P. Piganiol, on the Second Parliamentary and Scientific Conference, organized jointly with the Council of Europe, at Vienna, during May 23–27, 1965 (Pp. 177. Paris: Organization for Economic Co-operation and Development; London: H.M.S.O., 1965. 8 francs; 12*s*. 6*d*.; 2 dollars). Besides M. Piganiol's report on the debates and the formal conclusions of the Conference, it includes the text of the five addresses, of which those of Prof. G. Hess, Mr. K. Lindsay, Mr. Quintin Hogg and Mr. G. Palewski are outstanding. Annexes include a list of participants, the programme and the recommendations and resolutions for following up the Conference. The recommendations direct attention to the conclusion that there should be in each country a Minister charged solely, or together with other responsibilities, with the promotion and co-ordination of science policy in all its aspects. They invite the national Parliaments to set up, without delay, parliamentary and scientific committees where they do not exist; to examine, where the need arises, the advisability of changing their procedure and the organization of their work by setting up standing parliamentary committees for science; and to strengthen the machinery for informing their members about scientific questions. The first resolution is concerned with the development of European co-operation in the scientific field and advocates the organization of a general debate on the activities of international and European organizations in this field and their more effective co-ordination. The second suggests a study programme to follow up the Parliamentary and Scientific Conference. Prof. G. Hess's opening address dealt with "Science and Society"; Mr. K. Lindsay's survey, "Science and Parliament", included brief accounts of scientific policy in France, Belgium, Western Germany and the United Kingdom. Mr. Hogg discussed specific present and foreseeable impacts of science on political life; Mr. G. Palewski "A Scientific Research Policy in the World of Today"; and Mr. K. Czernetz described the common tasks of parliamentarians and scientists in drawing up a science policy.

Experimental Cartography

THE Natural Environment Research Council has awarded a grant of £11,035 to Mr. D. P. Bickmore, director of the Cartographic Department of the Clarendon Press, for a detailed survey of the needs and problems of the various users of cartographic methods and of the techniques being developed in Britain and overseas. Mr. Bickmore is a senior Research Fellow of the Royal College of Art. The survey, which will last 18 months, will be in two main parts; the first will be concerned with appraising needs, problems and present techniques, and the second with proposing the direction of further work. Besides the information traditionally converted into maps, the study will also be concerned with other information that could usefully be mapped; this includes oceanographic surveys, surface water records and information from satellites to archaeological surveys, population and health censuses and traffic planning probably using animated cartography. The survey will also be concerned to keep abreast of the flow of new information over the range of physical and social sciences. This will involve the application of automatic data processing techniques to cartography. A programme of experiments on the perceptual processes of map reading will be undertaken to assess the most efficient presentation of information. Animated maps showing changes with time will be necessary to aid, for example, planning and traffic studies.

Building Social Institutions

DR. H. V. PERLMUTTER'S essay, *Towards a Theory and Practice of Social Architecture: the Building of Indispensable Institutions*, is based on his experience at the Centre for International Studies at the Massachusetts Institute of Technology, at the Division of Industrial Mental Health of the Menninger Foundation, Yale University School of Industrial Administration, at the Institut pour l'Etude des Méthodes de Direction de l'Entreprise, and as consultant to some world organizations (Tavistock Pamphlet No. 12. Pp. vii+60. London: Tavistock Publications, 1965. 12*s*. 6*d*.). It is in three parts: the first outlines the need for a theory and practice of social architecture, starting from the assumption that men need to understand not only how to change the attitudes of individuals in desirable directions but also how to create social institutions which guarantee valued actions to meet the indispensable needs of society. It is also assumed that the measure and locus of enduring social progress and human welfare depend on the viability of indispensable social institutions. Such institutions, Dr. Perlmutter thinks, are themselves better understood and more easily created if increasing attention is given to the interdependence of the theory and practice of institution-building. The social part outlines the essential steps to be taken towards a theory of social architecture, including the conceptual requirements and the integration of the various dimensions involved. The third part is concerned with the actual practice of social architecture and describes the collaborative relation between the social architects, or persons trained in the applied behavioural sciences, and their clients, the actual institution-builders. The essay defines an area of study in which the theories and practices of building institutions could receive direct and concentrated consideration both by research workers and practitioners. In conclusion, Dr. Perlmutter defines social architecture as involving the development of concepts leading to research which guides men to create institutions that embody positive values and remain responsive to the needs of individuals within and without the institution, and the practical building of such institutions. In the contemporary situation, more than ever before, the hope for civilization lies in improving man's talent to create institutions which guarantee peace and security and promote economic and social development.

Organo-mineral Complex in Soils

FOR a full appreciation of the mechanism of the formation of complexes of clay and organic matter, a better knowledge of the structure and composition of the organic matter is required. The properties of this matter may, however, vary with soil or extraction technique. A further well-documented review of the present position, entitled "Interaction between Clays and Organic Compounds in Soils". Part 2: "Adsorption of Soil Organic Compounds and its Effect on Soil Properties", by D. J. Greenland, is presented in *Soils and Fertilizers* (28, 521; 1965). The humic acid fraction is composed of polymers of amino-acids and phenolic compounds. These are mainly of molecular weight 20,000–50,000, roughly spherical and relatively rigid, and give aqueous solutions of low viscosity. The fulvic acids are mainly phenolic sub-

stances, of lower molecular weight, and polysaccharides of molecular weight 120,000–400,000. They are linear and flexible, giving solutions of high viscosity. Humic acids are held at positive sites and do not penetrate the interlamellar regions as do the polysaccharides. The soil properties are changed by the formation of the complex. Organic material lining the coarse pores increases the strength of the aggregates. Organic cations are less strongly hydrated than inorganic cations and reduce the affinity of the surface for water; this may account for the common phenomenon of non-wetting sandy soils, certain soil regions becoming hydrophobic. Some organic compounds increase the swelling of clays, some organic anions cause flocculation of clay, some polysaccharides increase the stability of aggregates. Microbiological activity may be stimulated even though interlamellar protein is only slowly attacked by enzymes. This is of importance in relation to toxins added to the soil as pesticides, since positively charged organic matter is likely to be strongly adsorbed and liable to accumulation.

Manganese at Groote Eylandt, Australia

GROOTE EYLANDT is in the Gulf of Carpentaria, off the east coast of Arnhem Land, Northern Territory, Australia. Apart from a limited potential for development of fishery, pearl culture and some tourist trade, the principal asset of the island is now known to be manganese ore deposits, proved by an extensive geological survey in 1960 by P. R. Dunn (Bureau of Mineral Resources) and P. W. Crohn (senior resident geologist, Northern Territory Administration). The survey was followed in 1963 by an exploration programme, ultimately involving more than 300 pits and 600 drill holes. The deposit is regarded as a most important source of manganese, by far the largest yet known in Australia, and similar in many technical respects to those of the Ukraine and Georgia, in the U.S.S.R. (The latter contain the largest deposits and greater part of the world's reserves of manganese.) Under title "Manganese Ore Deposits at Groote Eylandt", W. C. Smith, assistant exploration geologist, The Broken Hill Proprietary Co., Ltd., has given a brief account of the Australian deposits (*The B.H.P. Technical Bulletin*, 24. The Broken Hill Proprietary Co., Ltd., Newcastle, N.S.W. 9, No. 3; November 1965).

Like the Russian manganese deposits (except in geological age), the Groote Eylandt deposits represent primary sediments, laid down under shallow-water, restricted marine conditions, on a platform, stable from Cambrian times until the beginning of the Lower Cretaceous marine transgression. The ore occurs as a single bed or zone within what are called the Mullaman Beds (Lower Cretaceous). It consists commonly of loose pisolites of pyrolusite and cryptomelane, ranging in size from 1 mm to 12 mm and associated with quartz sand, clay and fine pyrolusite. Another type comprises hard pebbles and boulders of the same minerals, again with pisolitic texture. There is wide variation in thickness, grade and distribution of these types of ore within the manganese zone; where both types are present together, however, the lump ore is found to overlie the loose, smaller, pisolitic material. The average grade of crushed and wet-screened lump ore ranges from 46 to 48 per cent manganese; the loose pisolite ore contains 44–46 per cent of manganese.

Royal Irish Academy

AT the stated meeting of the Royal Irish Academy on March 16 the following officers were elected: *President*, Dr. M. Dillon: *Treasurer*, Dr. V. C. Barry; *Secretary*, Dr. B. Ó Cúiv; *Secretary of the Science Committee*, Dr. W. Cocker; *Secretary of the Irish Studies Committee*, D. Greene.

The Scientific Estate

OXFORD UNIVERSITY PRESS has written to the Editor stating that it is the publisher in the United Kingdom of Prof. D. K. Price's *The Scientific Estate*, which was reviewed on p. 115 of the January 8 issue of *Nature*. The price of the book in the United Kingdom is 48*s*. net.

Appointments

MR. J. A. RATCLIFFE, formerly director of the Radio and Space Research Station of the Science Research Council, has been appointed by the Council of the Royal Society to be Rutherford Memorial Lecturer for 1966 in Australia, and to deliver the Lecture in Sydney on April 26.

DR. J. W. KING, a physicist at the Radio and Space Research Station, Slough, Bucks, has received the 1965 Wolfe Award for his work on "the analysis of results obtained from 'topside sounding satellites' and their interpretation in terms of ionosphere theory".

MR. RONALD W. CLARK is preparing a biography of the late Prof. J. B. S. Haldane and would be glad to receive any reminiscences or other material concerning him. Such information should be sent to 10 Campden Street, Kensington, London, W.8.

Announcements

THE new Engineering Research Station of the Gas Council is to be established at Killingworth near Newcastle upon Tyne.

A MEETING of the Biological Engineering Society will be held at the National Physical Laboratory, Teddington, on April 29. Further information can be obtained from W. J. Perkins, the National Institute for Medical Research, Mill Hill, London, N.W.7.

A MEETING on "Thermodynamics of Ceramic Systems" will be held by the Basic Science Section of the British Ceramic Society in London during April 19–21. Further information can be obtained from Dr. J. P. Roberts, Houldsworth School of Applied Science, University of Leeds, 2.

A MEETING on "Soil Moisture—Physical and Biological Aspects", organized by the British Society of Soil Science, will be held in University College, London, during April 19–20. Further information can be obtained from Dr. D. V. Crawford, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leicestershire.

THE twentieth annual Frequency Control symposium, sponsored by the Electronic Components Laboratory of the U.S. Army Electronics Command, will be held in Atlantic City, New Jersey, during April 19–21. Further information can be obtained from the Director, Electronic Components Laboratory, U.S. Army Electronics Command, Fort Monmouth, New Jersey.

AN international symposium on "The Radiological Protection of the Worker by the Design and Control of his Environment" will be held by the Society for Radiological Protection in Bournemouth during April 18–22. Further information can be obtained from D. B. B. Janisch, Production Group, United Kingdom Atomic Energy Authority, Risley, Warrington, Lancashire.

COURSES in metrology and quality control will be offered at the University of Aston in Birmingham during 1966. A course on "Measurement and Quality Control" will be held during April 25–May 6, and a course on "Statistical Quality Control and Acceptance Sampling" will be held during June 13–July 1. Further information can be obtained from the Department of Production Engineering, University of Aston in Birmingham, Gosta Green, Birmingham.

CORRIGENDUM. It was announced on p. 1073 of the March 12, 1966, issue of *Nature* that Dr. P. W. Kent had been appointed to the chair of biochemistry in the Queen's University of Belfast. It has been brought to the Editor's attention that Dr. Kent will not, in fact, be taking up this position.

ADMISSIONS TO UNIVERSITIES

IN the third annual report, for 1964–65, of the Universities Central Council on Admissions, its chairman, Dr. G. Templeman, reports a year of solid and encouraging progress*. The ten colleges of advanced technology entered the scheme in September 1964, while final preparations were made for the entry of the universities of Oxford and Cambridge into full membership in September 1965.

The medical and dental schools of the University of London have agreed to enter the scheme from September 1966, and from that date all university institutions in England and Wales will be full members of the Council. The technical difficulties of managing the multitudinous dealings between candidates and universities have been surmounted quickly and accurately, and the handling of these large numbers of decisions by universities and replies from candidates is now done by computer.

Beginning with the present report, the information at the disposal of the Council is being published in two parts. The main report, to be issued as soon as possible after the end of the period covered, contains essential information about applications and admissions, analysed in reasonable detail, as well as the customary survey of the operations of the Council, details of the participating institutions and of membership of the Council and the annual accounts. Supplementary tables, including analyses of the performances in the General Certificate of Education of those admitted in the year under review, will be published later and separately, probably in the early summer.

In May 1965 the universities hoped to admit a total of 49,000 candidates in October 1965, and in fact 45,500 were admitted. Except in science, where 430 fewer candidates were admitted than expected, the numbers of candidates actually admitted in October differed only marginally from the May estimate.

In the autumn of 1964 the Council for the first time received applications from all overseas students. The experience during the past winter suggests that the concern for the difficulties of overseas candidates in understanding the full United Kingdom procedure which had led the Executive originally to recommend separate procedure for handling their applications was unnecessary. After September, the separate overseas scheme will be abolished.

* The Universities Central Council on Admissions. Third Report, 1964–5. Pp. 19. (London: The Universities Central Council of Admissions, 1966) 6s.

In 1965 the number of candidates applying through the Central Council of Admissions increased by 37·2 per cent to 80,033, including applicants from colleges of advanced technology and all overseas applicants. The year 1964–65 was the first of full computer operation, and progress made in passing information to candidates was swift; more than 97 per cent of the candidates knew by September 10 how they stood with respect to their original applications. Every possible effort was made by the clearing panels to find alternative courses for candidates for whom it was certain that places would not be available in the subject of their choice. Some hundreds of candidates who had applied to read medicine but clearly had no chance of a place in a medical school had their applications considered for pure science or for a medical science. Transfers from pure science to technology were, however, successful in only a few cases because the unsuccessful candidates for science usually lacked the necessary qualifications in mathematics and physics to be acceptable for entry in technology. Of the 80,033 candidates, 8·7 per cent were from overseas, of whom 15 per cent were women, compared with 30 per cent of home candidates. According to their first preference, about 25 per cent of all candidates intended to read social, administrative and business studies, 25 per cent of all candidates opted for art subjects; about 20 per cent of the total chose pure science, and a further 15 per cent engineering, technology, and applied science.

Men candidates applied for pure science and technology in similar proportions, 22·1 and 21·8, respectively, but for women the corresponding proportions were 17·8 and 0·8 per cent, respectively. Of the 16,061 candidates considered by the Clearing Panels because they were not accepted by any university originally named by them, 9,051 did not possess the qualifications stimulated by universities for acceptance, and of the remainder 3,276, including 936 women, were offered a place at a university.

The universities of Great Britain received 480,342 applications for entry in October 1965, and if the colleges of advanced technology are omitted, this represents an increase of about 40 per cent overall between 1964 and 1965. Total admissions to universities in Great Britain in 1965–66 were 49,510, of whom 13,515 were admitted to pure science courses and 8,760 to technology courses.

THE PAST IN THE PRESENT

IN recent years considerable effort has been made by archaeologists and those anthropologists interested in material culture to trace and study groups of people who have maintained a hunting-gathering economy and to whom the use of metals is unknown. These groups may be said to represent a major source of information to the archaeologist about the methods of survival probably practised in Pleistocene and early post-Pleistocene times. Few prehistorians to-day would claim that these modern primitives represent to any appreciable degree the modes of existence in prehistoric times, when climate and environment may have been different from that of to-day. Nevertheless, the results of recent investigations on groups of people still practising a formally Stone Age existence have yielded valuable evidence, not so much on the social and political organization of the people, but on their hunting and gathering exploits, and on their techniques of production of stone, wood and bone tools. Such studies have occasionally resulted in specific interpretations of the functions of certain prehistoric tools on the basis of exact analogues with modern material, fortified of course by examination of traces of wear and utilization on both sets of tools.

A recent expedition to the northern Kaokoveld of South West Africa by the State Museum, Windhoek, has opened up a new field in these studies, and in a particularly valuable region*. It is well known that several groups of indigenous peoples in southern Africa were still formally in the Stone Age at the time of the arrival of the first Europeans; the recorded history of some of these groups is mainly concerned with their extermination by the whites, and there is a lamentable lack of information about the details of their basic hunter-gatherer existence.

* *Cimbebasia* No. 13: Preliminary report of two stone-working Ovatjimba groups in the Northern Kaokoveld of South West Africa. By H. R. MacCalman and B. J. Grobbelaar. Pp. 39 (16 plates). (Windhoek, South West Africa: Staatsmuseum, Posbus 1203)

The groups recently contacted and studied by the State Museum expedition probably represent the only stone-using indigenous peoples in the whole of southern Africa. Two groups calling themselves OvaTjimba were contacted; the first of these, the Okombambi group, consisted of fourteen individuals who normally lived in the heights of the Baynes Mountains. Details of their appearance, dress and decoration, and habitations were recorded by the expedition, and specific information about the equipment used for hunting and gathering was obtained. Nothing could be ascertained of their religion, and they appeared to have neither art nor music. The second group, called Otjinungua, consisted of only four individuals, but in general terms these seemed to conform to the Okombambi group in physical appearance and in material culture. Both extensively utilize the products of their limited high-altitude environment, using wood, skins, bone and stone, supplemented in both cases by scavenging in abandoned OvaHimba villages for cloth and iron. The stone-working methods observed consisted of direct percussion and block-on-block; the differences in detail between the two groups may have been due as much to the limited quantity of the evidence as to different traditions.

The authors of this report have also succeeded in establishing the true indigenous nature of these groups, by producing evidence which seems to show that these people do not represent OvaTjimba who have lost their cattle, but must be considered as a group whose economy has never been based on domestication. Further expeditions are planned, and it is to be hoped that this unique opportunity to investigate an isolated group living under conditions which must be relevant to those of prehistoric times will be fully exploited.

JOHN M. COLES

DRIFT REHABILITATED

AFTER many years of suspicion and acrimonious discussion the seal of respectability has firmly been set on the theory of continental drift by the publication of *A Symposium on Continental Drift** as a volume of the *Philosophical Transactions of the Royal Society*. For the first time Continental Drift is discussed with as little reference to Wegener as was formerly accorded to Taylor. It avoids the Scylla of inconclusive discussion of geological and palaeontological data on the Wegener–Du Toit models, and the Charybdis of inadequate mechanism. The first pitfall is avoided by discounting data so obviously insufficiently precise and relying on data from palaeomagnetism to establish a prima facie case for drift, and the second by concentrating on the most plausible mechanism so far proposed, mantle convection currents, and assembling the variety of data from modern petrology—petrochemistry, oceanographic geophysics, physics and geochemistry which have a bearing upon it.

The contributions to the Symposium are divided under four heads, each with discussion. These are "Continental Reconstructions", "Horizontal Displacements in the Earth's Crust", "Convection Currents and Continental Drift", and "Physics of Convection Currents in the Earth's Mantle".

The first section, largely introductory, opens with articles by P. M. S. Blackett, T. S. Westoll and S. K. Runcorn, which, if they present little that is new, nevertheless set the stage for the Symposium. This section is completed by Creer's survey of data from the southern hemisphere, much of it his own, and the article by E. Bullard, J. E. Everett and A. G. Smith on the fit of the continents around the Atlantic. In view of Westoll's exposure of the inadequacy of many of the geological data used in earlier discussions and the claim in each of the first three articles that there is good agreement between palaeoclimates and palaeomagnetism, it would perhaps have been of value if the quantitative work on palaeoclimates by Irving or Blackett had been recorded. For, in the fourth article, Creer's discussion of Devonian reconstructions overlooked the siting in polar regions of a North African Devonian fauna similar to one considered in Europe as a warm water fauna on palaeontological grounds and tropical on palaeomagnetic grounds, and results in the austral fauna generally considered as a cold water fauna in South Africa coming to rest in latitude 30°.

The article by Bullard, Everett and Smith removes once and for all from the realms of speculation the fit of the continents bordering the Atlantic. This fit for the North Atlantic is used by contributors to the third session (J. A. Miller and F. J. Fitch), whose assembly of radiometric and tectonic information would have gladdened the heart of F. B. Taylor. However, the attempt to reassemble the whole Atlantic, as J. L. Worzel points out, runs into difficulties as far as Central America and Mexico are concerned. The geological history of the Atlantic forms part of an article by J. T. Wilson.

The second section, on "Horizontal Displacements in the Earth's Crust", covers recent oceanographic work on transcurrent faults, ridge-rise systems, and rifting. The section title unfortunately precludes a review of the heat flow data. This section is completed by an article on continental transcurrent faulting. Mostly factual, the data are objectively described and tentative interpretation is attempted.

The final section, on "The Physics of Convection Currents in the Earth's Mantle", provides the geologist with present-day views of geophysicists on the state of the mantle, and although G. J. F. MacDonald shows that another interpretation in terms of mantle strength is possible, overwhelmingly, discussion is of convection. The conditions for thermal convection for a mantle of non-Newtonian viscosity are described by E. Orowan, while J. Verhoogen considers the effect of phase change on convection. Convection involving the whole mantle is proposed by Runcorn, and Tozer discusses convection confined to the upper part of the mantle. The former puts forward a general theory to account for "the most puzzling feature of continental drift; that it should have occurred in the last 5 per cent of the Earth's life", for it is hard to see why, if drift has been active during the past 250 m.y., it should not have occurred in earlier times. J. T. Wilson suggests, on the other hand, that "periodic break-up of continents . . . may have happened several times", a view with which the reviewer concurs. The reconstructed North Atlantic as shown by Bullard must have been broken through during much of the Palaeozoic by a geosyncline bordered by shelf seas. The steady sinking and later folding and uplift have to be explained in some way. If Wilson is right, the convection pattern suggested by D. C. Tozer may provide a better explanation than Runcorn's. Questions of this nature will continue to stimulate research and indicate how ideas are moving away from the initial simple pattern to the recognition of a more complex, and as yet uncertain, design.

In stimulating such interest, the Symposium succeeds, and thanks are due to the organizers and editor for the assembly of so much interesting material. If the price, at more than £2 per hundred pages, seems high, was respectability ever cheaply bought?

* *Phil. Trans. Roy. Soc.*, *A*, **258**, No. 1088, 1965: Organized for the Royal Society by Prof. P. M. S. Blackett, F.R.S., Sir Edward Bullard, F.R.S., and Prof. S. K. Runcorn, F.R.S. Pp. x+1–323+*A*1–*A*6. (London: The Royal Society, 1965.) 140*s*.; 21 dollars.

SOME NEW MATERIAL ON ROBERT DICK, BAKER OF THURSO, GEOLOGIST AND BOTANIST

IN December 1866, Robert Dick, baker of Thurso, geologist and botanist, died after a few weeks of illness. Forgotten and neglected by most of his townsmen, his name was a household word to many of the learned men of his day. He was the great shadow behind Hugh Miller (author of the *Old Red Sandstone*), and many of Dick's fossils were figured in later editions of this book. Dick discovered two new fishes: *Coccosteus minor*, now known as *Millerosteus minor* (Hugh Miller), and *Homosteus milleri*, erroneously ascribed to *Asterolepis* by Louis Agassiz, the Swiss naturalist. It has since transpired that the fish known to Dick as *Coccosteus decipiens* is, in fact, a new genus: *Dickosteus thrieplandi* (Miles and Westoll, 1963).

Sir Roderick Murchison, controller of the Geological Survey of Great Britain, visited Dick on several occasions and was instructed by him on the geology of Caithness, Dick making a geological map out of flour in his bakehouse. When Lucien Buonaparte called on Dick at his bakehouse he sent word that the prince must call at a more convenient time as the bread would wait for no man.

His entire collection of fossils had to be sold to pay for the loss at sea of a cargo of flour—he received £60 for them. He made a very thorough collection of botanical specimens from Caithness and confounded many experts with his knowledge. Among other things he discovered *Hierochloe borealis* (now called *Hierochloe fragrans*), the Northern Holygrass, growing near Thurso. He also made a collection of insects of Caithness which has, unfortunately, perished owing to neglect. A further collection was of native shells; the collection was lost.

Recent work by volunteer labour on the Dick collection has brought to light hundreds of botanical specimens, the existence of which was hitherto unknown, together with a collection of shells, some identified in Dick's handwriting, which must be the Dick collection of shells.

If further proof of the authenticity of the new material were required there are three envelopes containing botanical specimens addressed to Mr. Robert Dick, Baker, Thurso; two are postmarked 1859 and 1860, but the third is far older and bears an imperforate penny red. This must date from between 1841 and 1850. The letter was originally sealed with red wax and appears to have come from Prof. Owen.

There is also a parcel of grasses from a Mr. Notcutt, druggist, addressed to Mr. Dick, Thurso. Notcutt is mentioned in the biography by Smiles.

There are several pieces of paper which refer to correspondence in *The Witness*, and are concerned with a difference of opinion between a Mr. Rhind and Hugh Miller. They appear to be the draft of a reply to Rhind's criticisms and clearly refer to a press cutting signed "A Country Geologist". The subject is the Deluge and geological evidence that the sea level has not always been in its present position with regard to the dry land.

There are also notes on a stratigraphical succession concerning gneiss, quartzite, red conglomerate and limestone. This would seem to refer either to the area round Scaraben or to the vicinity of the Moine thrust plane.

J. SAXON
J. I. BRAMMAN
N. CAMPBELL

OBSERVATIONS OF SUDDEN IONOSPHERIC FREQUENCY DEVIATIONS AT WIDELY SEPARATED PLACES

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RAPID fluctuations are sometimes observed in the received carrier frequencies of ionospherically propagated high-frequency radio waves[1–4]. Some of these frequency fluctuations, which can be readily identified from the records, have been called sudden frequency deviations, and have been attributed primarily to electron content changes in the *E* and *F* regions of the ionosphere caused by ionizing radiation from solar flares[1,2]. Munro[5], however, has suggested that the observed effects may be the result of travelling ionospheric disturbances. The purpose of this report is to present a few examples as evidence to show that these frequency deviations are indeed produced directly by fluctuations in solar ionizing radiation at the time of a flare rather than by some form of local ionospheric disturbance.

Munro[5] pointed out: "that since the ionospheric effects of a solar flare are due to ultra-violet radiation they should all be observed simultaneously over the sunlit surface of the earth". This is a crucial point, and an opportunity has arisen to verify that the frequency-deviation effects are simultaneous over a large area of the Earth through a network of radio circuits established by the Institute for Telecommunication Sciences and Aeronomy. A list of the relevant circuits is given in Table 1, from which it

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† Formerly designated Central Radio Propagation Laboratory of the National Bureau of Standards.

Table 1. CIRCUITS OVER WHICH DOPPLER MEASUREMENTS HAVE BEEN MADE AND WHICH HAVE BEEN USED IN THE ANALYSIS OF THE SUDDEN FREQUENCY DEVIATIONS

Transmitter			Receiver			Path length (km)	Frequency (Mc/s)
Location	Latitude	Longitude	Location	Latitude	Longitude		
Monrovia, Liberia	6·22°N.	10·46°W.	Accra, Ghana	5·33°N.	0·15°W.	1,180	10·1018
Liberia	6·22°N.	10·46°W.	Natal, Brazil	5·46°S.	35·15°W.	2,990	20·2038
							10.1018
Washington, D.C.	39·02°N.	76·55°W.	Boulder, U.S.A.	40·02°N.	105·16°W.	2,430	15·0
							10.0
Sunset (Boulder)	40·03°N.	105·50°W.	Boulder, U.S.A.	40·02°N.	105·16°W.	25	5·054, 4.000
Maui, Hawaii	20·56°N.	159·29°W.	Honolulu, Hawaii	21·18°N.	157·40°W.	~100	5·0

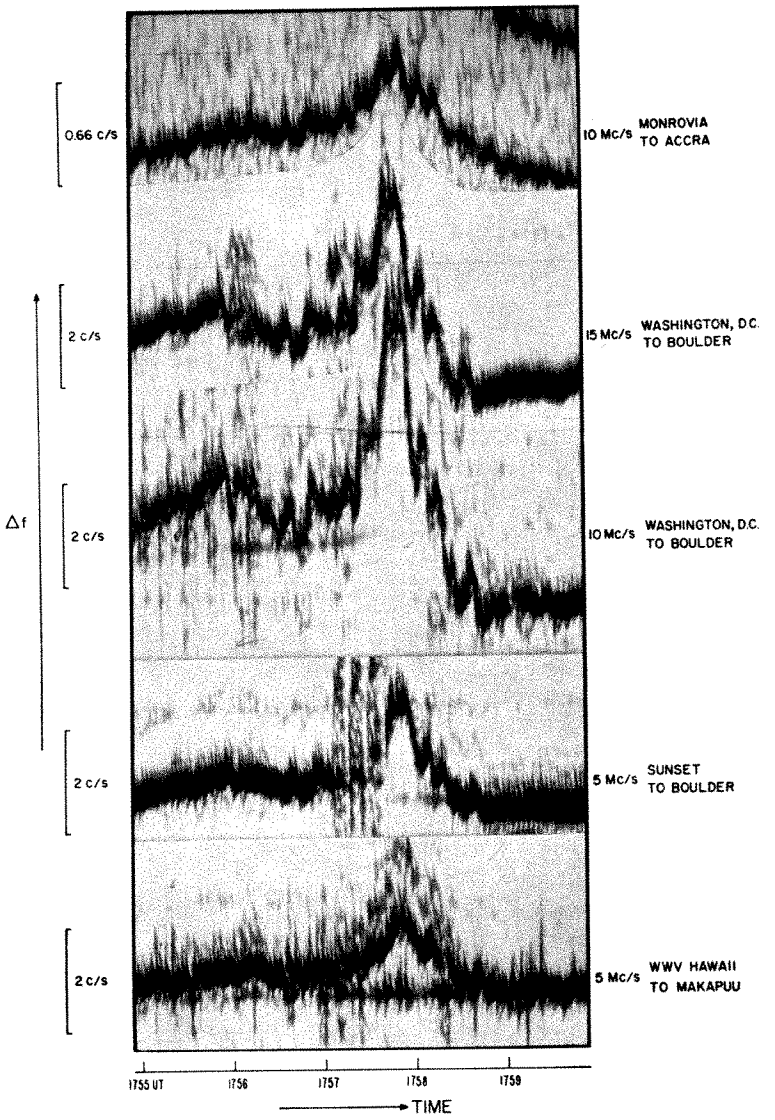


Fig. 1. Degree to which the fine structure of sudden frequency deviations is reproduced over a wide area of the sunlit hemisphere. Differences in magnitude are due to differences in solar zenith angle, path length, carrier frequency, reflexion height, etc. A solar flare of importance 1 began at 1756 U.T., reached maximum at 1800 U.T., and ended at 1837 U.T.

will be seen that the most widely separated receivers differ in longitude by nearly 160°.

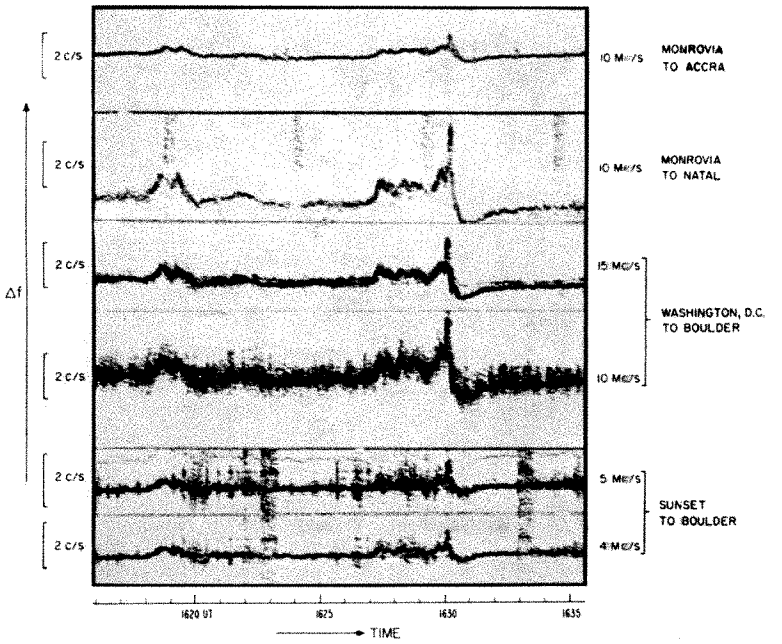
Figs. 1, 2 and 3 give examples of sudden frequency deviations observed over the paths listed in Table 1. The sudden frequency deviations shown in Figs. 1, 2 and 3 indicate that, in addition to the general increase in the frequency, there is a great deal of fine structure. The question which naturally arises is, are these fluctuations due to variations in the electron production or are they the result of some instability in the structure of the ionosphere? If the fluctuations are due to the incident solar radiation, they will be closely reproduced over the whole sunlit hemisphere; whereas if they are due to some ionospheric instability, the instantaneous frequency would largely depend on local atmospheric conditions which would scarcely be identical over the entire hemisphere.

Close examination of Figs. 1, 2 and 3 shows that the fine structure is reproduced, in remarkable detail, at stations as far apart as Accra, Ghana, and Honolulu, Hawaii, and at intermediate receiving locations. The exact magnitudes of the frequency shifts are, of course, different because they depend on such factors as solar zenith angle, path length, wave frequency, and ionospheric layer height. Detailed comparisons have been made of twenty-nine events recorded simultaneously on two or more different paths, and it has been found that in all cases the temporal fluctuations in the instantaneous frequency were reproduced over the various sunlit paths. Occasionally, the records were affected by local ionospheric conditions, for example moving irregularities[6,7], sunrise and sunset effects etc., but these were insufficient to obscure the salient features of the sudden frequency deviations.

Over the period October 1960—December 1962, 91 per cent of all observed sudden frequency deviations were accompanied by visible $H\alpha$ flares. This percentage is partly limited by the difficulty in seeing some of the small optical flares. Thus, it would appear that sudden frequency deviations are closely related to solar flare activity rather than to chance coincidence with ionospheric travelling disturbance.

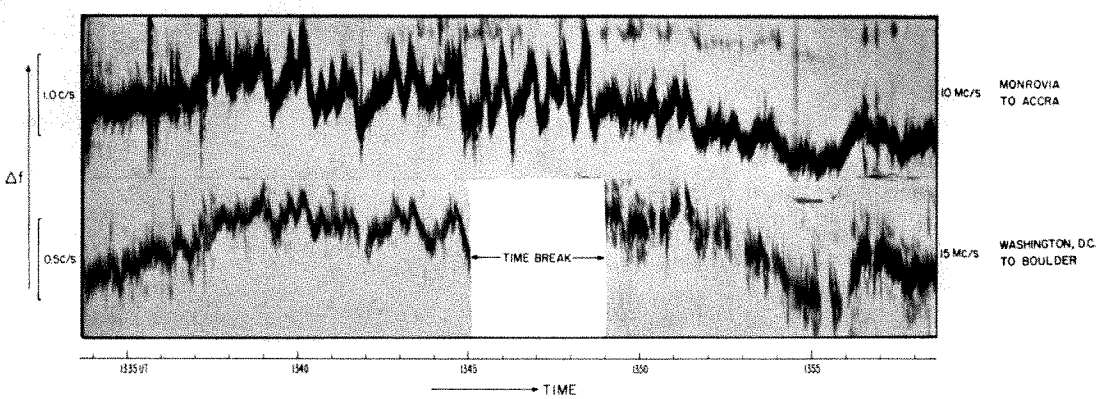
We conclude from our investigations that even the detailed features of sudden frequency deviations are the direct result of fluctuations in the flux of solar ionizing radiations.

So far as we know, the Doppler technique is the only one which has detected the fine structure in the iono-



TWO SUDDEN FREQUENCY DEVIATIONS
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Fig. 2. Agreement between sudden frequency deviations observed with different radio frequencies and geographical paths for two solar flares. Solar flares of importance 1 were reported at 1605–1623–1633 U.T. (beginning-maximum-end) and 1625–1628–1632 U.T.



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Fig. 3. Further illustration of the fine structure of sudden frequency deviations and its independence of geographical location. A solar flare of importance 2 began at 1329 U.T., reached maximum at 1350 U.T., and ended some time after 1530 U.T.

spheric effects of solar flares. However, somewhat similar structure has been observed in solar noise bursts[8].

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REACTION OF HYDROGEN ATOMS WITH *CYCLO*PROPANE AND WITH ALLENE AT 77° K

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WE have studied the reactions both by thermal generation of hydrogen atoms on a tungsten filament, following the method of Klein and Scheer[1–6], and by photolysis (2537 Å) of hydrogen iodide in admixture with hydrocarbon. No reaction could be induced with either hydrocarbon using the thermal technique. This finding, for *cyclo*propane, apparently conflicts with the observation of reaction between H atoms and *cyclo*propane by

Moser and Shores[7]. In recent studies[8], however, these authors indicate that, in their earlier work, there was probably direct radiative heating of the film, with consequent vaporization and cracking on the filament.

Both hydrocarbons reacted readily with photolysed hydrogen iodide. Details of apparatus and technique are given elsewhere[9]. Typical analytical results are given in Table 1.

Table 1. PRODUCTS OF PHOTOLYSIS OF FILMS OF *Cyclo*PROPANE OR ALLENE WITH HYDROGEN IODIDE AT 77° K ($\lambda = 2537$ Å)

Film composition (μ mole)	*Cyclo*propane = 35 hydrogen iodide = 40	Allene = 20 hydrogen iodide = 65
Photolysis time (min)	30	30
Products (mole per cent of total)		
Propane	16	14
Propene	—	25
Methyl acetylene	—	41
n-Propyl iodide	80	—
isoPropyl iodide	4	20
Trace products (< 1 per cent total majors)	Methane, methyl iodide, propene	C_1–C_3 iodides, a hexane
Absent (< 0·1 mole per cent)	Hexanes, allyl iodide	Allyl iodide

The failure to induce reaction by the thermal method may be due to (i) a high activation energy of the initial hydrogen atom addition or (ii) ready reversibility of the addition. The formation of $C_3H_5\cdot$ from allene is about 70 kcal mole^{-1} exothermic and it is possible that, following initiation, on account of this high energy, the reverse reaction occurs exclusively. However, even in great excess of carbon dioxide no reaction occurred and further, in the photochemical method, if the radical is directly formed, it would be even more energy-rich since hydrogen iodide is photolysed with 41 kcal mole^{-1} excess energy at 2357 Å. Finally, there is abundant evidence to show that thermalization of large excess energies is rapid in frozen films[1–6,10,11]. It thus appears most probable that the thermally induced reaction of allene is subject to an activation energy barrier in excess of 5 kcal mole^{-1} ($T_{fil} = 1800$ °K). Accepting this, the same explanation must hold for *cyclo*propane since an even higher activation energy would be anticipated because of the need to open the ring on addition of hydrogen. The reality of activation energy barriers in Klein–Scheer-type experiments is shown[3] by the lack of reactivity of 2-olefines whereas 1-olefines hydrogenate readily.

It might then appear that the postulate of 'hot' hydrogen atoms would explain the occurrence of reaction in the photochemical system. However, if an initial activation energy barrier of 5 kcal mole^{-1} can be overcome, other reactions, particularly hydrogen atom abstraction, in these and in olefinic matrices ($E = 5 - 10$ kcal mole^{-1}) would be likely to occur. We find no evidence for such processes in these or in olefinic matrices[9]. Further, we find no hydrogen atom abstraction from 1-olefines by photochemically generated methyl radicals (possibly 32 kcal mole^{-1} hot) in confirmation of the findings of Ayscough and Evans[10], who also showed that the energetically slightly more favourable abstraction from branched or 2-olefines occurs. Again an activation energy barrier is indicated. We thus conclude that the explanation of the reactivity observed in the photochemical experiments is not associated with 'hot' atoms.

Irrespective of the detailed mechanism of the processes occurring, one general conclusion, relevant to many alkyl radical-containing cryogenic systems, may be drawn from the results of Table 1 for *cyclo*propane. The addition of hydrogen to *cyclo*propane clearly offers the cleanest route to 1-propyl radical formation and perhaps the most nearly unambiguous means to generate any alkyl radical. The upper limit for the hexane yield (detectability limit) is about 0·1 mole per cent of total product and, accepting the largest estimate[1–6] of the relative rates of disproportionation to combination ($k_d/k_c \simeq 10$) at 77° K, admittedly for 2-propyl, no more than 1 per cent of the total products of propene could arise by radical-radical disproportionation. The experimental value is about half this amount and the propene, in fact, is highly likely to arise by way of secondary photolysis of *n*-propyl iodide since the trace products correspond to the products of that reaction. Since no products of hydrogen abstraction reactions are found, the large yield of propane must arise by successive hydrogen atom additions or reaction with hydrogen iodide. In the unlikely event that isopropyl iodide arises entirely by way of secondary processes involving addition of hydrogen iodide to propene, the hydrogenation reaction must still be three times as important as the sum of all disproportionations. The results thus show that combination and disproportionation reactions of radicals have only trivial importance in cryogenic systems such as those used in this work. This points clearly to immobilization of radicals.

Pimentel[11] has proposed that most if not all photochemically induced reactions of organic molecules in frozen films occur exclusively at the site of light absorption. These, and other investigations completed by us[9], lead us to accept this proposition with the single reservation that hydrogen atoms must usually be regarded as mobile and can therefore react outside the cage of formation. This view alone, however, does not offer a ready explanation of the discrepancy between the thermal and photochemical experiments described here, of the complexity of products often observed[11,14] or of other anomalies described elsewhere[9]. Predominant among the latter is the finding that at 77° K, conventional methyl radical sources in olefinic matrices yield products characteristic of reactions of methylene radicals. We thus extend Pimentel's hypothesis by suggesting that the primary photochemical act is either instantaneously followed by or directly involves formation of excited absorber-matrix complexes, their formation perhaps depending on excess energy of photolysis being available. It has several times been shown for individual reaction systems that reactant-matrix complexes must be formed, and Jacox and Milligan[12] have discussed features common to likely structures in some systems. In previous work when a complex has been proposed it has been formulated in terms of a matrix molecule and a photo-produced radical or atom. In contrast, we propose that all the nominal products of photolysis are involved in the complex which may contain also any species present in the matrix, and with which is associated the whole absorbed energy. In a loose complex, such as is usually proposed, relative motion of the constituent parts would give rise to numerous configurations (transition states) each of which on deactivation or decomposition could proceed via preferential paths to yield specific products which might not readily be visualized as arising from the obvious initial products of absorber photolysis. This unified view removes difficulties which arise in explaining the relative absence of hot atom or radical effects, the localization of reactive species at the absorption site, the unexpected reactivity of 'inert' matrices in some photolyses (for example, carbon dioxide and carbon monoxide in the photolysis[13,14] of CH_2N_2), the relative scarcity of free radicals and the kinetics of production of the often complex products of low temperature photolyses such as, for example, those of methyl iodide, ethyl iodide and nitromethane.

On this basis we suggest a tentative mechanism for *cyclo*propane + HI photolysis:

$$[c\text{-}C_3H_6 + HI]_c + h\nu \rightleftarrows [c\text{-}C_3H_6\cdots H\cdots I]_c \quad (1)$$

$$[c\text{-}C_3H_6\text{---H---I}]_c \longrightarrow [1\text{-}C_3H_7\cdot + I]_c \quad (2)$$

$$[c\text{-}C_3H_6\text{---H---I}]_c \longrightarrow [c\text{-}C_3H_6 + I]_c + H \quad (3a)$$

$$\longrightarrow [C_3H_6\text{---I}]_c + H \quad (3b)$$

$$\longrightarrow [C_3H_6\text{---H---I}]_c \rightarrow 2\text{-}C_3H_7I \quad (3c)$$

$$\longrightarrow C_3H_6 + HI \quad (3d)$$

$$[1\text{-}C_3H_7\cdot + I]_c \longrightarrow 1\text{-}C_3H_7I \quad (4)$$

$$[1-C_3H_7\cdot+I]_c+H(HI) \longrightarrow C_3H_8+[I]_c([I_2]_c) \quad (5)$$

$$[C_3H_6---I]_c+H \longrightarrow 2-C_3H_7I \quad (6)$$

$$2[I]_c \xrightarrow{\text{warm up}} I_2 \quad (7)$$

The subscript c designates the cage of initial absorption while bracketed entities connected by broken lines, represent activated complexes. Evidence for reaction (5) has been presented; reaction (3*a*) is included to account for the fact that $C_3H_6 \ll C_3H_8$; reaction 3(*b*) provides an energetically feasible route to what is in effect the radical $C_3H_6I\cdot$ (the thermalized equivalent is scarcely likely since $E > 5$ kcal mole^{-1}). Reaction (7) is supported by the observation that reacted films are of lighter colour at 77° K than test mixtures of *cyclo*propane and iodine, and darken more rapidly on warm-up. Reaction (3*d*) must obviously be trivial.

The corresponding mechanism for allene is:

$$[C_3H_4+HI]_c+h\nu \rightleftharpoons [C_3H_4---H---I]_c \quad (1)$$

$$[C_3H_4---H---I]_c \longrightarrow [CH_2=C-CH_3---I]_c \rightarrow [C_3H_5\cdot+I]_c \quad (2a)$$

$$[CH_2=C-CH_3---I]_c \longrightarrow CH_3-C\equiv CH+HI \quad (2b)$$

$$[C_3H_4---H---I]_c \longrightarrow [C_3H_4---I]_c+H \rightarrow C_3H_4+H+[I]_c \quad (3)$$

$$C_3H_5\cdot+I]_c+H(HI) \longrightarrow [C_3H_6+I]_c([I_2]_c) \quad (4)$$

$$[C_3H_6+I]_c+H \longrightarrow [2-C_3H_7\cdot+I]_c \quad (5)$$

$$[2-C_3H_7\cdot+I]_c+H(HI) \longrightarrow C_3H_8+[I]_c([I_2]_c) \quad (6)$$

$$[2-C_3H_7\cdot+I]_c \longrightarrow 2-C_3H_7I \quad (7)$$

The absence of allyl iodide from the products means that hydrogenation by way of (4) is very fast. Obviously, the predominant reaction is (2*b*) which accounts for nearly half the products, and this shows that hydrogen atom addition to allene is largely terminal. This reaction may be regarded as a disproportionation, the view usually taken in interpreting the formation of olefines in alkyl iodine photolysis, but it is to be noted that this reaction does not occur in the *cyclo*propane reaction although the radicals nominally present are those of the photolysis of *n*-propyl iodide. This may be attributed to the loss of energy consequent on the opening of the *cyclo*propane ring and for this reason reactions (2) in the two mechanisms are represented differently. Thus, reactions of the type (2*b*) are probably better regarded as hydrogen atom expulsion from the hydrocarbon radical in a fashion analogous to that known[15] to occur in the gas phase reaction of C_2H_4+T and in the reactions of halo-ethylenes with halogens in solution.

It is, of course, possible that methyl acetylene arises via alternative disproportionation reactions. Since no molecular hydrogen is formed these would have to involve radicals only. On the evidence of the *cyclo*propane $-HI$ reaction, the propyls of (6) and (7) would be immobile and thus would not disproportionate. Hence, at most, one disproportionation step, involving two $C_3H_5\cdot$ radicals, could occur (replacing (4)); there would, therefore, still have to be three direct hydrogen addition steps in forming C_3H_8. On the general evidence presented and because of the requirement of relatively high mobility of $C_3H_5\cdot$ for its self-disproportionation to compete with (4) (which itself is very fast compared with $C_3H_5\cdot+I \rightarrow C_3H_5I$) we reject this possibility.

We thank the Department of Scientific and Industrial Research for the award of a maintenance grant to A. N. H.

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A POSSIBLE RING STRUCTURE IN VITREOUS SILICA

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RECENT work on the structure of vitreous silica suggests that the Si–O–Si linkages differ considerably from the collinear form proposed in early work. Thus, neutron diffraction studies[1] lead to an average value for the Si–O–Si angle of 146° (ref. 2), and the X-ray work of Zarzycki[3] gives the value 143° ± 17°. On this basis it seems likely that the structure of vitreous silica contains, among the many possible local atomic configurations, almost planar six-membered silicon–oxygen rings similar to those which occur in various solid[4] (and possibly liquid[5]) silicates.

One can see the possibility for the existence of such rings in the following way. Consider any two SiO_4 tetrahedra connected through a common oxygen ($O_{(0)}$) as in Fig. 1. The atoms $Si_{(1)}$, $O_{(0)}$ and $Si_{(2)}$ are taken to be in the plane of the paper and, therefore, the remaining oxygen atoms of the $Si_{(1)}O_4$ and $Si_{(2)}O_4$ tetrahedra are confined to positions on circles C_1 and C_2, respectively, both perpendicular to the plane of the paper. Now, if all orientations of the $Si_{(1)}O_4$ and $Si_{(2)}O_4$ tetrahedra about their respective three-fold axes, $Si_{(1)}O_{(0)}$ and $Si_{(2)}O_{(0)}$, are regarded as more or less equally probable, then there is a small, but certainly non-negligible, probability that an oxygen atom ($O_{(1)}$) on the circle C_1 and another ($O_{(2)}$) on the circle C_2 will lie close to the plane $Si_{(1)}O_{(0)}Si_{(2)}$. We can be rather more specific. Let the angle between the line joining $O_{(1)}$ to the centre of C_1 and the projection of this line on the plane $Si_{(1)}O_{(0)}Si_{(2)}$ be ϕ_1, and the corresponding angle for $O_{(2)}$ in the circle C_2 be ϕ_2. Then the probability that ϕ_1 and ϕ_2 are both less than 15° is about 0·06, and the probability that both are less than 6° is 0·01 (assuming that all orientations of the tetrahedra are equally likely).

Let us now take Fig. 1 to represent a local structure (in a randomly connected network of SiO_4 tetrahedra) in which ϕ_1 and ϕ_2 are, in fact, fairly small angles. Then just one further condition—that the angle $Si_{(1)}O_{(0)}Si_{(2)}$ be about 130°—ensures that the distance $O_{(1)}O_{(2)}$ is of roughly the correct magnitude for these two oxygen atoms to be bridged by a single silicon atom, that is, to be members of the same SiO_4 tetrahedron. Moreover, if this silicon atom ($Si_{(3)}$, as in Fig. 1) lies close to the plane of $Si_{(1)}O_{(0)}Si_{(2)}$, then the angles $Si_{(1)}O_{(1)}Si_{(3)}$ and $Si_{(2)}O_{(2)}Si_{(3)}$ will both be about 130°. The three Si–O–Si angles of about 130° which occur in this configuration of atoms are a little below the suggested value for the mean Si–O–Si angle in vitreous silica[2,3], but they are almost certainly within reasonable bounds.

Some results of calculations on the geometry of this structure of adjacent SiO_4 tetrahedra are summarized in

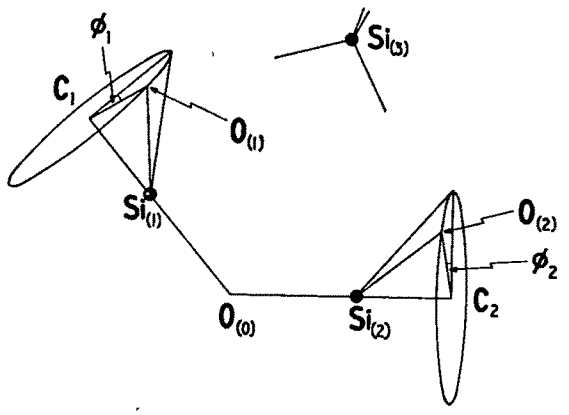


Fig. 1. Diagram illustrating the possibility of almost planar rings in vitreous silica

Table 1. θ_0, θ_1 and θ_2 are the Si–O–Si angles of the six-membered ring $O_{(0)}Si_{(1)}O_{(1)}Si_{(3)}O_{(2)}Si_{(2)}$ at $O_{(0)}$, $O_{(1)}$ and $O_{(2)}$, respectively. ϕ_1 and ϕ_2, defined earlier, have the same sign when $O_{(1)}$ and $O_{(2)}$ are both on the same side of the plane $Si_{(1)}O_{(0)}Si_{(2)}$. The distance d of $Si_{(3)}$ from the plane of $Si_{(1)}O_{(0)}Si_{(2)}$ is in terms of the Si–O bond length as a unit.

Table 1. TYPICAL DATA FOR ALMOST PLANAR SIX-MEMBERED RINGS

ϕ_1	ϕ_2	θ_0	θ_1	θ_2	d
0	±20 8	128·0	129·7	125 4	0 17
+ 5	−17·5	128·0	129·0	126·2	0·10
+10	−13 4	128·0	128·0	127·2	0·03
+15	− 8·2	128.0	126·8	128·4	0 05
+ 5	+23 3	128·0	127·9	119·8	0·08
+10	+24·9	128 0	127·7	120·2	0 16
0	±30·7	125·0	125·0	108·6	0

(All angles are in degrees; d is in terms of the Si–O bond length.)

These particular results have been chosen to illustrate various almost planar configurations of the six atoms of the ring. The calculations also show that other, less planar, arrangements are certainly possible but that these involve one or more Si–O–Si angles substantially smaller than about 130°. Although such structures may occur, the almost planar ring will be appreciably more likely if the overall distribution of Si–O–Si angles in the glass is one which falls away fairly rapidly to zero at angles very much smaller than the mean. We shall leave open, however, the question as to whether significantly non-planar six-membered rings actually occur; the object of this article is to put a case for the existence of the almost planar rings. In passing, we note that 8-, 10- and higher-membered rings are geometrically quite possible, even probable, but one would not expect these larger ring structures, in general, to be planar.

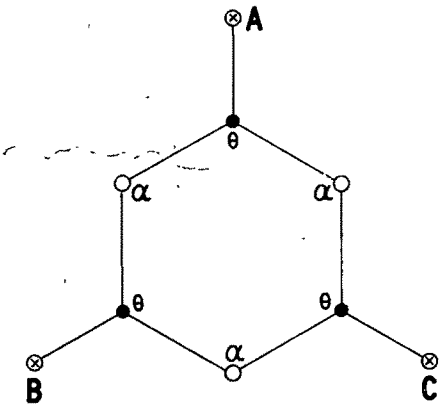


Fig. 2. A planar six-membered ring formed from three SiO_4 tetrahedra. Each of the open circles with crosses (at A, B and C) represents two oxygen atoms, one above and one below the plane of the paper. In the diagram $\theta + \alpha = 240°$, and for exact tetrahedra $\theta = 2\sin^{-1}\sqrt{6}/3$. ●, Silicon; ○, oxygen

Consider now a particular six-membered ring, as in Fig. 2. Let us first regard this ring as truly planar and symmetrical, and the SiO_4 structures as exact tetrahedra. Then, on a nearest-neighbour central force model, analysis shows that there exist two modes of vibration which are strictly localized to the atoms of the ring and involve no displacements from equilibrium of other atoms in the network. (The pairs of atoms represented by the points A, B and C in Fig. 2 are to be regarded as part of a large network of connected SiO_4 tetrahedra.) The characters of the localized modes are indicated diagrammatically in Fig. 3: one is an infinitesimal ring rotation, of zero frequency (ν_1), and in the other adjacent atoms vibrate out of phase as shown, with a frequency:

$$\nu_2 = \frac{\gamma^{\frac{1}{2}}}{2\pi}\left\{2m_1^{-1}\sin^2\frac{\theta}{2} + m_0^{-1}\left(\frac{1}{2} + \cos^2\frac{\theta}{2} + \sqrt{3}\sin\frac{\theta}{2}\cos\frac{\theta}{2}\right)\right\}^{\frac{1}{2}} \quad (1)$$

where m_1 is the mass of a silicon atom, m_0 that of an oxygen atom, γ the Si–O central force constant and θ the O–Si–O angle ($2\sin^{-1}\frac{\sqrt{6}}{3}$ for exact tetrahedra). The important point about these modes—and one which should be emphasized—is that they are strictly localized to the six atoms of the ring. This exact localization is an unusual property for vibrational modes in condensed matter. Strongly localized modes usually involve non-zero atomic displacements away from the centres of localization, although the amplitudes may fall away extremely rapidly with increasing distance.

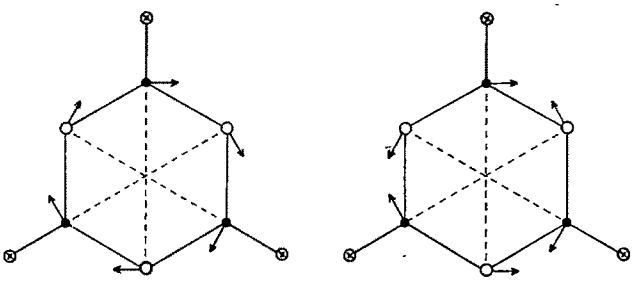


Fig. 3. Localized vibrational modes associated with six-membered rings. The arrows, which represent atomic displacements, are perpendicular to the (broken) lines joining the atoms to the centre of the ring

As so far described, this model (with exact localization) is an idealized one. In the real physical situation, there will be coupling of the modes to the network by way of the asymmetry in the ring, non-central—and also longer range—forces, and other effects. However, the fact remains that to a first approximation the normal vibrations of frequencies ν_1 and ν_2 involve only the displacements of the six atoms of the ring.

If we take the value of γ (Si–O bond stretching force constant) to be $4{\cdot}0 \times 10^5$ dynes/cm, then equation (1) leads to a value of ν_2, in wave numbers, of 1,010 cm^{-1}. A force constant of $\gamma = 4{\cdot}5 \times 10^5$ dynes/cm leads to a ν_2 of 1,060 cm^{-1}. The idealized symmetric ring structure of silicon-oxygen atoms has symmetry D_{3h} and the modes of interest are of species A_2', and therefore inactive in both the Raman and infra-red. However, in view of the distortions from symmetry which occur in the real physical situation one would expect infra-red activity, although over a fairly broad range of frequencies in view of the numerous possible ring environments.

The band at about 950 cm^{-1} in the observed infra-red spectrum of vitreous silica[6] can be accounted for by the ring mode ν_2. We have not attempted to identify observed infra-red activity with the low-frequency mode ν_1; this mode will be particularly sensitive to angular forces and local atomic geometry. However, if this mode does

retain some of its character in spite of coupling with the network, then it will contribute (and as a non-acoustic mode) towards the low-temperature specific heat of vitreous silica and thus may partially account for the well-known discrepancy between the observed heat capacity and that as calculated from elastic data[7-9].

I thank Drs. A. J. Leadbetter and T. H. K. Barron, of the University of Bristol, for a helpful discussion at the start of this work. I also thank Dr. G. W. Chantry and R. J. Bell, of the National Physical Laboratory, for their advice.

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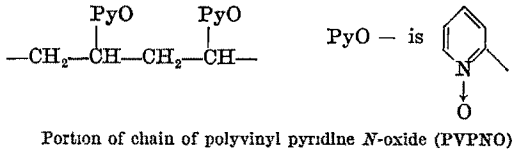
PHYSICO-CHEMICAL PROPERTIES OF SILICA IN RELATION TO ITS TOXICITY

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IT has long been recognized that silica dust is much more fibrogenic in man and experimental animals than is most other material. The fibrogenic activities of different substances are related to their capacity to kill the phagocytic cells ingesting them, as can be shown by observations of cytotoxic effects on cultures of macrophages[1,2]. Silica, in fact, provides a remarkable model of selective toxicity for one cell type by a substance of simple chemical composition and low chemical reactivity. Detailed investigations of the mechanism are of theoretical as well as practical interest in explaining the cause of the disease silicosis.

We have elsewhere presented evidence that silica and non-toxic particles such as diamond dust of comparable size are engulfed in the same way by macrophages[3]. Recently, engulfed particles have been located in phagosomes, into which hydrolytic enzymes from lysosomes are discharged. However, silica particles and lysomal enzymes soon escape from the phagosomes into the surrounding cytoplasm, rapidly causing cell damage, whereas non-toxic particles and the associated enzymes remain within phagosomes. It was suggested that the capacity of silica particles to react with, and make permeable, the lipoprotein membrane surrounding the phagosome is responsible for its toxicity. That silicic acid can react with lipoprotein or protein films has long been known[4,5], and the fact that colloidal silica is haemolytic[6] shows that it can induce changes of permeability in biological membrane systems.

It has also been found that a number of substances reduce the toxic effects of silica on macrophages *in vitro* and also its fibrogenic effect *in vivo*. Coating the silica particles with aluminium reduces these effects, and two other efficient protective substances are compound 48/80 (ref. 7) and polyvinyl-pyridine-*N*-oxide (ref. 8). The chemical structure of the latter is illustrated in (I); the basic unit, pyridine-*N*-oxide, has an oxygen atom with a high negative charge which is capable of forming strong hydrogen bonds without actually being able to abstract hydrogen from water to form ions. The polymer is very effective in suppressing silicosis in animals, where it reduces considerably the production of collagen. Macrophages are also protected by the compound against *in vitro* damage by silica

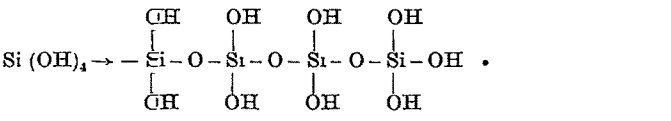


Portion of chain of polyvinyl pyridine *N*-oxide (PVPNO)
(I)

The aim of the present investigation was to determine the extent to which the toxicity of silica can be explained on the basis of hydrogen donor activity in the formation of complexes, and to find how far the protective effects of various compounds can be explained by their preferential formation of hydrogen bonds with silicic acid.

Hydrogen bonding. One of us has discussed hydrogen bonding in greater detail elsewhere[9]. Briefly, there are two classes of hydrogen bonding compounds. The larger class comprises hydrogen acceptors such as ethers and ketones and other compounds with active lone-pair electrons on oxygen or nitrogen. The smaller class comprises the hydrogen donors of which only the phenols are important among organic compounds, and silicic and one or two other very weak acids among inorganic compounds. Compounds of one class interact with those of the other, so that it is not surprising to find that there is one class (the former) the members of which are in general compatible with living cells, sometimes in very high concentration[9], whereas those of the other class are not. Large hydrogen donor molecules are, in fact, tanning agents, and it has already been suggested that silicic acid is one of them[5]. The hydrogen donor properties of tannic and of silicic acid are very similar, if measured by their acid dissociation constants[10].

State of solution of silica[10]. Silica (silicon dioxide) dissolves in water to form mono-silicic acid, probably $Si(OH)_4$, a saturated solution containing about 0·01 per cent. Much higher concentrations can be obtained by acidifying sodium silicate solution, or by ion-exchange, but the solution then polymerizes at a rate depending on temperature, *p*H, concentration, etc.:



The chains can cross-link by further elimination of water, to give a network (silicic acid jelly) and finally colloidal silica with —OH groups on the outer surface. The earlier products of polymerization resemble to some extent tannic acid as regards molecular weight and proportion of active hydrogen, and in view of this similarity the interactions of silicic and of tannic acids with 'tannable' compounds were examined.

Interactions of silicic and tannic acids with hydrogen acceptors and quaternary compounds. Silicic acid was prepared by treating 0·1 M sodium silicate with a sulphonic acid resin in the hydrogen form until the solution was slightly acid to litmus. Half was then diluted to 0·01 M in physiological buffered saline, and used immediately in the experiments, while the rest was made slightly alkaline and kept for a few days to allow the silicic acid to polymerize. It was then made weakly acid again and diluted in buffered saline to 0·01 M as before. The tannic acid solution used was 0·1 per cent strength.

Each solution, in lots of 6 ml., was treated in test-tubes, with gradually increasing amounts (up to 1 ml.) of 1 per cent (w/v) solutions of the following: polyethylene glycol 6000 (PEG), polyvinyl pyrrolidone 20,000 (PVP), poly-2-vinyl-pyridine-*N*-oxide (PVPNO, unknown molecular weight, kindly supplied by Prof. H. W. Schlipköter), and tetradecyl trimethyl ammonium bromide (TTAB). The maximum turbidities were read in an absorptiometer, calibrated in terms of optical density, and the results are shown in Table 1.

Table 1. TURBIDITIES DEVELOPED IN MIXTURES CONTAINING SILICIC OR TANNIC ACID AND HYDROGEN ACCEPTOR OR QUATERNARY COMPOUNDS, IN APPROXIMATELY 0·1 PER CENT SOLUTION IN PHYSIOLOGICAL BUFFERED SALINE

	PEG	PVP	PVPNO	TTAB
Fresh silicic acid	0 06	0 07	0·16	0·02
Aged silicic acid (sol)	0·015	0·02	0 07	0·04
Tannic acid	0·75	0·35	0·50	Zero

Figures are optical density read on 1·5 cm tubes with a green filter.

Relative strengths of interaction between various chemical groups and active hydrogen. In a study[9] of the chemical constitution of compounds able to protect living cells against freezing damage, it was shown that the common hydrogen acceptor groups showed a wide variation in their ability to interact with active (phenolic) hydrogen in aqueous solution. The strength of the interaction was estimated as the ratio of the solubility of a model compound in water to its solubility in 1 per cent phenol solution. It was not appreciated at the time of the study that there might be a significant difference in the behaviour of primary and secondary amide groups, so that the secondary amide (peptide) group was not tested; the expected large difference was found between primary amide and tertiary amide. The tests have now been completed and some of the results of the experiments are shown in Table 2. It can be seen that, in agreement with the results obtained by Holt and Went[5] for monolayers, there is some interaction between the peptide group and phenolic hydrogen, though apparently none between the latter and the primary amide group.

Table 2. A GRADED SERIES OF HYDROGEN-BONDING INTERACTIONS IN AQUEOUS SOLUTION

(*a*) Model for peptide group; (*b*) model for phosphate ester group; (*c*) model for protective compound PVPNO

Compound	Solubility in water	Solubility in 1 per cent w/v phenol	Ratio
Hexoamide	2·5	2·7 (approx.)	0·9
Ethyl ether	7·5	4·6	1·6
N-acetyl *cyclo*hexylamine	3·5	1·75	2·0 (*a*)
Diethyl ketone	5·5	2·5	2·2
s-Collidine	1·0	0·16	6
N-Dipropyl propionamide	5·0	0·3	17
Dibutyl sulphoxide	11·0	0·4	28
Tributyl phosphine oxide	4·0	0·05	80 (*b*)
Benzylpyridine *N*-oxide	∞	0·04	> 100 (*c*)

Solubilities are v/v per cent except for hexoamide which is solid at room temperature and was dispensed by weight.

Interaction between tannic or silicic acid and quaternary compounds. Both silicic and tannic acids will interact with quaternary compounds. The negative result shown in Table 1 for tannic acid simply means that the ionic conditions were in this instance not quite suitable. In the presence of sulphate ion (a micelle-promoting anion) tannic acid and the quaternary long-chain compounds show marked interaction. The fact that sulphate, and not halide, is the promoting agent shows that the interaction is probably between the micelles and tannic acid, rather than between individual molecules of the quaternary compound and tannic acid.

Silicic and tannic acids are only 0·1 per cent ionized in neutral solution, so that the interaction cannot be explained in terms of simple attraction between positive and negative charges. The interaction is of a secondary nature, possibly as a result of the 'structure forming' properties of quaternary groups in aqueous solution[11]. Compounds such as tannic acid are 'structure breaking' because they introduce plane trigonal regions in the normally tetrahedral structure of water. In other words, the factors causing the interactions under consideration are geometric rather than energetic, and are similar to those involved in the denaturation of protein by urea or guanidinium ion[12].

Tanning. We are probably near to being able to make a comprehensive definition of tanning. On one hand, there are the 'hydrophilic' polymers (hydrogen-attracting) such as gelatine, polyethylene glycol, polyvinyl pyrrolidone, some but probably not all of the polyamines (if they are too ionized hydrogen donor properties will begin to appear), and also the quaternaries and other structure-formers.

On the other hand, we have tannic acid and its analogues (silicic acid and any other polymers having active hydrogen with *pK* 9–11) and also large structure-breaking anions such as perchlorate, dichromate, hexafluorophosphate, tetraphenyl-boron and Nessler's reagent. These compounds are of very varied constitution but should have the common features of physical toxicity, and ability to precipitate or interact in some way with the first group of compounds[13].

With silicic acid the structure-breaking factor is probably size rather than shape, that is, the hydrogen bonds from silicic acid to water are probably tetrahedral in direction, but introduce disorder for some distance all the way around because none of the bond lengths fits into the water structure.

Most probable site of attack of silicic acid. From Tables 1 and 2 it appears that the interaction of silicic acid with the peptide group is likely to be considerably weaker than its interaction with phospholipid, which contains both quaternary and phosphate ester groups. Interactions with phospholipid are readily demonstrable experimentally. Egg lecithin forms an almost clear sol in water if mixed in from methanol solution. Both silicic acid and tannic acid cause a rapid increase in turbidity. Interaction of phospholipids with silicic acid in polar solvents contributes to the effectiveness of this system for chromatographic separation of phospholipids. Polymerized silicic acid on the surface of silica particles ingested by macrophages, or that reconstituted from soluble diffusing monomeric silicic acid in patches on the phagosomal membrane of the cell, could therefore react with the membrane to produce strong local modification of surface tension and increased permeability. Since PVPNO very readily forms hydrogen bonds with silicic acid it would be expected to protect biological membranes against the effects of the latter, and this protection is readily demonstrable experimentally.

Interaction of silicic acid with hydrogen acceptors. As silicic acid is a stronger acid than water, it will displace water from its bonds to lone-pair compounds such as those tested in Table 2. The interaction can be represented as follows:

$$Si - OH : \ddot{O} \quad \text{— lone-pairs on oxygen (as in pyridine } N\text{-oxide)}$$

$$Si - OH : N \quad \text{lone-pair on nitrogen (as in compound 48/80)}$$

When polymers interact with silicic acid there is the likelihood of multiple bonding making the whole process more efficient and leading to the more or less complete removal of the polymer from the aqueous phase. For example, PVPNO should adsorb on the surface of colloidal silica with so many uniformly strong hydrogen bonds that the complex should be stable. In accordance with Bronsted's principle[14] concerning the properties of polymers in solution, neutralizing agents of the type of PVPNO or PEG should show all-or-none properties, that is, PEG and PVP (polyvinyl pyrrolidone) can be expected to be completely inactive, if their affinity for active hydrogen is below a certain limit.

Haemolysis of red blood cells by colloidal silica, and the protective effect of a neutral polymer. A silica sol was prepared by passing 0·1 M sodium silicate solution through the hydroxyl form of a strongly basic ion-exchange resin, 'Zeo-Carb 225', until it began to come through alkaline, heating to 60° C in order to stabilize the sol and allowing to cool. The slightly alkaline solution was neutralized and diluted tenfold with buffered physiological saline. The same batch of sol was used both for tests with polymers and tests with human red blood cells.

The cells were washed three times in buffered saline, and suspended in saline. After exposure to various concentrations of silica for 1 h at room temperature, with repeated shaking to keep the cells in suspension, the liquid was centrifuged and the percentage haemolysis estimated by photometric measurement of haemoglobin in the supernatant. As shown in Table 3, concentrations of silica above 0·01 mM produce considerable haemolysis. As expected, good protection against haemolysis was ensured by first mixing equivalent weights of PVPNO solution with the colloidal silica, and then adding red cells to the mixture. Other experiments were carried out to discover if PVPNO can form relatively stable complexes with the cell membrane and so protect it against haemolysis. Washed red cells were pre-treated with a 0·1 per cent solution of PVPNO, spun down and washed twice, and then exposed to silica as before. The results in Table 3 show that no protection was afforded against high concentrations of silica, but that there was some against low concentrations which might have been due to the presence of small amounts of slowly released PVPNO. From these results it seems unlikely that PVPNO forms a stable complex with cell membranes.

Table 3. PERCENTAGE OF TOTAL HAEMOGLOBIN IN HUMAN RED CELLS FOUND IN SUPERNATANT AFTER EXPOSURE FOR 1 H TO VARIOUS CONCENTRATIONS OF SILICA SOL

Red cells	ml. 0·1 M silica/6 ml. final solution 0·3	0·1	0·03	0·01	0·003	0·001	Saline control
Untreated	51	75	75	81	49	10	0
Pretreated with PVPNO	64	83	73	61	31	1	0

Some cells were pretreated with 0·1 per cent PVPNO for 30 min and washed, before exposure.

Haemolysis was apparently less than 100 per cent at any silica concentration. This is because the cells were disrupted and the contents were partly precipitated; inspection of the precipitate showed that there were no intact cells. The reading on the supernatant was less than that which would have been obtained had the cells been haemolysed by distilled water, because some of the haemoglobin had been precipitated.

It is concluded that the toxic effects of silica are due to the capacity of polymeric silicic acid to act as a hydrogen donor in the formation of hydrogen-bonded complexes with active groups such as quaternary and phosphate ester groups of phospholipids or, to a lesser extent, secondary amide (peptide) groups of proteins. Polyvinylpyridine-*N*-oxide preferentially forms hydrogen bonds with silicic acid and prevents the permeability changes induced by the latter in biological membrane systems. Other substances protecting against the toxic effects of silica, such as compound 48/80, would be expected to act in an analogous way. Recently, Stalder and Stöber[15] investigated the haemolytic activity of various dusts and concluded that lipids are involved. They also found that PVPNO afforded good protection against haemolysis by powdered quartz, though possibly not so good as that which we found against haemolysis by chemically prepared silica sols.

From Table 2 it is clear why compounds such as polyethylene glycol or polyvinyl pyrrolidone should not be such good protective agents as PVPNO. The corresponding active groups, ether and dialkylamide, respectively, have less affinity for active hydrogen than has pyridine-oxide. Compound 48/80 has an even higher affinity for active hydrogen than PVPNO, but in this case the affinity is too high and some of the active centres are ionized. This is probably why the compound is rather toxic. The general aim in using or selecting neutral protective compounds would be to have as high an affinity as possible for active hydrogen without actually ionizing in water. A limit would be reached at about aliphatic amine oxide, R_3NO, the pK of which is 4, as compared with 1 for pyridine oxide.

Experiments are in hand to determine the effect on macrophages of water-insoluble derivatives of tannic acid which could be ingested in particulate form and then be slowly hydrolysed back to tannic acid inside the cells. In this way it should be possible to explore further the analogy between tannic and silicic acids. Finally, if the main suggestions of this article are correct, good protective action against the toxicity of silica should be afforded by polybetaines such as would result, for example, from the condensation of ethyl chloracetate with polyvinylpyridine and subsequent hydrolysis of the ester. Tests in this laboratory showed that long-chain monomeric betaines interacted with silicic acid but were haemolytic, as might be expected from their formal resemblance to lysolecithin. The polymeric betaines without any long alkyl chain should, on the other hand, be comparatively harmless, just as heparin is harmless compared with the long-chain alkyl sulphates.

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HIGH-SENSITIVITY POLARIMETRY AND ITS APPLICATION TO THE STUDY OF SMALL CONFORMATIONAL CHANGES IN PROTEINS

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THE measurement of optical rotation offers probably the most generally useful method for the observation of conformational changes in proteins in solution. The optical rotation is highly sensitive to solvent and other environmental perturbations[1], and the conformational changes which it will detect are therefore by no means limited to those involving α-helix ⇌ random coil transitions[2]. There is now abundant evidence, which is supported by the crystallographic studies on haemoglobin[3] and lysozyme[4], that when an enzyme combines with its substrate, a conformational change is frequently induced; it has indeed been suggested[5] that this might occur in all cases. It is supposed that small conformational changes are the general means whereby enzyme action is controlled by the non-competitive binding of activators and inhibitors[6–8]. A similar conformation-dependence may be anticipated in aggregation processes, such as are involved in the self-assembly of viruses, interaction of muscle proteins, and other systems. In consequence, increasing attention has been focused on the problem of detecting, following and interpreting such changes in conformation, which are expected to be small, and evidently do not in general involve significant breakdown or formation of α-helical structure. We describe here a technique for the application of polarimetry to the study of such small changes, and we give some examples of the type of information that can be obtained.

In a modern recording spectropolarimeter (Cary 60, Bellingham and Stanley 'Polarmatic', Jasco) full-scale rotations of the order of 0·1° can be measured. The precision of measurement varies with the circumstances, being under favourable conditions (stability of source and electronics, low strain in cell windows, constant sample temperature and clear solutions), generally about 2–4 per cent, outside the region of absorption bands. This makes it possible to detect a change in molar residue rotation $[m']$ of the same order.

When the Moffitt equation[9]:

$$[m']_\lambda = a_0\left(\frac{\lambda_0^2}{\lambda^2-\lambda_0^2}\right) + b_0\left(\frac{\lambda_0^2}{\lambda^2-\lambda_0^2}\right)^2 \qquad (1)$$

is applied to the optical rotatory dispersion curves, the constant b_0, which is considered to reflect the content of α-helix, can be determined with a precision of perhaps 5 per cent at best (depending on its absolute magnitude). Indeed, standard deviations in much of the published data are evidently a good deal higher. A similar limit can be set on measurements of the amplitude of the ultra-violet Cotton effects associated with the α-helical conformation, whether these be measured by optical rotation[10] or circular dichroism[11,12], depending on the relative magnitudes of absorption and rotation (that is to say in practice the actual α-helix content, and to some degree the proportion of aromatic amino-acids). Limits are thus set for the magnitudes of the conformational changes—whether reflected in b_0, or only in a_0 or a specific rotation or ellipticity—which can be observed.

In order to increase the sensitivity of polarimetric measurement of small changes, we proceed in the following manner. If the mean residue rotation of a protein $[m']$ be changed by some small amount $\delta[m']$, the measured rotation, α, will change by δα, such that $\delta[m']/[m'] = \delta\alpha/\alpha$ If the protein concentration (or the path-length) is now multiplied by a factor of say 10, δα will also be increased by the same factor; α, however, will now be immeasurably large. We therefore compensate out the rotation α with a dextrorotation of equal magnitude. This is most simply achieved by introducing into the beam, in series with the sample cell, a cell containing sucrose solution of appropriate concentration. Thus, a 5 per cent change in $[m']$, which would normally result in a measured rotation change of 0·005° (when α = 0·1°), will give instead a measured change of 0·05°. On the basis of the instrumental precision which we have indicated, this will make possible the measurement of a 0·2–0·4 per cent change in $[m']$ for a tenfold increase in protein concentration, and a 0·02–0·04 per cent change for a hundredfold increase. It will be noted that even such protein concentrations, giving, say, 5° actual rotations in the near ultra-violet region, are entirely reasonable. The limiting factor in measuring such small rotational changes ceases then to be polarimetric precision, and becomes instead one of volumetric accuracy, if reagents have to be added to effect the change in $[m']$. We may express the error in measured rotation, dα, resulting from an error dc in concentration in the following terms: $d\alpha/\delta\alpha = [m']\, dc/\delta[m']c$, where δα is the change in rotation to be measured. Thus, if it is proposed to measure a 0·1 per cent change in $[m']$, it follows that $d\alpha/\delta\alpha = 10^3\, dc/c$, so that if a 1 per cent precision of measurement is required, dc/c must be 10^{-5}. Such volumetric accuracy is, of course, entirely feasible, if small volumes of reagent can be added to large volumes of protein solution (for example, 10 μl. samples in 10 ml. samples of protein).

We have considered so far only rotations at single wave-lengths. For measurements of optical rotatory dispersion on a difference basis, the protein and the dextrorotator must remained balanced, or sufficiently nearly so to keep the rotation on scale, over a range of wave-length. This could be achieved by changing the sucrose concentration where the divergence becomes too great, or better by the use of a rotation compensator coil in place of the sucrose cell. We have found, however, that we can make use of the compensation from a single sucrose solution over the range 330–500 mμ. We are to some extent aided by the Verdet constant correction, which has to be applied to measured rotations in our instrument (Bellingham and Stanley 'Polarmatic' 62), to compensate for the wave-length variation of the Faraday cell rotation. This has the effect of flattening the recorded rotatory dispersion curves, and keeping the net apparent rotation on scale over such a large wave-length range. By determining $\delta[m']$ as a function of wave-length, we are now in a position to express our data in terms of 'difference' Moffitt plots, and to determine, within the limits of the Moffitt equation, to what extent differences in rotation result from changes in α-helix content. For a ten-fold increase in protein concentration, changes in b_0 corresponding to a change in apparent helix content of some 0·5 per cent should be measurable. (Clearly the measurements must not extend into the regions of absorption because of the very large absorbances which such concentrated systems would have.)

We give now some examples of applications of the difference method. We have observed first the activation of chymotrypsinogen with trypsin[13]. This gives rise to a

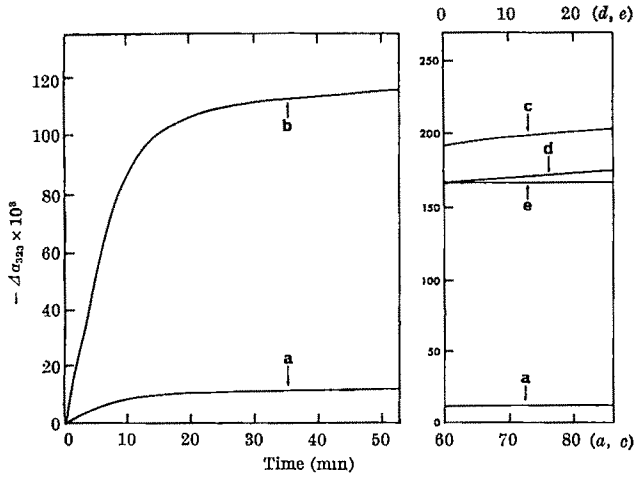


Fig. 1. Change in optical rotation (323 mμ) accompanying activation of chymotrypsinogen with trypsin: (*a*) observed directly, 3·5 mg/ml., 1 cm path; (*b*) 35 mg/ml. in 1 cm path, compensated by sucrose; (*c*) 26 mg/ml. in 1 cm path, compensated by sucrose, (*d*) α-chrymotrypsin under the same conditions as (*c*); (*e*) as (*d*), without sucrose. (Lower scale refers to (*a*), (*b*) and (*c*); upper scale to (*d*) and (*e*))

quite large rotational change, which is apparently not accompanied by any change in α-helix content, since both b_0 and $[m']_{333}$, as well as the far ultra-violet hypochromicity, remain essentially unchanged[14,15]. (The difference in specific rotation between chymotrypsin and its zymogen must be supposed, therefore, to arise from changes in anomalous optical rotatory contributions of aromatic residues, from environmental effects arising out of changes in the extent of exposure of residues to solvent, or from a combination of both.) The change of rotation as a function of time is shown in Fig. 1, measured both directly and by the difference method, at a ten-times higher protein concentration. It will be noted that after the initial steep change in rotation, there is a slower and smaller change, which soon becomes immeasurably small when observed directly, but can easily be followed by the sucrose technique. This slow change is inconsistent with the kinetics of the initial rapid change, and therefore represents a separate process. Fig. 1 shows also the time-course of the optical rotation of α-chymotrypsin in the presence of the same concentration of trypsin, and a similar slow change is observed. Over the same period no change could be detected in the absence of trypsin. We conclude that the activation of the chymotrypsinogen is accompanied by a perceptible proteolysis of the chymotrypsin which is produced on activation.

As a second example, we consider the slow denaturation of ovalbumin in urea solutions[16]. At low urea concentrations the rotational change is both slow and limited in extent. The inset in Fig. 2 shows the small changes measured by the difference technique as a function of time. The reaction at a urea concentration of 6 M was selected for more detailed study. We have measured the change in optical rotatory dispersion as a function of time by the following procedure: the wave-length is altered stepwise and at timed intervals, returning after the last wave-length to the first, and so on. By joining the rotations measured at each wave-length, we obtain the curves shown in Fig. 2, which then represent the time-course of the reaction at the indicated wave-lengths. The ordinates at a given time value now give the optical rotatory dispersion at that time, and from them Moffitt plots can be constructed. Fig. 3 shows the change of b_0 and a_0 with time: it is seen that b_0 after a rapid change reaches a constant value, whereas a_0 continues to fall. It will be noted that the overall b_0 change is about 25, and that one may accurately read off changes of about 1–2. In ovalbumin (*c.* 600 residues) this will correspond, on the scale of the Moffitt equation, to 1–2 residues leaving the α-helical conformation. Since we are here by no means working near the limit of the method (a 1·25 per cent ovalbumin solution in 1 cm path, and only a moderate instrumental scale expansion, were used), there seems little doubt that the effect of a single residue entering or leaving an α-helical segment would easily be observable. (In the case of short helices end-effects on anomalous rotatory dispersion become important; their magnitude may be assessed in terms of the data of Goodman *et al.*[17].)

We can further analyse the data of Fig. 3 with the aid of a variant of the Moffitt equation due to Doty[18], namely:

$$[m']_\lambda = (fa_0^H + \Sigma a_0^R)\left(\frac{\lambda_0^2}{\lambda^2 - \lambda_0^2}\right) + fb_0^H\left(\frac{\lambda_0^2}{\lambda^2 - \lambda_0^2}\right)^2 \quad (2)$$

where f denotes the fraction of residues in the α-helix, b_0^H the value of b_0 for a fully helical chain (usually taken as -630 for $\lambda_0 = 212$ mμ), and a_0 has been broken down into two components, a_0^H being the contribution of a fully helical chain arising only from the peptide chromophores in the helical state, and Σa_0^R the total intrinsic residue rotations, compounded of all side-chains, and modulated by the microscopic environment. Since, as has been noted, a_0 is changing regularly after b_0, and therefore the hypothetical fa_0^H, has ceased to change perceptibly, it appears that two denaturation processes are operating. If we assume these to be additive and independent, we may extrapolate back the change in a_0 which continues when b_0 has become constant. Towards shorter times the curve is seen to be not far from linear, and we therefore extrapolate linearly as shown in Fig. 3.

The difference between the extrapolated and observed a_0 curves should be fa_0^H, and this should be directly proportional to fb_0^H. The inset in Fig. 3 shows the two

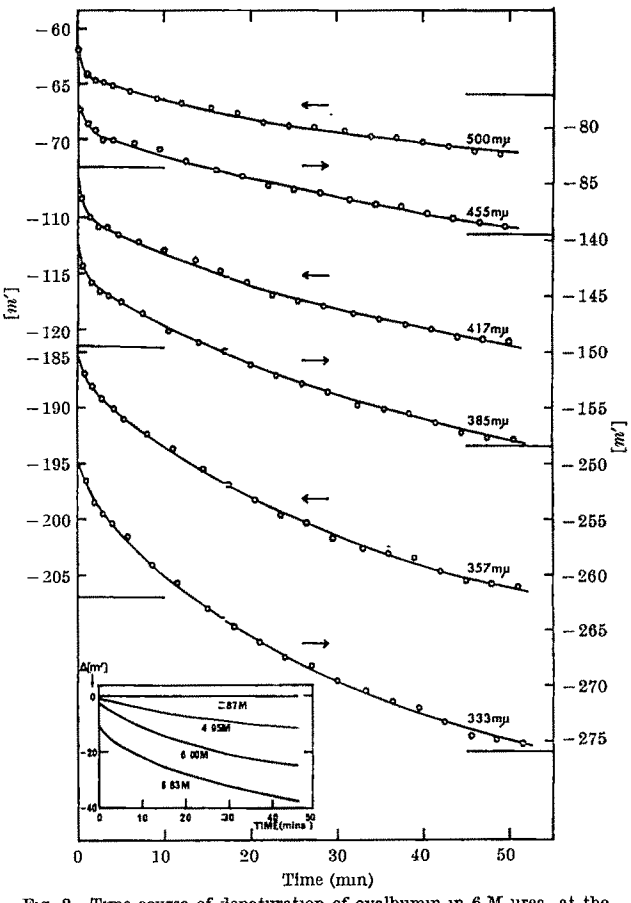


Fig 2. Time-course of denaturation of ovalbumin in 6 M urea, at the indicated wave-lengths. Protein concentration, 12·5 mg/ml., 1 cm path, compensated by sucrose. Readings were begun 1 min after mixing. Inset: denaturation rates as a function of urea concentration, all followed at 333 mμ using sucrose compensation to magnify the change. Protein concentrations, 25 mg/ml.

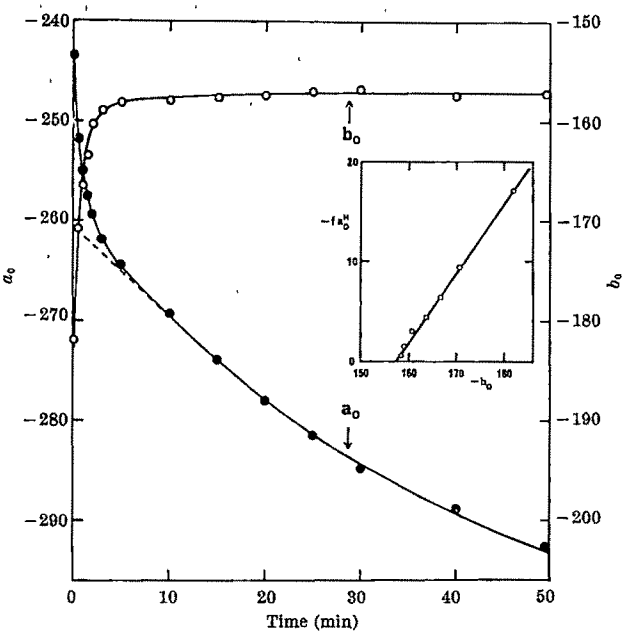


Fig. 3. Changes with time of optical rotatory dispersion parameters, a_0 and b_0, for ovalbumin, calculated from the Moffitt plots derived from the data of Fig. 2. Inset: plot of fa_0^H (equation 2) against b_0

functions plotted against each other, and a linear relation appears indeed to obtain. We deduce that the two steps in the reaction represent, respectively, disruption of an α-helical portion without appreciable change in Σa_0^R, and a much slower general unfolding of the molecule, with exposure of the internal residues to the new environment.

It is interesting to note that in 1954 Kauzmann[19] suggested from an investigation of the denaturation kinetics alone of ovalbumin in urea solutions that a helical segment was first unfolded, without major change in the molecule as a whole, and that this was then followed by a process of general expansion (as well as perhaps disulphide interchange), reflected in increased reduced viscosity. This interpretation, made before the advent of the Moffitt treatment of optical rotatory dispersion, is satisfactorily borne out by our experiments.

These examples illustrate the potentialities of the sucrose technique for the detection and elucidation of small conformational changes in proteins, and suggest possibilities in the sphere of enzyme function, where it is hoped the method will commend itself. Investigations of enzyme systems are in progress in this laboratory.

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DEOXYRIBONUCLEIC ACID VALUES IN DIVIDING AND NON-DIVIDING CELLS OF MALE AND FEMALE LARVAE OF THE HONEY BEE

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Galton Laboratory, University College, London

IN the honey bee (*Apis mellifera*), the males, or drones, normally arise from unfertilized haploid eggs, while the females, both workers and queens, originate from fertilized diploid eggs[1]. The chromosome numbers have been estimated as 32 and 16 in diploid and haploid cells respectively[1,2].

Recent work suggests that polyploidization occurs in the somatic cells of both male and female honey bees. Risler[3] concluded from chromosome counts as well as measurements of spindle sizes in dividing cells of larvae that during the first instar (that is, up to about 24 h after hatching[4]) the cells of the drones remain haploid and those of workers remain diploid, but that during subsequent instars different tissues become polyploid to varying degrees. Merriam and Ris[5] studied the deoxyribonucleic acid (DNA) contents of Feulgen-stained cells in interphase by microspectrophotometry and found that the DNA values of cells in both males and females fell into successive multiple classes.

We have carried out microphotometric studies on Feulgen-stained nuclei of young drone and worker larvae during the first instar. Even at this early stage some of the interphase nuclei showed considerable degrees of polyploidy, but the dividing cells were almost exclusively haploid in the males and diploid in the females.

The larvae, from an Italian hybrid strain, were removed from drone and worker cells of the hive and fixed in Carnoy's solution. For the present investigation we used younger larvae not more than 5 h after hatching, and older larvae aged 10–20 h. Feulgen staining was carried out in bulk, but whereas the younger larvae were stained *in toto*, the older larvae were first cut in half. Males and females which were to be compared were processed concurrently. The fixed larvae were hydrated and hydrolysed in N hydrochloric acid at 60° C for 10 min and stained in Feulgen solution for 1 h. They were then washed in water and placed in 45 per cent acetic acid kept on ice. The stained larvae were squashed gently between a siliconed slide and a clean cover slip, and the preparations were made permanent by the dry ice technique[6].

The DNA values, in arbitrary units, were measured on a Barr and Stroud integrating microdensitometer, type '*GN*. 2', at a wave-length of 5500 Å. In addition, the lengths of the two main axes of the nuclei were measured with an eye-piece micrometer and transformed into micra with the aid of a stage micrometer. The results were converted into nuclear areas using the formula Πab, where a and b are half axes. For diagrammatic representation, the readings of DNA values were converted into logarithms to the base 2.

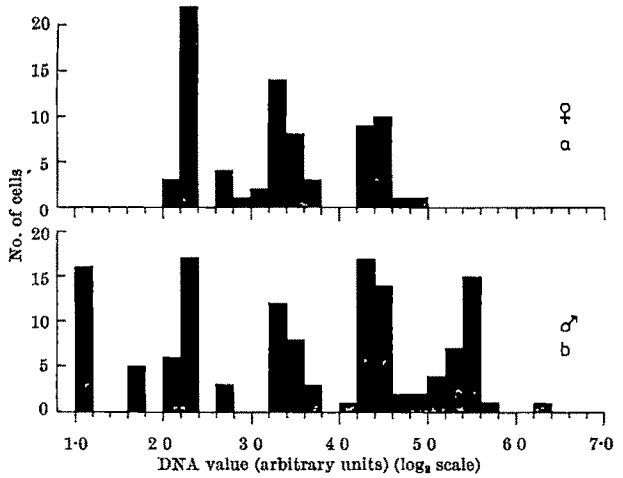


Fig. 1. DNA values of non-dividing cells of two larvae aged less than 5 h. (*a*) Female; (*b*) male

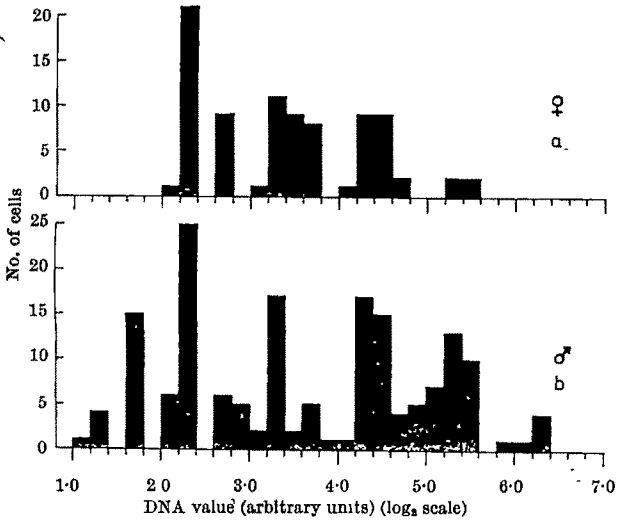


Fig. 2. DNA values of non-dividing cells of two larvae aged 10–20 h. (*a*) Female; (*b*) male

The distributions of DNA values of interphase nuclei of a male and a female larva aged less than 5 h are shown in Fig. 1 and those of a similar pair of larvae aged between 10 and 20 h are given in Fig. 2. All the histograms contain a number of peaks at distances of about one unit, corresponding to successive duplications of DNA values. Each of the two distributions of DNA values from males contains a peak on the left-hand side which is not present in those of the females, and we regard these peaks as corresponding to the haploid DNA values. In addition, the males also contained cells in the 32-ploid range, which were not found in the females. Although we cannot exclude the possibility that the males were slightly older than the females, the data suggest that the rate of polyploidization in drone larvae may be faster than that in worker larvae.

The DNA values of cells in mitosis taken from the same larvae are shown in Figs. 3 and 4. Bearing in mind that nuclei in prophase and metaphase contain the duplicated amount of DNA, it will be seen that the mitoses found in the female larvae were all diploid. By contrast, the mitoses in the younger male larvae were almost all haploid, although a few of the anaphases and telophases might have been diploid; in the somewhat older male, the majority of mitoses were still haploid, but a smaller proportion were undoubtedly diploid.

The nuclear areas of cells divided roughly according to their DNA values are given in Tables 1 and 2. Although there was considerable variation in nuclear sizes between the different larvae, the mean nuclear area of each individual is nearly doubled for each successive DNA class. A somewhat unexpected finding in the present data was that for each DNA class the nuclear sizes of the female larva from each pair was almost double that of the male, but this relationship was not borne out by some of our other material. Thus, while it is clear that successive duplications of DNA values are associated with correspondingly larger nuclei, the exact relationship between these two variables remains to be worked out.

Our results show that a large proportion of the non-dividing cells of both drone and worker larvae of the honey bee become polyploid soon after hatching. This may possibly be associated with their rapid growth, for it has been estimated that a worker larva increases its initial

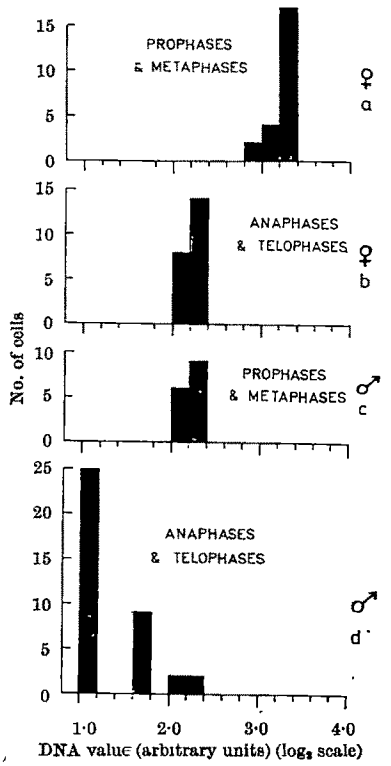


Fig. 3. DNA values of mitotic cells of two larvae aged less than 5 h. (*a*), (*b*) Female; (*c*), (*d*) male

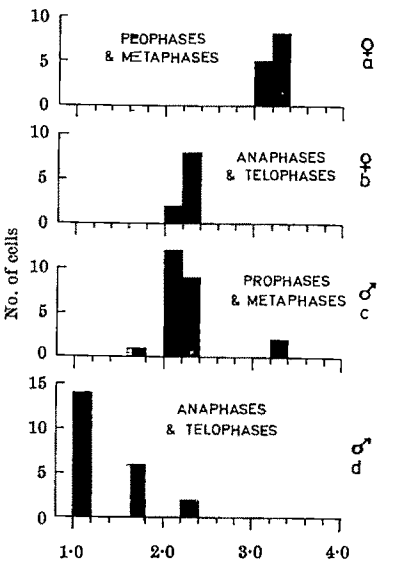


Fig. 4. DNA values of mitotic cells of two larvae aged 10–20 h. (*a*), (*b*) Female; (*c*), (*d*) male

Table 1. NUCLEAR AREAS IN CELLS WITH DIFFERENT DNA VALUES IN BEES 1 AND 2 (LARVAE AGED LESS THAN 5 H)

DNA value	Bee 1♂ No. of cells	Bee 1♂ Mean nuclear area (μ^2)	Bee 1♂ Standard deviation	Bee 2♀ No. of cells	Bee 2♀ Mean nuclear area (μ^2)	Bee 2♀ Standard deviation
Haploid	21	3	1·0	—	—	—
Diploid	26	6	1·7	30	12	4·7
Tetraploid	23	16	6·6	26	25	16·5
Octoploid	34	25	6·6	17	41	10·3
16 ploid	27	46	13·4	—	—	—
32 ploid	1	73	—	—	—	—

Table 2. NUCLEAR AREAS IN CELLS WITH DIFFERENT DNA VALUES IN BEES 3 AND 4 (LARVAE AGED 10–20 H)

DNA value	Bee 3♂ No. of cells	Bee 3♂ Mean nuclear area (μ^2)	Bee 3♂ Standard deviation	Bee 4♀ No. of cells	Bee 4♀ Mean nuclear area (μ^2)	Bee 4♀ Standard deviation
Haploid	20	5	1·6	—	—	—
Diploid	40	7	1·7	30	13	6·2
Tetraploid	28	17	9·8	29	26	8·4
Octoploid	42	27	10·9	21	46	14·7
16 ploid	31	40	10·4	4	81	7·3
32 ploid	5	106	16·8	—	—	—

weight 1,500 times within 4·5–5 days[4]. The nuclei become polyploid through the process of endoreduplication, which involves successive duplications of the chromosomal material in the absence of cell division. This process was originally discovered by Geitler[7–9] in insects belonging to the order Heteroptera and subsequently in other organisms and given the name 'endomitosis'. In young honey bee larvae, endoreduplication does not affect the dividing cells, for our results confirm those by Risler that during the first instar the mitoses of worker larvae are diploid while the majority of mitoses of drone larvae are haploid. Our results show that the ploidy in tissues of organisms originating by haploid parthenogenesis cannot be assessed by counting the chromosomes in dividing cells.

The contrast in ploidy number between dividing and non-dividing cells, which is so strikingly exhibited in honey bee larvae, occurs also, though to a lesser extent, in mammalian cells; and it has been suggested by Mittwoch *et al.*[10] on the basis of data obtained from human fibroblast-like cells in tissue culture that, whereas dividing cells have a strong tendency to retain the diploid chromosome number, a substantial proportion of cells become polyploid in interphase.

It is customary to distinguish between the germ line and the somatic cells. It may be useful to add a further category by subdividing the somatic cells into those which are likely to divide again and those which are not. We thus have three categories, namely (1) the meiotic line, (2) the mitotic line and (3) non-dividing or differentiating cells. Cells in the mitotic line have a strong tendency to retain their basic ploidy number, whereas differentiating cells may become polyploid to an extent which differs in different organisms and in different tissues.

We thank Dr. C. G. Butler, Head of the Bee Department of Rothamsted Experimental Station, for allowing us to use his colonies and Miss Elaine Fairey for her generous co-operation in providing male and female bees and larvae. The integrating microdensitometer was provided by the Central Research Fund, University of London.

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RAPID UPTAKE OF TRITIATED ANTIGEN BY MOUSE EOSINOPHILS

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THE association of eosinophils with a variety of immune reactions was summarized in a recent review by Litt[1]. Numerous studies have emphasized that eosinophils do not incorporate soluble antigen directly. An indirect function of eosinophils in mechanisms of antibody synthesis was suggested by autoradiographic investigations on the cellular localization of tritiated tetanus toxin in inflammatory peritoneal exudates of mice[2–4]. Eosinophils appeared to transfer tritiated material from disintegrating inflammatory cells to macrophages, which subsequently transformed into immature plasmacytes[2].

A significant eosinophilia was demonstrated in regional lymph nodes within 24 h after a single injection of heterologous protein antigen into the footpads of guinea-pigs[1,5,6]. Repeated injections of haemocyanin into the footpads stimulated a massive accumulation of eosinophils in the nearest draining lymph nodes[5]. Following footpad injections of fluorescein-labelled antigen, Litt[6] demonstrated the accumulation of eosinophils in regional nodes at the sites of antigen localization. The direct incorporation of antigen-antibody complexes by eosinophils has been shown with immunofluorescent techniques[7], electron microscopy[8] and phase microscopy[9]. Fluorescent techniques have failed to detect the uptake of soluble protein antigen by eosinophils in the absence of specific antibody[7]. These results indicated that immune complexes stimulate the eosinophil response.

Autoradiographic investigations of antigen localization revealed numerous tritium (^{3}H)-labelled eosinophils in draining lymph nodes 24 h after injections of a soluble ^{3}H-antigen into the footpads of mice. These observations initiated an investigation of the incorporation of antigen by eosinophils during the first 24 h of a primary immune response, and the results are presented in this article.

Sulphanilic acid was tritiated by the catalytic exchange method (New England Nuclear Corp., Boston) to a final activity of 2 mc./mg. A soluble, ^{3}H-labelled, heavily substituted azoprotein antigen (^{3}H-*S*-PγG) was prepared by coupling 12·5 mg sulphanilic acid-^{3}H to 80 mg of porcine gamma globulin (PγG, Pentex, Inc., Kankakee, Illinois)[10]. The final protein concentration of the ^{3}H-*S*-PγG was 8 mg/ml., and the total radioactivity content was 14 mc./80 mg PγG. Radioactivity determinations were performed in a liquid scintillation spectrometer[11]. An unlabelled, heavily-substituted azoprotein antigen (*S*-PγG) was prepared by coupling 12·5 mg sulphanilic acid to 80 mg PγG and was dialysed to a final protein concentration of 8 mg/ml. Mice injected with the *S*-PγG provided tissues for control autoradiograph preparations.

Mouse serum antibodies specific for the sulphanilazo determinant were titrated by the passive haemagglutination reaction using sheep erythrocytes coupled with diazotized sulphanilic acid[12]. Tests for serum antibodies against the PγG carrier utilized the passive tannic acid haemagglutination technique[10]. Serum from each experimental mouse was tested by the two haemagglutination methods.

Adult albino mice (*NLW-CFW*-Webster Swiss origin) were investigated in three groups. In groups 1 and 2, each mouse received a subcutaneous injection of 80 μg

^{3}H-*S*-PγG (14 μc./0·01 ml.) into the right hind footpad. In group 1, four mice were studied at each of ten time intervals following injection: 1, 2, 3, 4, 5, 6, 7, 8, 16 and 24 h. Eight right and left lymph nodes (popliteal, inguinal, ventral and dorsal axillary) were removed from each mouse and prepared for autoradiography. Lymph node sections were prepared from two of the four mice, using one mouse for each of two staining techniques: neutral formalin fixation for the haematoxylin and eosin stain and Bouin-Hollande fixation for the Dominici stain[13]. Lymph node imprints were prepared from the other two mice and observed with the haematoxylin and eosin stain and Dominici stain. All tissue preparations were stained following development of the autoradiographs. Details of the histological and autoradiographic techniques have been described[10].

Twenty-one mice in group 2 were investigated for serum antibody formation on days 1–7 after the footpad injections. Sera were collected from 3 mice/day and were tested by the haemagglutination techniques described earlier.

In group 3, each of ten control mice received a subcutaneous injection of 80 μg *S*-PγG (0·01 ml.) into the right hind footpad. One mouse was killed at each of the ten time intervals scheduled in the group 1 investigation, and the lymph nodes were sectioned for autoradiographic background determinations[10].

The quantitation of tritium-labelled eosinophils and observations on the concentrations of tritium-exposed emulsion grains over the labelled cells were performed on autoradiographs of lymph node sections after a 6-week exposure period. The intracellular tritium concentrations were graded as light (3–10 grains/cell), moderate (10–20 grains) and heavy (>20 grains). The average number of background grains over unlabelled control tissue sections (mouse group 3) and emulsion-coated, non-tissue areas of experimental slides was 1 grain/1,000 μ² (ref. 10).

Autoradiographs revealed no tritium-labelled cells in the left popliteal, inguinal and axillary nodes of group 1 mice through the first 24 h following ^{3}H-*S*-PγG injections into the right hind footpads. The basal level of eosinophils in these lymph nodes was determined by counting the total number of eosinophils in 1,920 sections of eighty nodes. The mean of the eosinophil counts per section was thirteen, with a range of 0–61. Only eighteen of the 1,920 sections contained more than nineteen eosinophils, and many of the left popliteal node sections contained no eosinophils. The right dorsal axillary lymph nodes also remained unlabelled throughout the interval investigated. Counts of unlabelled eosinophils in 980 sections of twenty right dorsal axillary nodes revealed a mean of thirteen eosinophils to each section (range 1–47).

Autoradiographic observations detected ^{3}H-labelled eosinophils in the right popliteal, inguinal and right ventral axillary lymph nodes of group 1 mice following injections of ^{3}H-*S*-PγG into the right hind footpads. The total number of ^{3}H-labelled and unlabelled eosinophils, and the concentration of ^{3}H-exposed grains over the labelled cells, were determined in a minimum of twenty-four sections of each node, and the results are presented in Table 1. Labelled eosinophils were scattered in the regional popliteal nodes 1 h after footpad injections, but no significant increases were evident in the eosinophil counts of each section. An increase in eosinophil counts between 1 and 4 h was accompanied by a 40-fold increase in the percentage of labelled eosinophils. The highest eosinophil counts in the draining popliteal nodes occurred between 4 and 8 h, during which time over 90 per cent of the eosinophils had incorporated ^{3}H-antigen. Although the eosinophil counts decreased to normal means by 16 and 24 h, the proportion of labelled cells remained above 90 per cent.

Autoradiographs of the right inguinal and right ventral axillary lymph nodes of group 1 mice showed many heavily tritiated eosinophils 1 h after the footpad injections (Table 1). As in the regional popliteal nodes, the appearance of ^{3}H-labelled eosinophils in the inguinal and axillary nodes preceded significant increases in the number of eosinophils per section. Eosinophil counts in these lymph nodes attained very high levels at 4, 6 and 7 h, and the autoradiographs revealed a high percentage of heavily labelled eosinophils at 1, 6, 7, 8 and 24 h. The inguinal and axillary nodes which contained tritiated eosinophils usually had eosinophil counts significantly above the normal means derived from the contralateral nodes. Exceptions to this relationship occurred at 1 and 24 h. Individual mouse variation was noted in the incorporation of ^{3}H-antigen by cells of the inguinal and axillary nodes[14]. Autoradiographs failed to detect radioactivity in the right ventral axillary node sections examined at 2 h and in the inguinal and axillary node sections studied at 5 and 16 h (Table 1). Autoradiographs of lymph node imprints from other group 1 mice, however, demonstrated ^{3}H-labelled eosinophils in the right inguinal and right ventral axillary nodes at 2, 5 and 16 h. The uneven distribution and irregular depth of the cells in these tissue imprints prevented any quantitative evaluation of the eosinophil responses. Of the 40 group 1 mice which received footpad injections of ^{3}H-*S*-PγG, all responded with antigen incorporation by cells of the draining popliteal nodes, but six failed to show any ^{3}H-antigen in the right inguinal and right ventral axillary nodes. The results from the latter six mice indicated variation in the distribution of antigen from the injection site.

The appearance and distribution of labelled eosinophils in the popliteal, inguinal and axillary nodes were similar. The efficient incorporation of ^{3}H-antigen by eosinophils was emphasized by the high percentage of heavily labelled cells which persisted [illegible] 24 h of the investigation (Table 1). The ^{3}H-grains were densely packed over the cytoplasm of the labelled eosinophils, closely surrounding the nuclear lobes, but no intranuclear localization was observed (Fig. 1). More than 90 per cent of the eosinophils were mature cells with

Table 1. INCORPORATION OF TRITIATED ANTIGEN BY MOUSE EOSINOPHILS DURING EARLY PRIMARY IMMUNE RESPONSE

Time after injection* (h)	Lymph node†	Mean eosinophils per section (range)‡	Labelled eosinophils (per cent)	^{3}H concentration in labelled eosinophils§ Heavy (per cent)	Moderate (per cent)	Light (per cent)
1	Popliteal	4 (0–17)	2·4	0	100	0
	Inguinal	55 (11–89)	83·6	82·5	14·3	3·2
	Axillary	19 (11–26)	96·0	82·5	9·8	7·7
2	Popliteal	12 (3–20)	78·7	81·0	9·5	9·5
	Inguinal	225 (85–370)	65·2	70·4	15·7	13·9
	Axillary	31 (21–38)	0			
3	Popliteal	34 (14–55)	90·3	88·1	9·0	2·9
	Inguinal	68 (29–106)	49·8	58·5	17·6	23·9
	Axillary	79 (35–172)	78·7	50·1	26·5	23·4
4	Popliteal	158 (128–211)	99·5	79·6	15·9	4·5
	Inguinal	472 (295–556)	73·5	58·7	25·2	16·1
	Axillary	466 (356–626)	75·4	45·7	31·5	22·8
5	Popliteal	44 (7–86)	98·1	95·7	3·3	1·0
	Inguinal	32 (24–41)	0			
	Axillary	22 (24–29)	0			
6	Popliteal	88 (66–114)	94·7	82·7	14·9	2·4
	Inguinal	227 (167–300)	84·6	86·2	10·4	3·4
	Axillary	332 (188–506)	88·8	80·6	13·4	6·0
7	Popliteal	42 (21–72)	93·0	78·1	15·6	6·3
	Inguinal	451 (297–499)	98·5	88·5	8·3	3·2
	Axillary	538 (319–554)	97·1	88·5	7·9	3·6
8	Popliteal	233 (42–525)	97·8	90·3	9·0	0·7
	Inguinal	78 (41–142)	83·0	78·7	17·8	3·5
	Axillary	92 (65–116)	93·0	89·8	7·1	3·1
16	Popliteal	19 (9–27)	91·4	95·0	3·6	1·4
	Inguinal	10 (2–17)	0			
	Axillary	22 (10–42)	0			
24	Popliteal	10 (2–14)	100	97·6	2·4	0
	Inguinal	12 (7–19)	89·1	75·6	14·6	9·8
	Axillary	23 (9–42)	84·9	91·1	6·4	2·5

* 80 μg (14 μc.) of ^{3}H-sulphanilazo porcine γ-globulin was injected subcutaneously into each right hind footpad of twenty mice.

† Cells of the right popliteal, right inguinal and right ventral axillary lymph nodes incorporated ^{3}H-antigen.

‡ Mean of the total eosinophil counts per section in a minimum of twenty-four sections per lymph node from each of two mice.

§ Intracellular ^{3}H concentrations were scored as: heavy = >20 ^{3}H-exposed emulsion grains/cell; moderate = 10–20 grains; light = 3–10 grains.

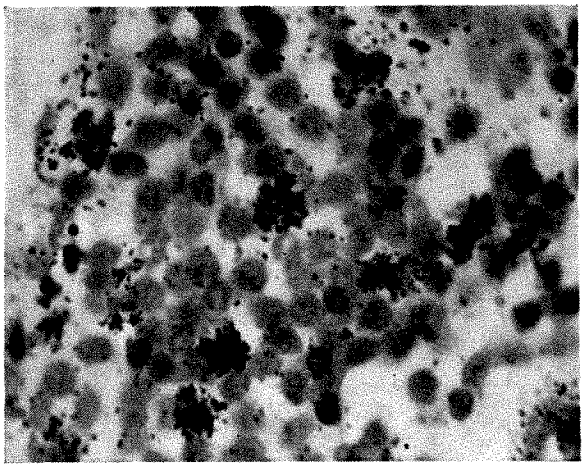


Fig. 1. Autoradiograph of right ventral axillary node section 1 h after ^{3}H-antigen injection into right hind footpad. Note the concentration and intra-cytoplasmic localization of tritium in the eight heavily and moderately labelled eosinophils. (×c. 667)

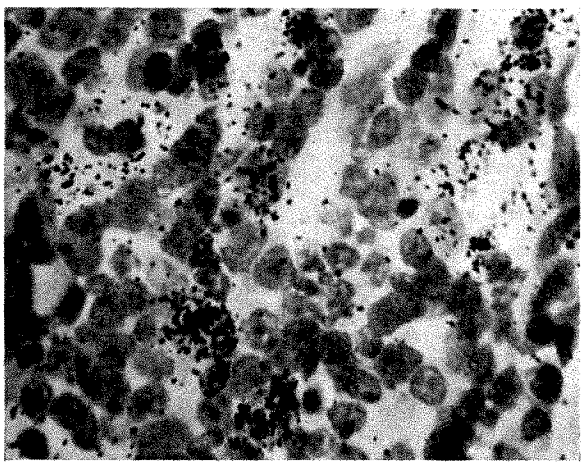


Fig. 2. Autoradiograph of right inguinal node section 3 h after ^{3}H-antigen injection into right hind footpad. This shows the distribution of eight heavily tritiated eosinophils in areas of unlabelled lymphoid cells (×c. 667)

^{3}H-labelled mononuclear eosinophils were observed in the lymph nodes, but these immature cell forms were never prevalent.

From 1–3 h after injection, the labelled eosinophils were scattered in the medullary regions, interfollicular cortex and polar regions of the nodes. At 3 h, labelled eosinophils were distributed in areas of heavily labelled macrophages. No distinct association was observed, however, between the two cell types[14]. Many heavily labelled eosinophils were also scattered generously in areas of unlabelled lymphoid cells (Fig. 2). At 4, 6 and 7 h, large clusters of heavily labelled eosinophils were demonstrated in the right inguinal and right ventral [illegible]llary nodes. These clusters of thirty to fifty cells [illegible]od in the interfollicular medullary areas, inter-[illegible]ortex and polar regions of the nodes. The 8-h [illegible]ode sections presented a similar appearance, [illegible]ers of heavily tritiated eosinophils localized [illegible]follicular medullary areas (Fig. 3). Although [illegible]counts in the three nodes had returned to [illegible] 16 and 24 h, the tissue localization of the [illegible]was unchanged.

[illegible]nice in groups 1 and 3 contained no [illegible]s specific for the sulphanilazo deter-[illegible]arrier protein when tested by the [illegible]tination methods. Sera from group 2 mice yielded negative haemagglutination results on days 1–6 after antigenic stimulation. The three mouse sera studied on day 7 demonstrated low antibody titres of 1 : 8 to 1 : 16 against the sulphanilazo determinant and remained negative (< 1 : 2) for the carrier protein. These serological results indicated a primary immune response.

The present investigations combined the specificity of a heavily tritiated antigen (175 μc./mg) labelled only in the determinant group, the autoradiographic sensitivity of ^{3}H localization and a regional primary response stimulation. This allowed us to quantitate early antigen incorporation by lymph node eosinophils. The demonstration of ^{3}H-antigen in eosinophils contrasts with the results of other investigations on antigen localization[2–4,10,15] and experimental eosinophilia[1,5–7]. Previous investigations did not reveal antigen incorporation by eosinophils following the intravenous injection of ^{3}H-aniline-azoprotein into mice[10]. The absence of ^{3}H-labelled eosinophils in sites of antigen deposition could have resulted from the low radioactivity of the antigen (64 μc./mg), the route of injection and the lack of observations within the first 24 h. Studies on the localization of tritiated tetanus toxin in mouse peritoneal exudate cells failed to detect the direct incorporation of ^{3}H-toxin or ^{3}H-toxin-antitoxin complexes by eosinophils[2–4]. However, this lightly labelled toxin (2 μc./mg) provided very low sensitivity for autoradiographic observations. Nossal *et al.*[15,16] observed the cellular localization of iodine-125 and iodine-131 labelled antigens following a primary response stimulation in the footpads of rats. Although autoradiographs of draining lymph nodes did not reveal antigen incorporation by eosinophils, the use of the methyl green-pyronin stain for all histological preparations could have hindered recognition of these cells.

In this investigation, the appearance of eosinophilia in lymph nodes draining the sites of antigen injection correlates with the results of Litt[1,5,6]. Previous observations on experimental eosinophilia indicated, however, that antigen-antibody complexes were the prominent mediators of the eosinophil response[1,5–7]. *In vivo* and *in vitro* investigations of the interaction of cells with fluorescent-tagged antigen, antibody and antigen-antibody complexes detected the intracytoplasmic localization of only the fluorescent immune complexes in eosinophils[7]. From these results, Litt[1,6,7] proposed that following a primary antigenic stimulus, the draining lymph node responds with the very early formation of antibody which complexes with antigen and subsequently stimulates eosinophilia. Recent indirect evidence has suggested that antibody formation begins within 24 h following a primary

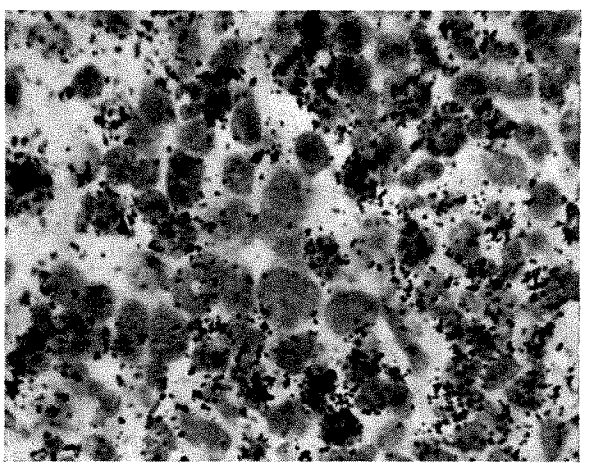


Fig. 3. Autoradiograph of right popliteal node section 8 h after ^{3}H-antigen injection into right hind footpad. A large cluster of heavily labelled eosinophils in the interfollicular medullary area. (× c. 667)

response stimulation[17]. However, previous observations by Nossal *et al.*[18] did not detect significant antibody formation prior to day 3 with the highly sensitive immune adherence phenomenon.

The present investigations have demonstrated the rapid uptake of ^{3}H-antigen (or antigen fragments containing the ^{3}H-sulphanilazo determinant groups) by eosinophils and the subsequent eosinophilia in draining lymph nodes within 4 h of primary antigenic stimulation. The early, heavy accumulation of ^{3}H in eosinophils indicates that the soluble, heterologous protein induced the eosinophil responses in the absence of specific antibody. It is unlikely that antibodies were synthesized and released from lymph node cells in quantities sufficient to complex with the ^{3}H-antigen prior to its incorporation by eosinophils. These results suggest, therefore, an important function of eosinophils in mechanisms of the primary immune response.

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NEURONAL MODELS FOR CONDITIONED REFLEXES

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THE large number of neural models of learning[1–9] is a reflexion of our lack of understanding of the learning process at the cellular level. Nevertheless, model-building may be of value, particularly if the model is sufficiently precise to be tested experimentally. The argument for making it precise rests on the assumption that learning involves a change in the quality of synaptic transmission at specific synapses. In this discussion attention will be directed mainly to the classical conditioned reflex[10].

The essential change occurring in the formation of a classical conditioned reflex is that a given stimulus now produces a certain response whereas previously it did not do so. In neuronal terms, somewhere along the chain of neurones connecting stimulus receptor to effector facilitation has occurred. The facilitation arises because of a temporal association between the unconditioned stimulus (UCS) and the test stimulus (CS). Although the conditioned response (CR) eventually obtained to CS is usually not identical with that resulting from the UCS (UCR), it is clear that at least some of the effector pathways must be common to the two reflexes. In other words, there must be convergence of the CS and UCS at some point in the chain.

Because of the extremely large number of conditioned reflexes which can be formed, in the primate at least, it is improbable that there is a growth of nerve terminals under specific chemotactic influences. Because of the speed with which conditioned reflexes can be established it is unlikely that there is any extensive growth of nerve terminals. The assumption is made here that all pathways are laid down by early life and that learning consists in selective facilitation of certain synapses already present.

Fig. 1*a* summarizes the situation described here. However, this model is inadequate and in conflict with accepted concepts. It is inadequate because a prediction from the model is that learning should occur by simple contiguity of CS and UCS (in the absence of reinforcement), but it is doubtful if this ever occurs. The model also conflicts with our concept of the independence of afferent terminals on a cell. Phenomena such as post-tetanic potentiation, once considered to provide a basis for learning, seem to be confined to the pathway being tetanized.

A model which overcomes the second objection is shown in Fig. 1*b*. For the purpose of these diagrams the convention is adopted that any cell is discharged by simultaneous activity in two terminal knobs but activity in one is not enough, unless it is 'facilitated'. Thus B is discharged only by concurrent action of CS and UCS. B synapses with a CS terminal on A and discharge in B produces presynaptic facilitation of the terminal so that after a certain number of CS/UCS pairings CS alone will fire A. However, this model is also unsatisfactory as an explanation of permanent learning. If CS is given without UCS, B does not fire, facilitation gradually disappears and CS becomes ineffective.

The model in Fig. 1*c* includes the element of reinforcement which seems to be necessary for successful conditioning. The model functions as follows. Neurone C, discharged only by concurrent action of CS and UCS, is capable of activating A, leading to the response. Neurone B is discharged by concurrent activity in C and in some reinforcement pathway. B feeds back on to a CS terminal to produce presynaptic facilitation. After an appropriate number of CS/UCS pairings, the degree of facilitation is adequate to allow CS alone to discharge neurone C and thus neurone A and so to elicit the response. If reinforcement is withdrawn, neurone B is not discharged and there is no further facilitation, so that eventually CS fails and the conditioned reflex is extinguished. Differences between CR and UCR can be accounted for by assuming different degrees of facilitation at individual synapses.

The diagram is for positive reinforcement (reward or avoidance of injury) but could be the same for negative reinforcement (injury) if the action of B on the CS terminal is inhibitory. The model is the simplest which incorporates reinforcement. The axon of B is shown forming an axo-axonal contact with the CS terminal on C. It does not synapse with the cell-body of the CS terminal because this would not give facilitation at specific nerve terminals. Axo-axonal synapses have been reported in various parts of the central nervous system[11] and so far it has been assumed that they subserve a process of presynaptic inhibition[11]. This assumption should be re-examined. Presynaptic hyperpolarization has been demonstrated in the mammalian spinal cord[12] and a process of presynaptic facilitation has been postulated at the giant cell of the abdominal ganglion of the sea slug[13,14].

In the process of presynaptic inhibition a depolarization of afferent terminals occurs[11]. One might imagine that pre-

synaptic facilitation would involve a hyperpolarization of the terminals; however, an action potential falling during the active phase of such a hyperpolarization would tend to be inhibited rather than enhanced. Since a conditioned reflex, once established, may persist for years in the absence of CS, the process of facilitation must involve more than a mere change of membrane potential. It therefore seems probable that the action of neurone *B* on the CS terminal concerns not only membrane potential changes but also the synthesis and storage of transmitter by that terminal.

We may suppose that facilitation involves both stimulation of synthesizing processes and an increased store of transmitter. If no presentation of CS is made the store remains unchanged. If CS is presented without reinforcement the store is depleted, but the facilitated synthesizing processes partially restore the level so giving 'spontaneous recovery'. Eventually the absence of reinforcement will lead to a depression of the synthesizing process.

Experimentally, CS must be given slightly before UCS or overlapping in time. Therefore it must be assumed that the CS pathway to *C* involves a longer and more complex route than UCS so that there will be synchronous bombardment of neurones *C* and *B*. 'Orientation' may provide a background depolarization of all *C* neurones non-specifically but decrementing (habituation) unless facilitated.

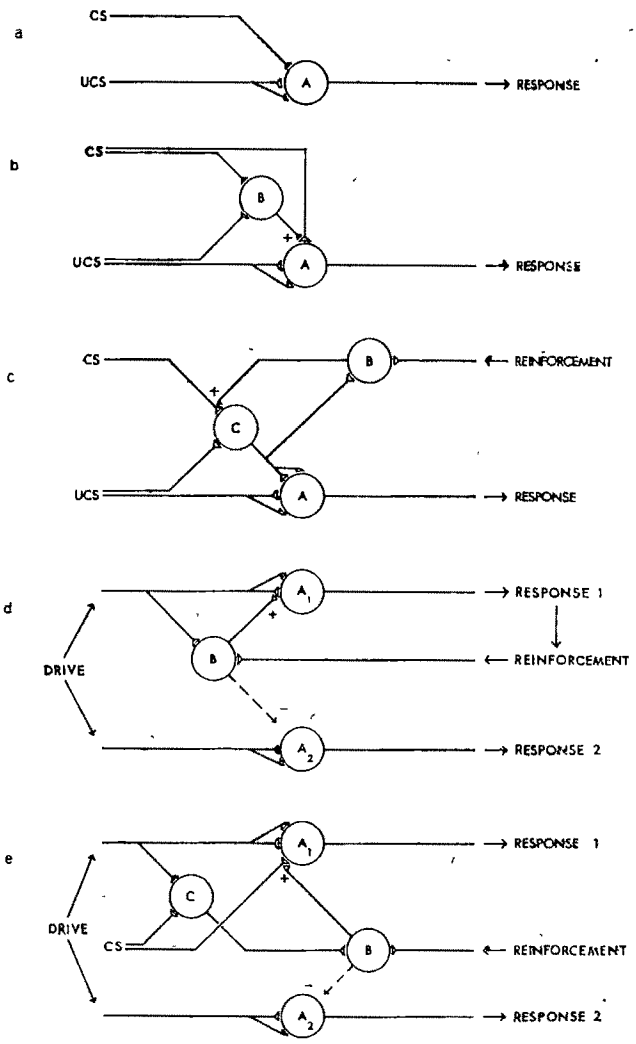


Fig. 1 Possible models for conditioned reflexes In all cases the convention is that the cell-body (represented as a circle) is discharged only by concomitant activity in two terminals; a single terminal is ineffective unless 'facilitated'. CS, Conditioned stimulus. UCS, Unconditioned stimulus +, Facilitation. −, Inhibition. *a*, *b*, *c*, Models for classical conditioning. *d*, *e*, Models for instrumental conditioning. Further explanation in text

'Backwards conditioning' in which UCS precedes CS is much less effective because UCS activity is finished by the time CS arrives at *C*. It may be necessary, however, to suppose that UCS or UCR prevents CS reaching *C*. It is also necessary to suppose that *C* cells, once they discharge, continue to fire for some time, at least seconds, without further activation by CS and UCS. 'Disinhibition' (a strengthening of the response during extinction as a result of applying a new stimulus) may operate in a similar way to orientation, that is by a generalized depolarization of *C* neurones.

An important inference from the model is that neurones *B* and *C* are private to the particular reflex set up. In other words, these neurones might never function in the entire life of the individual. They may be allowed only low-frequency 'spontaneous' activity. This idea may not be so improbable. Learning ability in different species seems to be a function of the number of neurones available; at least in the mammalian central nervous system the numbers involved are extremely large. Furthermore, because of higher-order conditioning and stimulus generalization, *B* and *C* neurones may participate in many different reflexes. It would also seem to be necessary that any given *B* neurone be reciprocally connected with a *C* neurone in the manner shown, and that *A* and *C* neurones lie close together so that there will be a high probability of UCS innervating *C* neurones which project to *A* neurones also innervated by UCS. Any departure from strict correspondence of innervation will permit a stimulus generalization, assuming that there is an orderly topographical projection within any given modality. Conversely, the more precise the correspondence of innervation the finer will be the discrimination. Thus, parts of the central nervous system may consist of aggregates of *A*, *B* and *C* neurones; in vertebrates the reticular formation[15–18] and parts of the cerebral cortex are possible sites although axo-axonal contacts seem to be absent in the cerebral cortex[19].

Figs. 1*d* and 1*e* show models for instrumental conditioned reflexes[10], for the free-responding and signalled situations respectively. For the establishment of any conditioned reflex, classical or instrumental, 'drive' ('motivation') seems to be necessary. 'Drive' includes already established unconditioned and conditioned reflexes, for example, hunger and fear. In any given situation 'drive' leads to a limited number of responses. In Figs. 1*d* and 1*e* only two possibilities are shown. In the free-responding situation the 'correct' response produces facilitation of synapses in the neuronal pathway leading to that response and inhibition of other responses. The mechanism for inhibition is not shown but might be a form of presynaptic inhibition. Fig. 1*e* shows a possible circuit for a situation in which a particular stimulus (CS) is the signal for a certain response, only the combination of CS and correct response being reinforced.

For 'stimulus–stimulus' learning it must be supposed that, although there is no overt response, the combination of two stimuli leads to a reinforcement, for example, intellectual satisfaction, which feeds back and facilitates certain synapses coupling the two stimuli together in some way.

There is not space here to discuss all the features of conditioned reflexes in terms of the models. For many of these features, for example, external inhibition, additional circuitry is necessary. It may be objected that the models contain no inhibitory pathways whereas the evidence for these is strong; the models, however, provide a core around which accessory features can be built. It is a reasonable assumption that the basic mechanism for learning will be the same for all organisms possessing nervous system. If this is so, it may be most rewarding to study the simplest organisms. The recent demonstration[20] in *Aplysia* of heterosynaptic facilitation at the cellular level produced by a classical conditioning procedure is most encouraging.

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FUNCTION OF NEUROSECRETION IN ENDOCRINE REGULATION

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THE theory of neurosecretion has undergone modification during the past decade, and it is perhaps now time to restate the original postulate in slightly different terms[1]. In his last major paper, the late Ernst Scharrer[2] began a reconsideration of the contribution of the nervous system to endocrine regulation. In extending the re-estimation of the role of neurosecretion, we wish to continue the distinction between the endocrine role characteristic of neurosecretory neurones and the secretory activity characteristic of neurones generally[3,4]. Although secretion is a general property of neurones at the cellular level, at the level of the organism neurosecretion occupies a specific and central position in the developing field of neuroendocrinology.

At the beginning, the neurosecretion theory was based on the selective staining of secretory materials within some neurones of the central nervous system. The quantity of secretory material thus demonstrated was large, and changes in the amount and nature of the stainable inclusions often suggested the existence of secretory cycles as in gland cells. Later these neurones were associated with the production of hormones (for example, antidiuretic hormone in vertebrates, chromactivating hormones in crustaceans, prothoracotropic brain hormone in insects). Later, the neurohaemal concept[5] provided a useful criterion for assessing whether a neurone was truly neurosecretory. According to the neurohaemal concept, neurosecretory neurones are engaged in hormonogenesis and liberate their products into the blood stream at a neurohaemal organ.

They are thus distinguishable from neurones engaged in transmitter activity. This definition of neurosecretion has been widely accepted[6]. Recent investigations with the electron microscope, however, indicate that an autochthonous endocrine activity may not be the sole function of neurosecretion, and that the concept of neurosecretion should be extended to include other aspects of neuroendocrine regulation besides the production of blood-borne hormones. The present communication attempts to develop the concept of neurosecretion in the light of recent discoveries.

The neurohaemal concept implied the absence of transmitter activity of neurosecretory neurones and thus of synaptic termination. Some neurosecretory axons, however, would seem to make contacts with endocrine cells, notably in the corpora allata of insects[7] and in the pars intermedia of the vertebrate pituitary[8–10]. The physiology of these junctions has not yet been studied, but morphologically many of the contacts described resemble ordinary secretomotor junctions between nerve fibres and gland cells. A close association between neurosecretory fibres and ependymal cells has also been demonstrated[11], and junctions between neurosecretory fibres and ependyma-like pituicytes, with the morphological features of synapses, have been seen[12]. Contacts such as have been observed raise the possibility that some ependymal cells, functionally related to neurosecretory systems, might have a humoral function concerned with communication within the central nervous system[13]. It is evident that discharge of hormones into the blood stream can no longer be considered as the sole function of neurosecretory neurones.

During the past decade certain staining techniques, notably the Gomori chrome-alum-haematoxylin (CAH) method, the Gabe paraldehyde fuchsin method and the Adams-Sloper alcian blue technique, have proved useful for the recognition of neurosecretory systems. Indeed the term 'Gomori-positive' has often been used to denote material stained blue by the basic component of the CAH method, and 'Gomori-negative' to specify material coloured red by the acid component. Although these terms have a certain convenience and persist in the literature it should be recognized that the foregoing staining methods are not sufficiently specific to be diagnostic of neurosecretory systems, and especially that the term Gomori-negative is confusing since it could be interpreted as a failure to stain with either component of the Gomori CAH method.

A brief survey of the findings by workers who have used the Gomori CAH method to identify neurosecretory systems will show how varied is the staining reaction of neurosecretory neurones. Among those which stain with the basic stain are: (1) the pars intercerebralis–corpus cardiacum–corpus allatum system of insects; (2) the eyestalk and other neurosecretory systems in crustaceans; (3) the principal hypothalamus–pars nervosa system of higher vertebrates; (4) elements in the innervation of the pars intermedia of lower vertebrates.

Some other neurosecretory systems, however, are stained by the acid component of the Gomori technique, notably: (i) the hypothalamic nucleus lateralis tuberis innervating the pars distalis of teleost fishes[14]; (ii) some elements in the pars intermedia of elasmobranch fishes[8,9]; (iii) neurosecretory cells in the brains of many inverte-

brates. Yet other neurosecretory systems are relatively unreactive to the Gomori CAH technique, notably: (*a*) the caudal neurosecretory system of some fishes[15]; (*b*) neurosecretory neurones in the median eminence of tetrapods[16].

Most but not all of the foregoing neurosecretory systems are equipped with elementary granules on the ultrastructural level. The typical elementary neurosecretory granule, electron-dense and measuring 1000–3000 Å in diameter, originally described by Bargmann and by Palay[17], is characteristic of most established neurosecretory systems where hormonogenesis is unquestionably involved. However, these granules are apparently not diagnostic of every neurosecretory system.

The median eminence of the amniote neuro-hypophysis, for example, shows vesicles and granules which more frequently resemble those associated with cholinergic and adrenergic nerve endings[16]. It has been suggested that two biochemically distinct groups of neurosecretory products—peptide and non-peptide respectively—may be represented in neurosecretory systems[18]. It appears evident that more than one kind of neurosecretory system may be concerned in the regulation of other endocrine activity. Whereas the '*A*' fibres in contact with pars intermedia cells in elasmobranchs contain typical elementary neurosecretory granules of diameter of about 1800 Å, the '*B*' fibres (with vesicles of diameter of about 700 Å) resemble adrenergic fibres[8]. '*B*' type fibres also form the major part of the innervation of the pars distalis of some teleosts[13].

Neurosecretory fibres, then, may influence endocrine gland cells, either by blood-borne hormones, or through neurosecretomotor junctions morphologically not unlike typical synaptic connexions, but possibly involving 'transmitters' with special biochemical and physiological features. Another possibility can also be envisaged, namely, the release of chemical agents from what might be termed a neurosecretory neuropile[19]. Such agents would reach adjacent glandular tissue by diffusion and exert control in a manner indistinguishable from that of blood-borne hormones. The concept of 'chemical addressing'[20], which in this case means the development of specific tissue sensitivity to a diffusing neurohormone, can thus be extended to this direct neurosecretory influence.

The present evidence indicates, therefore, a diversity in neurosecretory form and function, in the spatial relations between neurosecretory neurones and other tissues, and in the biochemical nature of the neurosecretory hormones. Yet it is possible to recognize one unifying feature of neurosecretion, namely that all the neurosecretory neurones specified above participate either directly or indirectly in endocrine control and form all or part of an endocrine organ. They may directly control bodily function by releasing hormones into blood vessels or into a haemocoel (or even into the cerebrospinal fluid[11,13,21]) to regulate particular activities (for example, water balance in vertebrates, colour change in crustaceans, tanning in insects). In other instances the action of the neurosecretory neurones is indirect and involves control of other endocrine tissues. This control may be exerted through the systemic circulation at some distance from the point of liberation of the neurosecretory hormone (for example, the prothoracotropic 'brain hormone' of insects, the moult-inhibiting hormone of crustaceans), through a short special circulatory pathway (for example, the portal transport of adenohypophysial releasers and inhibitors), through diffusion from a neurosecretory neuropile, or through direct neurosecretory contact (for example, with the pars intermedia of lower vertebrates, with the corpus allatum and the intrinsic cells of the corpus cardiacum in insects).

One striking feature of neurosecretory systems is their ability to sustain an inhibition or excitation of endocrine tissue or other effector organs. The pars intermedia of fishes and amphibians and the corpora allata of insects appear to be under a sustained inhibitory control; the control of colour change and water balance requires a regulatory system which can maintain a steady stimulus without fatigue. This temporal distinction between neurosecretory and non-neurosecretory neurones would seem to be fundamental; neurosecretory control can be viewed as 'tonic', depending on a slow continous release of a slowly degraded product. In analysing the nature of the physiological contacts between neurosecretory fibres and endocrine cells, this special temporal feature needs to be considered; it has been suggested, moreover, that different forms of neurosecretory control of endocrine function might be distinguishable on a temporal basis[8].

It must be admitted that there are still problematical areas in the delineation of neurosecretion. For example, the adrenal medulla (chromaffin) cells receive cholinergic innervation from preganglionic fibres of the sympathetic nervous system, which are therefore indirectly engaged in endocrine control. The frequently reported occurrence of stainable 'neurosecretory' fibres extending to non-neurohaemal and non-endocrine tissues also requires further investigation. However, controversies as to the possible neurosecretory nature of neurones do become simplified for the time being by our suggested definition that neurosecretory neurones are engaged directly or indirectly in endocrine control and may form all or part of an endocrine organ.

According to this concept, nerve fibres which innervate muscles or exocrine glands would not be classified as neurosecretory, even if they possess special staining properties or contain granules resembling those of neurosecretory cells. In our opinion the finding, by itself, of electron-dense granules at the level of ultrastructure cannot at the present time be more diagnostic of neurosecretion than is the presence of droplets staining by the Gomori or other 'neurosecretory' stains.

Morphologically, neurosecretory neurones combine neuronal and glandular characteristics. Physiologically also they provide an essential link between the nervous and endocrine systems. As such they provide the final common pathway for neuroendocrine regulation[2].

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EFFLUX OF PROSTAGLANDIN FROM ADRENAL GLANDS STIMULATED WITH ACETYLCHOLINE

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THE prostaglandins, a group of pharmacologically active, unsaturated hydroxy-carboxylic acids, have been found in a variety of tissues in many different species, but their physiological significance is uncertain[1,2]. The fact that these acids have been observed to escape from various tissues on nervous stimulation[3–5] led us to suspect that their discharge could be related to the release of neurohumours. This conjecture prompted us to test whether prostaglandin might escape during release of catecholamines from the chromaffin cells of the adrenal medulla which are developmentally related to sympathetic nerves and offer the experimental advantage of a more massive secretory activity. Our results show that prostaglandin $F_{1\alpha}$ appears in the venous effluent from perfused cats' adrenal glands when catecholamine secretion is evoked by acetylcholine (ACh).

Cats' adrenal glands, acutely denervated, were perfused *in situ* with Locke's solution by the method previously described[6]. Venous effluents were collected from one or both glands. Acetylcholine (10^{-5} g/ml.) was introduced for 20–40 sec five to ten times at 6-min intervals and the effluents collected during the stimulation periods were pooled. Effluents collected during the 5–10 min period preceding the first exposure to acetylcholine in each experiment served as controls. Catecholamines were measured fluorimetrically[7]. The prostaglandin content of control glands was determined after brief perfusion with Locke's solution, and that of stimulated glands after the aforementioned treatment with ACh. The prostaglandin content of effluents and glands was determined as follows: the effluents were acidified to *p*H 2 and extracted with petroleum ether followed with diethyl ether and the diethyl extracts were dried and biossayed on the rat uterus[3]. The glands were homogenized in saline and the homogenates extracted and assayed as above except that extraction was preceded by three washes each with equal volumes of petroleum ether and diethyl ether at *p*H 7·0 to remove substances likely to interfere with the bioassay. Control experiments with prostaglandin E_1 (PGE_1) or prostaglandin $F_{1\alpha}$ (PG$F_{1\alpha}$) showed that 80–90 per cent of these substances were recovered in the acid–diethyl ether extracts. Acetylcholine, catecholamines and other polar substances are not extracted by the procedure used and thus did not interfere with the bioassay; interference from spasmogenic unsubstituted C_{18} and C_{20} carboxylic acids and phospholipids was eliminated due to their solubility in acid–petroleum ether. When acid–diethyl ether extracts contained oxytocic activity equivalent to 100 ng or more of PG$F_{1\alpha}$, thin-layer chromatography in the *A*I and *A*II systems of Gréen and Samuelsson[8] was performed as described previously[4]. In all experiments the R_F of the oxytocic activity obtained from the adrenal glands and effluents after stimulation of the glands with acetylcholine coincided with the R_F of the PGF compounds in the *A*I system and PG$F_{1\alpha}$ in the *A*II system; PGE_1, PGE_2, PG$F_{1\alpha}$, and PG$F_{2\alpha}$ were used as standards. No evidence for the presence of prostaglandins other than PG$F_{1\alpha}$ was obtained. PG$F_{1\alpha}$ was therefore used as the standard in all assays, and results are expressed in terms of PG$F_{1\alpha}$ equivalents. Since the number of glands used in different experiments varied, all values for prostaglandin or catecholamines in perfusates or homogenates are expressed in units per gland used.

In the absence of stimulation, prostaglandin was detected in only two of seventeen samples of venous effluent collected from eleven different adrenal preparations. These two samples contained the equivalent of 12·5 and 18·8 ng PG$F_{1\alpha}$/gland perfused. The corresponding values for catecholamine output, 0·075 and 0·25 μg/gland perfused, were within the normal range found in effluents from unstimulated glands. In six preparations ACh (10^{-5} g/ml.) was added to the perfusion medium for periods from 100 sec to 7 min and increased catecholamine secretion to high levels (37·7–175 μg/gland perfused). In each instance prostaglandin was detected in the effluent: the range was 18·8–250 ng PG$F_{1\alpha}$/gland perfused (Table 1).

Table 1. STIMULANT EFFECT OF ACETYLCHOLINE ON EFFLUX OF CATECHOLAMINES AND PROSTAGLANDIN FROM PERFUSED ADRENAL GLANDS

Experiment No.	Spontaneous release: Catecholamines (μg/gland)	Spontaneous release: PG$F_{1\alpha}$ equivalents (ng/gland)	ACh-evoked release: Catecholamines (μg/gland)	ACh-evoked release: PG$F_{1\alpha}$ equivalents (ng/gland)
(1)	0·58	< 5	37·7	30
(2)	0·39	< 10	67·9	250
(3)	0·25	< 5	148	100
(4)	0·80	< 2·5	175	18·8
(5)	0·25	18·8	97	33
(6)	0·17	< 10	140	250

Since catecholamines have been found to release prostaglandins from some tissues[3], experiments were performed to determine whether the efflux of PG$F_{1\alpha}$ from the adrenal gland during exposure to ACh was the result of some action of the catecholamines that were released. Six preparations were perfused with epinephrine, norepinephrine, or mixtures of equal parts of the two, in a concentration (50 μg/ml.) which exceeded that found in the venous effluents from adrenal glands stimulated with ACh. No prostaglandin was detected in the effluents except in the one experiment where prostaglandin was present in the effluent obtained during the preceding control period.

Prostaglandin was found in adrenal glands in each of eleven experiments: The mean content was 15·5 ng PG$F_{1\alpha}$/gland. This is a much smaller amount of prostaglandin than was found in the venous effluents (mean = 114 ng PG$F_{1\alpha}$/gland perfused). The prostaglandin content of ACh-stimulated glands did not differ significantly from that of unstimulated glands (Table 2). In four experiments cortices and medullae were assayed

Table 2. PROSTAGLANDIN CONTENT OF ADRENAL GLANDS

Experimental treatment	PG$F_{1\alpha}$ equivalents (ng): Medulla	Cortex	Total	Total/gland
Control	15	100	115	28·8
Control	< 20	50	50–70	12·4–17·5
Control	—	—	12·5	6·25
Control	—	—	15	7·5
ACh†	< 10	50	50–60	25–30
ACh	10	30	40	20
ACh	—	—	25	12·5
ACh	—	—	12·5	6·25
ACh	—	—	12·5	6·25
ACh*	—	—	60	30
ACh	—	—	30	15

Two glands were used for each measurement except in each of the first two (control) experiments where four glands were used. The glands designated by the symbols † and * were obtained from the animals used in experiments 5 and 6, respectively, of Table 1.

separately: the bulk of the prostaglandin was present in the cortex, where its concentration ranged from 115 to 197 ng $PGF_{1\alpha}$/g. The concentration of prostaglandin in the medulla in the two experiments where values were obtained was higher: 370 and 430 ng $PGF_{1\alpha}$/g.

Previous experiments have shown that analysis of the venous effluent from the adrenal gland for substances other than the released catecholamines is a useful means of analysing cellular events involved in catecholamine extrusion. Thus the finding that the catecholamines in the effluent are accompanied by equivalent amounts of ATP metabolites indicates that the immediate source of these amines is the nucleotide-rich chromaffin granules[9,10]; and the demonstration that massive amounts of ATP may escape unhydrolysed in appropriate conditions shows that hydrolysis of the ATP within the chromaffin granules is not a critical step in catecholamine release[11]. Furthermore, these results, as well as the detection in the adrenal effluent of protein characteristic of the chromaffin granules[12], lend support on chemical lines to the view advanced on electron-microscopical grounds that the chromaffin granules empty directly to the cell exterior by reverse pinocytosis or some related process[13,14].

The present finding that prostaglandin escapes from the adrenal gland during exposure to ACh—but not when catecholamines are perfused through the gland—suggests that the efflux of these lipid acids may offer an additional clue to the mechanism of medullary secretion. Since the mechanism of catecholamine secretion described here must involve momentary disruption of the membranes of the chromaffin granule and the chromaffin cell, it is conceivable that prostaglandin could be formed from breakdown of these lipid membranes. That prostaglandin is indeed formed during the action of ACh is indicated by the fact that the amount of prostaglandin escaping on stimulation greatly exceeded the amount endogenous to the adrenal glands.

It seems pertinent that the formation and escape of a group of spasmogenic acid lipids (SRS; slow reacting substance) closely related to prostaglandins[15] also occurs during the release of another biogenic amine, histamine; and, moreover, that this effect has been attributed to phospholipase *A* activity[16,17]. Possibly ACh activates an endogenous phospholipase *A* in the adrenal gland which splits off prostaglandin from membranes and thus allows catecholamines to escape. Although there is, as yet, no evidence that prostaglandins are incorporated in phospholipids and can be split off by the activity of such an enzyme, the postulate that stimulation with ACh involves activation of an endogenous phospholipase *A* is consistent with the following facts: first, that phospholipase *A* activity is reportedly present in adrenal glands[18]; second, that most preparations of phospholipase *A* are activated by calcium which is believed to mediate the stimulant effect of acetylcholine on catecholamine secretion[6,19,20]; and third, that a possible end-product of phospholipase *A* activity, lysolecithin, has been detected in the adrenal medullae of cattle[21] and cats and dogs[22]. This line of speculation is, of course, based on the assumption that it is from the medulla, the classical site of action of ACh, that the prostaglandin escaped. However, our results do not exclude the possibility that some other action of ACh within the gland is responsible, for example an action on cortical cells[23].

Finally, our conjecture that prostaglandin release may be an accompaniment of secretory activity in the adrenal gland offers a possible explanation for the occurrence of prostaglandin in other systems. Thus nerves, the stimulation of which also results in prostaglandin release, are cells modified to 'secrete' in a specialized way; furthermore, the classical source of prostaglandin, the prostate, is an exocrine gland. Perhaps prostaglandin formation is a common accompaniment of secretory activity.

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AUTORADIOGRAPHY *IN VIVO* OF HUMAN CANCERS

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AUTORADIOGRAPHY in the human *in vivo* has only rarely been carried out[1,2], because the doses necessary for satisfactory results are dangerous to the patient. We have found an effective isolated isotopic perfusion technique[3] which allows us to decrease the quantity of circulating radioactivity so that the investigation becomes innocuous to the patient. We have applied this method of autoradiography *in vivo* to patients with a variety of cancers in order to investigate the precise kinetics of malignant human cells.

The life of a cell can be divided into four periods: (1) Cell division by mitosis or amitosis over a period of 30–60 min. (2) First resting phase (G_1) which varies between several hours and several years depending on the type of cell. The cell matures and its physiology is characterized. (3) A 6–10-h phase of synthesis (S), during which the cell doubles its DNA reserve. (4) Second resting phase (G_2) of 2–6 h, at the end of which the cell division begins and the cycle begins anew[4].

We introduced a radioactive DNA precursor during the synthesis phase in order to study this period and the second resting phase (S and G_2). The DNA molecule is composed of two purine bases (adenine and guanine), two pyrimidine bases (cytosine and thymine), a pentose (desoxyribose), and inorganic phosphates. If the synthetizing cells are tagged with tritiated thymidine or ^{32}P, the newly formed DNA will incorporate the isotope. The autoradiographic technique will then identify the labelled cells.

We studied five cancers of the colon, one of which had metastasized to the liver and the periaortic lymph nodes; five gastric cancers, two of which had metastases; one uterine paramalpighian carcinoma; one metastatic adrenal gland from a primary digestive cancer; and three osteosarcomata, two of which were from patients with Paget's disease.

This procedure was incorporated into the surgery of the cancer and did not cause the patient any additional ordeal. The vascularizing artery of the pedicle of the tumour is isolated and then denuded and cannulated with a No. 1 or 2 cannula which is tied in place. The normal arterial flow and venous drainage are not disturbed. A 1-h perfusion of 100 c.c. of isotonic saline containing 0·5–1 mc. of tritiated thymidine or ^{32}P (specific activity 2·7 c./mmole) is begun. Sometimes at the beginning of the perfusion a slightly increased perfusion pressure is necessary. This procedure varies with the type of tumour. In carcinomata of the digestive tract, the tumour is completely isolated except for the vascular pedicle (vein and artery) to be perfused. The pyloric pedicle is chosen for the stomach, the inferior mesenteric pedicle for the descending colon and rectum. The tumour is resected and the continuity re-established. Perfusion through the vascular pedicle in bone tumours is followed by resection, amputation, or disarticulation as the case may be. In this case arteriographic verification is sometimes necessary.

Six to ten samples are taken from different zones (with two control pieces from normal tissue) for histological examination. The pieces are fixed in Bouin's solution, passed through alcohols, and embedded in paraffin. For best results the sections must be as thin as possible; sections 2·5–5μ in thickness give best results. The sections are deparaffined and placed in running tap water. After this, all subsequent operations are carried out in darkness.

An emulsion of Ilford '*NRE* K_2' is diluted in the proportion of one part emulsion to 10 parts distilled water. This is kept in a water-bath at 40° C. The slides are 'dipped' in this emulsion for 5 sec, then drained in the vertical position for 1 min. They are then placed in a black box, which is in turn placed into a second black box. The boxes are refrigerated at 4° C for 14 days when ^{32}P is the isotope; and for a period of 40 days when the tissues were prepared with tritiated thymidine. At the end of the cold-storage period the slides are developed in Kodak '*D* 76' during an 8-min period. They are fixed in a 10 per cent hyposulphite bath for 15 min. Finally, they are washed in running water for a minimum of 20 min.

The primary interest of this procedure lies in the possibility of combining the standard histological staining methods with autoradiography, thereby permitting a precise study of the pathological microstructure of a malignant tumour including changes in the points of reference normally found in non-cancerous tissue.

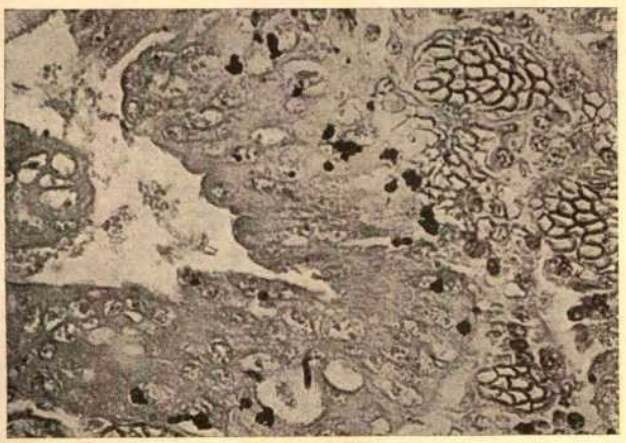


Fig. 1. Gastric carcinoma, basal and axial labelled cells. Blood vessels on the right. (×300)

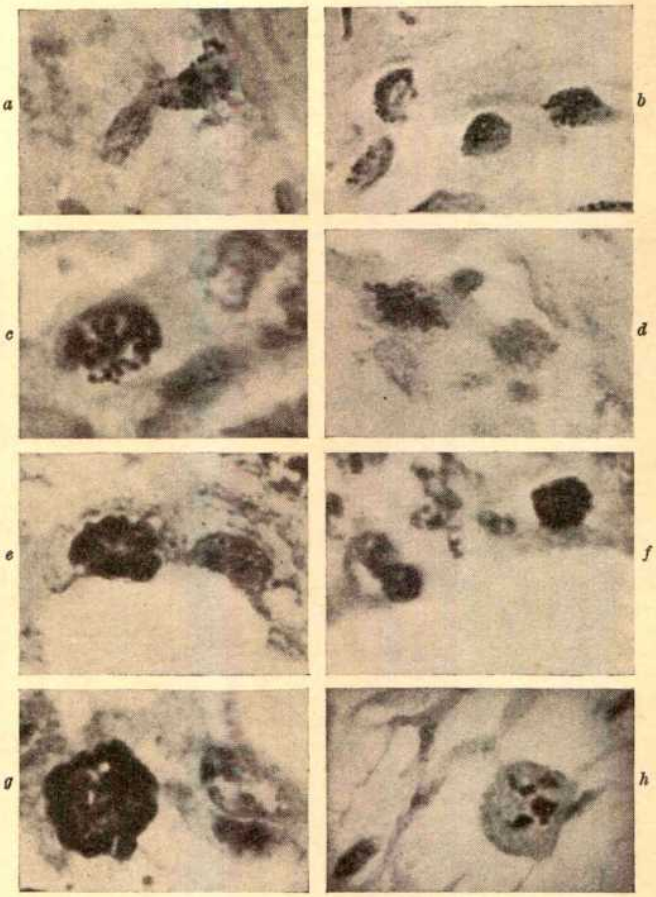


Fig. 2. (*a*) Gastric carcinoma (stroma); (*b*) osteosarcoma (elegant argyrophilic granulations) (×550); (*c*) cancer of colon, confluent silver granules (×700); (*d*) osteosarcoma, confluent silver granules (×700); (*e*) gastric carcinoma, a 'diamond-studded ring' nucleus (×700); (*f*) cancer of colon, flecked labelling in two nuclei (×700); (*g*) cancer of colon, a confluent labelling giving the impression of a 'fleck' (×800); (*h*) osteosarcoma, 'irregular moats' in a nucleus (×800)

Feulgen's method (with or without Lumière green) and Masson's trichrome stains are excellent, but we have found it an advantage to decrease the staining time. We prefer a staining technique without haematoxylin because of the blue-black spotted artefacts which sometimes appear. For this reason we used carmine alum 1 per cent, eosin 1 per cent, and safran 1/300.

The labelled cells are clustered, suggesting the division of the tumour into active and resting zones. The labelled zones are usually found to be in close proximity to, or surrounding, a blood vessel (Fig. 1) in the form of a rosette. Sub-groups in which DNA synthesis is synchronous can be seen in the actively dividing zones. Low-power examination of these sub-groups or moats is often misleading, because of their resemblance to artefacts (Fig. 1). This type of zone-distribution is characteristic of the malignant process, and can be explained by loss of the 'normal structure synthesis model'. In glandular epitheliomata, the labelled cells are numerous adjacent to the basement membrane and in the zones peripheral to the polystratified lining (Fig. 1). This last finding emphasizes the loss of homoeostasis at the cellular level. In addition to the perivascular region, marked cells can be plainly seen in the extensive dedifferentiated areas. The perivascular labelling is not observed in infiltrating tumours; the tagged cells are found in groups of two or three cells situated close to the limit of the normal tissue.

The stroma and monocytes are labelled with varying intensities and the conjunctivo-vascular tissue is sometimes labelled, whereas the sclerotic zones are never tagged with the isotopes. Normal muscular, connective, or epithelial tissues were almost never labelled under our

experimental conditions. We have found on rare occasions a lightly labelled cell in the region of the fundus of a crypt of Lieberkühn, but there was no confusion between the frankly labelled tumour cells and the rare cells found in normal tissue which showed a very slight affinity for the isotope.

Cytological examination revealed several types of labelling (Fig. 2, *a–h*). Nuclei of the stromal cells (Fig. 2*a*) and some well-differentiated malignant cells (Fig. 2*b*, *c*) had an elegant argyrophilic granulation which was often distributed in a homogenous manner. The least typical of the differentiated malignant cells were labelled in irregular moats (Fig. 2*h*), almost always found under the nuclear membrane, giving the impression of a diamond-studded ring (Fig. 2*e*).

Massive zones containing few or no differentiated cells, the basal cells of the tubules, and the atypical sarcoma cells have a flecked labelling (Fig. 2*f*, *g*) due to the confluence of numerous silver granules (Fig. 2*c*, *d*). Some nuclei were partly labelled by the isotope as though only a part of the nuclear DNA had been synthesized (Fig. 2*h*). This was observed with greatest frequency in the osteosarcomata. In very proliferative osteosarcomata and in metastasized epitheliomata we observed some labelled mitotic figures. Sometimes, two contiguous nuclei in the same chondroplast or osteoplast are intensely labelled, suggesting that one nucleus had divided into two.

This technique gives excellent autoradiographic labelling and is innocuous to the patient. The weak dose of isotope (0·5–1 mc.), the rapid transformation of the tritiated thymidine to heavy water[2], and the short half-life of ^{32}P are evidence that the method is not more dangerous than thyroid cartography or other routine *in vivo* isotopic studies. Moreover, its application in patients whose treatment requires much higher follow-up doses of radiotherapy justifies our isotopic explorations.

Up to now it has been difficult to draw prognostic conclusions from the usual histopathological study. On the other hand, knowledge of the rates of cell division makes possible a decisive prognosis[5]. The presence of numerous labelled elements shows that cellular divisions are frequent. The fact that healthy tissues are not labelled proves that the malignant cells have a turnover which is clearly superior to that of normal cells[6,7]. The presence of numerous nuclear flecks attests to the rapid uptake of the isotope, which in turn is responsible for the over-exposure of the photographic emulsion. The fact that many marked molecules were incorporated in a relatively short period of time shows that the duplication of DNA is more rapid in these cells.

Finally, one can conclude from the presence of the labelled mitotic figures that in some malignant cells a part of the S phase, all the G_2 phase, and a part of the cell division period took place in a 1-h period (perfusion time), whereas this normally requires 6–12 h. We think it possible that this method could be used to calculate the rate of tumour growth, and with this information an accurate prognosis could be arrived at.

When surgical exploration is not complicated it is possible to carry out several successive autoradiographic studies with ^{32}P. Well-chosen intervals between studies would eliminate a cumulative radioactivity at the level of the tumour. One could then evaluate the therapeutic effect of various drugs and even of radiotherapy. It is feasible in certain cases to use ^{32}P as the therapeutic agent. Since the least differentiated cells take up more of the isotope, such action might have the effect of redifferentiating tumours about to become anaplastic (dedifferentiated).

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IN VITRO CELL TRANSFORMATION BY X-IRRADIATION

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CARCINOGENESIS can be induced by viral, non-viral chemical, and physical agents. In order to elucidate the mechanism of carcinogenesis, it would be of value to be able to induce by these different agents transformation of the same normal cells to tumour cells in tissue culture. It has previously been shown that normal hamster cells can be transformed to tumour cells *in vitro* by a virus such as polyoma[1], and by carcinogenic hydrocarbons[2]. The present experiments were undertaken to determine whether *in vitro* transformation of normal hamster cells can be directly induced by X-irradiation, and if so, to characterize some of the conditions required for this transformation to occur.

Short-term cultures from golden hamster embryos were used as a source of normal cells. The cells were grown in plastic Petri dishes (Falcon Co.) with Eagle's medium containing a four-fold concentration of amino-acids and vitamins plus 10 per cent calf serum, in a humified incubator with a constant flow of 10 per cent carbon dioxide in air. Primary cultures were seeded at 1×10^7 cells per 100 mm Petri dish, and subsequent passages were usually seeded at 1×10^6 cells per 50 mm Petri dish. Cells were plated for cloning at 5 or 7×10^2 cells per 50 mm plate. The cells were cloned on X-irradiated (4,000 r.) rat embryo feeder cells, and for the assay of transformed clones the plates were fixed, and stained with May–Grünwald–Giemsa, after 10 days of incubation[3].

Two types of X-ray machines were used. One type, which gave hard X-rays, was from a Picker Vanguard X-ray machine operating at 250 kV and 15 m.amp, filtered with 1·0 mm of aluminium and 0·5 mm of copper. This was used at a dose rate of 60 r./min. The second type, which gave soft X-rays, was from an R. Seifert Derm ovolt X-ray machine operating at 56 kV and 15 m.amp, filtered with 1·5 mm of aluminium. This was used at a dose rate of 280 r./min. The dose was measured with a Philips standard dosimeter, and for the dose measurement the meter was covered with a plastic Petri dish lid. The cells were irradiated in covered plastic Petri dishes in 4 ml. of medium per 50 mm and 10 ml. of medium per 100 mm plate. The medium in the cultures was changed before irradiation.

The first experiments were carried out with mass cultures. One-day-old primary cultures were irradiated with 300 r. Three days after irradiation the cells were seeded at 1×10^5 cells per plate on rat feeder layers, and they were then passaged when confluent, without a feeder layer, at 1×10^6 cells per plate. The growth rate of the controls decreased with further passaging,

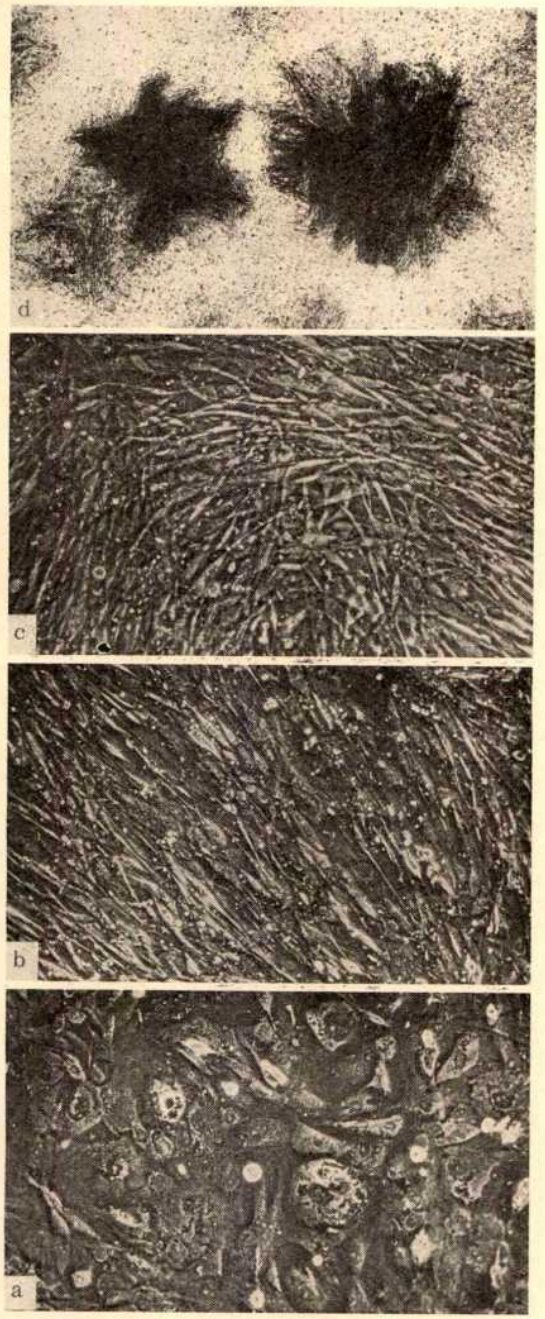


Fig. 1. Photographs of cells derived from hamster embryos. Control mass culture after 28 days (*a*), transformed cells in X-irradiated mass cultures after 25 days (*b*) and 58 days (*c*), and clones of transformed cells from mass culture 59 days after X-irradiation (*d*). *a–c*, Living cultures, phase contrast (× 72). *d*, Fixed and stained 10 days after seeding (× 4)

until at about 40–80 days after seeding there was no further growth and the cultures consisted only of giant cells. In contrast to this degeneration of the controls, in seven experiments a considerable increase in mitoses was found in the irradiated mass cultures at 17–41 days (average = 34 days) after irradiation, and at 23–54 days (average = 44 days) piled-up fusiform cells were observed on the background of flat cells. These piled-up fusiform cells had a selective advantage in the mass cultures, and on continuing passages they outgrew the other cells. The hereditary multi-layering shown by these cells indicated that transformed cells with a decrease in contact inhibition[4] had been obtained in the X-irradiated cultures (Fig. 1). The transformed cells could be continuously cultured *in vitro*, and some of the cell lines have now been cultured for 15 months. Owing to their rapid rate of growth, the cultures with transformed cells were passaged at $2{\cdot}5 \times 10^5$ cells per 50 mm plate at 35–76 days (average = 62 days) after irradiation. An examination of the clones indicated that although the X-ray transformed cells were piled up on one another (Fig. 1), they were less randomly arranged than hamster cells transformed *in vitro* by polyoma or carcinogenic hydrocarbons.

Transformed cells were found in seven out of seven experiments after irradiation of mass cultures of one-day-old primaries and further passaging as already described. Similar results were obtained in three experiments with, and four without, a medium change immediately after irradiation, and with irradiation at either 250 kV or 56 kV.

In addition to their ability for multi-layering and continuous cultivation *in vitro*, the X-ray-transformed cells possessed some other properties that have also been observed in cells transformed by polyoma or carcinogenic hydrocarbons[1,2]. Thus the X-ray-transformed cells made the medium more acid than normal cells, they could not be as easily removed from the surface of the Petri dish by trypsin, and were more resistant to nutritional conditions unfavourable for normal cells and to the toxic action of benzo(*a*)pyrene[2,5]. The transformed cells continued to grow well on passage when cells were maintained without a medium change for 20 days, and when tested after 9 months *in vitro* gave no decreased cloning efficiency after the addition of 25γ per ml. of benzo(*a*)pyrene.

In contrast to cells transformed by other agents[6], the X-ray transformed cells after 1 year in culture did not form colonies in 0·30–0·34 per cent agar when seeded at $1{-}5 \times 10^5$ cells per 50 mm Petri dish and observed for 60 days. Although they did not grow in the agar, cells isolated after 14–40 days in agar again grew well in fluid medium.

The ability for tumour formation *in vivo* was tested by subcutaneous inoculation of $6{\cdot}5 \times 10^6$ cells per animal into 2–6-week-old hamsters. Thirty-five hamsters were inoculated with transformed cells taken at about 2 or 8 months after X-irradiation. In all animals growths were formed after several days at the site of inoculation; these reached a maximum size of about 7–10 mm in diameter, and then regressed beginning at about 10–30 days (average = 20 days) after inoculation. No such growths were formed after inoculation of the same number of cells from normal hamster embryo cultures. Histologically these growths, before regression, were classified as fibrosarcomata. Cells taken from such growths before regression, and transplanted into adult hamsters either without or with a further growth *in vitro* for 1 month, again gave growths after several days that reached a maximum size of about 8–10 mm diameter and then regressed. When animals in which growth had regressed were inoculated a second time with the same transformed cells, the second inoculation gave a smaller growth that regressed more rapidly than after the first inoculation. These results indicate that, although the transformed cells had a better growth potential *in vivo* than normal cells and formed small growths—these did not grow progressively—and that the regression was presumably partly or wholly due to an immune reaction owing to the acquisition of a new antigen by the transformed cells.

After the foregoing results had shown *in vitro* cell transformation by X-irradiation and some properties of the transformed cells, the following experiments were carried out to determine some of the conditions required for the transformation to occur, and the percentage of transformed clones. In these experiments the percentage of transformed clones was determined after 300 r. irradiation of confluent mass cultures, sparse mass cultures, and cells plated for cloning, by scoring the percentage of piled-up transformed clones (Table 1). Irradiation of confluent cultures gave 0·7–0·8 per cent transformed clones in three out of three experiments when the cells were cloned immediately after irradiation, but no trans-

Table 1. PERCENTAGE OF *in vitro* TRANSFORMED HAMSTER EMBRYO CLONES AFTER X-IRRADIATION WITH 300 r.

Condition of cultures at time of irradiation*	X-irradiation (kV)	Days after irradiation when cells plated for cloning	No. of experiments	No. of experiments with transformation	Total No. of clones†		Cloning efficiency† (per cent)		Transformed clones† (per cent)	
					Irradiated	Control	Irradiated	Control	Irradiated	Control
Confluent	250	3–5	6	0	5,716	6,054	0·7–0·9	0·8–1·0	0	0
Confluent	56	3–5	6	0	4,931	5,004	0·9–1·0	0·9–1·0	0	0
Confluent	250	0	2	2	5,310	4,595	0·7, 0·9	0·9, 1·1	0·7, 0·7	0
Confluent	56	0	1	1	1,921	2,971	1·0	1·0	0·8	0
Sparse‡	250	3	1	1	1,136	1,230	1·1	1·0	0·5	0
Cells plated for cloning	250	—	2	2	2,350	3,614	2·1§, 0·5	2·7§, 1·0	0·6§, 0·5	0
Cells plated for cloning	56	—	3	3	4,525	3,745	1·9§, 2·0§, 0·7	2·1§, 2·7§, 1·0	0·7§, 0·5§, 0·5	0

* Confluent primary cultures were irradiated 3–4 days after seeding 1 × 10^7 cells per 100 mm Petri dish, and sparse primary cultures 1 day after seeding the same number of cells. Cells plated for cloning were secondary cultures seeded 1 day before irradiation.

† Plates were fixed and stained 10 days after cells were plated for cloning.

‡ Transformed cells were also obtained in seven out of seven experiments in which sparse cultures were irradiated with either 250 or 56 kV and passaged 3 days later as mass cultures (see text).

§ 10 per cent foetal calf serum. All other experiments with non-foetal calf serum.

formed clones were observed in six experiments when the cells were cloned at 3–5 days after irradiation. In these six experiments further passaging of the cultures resulted in cell degeneration at about 40–60 days in the same way as in the controls. About the same frequency of transformed clones was found, with 250 kV or 56 kV, after irradiating confluent primary cultures cloned immediately after irradiation, sparse primary cultures irradiated 1 day after seeding and cloned 3 days later, and secondary culture cells 1 day after they were plated for cloning. The results suggest that the recovery of transformed cells requires the irradiation either of cells that are actively growing or of those able to do so soon after irradiation. In all experiments the transformed clones were on the average larger than the normal, indicating that they had acquired an increased rate of cell multiplication.

The small decrease in cloning efficiency in the irradiated cultures, and the absence of transformed clones in the controls, indicate that the transformation was directly induced by the X-irradiation and not merely the result of a selection of spontaneously occurring transformed cells. The existence of transformed clones at 10 days after irradiation of cells plated for cloning indicates, as with polyoma and carcinogenic hydrocarbons[2,3,6], that the expression of X-ray induced transformation is a rapid event, presumably within the time required for one cell generation. The frequency of transformation, as with these other two agents[2,3,6], is also much higher than that expected from a randomly occurring mutation. Further experiments are required on the properties after passage of apparently normal-looking clones in the X-irradiated cultures, to show whether there may also be X-ray induced transformation other than the type scored in the present experiments.

The foregoing results showing that transformation can be induced by X-irradiation in culture now make it possible to transform hamster cells *in vitro* by a virus such as polyoma, non-viral chemical carcinogens, and a physical agent such as X-rays. This allows a comparison *in vitro* of the molecular mechanism of transformation by these three different agents. The decrease in contact inhibition in X-ray induced transformation suggests that, as in the case of transformation by polyoma and carcinogenic hydrocarbons, the change of cell surface may be responsible for the change in the regulatory mechanism of the cell[7]. The lack of growth in agar by the X-ray transformed cells could be interpreted to suggest that the change induced in normal cells need not necessarily lead to the same final results with all three agents. In order to analyse possible similarities in mechanism at the molecular level, further investigations are aimed at determining to what extent transformation by X-irradiation and non-viral chemicals can result in a change in the regulation of normal cellular DNA synthesis of the same type as that induced by polyoma[8]. It is of interest in this connexion that, in addition to the chromosome abnormalities and reversible mitotic lag[9] observed after treatment with X-rays, there is also a report of an increase in the number of cells synthesizing DNA soon after X-irradiation[10].

We thank Mrs. Nava Shtacher for her assistance, and Mr. Aaron Yerushalmi and his staff for the X-irradiation. This investigation was supported by a research grant from the National Cancer Institute, U.S. Public Health Service.

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HETEROGENEITY OF ENDOTOXIC BACTERIAL LIPOPOLYSACCHARIDES REVEALED BY ION-EXCHANGE COLUMN CHROMATOGRAPHY

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FREE boundary electrophoretic analysis and sedimentation in the ultracentrifuge or by the density gradient technique are most commonly used for the detection of heterogeneity in bacterial lipopolysaccharides. Gel-diffusion or immunoelectrophoretic patterns also reveal heterogeneity if the antiserum used contains precipitating antibodies against all components of the preparation. These procedures usually do not detect all substances

present in such materials. Paper chromatography or paper electrophoretic analysis, other important methods used in exposing heterogeneity, could not be applied satisfactorily in this laboratory. No sensitive and specific method could be found which would detect lipopolysaccharides on paper sheets. In addition, the very large molecular weight of the components to be separated prevents the application of such procedures.

Column chromatographic procedures were applied in our laboratories using highly porous tertiary amine anion exchangers. Best results were obtained with 'Amberlite *XE* 220' resin (generously supplied by Rohm and Haas Co., Philadelphia, Pennsylvania).

First, an analytical procedure was developed. A column 12 mm in diameter by 180 mm in height was prepared from a slurry of resin in the HCO_3^- form. 5 mg bacterial lipopolysaccharide, dissolved in 0·5 ml. water, and then adjusted to *p*H 7·5, was applied on the column. Approximately 5 ml. of the resin slurry was then carefully layered on top of the sample, followed by elution with 20 ml. water. Thereafter, linear gradient elution was carried out by mixing 100 ml. water and 100 ml. saturated sodium hydrocarbonate in a gradient device, and was continued by mixing 50 ml. saturated sodium hydrocarbonate and 50 ml. 2 N sodium hydroxide, producing a 0–2 N sodium hydroxide gradient.

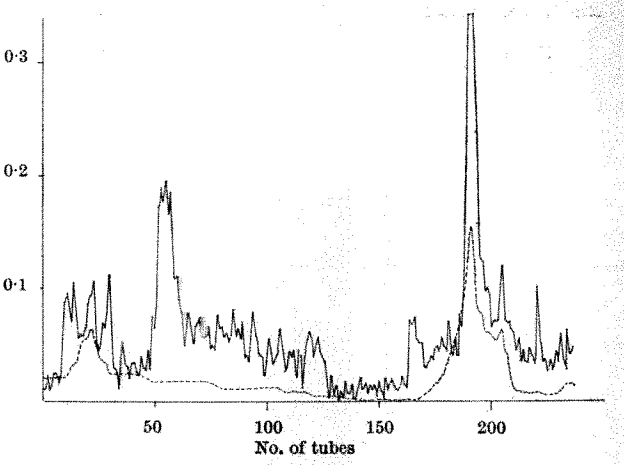


Fig. 3. Preparation obtained from *Serratia marcescens* by the aqueous ether procedure (ref. 2). The dialysed product was precipitated with methanol. LD_{50} is approximately 1,000 μg/mouse. Solid line shows the optical density of the phenol-sulphuric acid carbohydrate determination. Broken line indicates the automatic monitoring of the effluent at 260 mμ

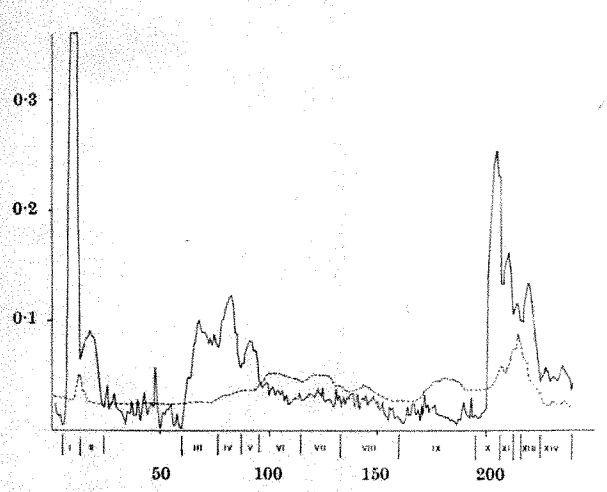


Fig. 1. Lipopolysaccharide isolated from *Serratia marcescens* by cold trichloroacetic acid. Crude product was purified by repeated methanol extraction and sedimentation at *p*H 10 at 5,000*g*. The LD_{50} of the preparation is 420 μg/mouse. Roman numerals designate the isolated fractions obtained by pooling the collected tubes as marked on the baseline of the figure. Solid line shows the phenol-sulphuric acid carbohydrate determination. Broken line indicates automatic monitoring of the effluent at 260 mμ

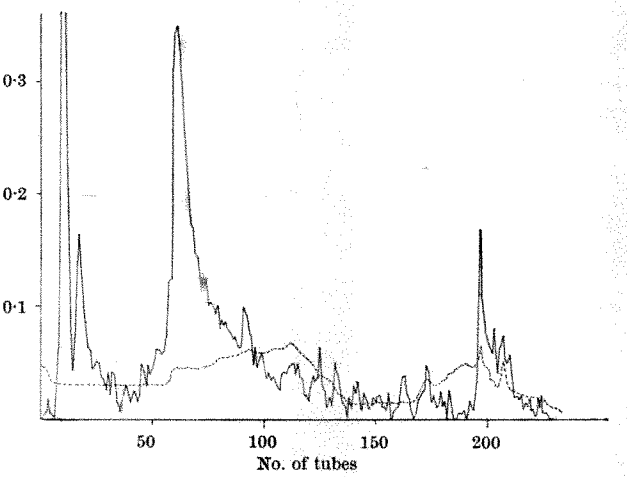


Fig. 4. The same preparation as in Fig. 1, but after detoxification with CH_3OK (ref. 5). The LD_{50} of this preparation is 4,000 μg/mouse. Solid line shows the optical density of the phenol-sulphuric acid carbohydrate determination. Broken line indicates the automatic monitoring of the effluent at 260 mμ

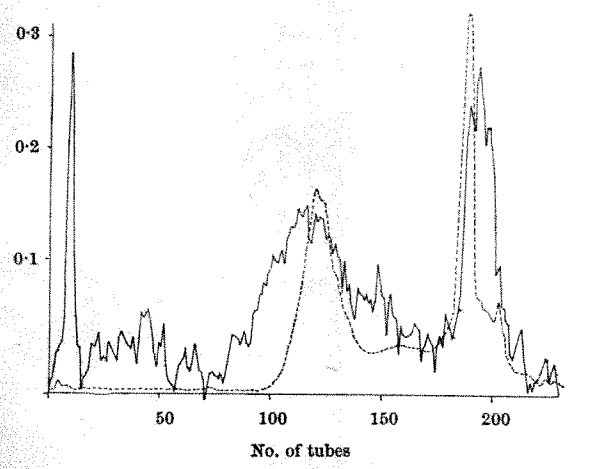


Fig. 2. Lipopolysaccharide isolated from *Serratia marcescens* by the phenol-water method at 68° C (ref. 2). The LD_{50} of this material is 670 μg/mouse. Solid line shows the optical density of the phenol-sulphuric acid carbohydrate determination. Broken line indicates automatic monitoring of the effluent at 260 mμ

Effluent was channelled through a micro-flow cell of a spectrophotometer and the optical density was automatically recorded at 260 mμ. 1·5 ml. samples were collected from the effluent of the flow cell in chromic acid washed and pre-calibrated test-tubes. The collected samples, approximately 250 tubes in each run, were used for the determination of carbohydrate content. 1·5 ml. 5 per cent phenol in water was added to each tube, followed by 7·5 ml. concentrated sulphuric acid (all chemicals were reagent grade). Reagents were dispensed from all-glass, automatic pipettes. After 60 min at room temperature the developed colour was read at 490 mμ in a spectrophotometer supplied with the proper test-tube adapters.

The following figures show the results obtained by analysing different bacterial lipopolysaccharides. Brief descriptions of the preparations are given under each figure.

By using merely two parameters for chemical analysis, that is, ultra-violet monitoring and carbohydrate analysis by the phenol–sulphuric acid procedure, it became obvious that endotoxic lipopolysaccharide preparations consist

Table 1. SEROLOGICAL AND BIOLOGICAL REACTIVITY OF THE ISOLATED FRACTIONS

No. of fraction	Shwartzman reactivity*	Lethality $L.D._{50}$† (μg)	Gel-diffusion ‡
I	0	> 200	+
II	0	> 200	0
III	2·8	55	+ +
IV	2·4	55	+ + +
V	2·7	46	+ +
VI	2·4	58	+
VII	0	> 200	±
VIII	0	> 200	0
IX	0	> 100	+
X	1·5	> 100	+ +
XI	3·6	> 100	+ +
XII	0·4	> 200	+ + +
XIII	0	> 200	+ + + +
XIV	0	> 200	±
Control	3·2	32	+ + + +

* The figures represent the extent of haemorrhagic necrosis. They are the products of two diameters (in centimeters) taken at right angles.

† Lethality was measured in 10-day-old chick embryos by chorioallantoic inoculation. > 200 or > 100 means that none or less than 50 per cent died from this dose.

‡ The intensity of the formed precipitation lines was compared in Ouchterlony agar gel-diffusion. The antiserum used was hyperimmune rabbit O-antiserum.

of several components. Amino-acid, hexosamine, or carboxylic acid analysis of the collected tubes most probably will reveal additional components.

Enlarged operation on a preparative scale for the separation of components of a partially purified and trichloroacetic acid extracted endotoxin from *Serratia marcescens* cells was also elaborated (the supply of freeze-dried *Serratia marcescens* cells was obtained through the courtesy of Merck, Sharp and Dohme Co., Rahway, N.J.). The profiles obtained in preparative scale were similar to those obtained in analytical operations.

The isolated fractions showed similar inhomogeneity in their biological activities. Serological reactivity with the homologous O-antiserum of some of the fractions was investigated by micro-gel diffusion. It could be shown that not all fourteen isolated components formed precipitin lines with O-antiserum. Shwartzman skin tests on rabbits and chick embryos were used to check the toxic effects of the fractions. It was observed that several fractions which exhibited reactivity with O-antiserum were non-reactive in the skin test, and some fractions showed no reactivity in either system. The serological and biological activity of the isolated components is indicated in Table 1. The skin reactivity and lethality of fractions XII–XIV could be destroyed by the strong alkaline eluting agent. The preparations are being subjected to further chemical and biological analysis[4].

During these experiments some major endotoxin fractions manifested an extremely high affinity to anion exchangers. This adhesion to the resin is so strong that it could not be displaced with 2 N ammonia or similar less degradative eluting agents. This remarkable property is a common feature of serologically unrelated but similarly toxic bacterial lipopolysaccharide preparations. As shown in Fig. 4, endotoxoid[5] obtained through chemical detoxification of endotoxin does not show the same affinity to ion exchangers.

An especially noteworthy result of the analysis of the curves shows that phenol–water extraction isolates a very large amount of polysaccharides from the bacteria (see Fig. 2, tubes 80–180), which are almost absent from similarly toxic preparations obtained by trichloroacetic acid. Aqueous ether procedure, in addition to dissolving very large amounts of proteins, also elutes some polysaccharides from the whole cell (see Fig. 3, tubes 15–35 or 160–170), which are not present in appreciable amount in other preparations. The above results support our earlier report, which described that the different extraction methods, applied to the same strain, will yield preparations with chemically different components.

While chromatographic procedures seem to be appropriate tools in isolating endotoxins and a large number of different cell constituents, further elaboration of the method is required. Nevertheless, it should be pointed out that results of previous investigations on the endotoxic lipopolysaccharides should be re-evaluated in the light of the foregoing observations.

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SOME RELATIONSHIPS OF FISHES TO BENTHIC ORGANISMS ON CORAL REEFS

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RECENTLY, certain tropical fishes have been suspected of influencing the sedentary biota. Stephenson and Searles[1], through experimentation in the field, showed that grazing by reef fishes accounts for the scarcity of intertidal algae at Heron Island, Queensland, Australia. Further work on the feeding habits of tropical fishes was reported by Newman[2], Hiatt and Strasburg[3], Stephenson[4], Bardach[5], Randall[6,7] and Bakus[8].

Perhaps one of the most common characteristics of a number of tropical coral reefs is the lack of exposed organisms (that is, those organisms living on the surface of a coral substratum where they can readily be consumed by predators and grazers), with the major exceptions of corals, organisms with skeletal protection (for example, shelled gastropods and calcareous algae), and organisms that are poisonous, venomous or distasteful to predators or grazers (for example, sea cucumbers). Tropical coral reefs may be unique in that more species are concealed within the reef complex than are usually observed in substrata of non-coral-dominated tropical, temperate, and boreal shallow waters[3,9–13]. Even subtropical coral reefs may harbour a rich and varied infauna[14]. Quantitative data to support this are extremely difficult to obtain. All but about six of approximately forty species of Porifera collected by me at Fanning Island, Line Islands, central equatorial Pacific, were concealed within reefs (only two of the six exposed species were common to abundant) whereas in the San Juan Islands, Washington, it would be safe to state that at least half the species of sponges were exposed in sublittoral waters. From a collection of intertidal sponges made on April 14–15, 1965, at Punta Peñasco, upper Gulf of California, it was found that representatives of ten out of a total of fourteen species were growing exposed. Moreover, numerous

tunicates were also growing exposed on the firm sandstone substratum. Glynn[10] indicated the species composition of *Porites furcata* reefs in Puerto Rico, but quantitative data were not available at that time. Garth concluded that the overwhelming representation in the atoll fauna of the family Xanthidae, which alone constituted 45 per cent of the genera and 56 per cent of the species of Brachyura collected at Eniwetok, is believed in large part due to the protection afforded its smaller members by the branching corals[15].

Fishes appear to be faced with numerous modes of concealment and protection on the part of coral reef invertebrates that serve as a potential food source. For example, there are boring sponges, barnacles, bivalved molluscs, polychaetes and sipunculids. Some organisms are concealed by means of their biological associations (for example, haplocarcinid crabs within protective 'coral galls', numerous invertebrates are inquilines in certain tropical sponges, etc.[12,16,17]). Pearse[16] found that specimens of the loggerhead sponge (*Spheciospongia vesparia* (Lamarck)) collected in the Dry Tortugas, off southern Florida, contained from 6,282 to 17,128 animals (the latter number represented by 22 species). During my investigations on biological associates within Porifera of Washington[18], the most capacious sponges seldom contained more than about a hundred metazoan inquilines represented by several species. Certain marine organisms are poisonous or venomous[19,20]; the incidence of this characteristic may be higher in the tropics, according to Dr. Bruce W. Halstead (personal communication). If one quantifies Southcott's and Halstead and Mitchell's information (in ref. 20) it is found that at least 74 per cent of the coelenterates (out of a total of 34 species) and 68 per cent of the fishes (out of a total of 101 species) of medical importance to man live in tropical and subtropical waters. However, it should be emphasized that many marine organisms are immune to ciguatera- and tetraodon-type toxins that afflict man[21]. With the large number of species of carnivores and omnivores associated with coral reefs one would assume that numerous protective devices (for example, venomous spines) would have evolved over a long geological time period (see following).

Perhaps the most significant method of concealment occurs in the numerous organisms that live in fissures and crevices of corals, on the undersides of coral slabs, and under the bases and among the branches of corals. This concealed fauna includes such a large number of organisms that it may be preferable to consider the animals a more or less ecologically distinct fauna. Gerlach[11] called this the 'phytalfauna'; I prefer to use the term 'cryptofauna', because it is more descriptive. In certain regions of the Indian Ocean the lack of exposed organisms may be in part the result of the destructive annual scouring due to subtidal surge which is caused by the action of winds (monsoon) on shallow waters (Dr. John S. Garth, personal communication). The shortage of organisms was noted earlier by Gardiner (in ref. 22), who stated: "The reefs of the Chagos are in no way peculiar, save in their extraordinary paucity of animal life", but Kohn[23] discovered that on the Isle du Coin organisms such as cup sponges (*Phyllospongia foliascens*) and cone snails (*Conus*) were abundant on the exposed seaward reef flat. He found that the density of *Conus* on a reef at Peros Nahos was considerably higher than in most populations he studied in Hawaii (Dr. Alan J. Kohn, personal communication).

There are very few reports of direct observations on feeding behaviour of fishes in extra-tropical latitudes. Information from Elegvad[24] in Denmark, Suyehiro[25] in Japan, and Limbaugh[26] in southern California suggests that fish predation and fish-grazing on shallow-water benthic invertebrates are widespread. However, direct observations made by Limbaugh[26] during a 5½ year investigation on feeding fishes in southern California suggest to me that the intensity of fish-rasping is considerably less in temperate latitudes than in the tropics. This conclusion appears to be supported by Neushul and Haxo[27]. Although they report the effects of grazing on *Macrocystis* and mention three species of kelp-eating fishes, there seem to be no direct observations on intense fish-rasping activities at La Jolla, California. In contrast, the effects of fish-grazing and rasping on algae, sponges and didemnid tunicates at Fanning Island, central Pacific, during July and August 1963[8] are presented in Figs. 1 and 2.

The previous statements lead to the suggestion that there has been an accentuation towards protective mechanisms and habits in certain coral reef invertebrates and that this may be in part an evolutionary response to the predatory and grazing habits of the diverse tropical reef fishes over a long geological period. Scleractinian reefs have persisted since the Mesozoic[28,29], and coral reef fishes apparently have had a long geological history[30]. That the influence on invertebrates by fishes perhaps is significant may be inferred from the numbers of genera and species of tropical fishes that are grazers and raspers

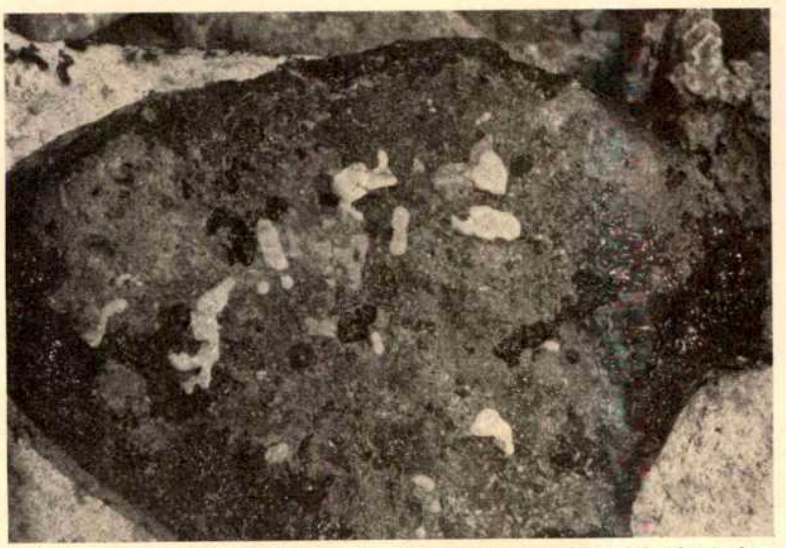


Fig. 1. View of the underside of a coral slab; the slab was collected from 3 ft. of water in English Harbor Channel, Fanning Island, Line Islands, central Pacific. Note the dark sponges, white didemnid tunicates and peripheral brown algae (see ref. 8)

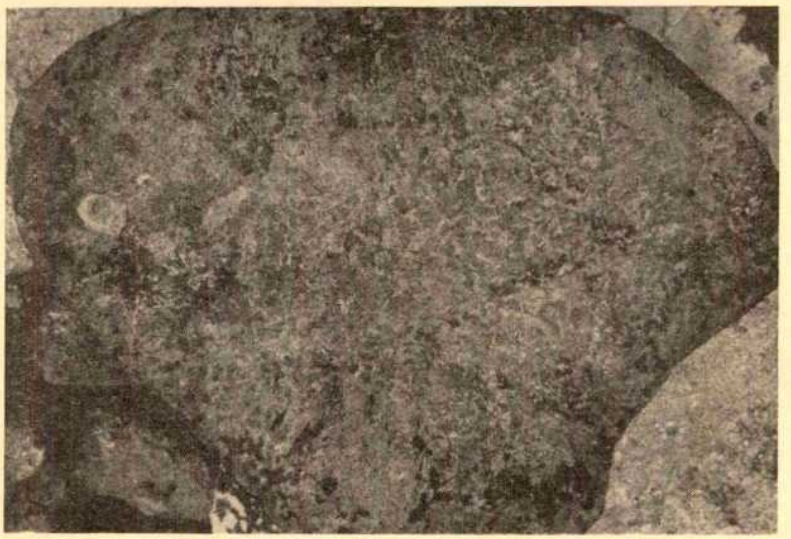


Fig. 2. View of the underside of the coral slab shown in Fig. 1, after placing it in 2 ft. of water in English Harbor Channel with the underside exposed to rasping fishes for only 1 h. Note the numerous tooth marks (see ref. 8)

and the high overall percentage of carnivores[3,30–34] (see also following refs.).

It appears that the standing crop of exposed foliaceous shallow-water marine algae in the tropics is often considerably less than that of similar benthic algae at comparable depths in temperate and boreal latitudes[28,35–37]. This may be true of Fanning Island and perhaps Christmas Island, central equatorial Pacific, but much additional work is needed to verify this general impression. Odum, McConnell and Abbott[38] and McFarland and Prescott[39] indicated that the standing crops of a *Macrocystis* bed, coral reef, and littoral algal community at Woods Hole, Massachusetts, based on the number of grams of chlorophyll *A* per m² of surface, are of the same order of magnitude, but it should be emphasized that much of the chlorophyll of coral reef communities is buried within the living and dead coral substratum. There is a difference in the composition of the algae. There is a predominance of red algae (especially calcareous algae) and a reduction in brown algae in the tropics when compared with coastal algae at higher latitudes[22,28,35,36,40–43]. In fact, certain reefs may consist almost entirely of calcareous algae[12,22,44]. Certain reefs in the Caribbean and in the Cocos-Keeling Islands are reportedly exceptions since reef-building algae of the *Porolithon*-type are poorly represented in comparison with that of Pacific reefs[45,46]. Odum and Odum[47] found *Porolithon* only in small patches beneath the other algae or down in crevices on Japtan reef, Eniwetok, but it should be noted that zonation of this inter-island reef is very different from zonation on other island reefs described in the literature. It is of interest to note that Smith[48] reported twenty-seven species of Corallinaceae from the Monterey Peninsula, California, whereas Taylor[35] listed only thirteen species of Corallinaceae from Bikini and Rongerik atolls, Marshall Islands. This emphasizes the point that only a small number of algal species (*Porolithon*-type) play a major role as cementing agents on tropical coral reefs.

There have been reports of low species diversity in tropical Pacific benthic reef algae. Taylor[35] attributed the lack of species diversity in places such as Bikini and Rongerik atolls to intense isolation (154 species of benthic marine algae were reported from the two atolls), but Dawson[49] reported some 220 species of benthic algae from Eniwetok Atoll, Marshall Islands (12° N. Lat.). In contrast, Smith[48] described 490 species of benthic marine algae from the Monterey Peninsula, California (36° N. Lat.). Wilse[50] indicated that there are fewer species of benthic marine algae in the Arctic than in cool temperate latitudes. It would appear that where algae may be less available or less desirable as fish food (that is, tropical calcareous algae) fishes may have specialized in the direction of predation and grazing on invertebrates. Table 1 gives comparative data on the feeding habits of marine fishes living at different latitudes. There is general agreement in that tropical coral reef fishes consist of roughly 25 per cent herbivores and 65 per cent carnivores. I had thought that the percentage of carnivorous-omnivorous fishes in waters of tropical coral reefs was greater than that of fishes in temperate or boreal shallow waters. The data in Table 1 suggest the opposite, but it should be emphasized that so far as is known no truly comprehensive investigations have been published on the feeding habits of nearshore fishes in temperate and boreal latitudes and even the information offered here is unsuitable for an adequate comparison.

If the assumption is made that the percentages of carnivorous-omnivorous nearshore fishes are approximately the same throughout the world it is still likely that the effects of predatory and grazing fishes on benthic invertebrates are more marked in tropical reefs. Le Danois[30] listed forty-one families of reef fishes; seven families are grazers. Table 2 indicates the diversity of nearshore fishes in different latitudes. Although the number of fishes given for southern California may appear great, this is somewhat misleading in that rock-fishes (Family Scorpaenidae) are atypical since they are represented by about forty-five species. Moreover, the total number includes peripheral representatives from cool temperate and sub-tropical waters. Therefore, not only are there a large number of species of carnivorous-omnivorous fishes in waters of tropical reefs, but Randall[7] indicated that the Scaridae (rasping parrot fishes) is the largest family by weight at St. John's reef, Virgin Islands, and that this dominance may be the rule throughout the West Indies. This may also be characteristic of the Indo-Pacific region (Dr. Philip Helfrich, personal communication).

The question now arises: Are there major exceptions in regard to the lack of exposed fleshy algae and animals on tropical coral reefs? Two large equatorial regions that have a diverse and moderately abundant algal flora are south-east Asia[12,36,51] and the Caribbean[28,52,53]. However, neither certain Vietnamese nor Caribbean reefs appear to harbour a standing crop of exposed fleshy algae comparable to that of coastal regions in temperate and boreal latitudes (Dr. E. Yale Dawson, personal communication) although they may approach that of the Arctic. It should

Table 1. FEEDING HABITS OF NEARSHORE MARINE FISHES IN DIFFERENT LATITUDES

Investigator	Region	Method of estimation	Herbivores (per cent)	Omnivores (per cent)	Carnivores (per cent)
Randall (ref. 7)	St. John, Virgin Islands	Percentage by weight from natural reefs	24	16	60
Hiatt and Strasburg (ref. 3)	Marshall Islands	Percentage by No. of species, 212 species considered	25	8	67
Bardach (ref. 60)	Bermuda	Percentage by weight	—	32 (mostly herbivorous)	68
Al-Hussaini (ref. 61)	Ghardaqa, Red Sea	Percentage by No. of species, 56 species considered	23	23	54
* Suyehiro (ref. 25)	Japan	Percentage by No. of species, 85 species considered	5?	21?	74?
† Limbaugh (ref. 26)	Southern California	Percentage by No. of species, 49 species considered	0?	6?	94?

* Certain species were not collected from nearshore waters.

† Food habits were incompletely investigated, especially in regard to algae consumed.

Incidental hervibores and omnivores, detritus and plankton feeders, and scavengers are excluded. Percentages are approximate values. Question marks are placed after the data taken from Suyehiro and Limbaugh, because the investigations were incomplete.

Table 2. DIVERSITY OF MARINE FISHES IN DIFFERENT LATITUDES

Investigator	Region	Latitude	Longitude	Approx. depth	Approx. No. of species of fishes
Scott (ref. 62)	South Australia	32–38° S.	128–141° E.	Nearshore and deep waters	341
Fowler (ref. 63)	Fiji Islands	15–22° S.	178° W.–177° E.	Nearshore and deep waters	439*
Scott (ref. 62) and Marshall (ref. 64)	Queensland	10–28° S.	143–154° E.	Nearshore and deep waters	>1,000
Hiatt and Strasburg (ref. 3)	Marshall Islands (3 atolls)	7–12° N.	163–172° E.	0–10 ft.	233†
Gosline and Brock (ref. 65)	Hawaiian Islands	17–28° N.	155–178° W.	Inshore or surface-living fishes	448†
Robert Lavenberg, Ichthyologist, Los Angeles County Museum, 1965 compilation	Southern California (Point Concepcion to Mexico)	32–34° N.	117–121° W.	0–100 ft.	180 (45 of these were rock-fishes)
Clemens and Wilby (ref. 66)	Pacific coast of Canada	48–55° N.	123–133° W.	Nearshore and deep waters	272
Backus (ref. 67)	Labrador	51–60° N.	56–65° W.	Nearshore and deep waters	59
Walters (ref. 68)	Western Arctic America to eastern Arctic Siberia	66° N. to longitudinal coordinates	105° E–105° W.	Nearshore and deep waters	50

* The total No. of species of fishes in the Fiji Islands is estimated to be roughly twice this figure.

† The total No. of species of fishes in the Marshall and in the Hawaiian Islands is estimated to be about 600 each.

be emphasized that the gross primary productivity of coral reefs is very high compared with other habitats on the Earth's surface and that a low standing crop is not indicative of the level of primary production[54]. However, Odum and Odum[47] indicated that a large amount of green plant material on Japtan reef, Eniwetok, is sub-surface. This suggests that much energy from primary producers may be available only to those fishes that are raspers or to carnivores and omnivores that consume animals containing zooxanthellae. However, Hairston, Smith and Slobodkin[55] report that herbivores are seldom food-limited, at least in terrestrial environments. Hayne and Ball[56], who worked with freshwater fishes in southern Michigan, found that in the presence of fish, the standing crop of benthic fish food was depressed and the rate of production increased. Whether or not this phenomenon is characteristic of tropical reefs is unknown. The lack of exposed fleshy algae and certain animals coupled with high primary production on certain tropical coral reefs may be associated with low nutrient concentrations and rapid local recycling of available nutrients. If the coastal Caribbean and south-east Asian regions are compared it is evident that they share the following characteristics: (*a*) They have partially land-locked waters and (*b*) they receive an appreciable quantity of nutrients during the year from land run-off[57]. This nutrient reservoir could support a greater standing crop of productive fleshy algae and presumably make more plant food available for animals ('island' or 'land effect'). Odum, McConnell and Abbott[58] found a strong relationship between nutrient concentration and the standing crop of benthic primary producers in shallow-water communities at different latitudes. Dr. Alan J. Kohn (personal communication) observed that a large proportion of the area of some reef flats in the Seychelles and Ponape (high islands with considerably more land run-off than that of atolls) is covered with plants (for example, *Cymodocea* in the Seychelles). In conclusion, the predatory, grazing and rasping behaviour of tropical coral reef fishes may be reflected in the protective mechanisms and habits of certain invertebrates associated with these reefs (for example, certain sponges, didemnid tunicates, brachyuran crabs, etc.).

The information offered here immediately evokes many questions, some of which may be answered in part during the next decade:

(*A*) Have the feeding habits of carnivorous, grazing and rasping tropical fishes led to the evolution of protective behaviour in certain reef invertebrates? If so, in which ways?

(*B*) Is the intensity of fish grazing at Fanning Island related to the apparent low diversity and low standing crop of certain invertebrate taxa (for example, bivalved molluscs, thoracican barnacles) coupled with an apparent diversity and abundance of fishes (see refs. 8 and 58)?

(*C*) What are the factors that restrict the standing crop of exposed fleshy benthic algae in shallow waters of the tropics?

(*D*) Have the encrusting coralline red algae (for example, *Porolithon*) evolved their habitus in part as a response to the grazing and rasping habits of reef fishes?

(*E*) How significant are algae (for example, *Ostreobium*) living within coral in regard to the food requirements of certain rasping fishes (for example, parrot-fishes) that consume them?

(*F*) Which factors have led certain tropical fishes to specialize in grazing and rasping behaviour in order to exploit the sessile benthic biota as a food source?

These and numerous other problems await solution.

Dr. E. Yale Dawson (personal communication), while in the Galapagos Islands during early February 1964, observed intense grazing on shallow-water fleshy benthic

algae by crabs, fishes and marine iguanas. Mr. Andrew A. Pilmanis investigated the grazing habits of shallow-water fishes in the region of Los Angeles, California, during the first half of 1964 and found no evidence of intense rasping such as that described for certain tropical reefs. Squires[59] presented evidence that certain fishes may feed on corals at depths of from 35 fm to 90 fm off Mayor Island, New Zealand. These observations again suggest that fish-grazing may influence marine invertebrates more than formerly thought.

There is little reason to suspect that fish predation and fish-grazing have been solely responsible for what may prove to be rather widespread protective mechanisms and habits in certain coral reef invertebrates. Larval settling behaviour and the possible effects of surge on the survival of reef invertebrates must also be considered. Experiments I performed at Fanning Island indicate that sedimentation (that is, specifically siltation) is another environmental factor of major significance in regard to the distribution and survival of certain sponges and tunicates[8]. Invertebrate predators (for example, *Conus*) may be found to exert a strong influence on the habits of certain of their invertebrate prey on coral reefs, but very little seems to be known about this (Dr. Alan J. Kohn, personal communication). Further work is needed to determine how significant is each of these factors in regard to the biology of reef invertebrates.

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Addendum. An exception to the statement that benthic red algae predominate in tropical waters is offered by Knox[69], who indicated that the majority of genera and species of benthic algae in southern Australia and New Zealand are Rhodophyta. Andriashev[70] reported that there are about seventeen species of fishes in the upper 100 metres of eastern Antarctic waters. The greatest diversity of fishes (thirty-one species) apparently occurs at a depth of about 400 metres.

Randall[71] found that bands of bare sand separating reefs and beds of sea grasses (*Thalassia* and *Cymodocea*) in the Caribbean appear to be the result of heavy grazing by parrot fishes (*Scarus* and *Sparisoma*) and surgeonfishes (*Acanthurus*). Talbot[72] reported that the fishes of Tutia Reef, Tanganyika, East Africa, consist of about 20 per cent herbivores and 80 per cent carnivores by weight and 16 per cent herbivores and 84 per cent carnivores by number of species. Approximately 20 per cent of the fishes (by weight) are coral feeders and 49 per cent consume invertebrates. These data are based on 96 species of fishes, excluding plankton feeders. The category 'herbivores' includes a few facultative omnivores. Stasek[73] concluded that behavioural responses of the giant bivalve *Tridacna*, such as the aiming of the excurrent siphon and spurting of water, are probably defensive adaptations to the presence of grazing fishes.

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LETTERS TO THE EDITOR

RADIO ASTRONOMY

Magnetic Field within some Quasi-stellar Radio Sources

THE origin of the energy in the powerful radio sources presents one of the major problems in present-day astrophysics. It is generally accepted that the radio emission arises from the synchrotron mechanism, and Burbidge and Burbidge[1] have shown that the energy requirements are at a minimum when there is approximate equipartition between the magnetic and particle energies.

Making reasonable assumptions about the upper and lower limits in the energy spectrum of the relativistic electrons responsible for the emission, the total energy may then be computed if the volume of the source is known. In the case of the compact sources associated with quasi-stellar objects the energy needed to produce a source of given radio luminosity is considerably less than that required for the more extensive radio galaxies if equipartition occurs in both types of sources; the radio emission in the latter case will, however, persist for a longer period.

In order to establish the energy requirements it is, therefore, essential to decide whether or not the particle and magnetic field energy approximate to the condition of equipartition, for if they do not the total energy must be correspondingly increased.

In a recent paper, Hornby and Williams[2] have shown that for quasi-stellar radio sources of small angular size it is often possible to establish an upper limit for the magnetic field within the source. Many of these sources show a cut-off in the flux density at low frequencies. The most probable cause of this cut-off is self-absorption by the synchrotron mechanism itself, and in this case the magnetic field B can be related directly to the observed angular diameter θ. If the number of relativistic electrons per unit volume, with energies lying between E and $E+dE$, is represented by $N(E)\,dE = \text{const.}\ E^{-\gamma}\,dE$, it can be shown that:

$$B^{-\frac{1}{2}}\theta^2 = \frac{S_h}{\nu_h^{(1-\gamma)/2}} \left\{\frac{e}{2\pi m_0^3}\right\}^{\frac{1}{2}} \frac{(1+z)^{\frac{1}{2}}}{\nu_m^{(\gamma+4)/2}}\, G(\gamma)$$

where S_h is the observed flux density of the source at frequency ν_h higher than the frequency at which the cut-off begins; ν_m is the frequency at which the observed flux density is a maximum; z is the red-shift of the source; and $G(\gamma)$ is a numerical factor derived from Le Roux[3] and is approximately equal to 5 for $1{\cdot}6 < \gamma < 3$ (using MKS units throughout, and measuring θ in radians).

ν_m can be determined directly from the observations of the spectrum so that the measured angular diameter, θ, or an upper limit to θ, allows an upper limit to B to be found.

It is, of course, possible that some other mechanism may be responsible for the observed low-frequency cut-off in the spectrum; in these circumstances the frequency at which synchrotron self-absorption would occur must be even smaller than the value of ν_m observed. The calculated upper limit for B is, therefore, still valid. A similar argument can be used for sources which show no sign of a cut-off over the range of frequencies observed.

It is important, however, to consider the possibility that in sources such as 3C 273 a compact component showing strong self-absorption even at high frequencies may be associated with a more extensive component with a normal spectrum. In such circumstances information on the angular size obtained at high frequencies where the compact source may contribute most of the total intensity might erroneously be associated with a normal low-frequency spectrum associated with the more extensive component. Ideally, the angular structure should be determined at comparatively low frequencies.

In the present communication, six sources are considered for which interferometric (Int) measurements at long baselines have revealed values of $\theta < 0{\cdot}6$ sec arc. The measurements were made at 408 Mc/s with a baseline of 180,000 wave-lengths[4] and at 1,420 Mc/s with a baseline of 600,000 wave-lengths[5]. Evidence of the angular diameter at low frequencies was obtained by observing interplanetary scintillations (IPS) at a frequency of 178 Mc/s[6], and from such evidence the source 3C 208 was also included for consideration. The value of the angular diameter actually used in the calculation is indicated by an asterisk. The spectra of these sources were also determined and the value or limiting value of ν_m measured. These values together with the derived values of B are listed in Table 1.

On the assumption that the observed red-shifts are cosmological, the distance of each source can be determined and hence the volume. From this the value of the magnetic field, $B_{eq.}$, which would correspond to equipartition between magnetic field and particle energy may be computed. The values of $B_{eq.}$ are given in Table 1. If it is supposed that the total particle energy is 100 times that of the electrons[7] then the lower limits of $B_{eq.}$ should be increased by a factor of 3·7; the increased values are given in brackets.

It may be seen that for three of the sources listed (3C 48, 286 and 287) the derived upper limit of B is considerably lower than $B_{eq.}$, and for these sources the total energy of the relativistic electrons is far greater than the energy of the magnetic field. The minimum values of the total energy, U, needed for each source are included in Table 1; in brackets are given the corresponding values of the total energy if it is assumed that the total particle energy is 100 times that of the electrons. Finally, for comparison, the minimum total energies for equipartition are listed.

Table 1

3C	z	θ_{178} (IPS)	θ_{408} (Int)	θ_{1420} (Int)	ν_m (Mc/s)	B (gauss)	B_{eq} (gauss)	U (erg)	U_{eq} (erg)
48	0·37	$<0{\cdot}7''$	$0{\cdot}4'' \times *$ $\leqslant 0{\cdot}3''$	—	70	$\leqslant 1{\cdot}8\ 10^{-4}$	$\geqslant 4{\cdot}5\ 10^{-4}$ $(1{\cdot}7\ 10^{-3})$	$\geqslant 1{\cdot}2\ 10^{57}$ $(1{\cdot}1\ 10^{59})$	$\leqslant 6{\cdot}10^{56}$ $(8\ 10^{57})$
119	—	$<0{\cdot}5''$	—	$0{\cdot}16'' \times *$ $\leqslant 0{\cdot}1''$	70	$\leqslant 4\ 10^{-5}$	—	—	—
138	—	$\leqslant 0{\cdot}15''*$	$\sim 0{\cdot}5''$	—	80	$\leqslant 1{\cdot}7\ 10^{-4}$	—	—	—
147	0·55	$<0{\cdot}7''$	$0{\cdot}6'' \times *$ $\leqslant 0{\cdot}4''$	—	105	$\leqslant 5{\cdot}10^{-3}$	$\geqslant 3{\cdot}6\ 10^{-4}$ $(1{\cdot}3\ 10^{-3})$	—	$\leqslant 2{\cdot}0\ 10^{57}$ $(2{\cdot}8\ 10^{58})$
208	1·11	$\sim 1''*$	$>4''$	—	$\leqslant 30$	$\leqslant 1{\cdot}1\ 10^{-4}$	$\geqslant 1{\cdot}8\ 10^{-4}$ $(6{\cdot}7\ 10^{-4})$	$\geqslant 1{\cdot}1\ 10^{58}$ $(1{\cdot}0\ 10^{60})$	$\sim 9\ 0\ 10^{57}$ $(1{\cdot}3\ 10^{59})$
286	0·85	$<1''$	$\leqslant 0{\cdot}4''$	$0{\cdot}12'' \times *$ $\leqslant 0{\cdot}1''$	$\leqslant 80$	$\leqslant 1{\cdot}0\ 10^{-5}$	$\geqslant 6{\cdot}0\ 10^{-4}$ $(2{\cdot}2\ 10^{-3})$	$\geqslant 2{\cdot}5\ 10^{59}$ $(2{\cdot}5\ 10^{61})$	$\leqslant 1{\cdot}0\ 10^{57}$ $(1{\cdot}4\ 10^{58})$
287	1·06	$<1''$	$\leqslant 0{\cdot}4''$	$0{\cdot}14'' \times *$ $\leqslant 0{\cdot}1''$	$\leqslant 80$	$\leqslant 5\ 10^{-5}$	$\geqslant 6{\cdot}0\ 10^{-4}$ $(2{\cdot}2\ 10^{-3})$	$\geqslant 1{\cdot}7\ 10^{58}$ $(1{\cdot}7\ 10^{60})$	$\leqslant 8\ 0\ 10^{56}$ $(1{\cdot}1\ 10^{58})$

The most striking of these sources is 3C 286, and for this source especially further evidence is needed to determine the angular diameter at low frequencies and so eliminate the possibility of a structure similar to 3C 273. Some evidence has been obtained by observations of interplanetary scintillations which show that at 178 Mc/s the source has a diameter which is certainly less than 1 sec arc[6].

The uncertainty in the value of ν_m for 3C 287 is due to confusion by the neighbouring source 4C 25·44; ν_m could indeed be considerably less than 80 Mc/s, in which case the magnetic field would be even weaker and the total energy even greater than the values quoted.

These results suggest that very compact sources contain an intense flux of relativistic electrons in a comparatively weak magnetic field; the total energies involved are then comparable with those of the extensive radio galaxies. The magnetic field is of course too weak to contain the electrons. The association between quasi-stellar sources and radio galaxies has already been suggested by the observations of 3C 47 (ref. 8), which showed that a source with large total energy requirements was associated with a compact optical object showing strong ultra-violet excess.

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GEOPHYSICS

Breakdown of the Biennial Variation of Ozone and of Lower Stratospheric Temperatures in the Middle Latitudes of the Southern Hemisphere

A BIENNIAL variation in atmospheric ozone has recently been reported at Australian, Indian, Japanese and Italian stations[1,2]. This phenomenon was tentatively connected with the biennial variation of zonal winds in the stratosphere of low latitudes[2,3], which was elucidated by several other workers[4–6]. Evidence was also put forward to show that there existed at Laverton (37·9° S., 144 8° E.) and Macquarie Island (54·5° S., 159·0° E.) in middle latitudes of the southern hemisphere a biennial variation of temperature at 60 mbar with higher temperatures in even years when the spring level of ozone was high[3].

An examination of the recent ozone data at Brisbane (27·5° S., 153·0° E.), Aspendale (38·0° S., 145·1° E.) and Macquarie Island (Fig. 1) brings out the following: (1) since the beginning of the ozone observations at Aspendale and Brisbane in 1955 and 1956 respectively up to 1963, the spring maximum (July–October) was higher in even years. However, in 1964 the level of ozone in July–October was more or less the same as in 1963 and 1965. (2) At Macquarie Island the level of ozone was slightly higher in 1958 than in 1957 and also was higher in 1964 than in 1963. But in 1965 the level seems to be the same as in 1964.

By taking 12-month running means the breakdown of the biennial cycle is clearly shown after the years 1962–1963 (Fig. 2). Although the total ozone amount in 1964 was not higher than in 1963 the ozone content between 24–36 km as calculated from 'umkehr' observations made at Aspendale continued to be in agreement with the biennial oscillation with the ozone in 24–36 km greater in 1964 than in 1963 (ref. 7).

It is interesting to note that the earliest ozone observations made with the old type of Dobson's spectrograph in the years 1929–32 at Mt. Stromlo, Canberra (35·3° S., 149·0° E.), also showed no biennial variation in the spring level of ozone of the type observed from 1955 to 1965. 1929–32 was a period of low sunspot activity.

Fig. 3 gives the mean monthly temperatures at 100 mbar at Laverton, Hobart (42·8° S., 147·5° E), and Macquarie Island from 1951 to 1964. These data were kindly supplied by the Commonwealth Bureau of Meteorology, Melbourne. The following points are interesting: (1) the level of maximum temperature in July–October at Laverton and Hobart seems to vary in alternate years from 1954 to 1962 with higher temperatures in even years. However, in the years preceding 1954 and after 1962 there is no such oscillation. (2) At Macquarie Island the level of minimum temperature in June–September seems to oscillate with a 2-year cycle from 1954 to 1963, whereas the cycle is somewhat uncertain prior to 1954.

These facts are brought out more clearly in Fig. 4, which gives the average 100 mbar temperature for the months July, August and September (Fig. 4). As can be seen from the diagram, between 1954 and 1962 there existed a biennial oscillation in the 100 mbar temperatures at Laverton, Hobart and Macquarie Island. The maximum temperatures at Laverton and Hobart and the minimum temperatures at Macquarie Island were higher in even years. Prior to 1954 and after 1963 there appears to be no oscillation (excepting Macquarie Island where the oscillation continued even after 1963). It should be remembered that the years 1954 and 1963 are years of low solar activity. It can also be seen that there is a suggestion of a gradual cooling at 100 mbar from 1955 to 1961.

Almost all the work done on the biennial oscillation of zonal winds over low latitudes and similar temperature and ozone oscillations is based on observations made since 1953.

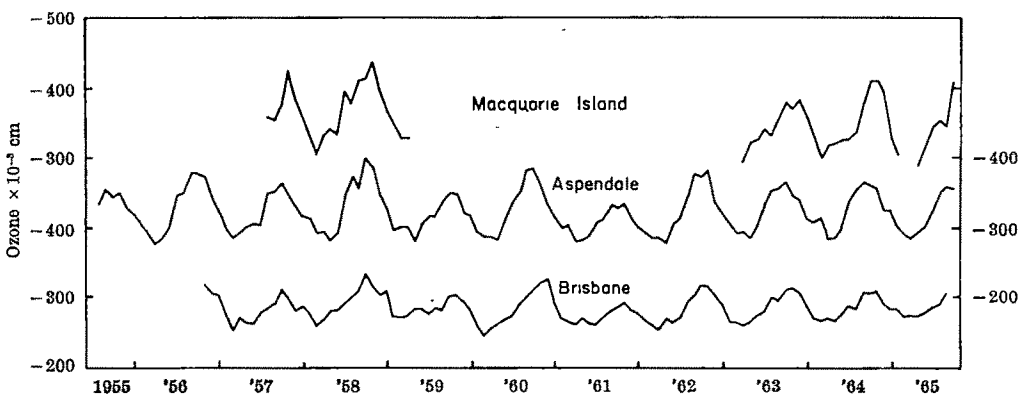


Fig. 1. Monthly mean ozone values at Brisbane, Aspendale and Macquarie Island

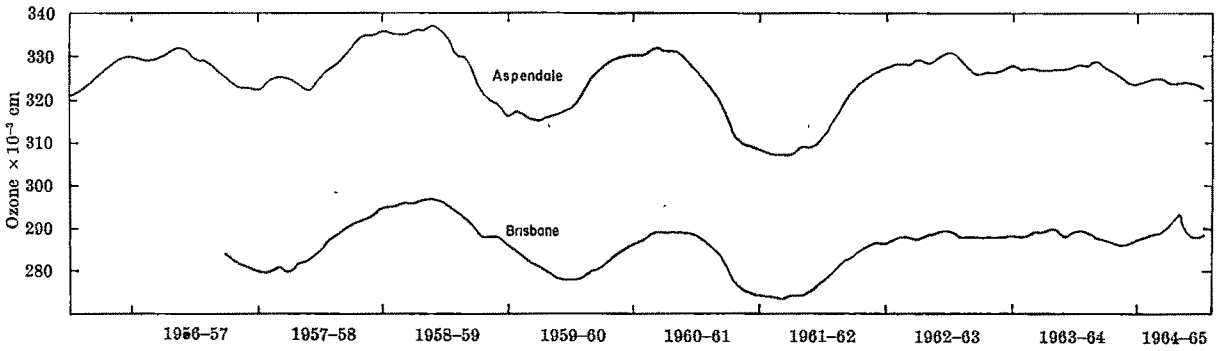


Fig. 2. Running 12-month means of ozone for Aspendale and Brisbane

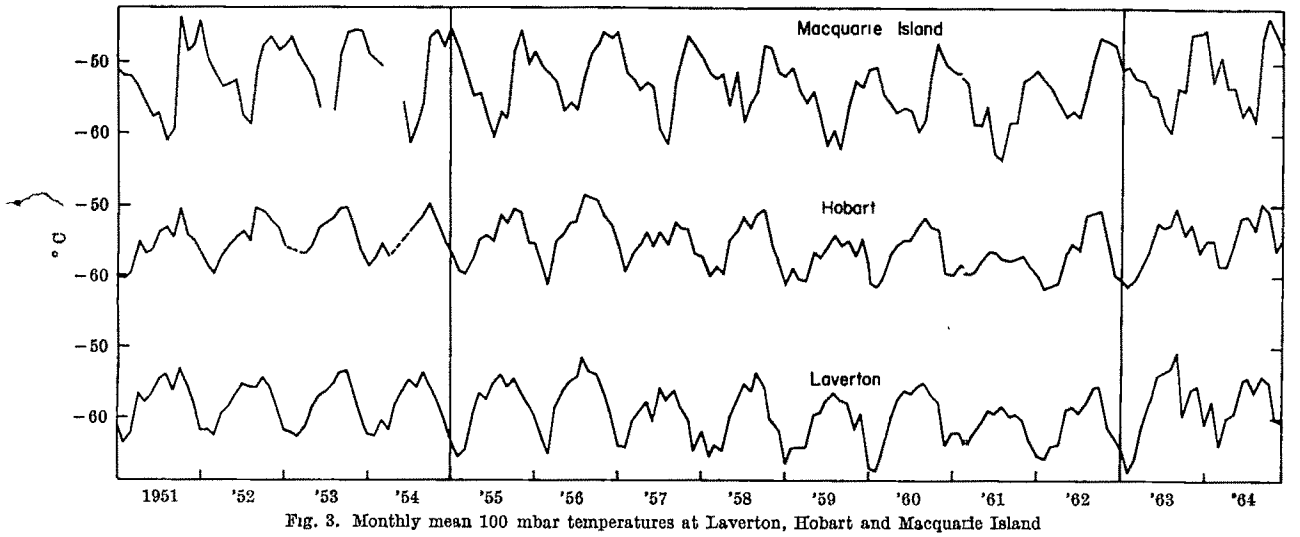


Fig. 3. Monthly mean 100 mbar temperatures at Laverton, Hobart and Macquarie Island

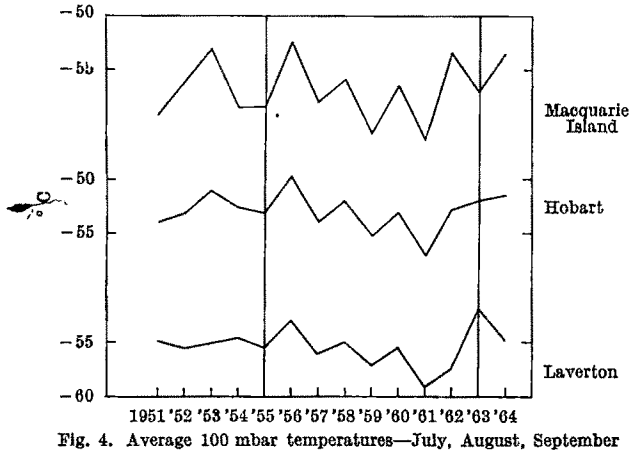


Fig. 4. Average 100 mbar temperatures—July, August, September

The above analysis tends to show that a biennial oscillation existed between 1954 and 1963 and not prior to 1954 and after 1963. It is interesting that the cycle should have been observed between periods of low sunspot activity, though with such a limited amount of observations any suggested connexion can only be tentative. The other possible explanation that the period of the cycle is not exactly 24 months, with its consequent disappearance after some time, gains little support from the fact that the occurrence of the breakdown is rather abrupt. It is, however, possible that the process responsible for the biennial oscillation could have descended to lower levels during 1954–63 to effect a temperature oscillation at the 100-mbar level. The observations that ozone above 24 km was higher in 1964 than in 1963, but not the total ozone, and that there was a breakdown of the cycle in 100-mbar temperature in 1963 imply that the influence of the process did not extend down to lower levels after 1963.

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PHYSICS

Application of the Principle of Extremum to a Submerged Discharge

IN hydraulic problems the use of the principle of extremum has produced results in good agreement with the results of experiments for some cases in which streamlines are nearly rectilinear and the flow passes from subcritical to supercritical flow. This type of flow occurs in hydraulics when water flows over a broad-crested weir, in a drop structure, or in a channel with side constriction. Flow over a sharp-edged weir, however, or over a curved spillway crest, involves curved streamlines which have the effect of increasing the flow considerably. It has been shown that the principle of extremum can be applied to curved streamlines[1] and this method has also been used for the calculation of discharge over a sharp-edged weir, giving values which agree very well with experiment[2]. In all the foregoing cases the principle of extremum has been applied to free surface flow, in which the boundary

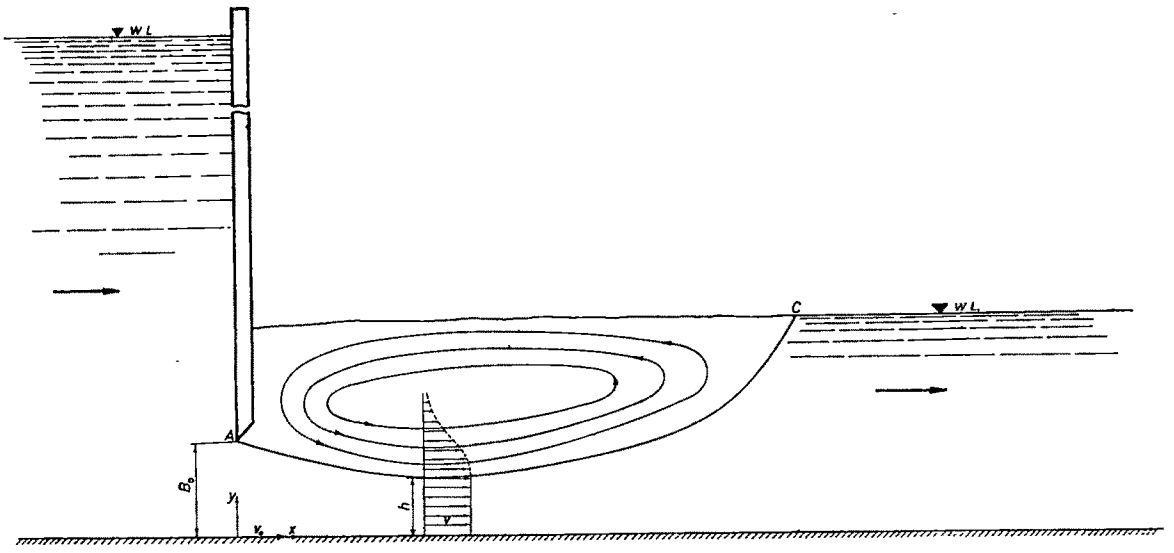


Fig. 1. Jet from a sluice gate with velocity distribution at critical section

streamlines are free to adjust for the condition of a minimum of energy head. However, the principle can also be used for the determination of the contraction coefficient when the boundary streamline is submerged, as in the case of submerged jet issuing from a sluice gate; in this case there is a separation-line (defined later) which also adjusts to a minimum of energy head.

It is not necessary to know the exact velocity distribution in a section for the application of the principle of extremum. The true distribution will be determined by its use when the velocity is given at known boundaries. The two following conditions can be derived from the principle of extremum for its application to hydraulics: (1) Discharge through a critical section is a maximum when the energy head upstream of the critical section is not influenced by the change of depth downstream from the section. (2) Discharge through a critical section requires the minimum energy with which it is possible to fulfil the theorem of Bernoulli. Conditions 1 and 2, which indicate Q_{max} and H_{min}, respectively, must simultaneously be fulfilled in the critical section[2,3].

A submerged jet issuing from a vertical opening of a sluice gate does not immediately spread in the upward direction but forms a compact stream for some distance, which is bounded by the floor and to the curved boundary defined by line AC in Fig. 1. Furthermore, the water is supplied from a large reservoir of high head. It is assumed for this analysis that the floor is a smooth rigid boundary and does not exert any resistance on the flow. Moreover, the rigid boundary will be considered as a streamline with velocity V_0 (Fig. 1). The assumption of a frictionless rigid boundary is justified, because the section under consideration, where the compact stream reaches its minimum depth, lies at a distance from the gate of about twice the height of the gate opening, as has been demonstrated by model tests[3], and consequently for large-scale flows frictional forces over this length are negligible. The upper boundary of the compact stream is separated from the rest of fluid by the line AC, which is defined as the separation-line. The fluid in the region above this line does not take part in the motion of the jet. It is either at rest, or it has a strong closed circulation which does not affect the main motion of the jet.

According to Strscheletzky[4], the separation-line AC can be identified as a separation-line with a second-order discontinuity, in which velocities, accelerations and pressure of fluid particles in an incompressible and inviscid fluid are equal on both sides of the separation-line. It can further be shown that the derivative of velocity along the streamlines, and all components of acceleration and pressure, are continuous across the separation-line. But the derivative of the velocity in a normal direction indicates a discontinuity (Fig. 2). The fluid particles of an inviscid fluid which are separated by the line AC do not slide on each other, but the first-order partial derivatives of velocity components across the separation-line are related to each other. Viscosity in a real fluid introduces an additional limiting condition to the foregoing partial derivative, but which does not nullify the existence of a distinct separation-line.

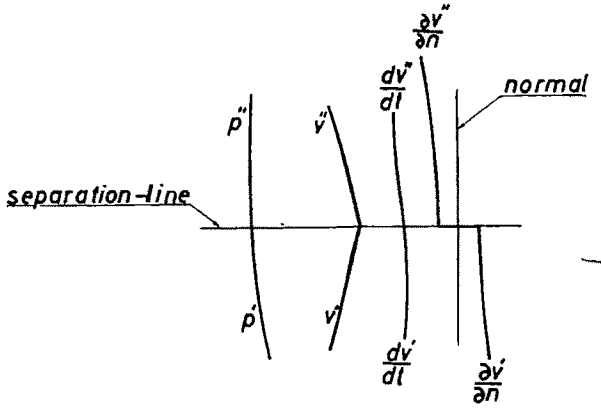


Fig 2. Second-order discontinuity in an incompressible fluid (according to Strscheletzky)

The form of the separation-line can be seen in practice by injecting dye to one side or the other, but the separation-line cannot easily be distinguished because of the turbulent diffusion of the flow across it. There is, however, a separation-line in a real fluid which forms in almost the same manner as in an incompressible inviscid fluid. A submerged jet issuing from a sluice gate, therefore, taking into account all forces acting on it, can be considered as a conservative system in which the principle of extremum is applicable for determining the contraction coefficient.

A submerged jet issuing from a sluice gate can be represented by substituting a rigid boundary for the plane of symmetry of a completely submerged jet (free turbulent flow). This has been studied by Tollmien[5] and other investigators. The velocity distribution of such flow is expressed as a term of the error function, but the use of the error function in this analysis introduces unnecessary complications. Moreover, as already mentioned, the principle of extremum does not require an

exact velocity distribution. For simplicity, we take an approximate equation for the velocity distribution:

$$\frac{V}{V_0} = \frac{a^2}{\left(\frac{y}{h}\right)^2 + a^2} \quad (1)$$

where h is the height of the submerged jet (Fig. 1), a being a constant which will be determined from the boundary condition

$$\frac{V}{V_0} = \alpha \text{ when } y = h$$

in which α is given by Albertson and others[6]:

$$\alpha = \exp\left(-\frac{(h + \sqrt{\pi}\, c_1 x - B_0)^2}{2(c_1 x)^2}\right)$$

where B_0 is the gate opening and x is the distance of the critical section from the gate. The value of c_1 has been taken as 0·109 since it has been assumed that the vena contracta lies in the 'zone of flow establishment' in which the core of the issuing jet of constant velocity, V_0, steadily decreases with distance from the gate. Further, since the contraction is gradual, the transverse components of velocity and acceleration may be neglected near the vena contracta. As shown by Henry[7], this assumption is justified, because the jet issuing from a sluice gate is similar to that of a free jet discharging from a nozzle or slot. It follows that:

$$a = \sqrt{\frac{\exp(-X)}{1 - \exp(-X)}}$$

where

$$X = \frac{(h + \sqrt{\pi}\, c_1 x - B_0)^2}{2(c_1 x)^2}$$

Hence, equation (1) can be written:

$$\frac{V}{V_0} = \frac{1}{\left(\sqrt{\frac{1 - \exp(-X)}{\exp(-X)}} \cdot \frac{y}{h}\right)^2 + 1} \quad (2)$$

and discharge per unit width can be obtained from equation (2) as follows:

$$q = \int_0^h v\, dy$$

$$= V_0 \sqrt{\frac{\exp(-X)}{1-\exp(-X)}}\, h \text{ arc tan} \sqrt{\frac{1-\exp(-X)}{\exp(-X)}} \quad (3)$$

According to condition 1, already defined:

$$\frac{\partial q}{\partial h} = 0$$

and by its application in equation (3) the following equation is obtained:

$$\left(1 - \frac{2\sqrt{X}\, h}{2c_1 x\,(1-\exp(-X))}\right) \text{arc tan} \sqrt{\frac{1-\exp(-X)}{\exp(-X)}} + h\, \frac{\sqrt{2X}\, \exp(-0{\cdot}5\, X)}{2c_1 x \sqrt{1-\exp(-X)}} = 0 \quad (4)$$

Equation (4) has been solved graphically to determine the value of h, when x, the distance of the vena contracta downstream of the gate, has been taken as twice[3] the gate opening. When there is a large head upstream of the gate, calculation gives the value for the contraction coefficient C as 0·61 for all gate openings. Hsin-Kuan Liu's measurements in a small-scale model gave C constant for all gate openings but with a mean value of approximately 0·67. The discrepancy is probably due to the low Reynolds number of his tests. The velocity distributions obtained from equation (2) have similar forms (Fig. 1) to some of those given by the measurements of Henry[7].

Thus the use of the principle of extremum allows the calculation of the contraction coefficient, which is in reasonable agreement with measured values for a case of submerged flow.

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Study of Correlation of the Three Basic Indices of Ionospheric Propagation: R_{12}, I_{F2} and Φ

SOON after the introduction of regular ionospheric measurements in 1938, it was found that the critical frequency of the $F2$ layer is closely correlated with the relative sunspot-number R. Further investigation has led to the introduction of the 12-months' running average of this number, R_{12}, as a basic index of ionospheric propagation[1].

About 1947, Medd and Covington[2] began regular measurements of the solar noise flux at 10·7 cm, Φ, which was also found to be correlated with ionospheric characteristics.

Later, about 1955, Minnis and Bazzard[3] introduced the index I_{F2}, now based on measurements at nine long-established ionospheric observatories, and monthly values of I_{F2} are now available from 1938 onwards.

In 1963, the International Radio Consultative Committee introduced these three indices, R_{12}, I_{F2} and Φ, as basic indices for ionospheric propagation, which are at present regularly published in the *Telecommunication Journal* of the *International Telecommunication Union*.

While the values of Φ and of I_{F2} are known and published the month following the month in question, the value of R_{12} is known only after a delay of 7 months.

The existing series of values of R_{12}, I_{F2} and Φ permits the establishment of correlation curves of these three indices and the use of these curves for the prediction of the other two indices, using the prediction of one index as a base. The form of these curves, their approximate analytical formulae and numerical values can be found in Figs. 1 and 2 and Table 1. The points in the figures show averages of the respective values taken in intervals of 5.

Table 1

R_{12}	I_{F2}	Φ
0	− 20	69
10	− 1	70
20	+ 16	75
30	+ 30	82
40	+ 41	88
50	+ 54	97
60	+ 67	106
70	+ 80	116
80	+ 91	126
90	+103	136
100	+114	146
110	+124	156
120	+133	166
130	+142	176
140	+150	186
150	+158	196
160	+166	206
170	+172	216
180	+177	226
190	+182	236
200	+187	246
210	+192	256

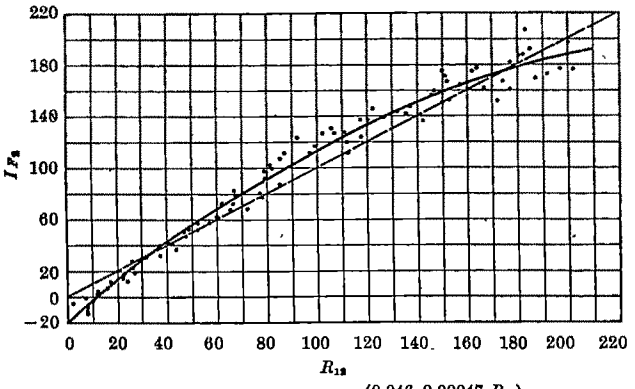


Fig. 1. $I_{F_2}=(2{\cdot}05+0{\cdot}001\ R_{12})R_{12}^{\ (0{\cdot}946-0\ 00047\ R_{12})}-20$

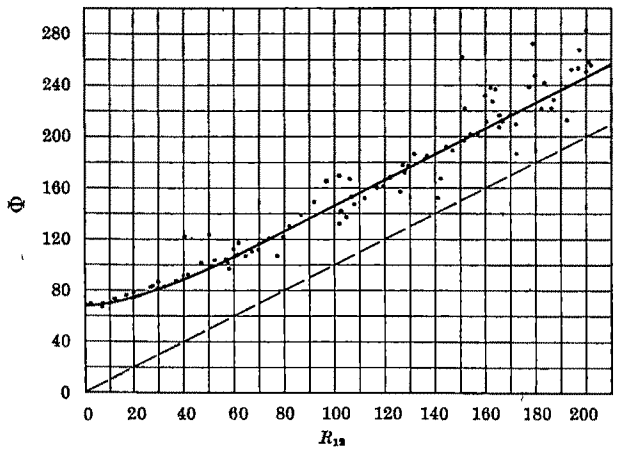


Fig. 2. $\Phi=R_{12}+46+23^{-0\ 05\ R_{12}}$

Since the index I_{F2} can be calculated with only a short delay and since, at the same time, it is possible to evaluate the preceding prediction up to 6 months ahead, this index is used as a basis for predicting R_{12} and Φ. The prediction of R_{12}, according to that procedure, can be considered only as an additional attempt, as predictions of R_{12} by other means are already well established. For the values of Φ, this method presents the first attempt at prediction and can be used in the evaluation of the 'planetary' F_2-layer ionization, quite recently proposed by Chaman Lal[4], to constitute another basic index of ionospheric propagation.

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GEOLOGY

Strontium Isotopes and Trace Elements in Carbonatites and Limestones from Ice River, British Columbia

HIGH in the westerly ranges of the Canadian Rockies the Ice River alkaline complex, described in a classic memoir by J. A. Allan[1], intrudes the Ottertail Limestones (of Cambrian age) forming an extensive metamorphic aureole of contact skarns and hornfels. In the heart of the complex, on Aquilla Ridge, is a large mass of carbonate rocks which Allan described as an "abnormally large xenolith" of metamorphosed Ottertail Limestone "at least ¾ mile long", but which J. E. Rapson[2,3] has recently shown to be even more extensive and to consist of intrusive carbonatites. This locality is therefore a particularly favourable one in which to test certain isotopic and chemical criteria which are used to differentiate carbonatites from metamorphic limestones, and for this purpose we have examined a small series of specimens provided by J. E. Rapson. They comprise five carbonatites from Aquilla Ridge, three metamorphosed limestones from the contact of the nepheline syenites and ijolites with the Ottertail Limestone, and four specimens of unaltered Ottertail Limestone, one from nearby, and three from the Palliser River area 60 miles to the south-east, far from any possible effects of the intrusion. The Palliser River specimens, collected by Dr. G. B. Leach and donated by the Geological Survey of Canada, are from three well-spaced points in a stratigraphic section 1,450 ft. in thickness.

Petrographically the Ottertail Limestone specimens are fine-grained laminated rocks, predominantly calcitic, but with a variable dolomite content which is sometimes substantial. They are somewhat argillaceous, and all contain a few per cent of authigenic albite. The metamorphosed limestones, from close to the contact with the syenites, are calcite marbles containing phlogopite, tremolite and minor amounts of albite and pyrite. The carbonatites comprise both calcitic and ankeritic rocks containing minor amounts of phlogopite, chlorite, feldspar, apatite, pyrite, barite and other accessories, which in one sample (*G*.II 7) include very minor amounts of pyrochlore.

Strontium was extracted from the carbonates in dilute acid and separated as the nitrate. The isotope ratios were determined at Oxford on an A.E.I. *MS*.2 solid-source mass spectrometer (6 in. radius tube) and all $^{87}Sr/^{86}Sr$ ratios were normalized to an $^{86}Sr/^{88}Sr$ ratio of 0·1194. Calibration of this machine against the *MS*.5 mass spectrometer (12 in. radius tube) on which earlier determinations by Hamilton and Deans[4] were made has not yet been completed, but so far determinations of the $^{86}Sr/^{88}Sr$ ratio on 48 different samples (limestones, carbonatites, granites, micas and feldspars) have given an average of 0·1198. Selected trace elements were determined spectrographically by Miss D. Hosking in the laboratories of the Mineral Resources Division, London. The results, shown in Table 1, confirm the validity of these criteria for distinguishing carbonatites from limestones, and merit some brief comments.

Powell[5] has tabulated the $^{87}Sr/^{86}Sr$ ratios of thirty-two carbonatite samples, all from different complexes in America, Europe and Africa, and his results show that in this class of rocks the ratio is remarkably constant, with a mean value of 0·7032, and the standard deviation of this mean $\sigma = 0{\cdot}0002$. The Aquilla Ridge carbonatites with a mean ratio of 0·7029 ($\sigma = 0{\cdot}0006$) conform to Powell's mean, and we believe that the rather wider scatter of our

Table 1. ANALYTICAL RESULTS FOR ICE RIVER AND PALLISER RIVER CARBONATE ROCKS

Sample reference	Principal carbonates	Carbonate content %	$^{87}Sr/^{86}Sr$ ratio	Trace element content (%) Nb	La	Sr	Ba	Zr
Ottertail Limestones, Palliser River								
LDB 27.7	Calcite	75	0·7118	*a*	*b*	0·28	0·05	*c*
LDB 27.11	Calcite and dolomite	75	0·7188	*a*	*b*	0·31	0·04	*c*
LDB 27.25	Calcite	90	0·7131	*a*	*b*	0 22	*c*	*c*
Ottertail Limestone, Ice River								
A	Calcite	96	0·7121	*a*	*b*	0·13	*c*	0·02
Contact metamorphosed limestone, Ice River								
GN.24	Calcite	70	0·7088	*a*	*b*	0·14	0·02	0 007
J.2	Calcite	88	0·7131	*a*	*b*	0·23	0·10	*c*
SB.10	Calcite	90	0·7119	*a*	*b*	0·16	0·04	*c*
Carbonatites, Aquilla Ridge, Ice River								
G. II 7	Calcite	92	0·7021	0·22	0·15	1·1	0·71	*c*
G. III 17*c*	Calcite	90	0·7047	0·005	0·05	0·27	0·10	0·007
G. III 16	Ankerite	92	0 7014	0·004	0·20	0·94	0·88	0·005
T.36	Ankerite	99	0·7037	0 003	*b*	0·39	0·03	*c*
T.37	Calcite and ankerite	92	0 7026	0 003	0·10	0·84	0·35	*c*

$a = <0{\cdot}002$. $b = <0\ 01$. $c = <0{\cdot}005$.

results is probably due to lack of certain experimental refinements. In contrast the limestones and metamorphosed limestones show substantially higher $^{87}Sr/^{86}Sr$ ratios, with mean values of 0·7113 (σ = 0·0013) and 0·7146 (σ = 0·0021) respectively. Applying the statistical 't' test to compare these three groups (omitting the single specimen A), we find:

Carbonatites/metamorphosed limestones, t = 6·8
Carbonatites/unaltered limestones, t = 6·7

whereas the critical value of 't' for 1 per cent probability is 3·7 with six degrees of freedom. In marked contrast, for:

Metamorphosed limestones/unaltered limestones, t = 1·3 (critical value of 't' for 25 per cent probability is 1·34 with four degrees of freedom).

These calculations substantiate our contention that the $^{87}Sr/^{86}Sr$ ratio of the carbonatites is significantly different from that of the Ottertail Limestones, whether metamorphosed or not, and demonstrate also that metamorphism has not altered this ratio.

The trace element data clearly reveal that the carbonatites are characterized by the presence of niobium and lanthanum, elements not detected in the limestones and marbles, and by a markedly greater abundance of strontium and barium. These results are completely in keeping with experience elsewhere. It may be noted that in four out of five cases the concentrations of niobium, lanthanum and strontium in the carbonatites are much below the levels associated with the appearance of distinctive minerals, such as pyrochlore, so that this characteristic might easily pass unnoticed if a mineralogical examination of a few specimens was solely relied on. The absence of niobium and lanthanum from the contact metamorphosed limestones is also noteworthy, and in keeping with general experience, for although the alkaline silicate rocks associated with carbonatites commonly carry the same suite of trace elements as the latter, no cases are recorded of these elements having diffused from the alkaline rocks into adjacent limestone skarns.

Hayatsu *et al.*[6] have recently expressed doubts regarding the significance of strontium isotope ratios in investigations of carbonatite, citing new preliminary results from eastern Canada where Grenville Marbles have $^{87}Sr/^{86}Sr$ ratios ranging from 0·7072 where no alkaline metasomatism has occurred, to 0·7030 (= average for carbonatites) where the marble has reacted with calcite-free syenite. The contact metamorphism of the Ottertail Limestones by the Ice River syenites was clearly much less intense than that in the Grenville province, so that the conflicting evidence arises in very different circumstances. More extensive and detailed studies of the Grenville rocks might lead to a better understanding of this problem.

We thank those of our colleagues who helped us in the laboratory work, and Mr. E. H. Beard and the late Prof. L. R. Wager for their support in this research.

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Mechanisms of Cauldron Subsidence

WILLIAMS'S subdivision of calderas into 'Krakatoan' and 'Glen Coe' types[1] has been the subject of recent discussion[2–4]. Williams's concept of the 'Krakatoan' mechanism is that explosive eruption of pumiceous volcanic products from the vents of a central-type volcano produces a void in the underlying magma chamber; the unsupported chamber roof then collapses, producing a caldera at surface level bounded by a ring-fault escarpment. The 'Glen Coe' type, on the other hand, is said to involve the stoping of magma up ring fractures and the coeval descent of the enclosed cylindrical block.

From gravity surveys of seven Japanese calderas Yokoyama[3] claims that four of these showing negative Bouguer gravity anomalies are of 'Krakatoan' type, and one is of 'Glen Coe' type and shows a positive anomaly; the lack of any anomaly on the remaining two is not commented on. McCall[4] has revised an earlier interpretation of the Menengai caldera in Kenya[5] and describes it as "primarily of 'Glen Coe' type" and not of the 'Krakatoan' type which he previously supposed. Due to the presence of an extensive bedded pumice mantle on the western flank of the volcano, however, McCall further amends its position in the classification of calderas by claiming it has "weak 'Krakatoan' characteristics" and is of a "weakly 'Krakatoan' sub-type".

I have spent several months in the detailed field investigation of Mount Suswa, a Pleistocene phonolite-trachyte volcano which lies 90 miles south of Menengai in the Rift Valley of Kenya. These two alkaline volcanoes show clearly defined calderas in the summits of primitive shield-shaped cones, and in both cases the formation of the calderas has been accompanied by the eruption of large quantities of pumice which mantle their western flanks. Unique to Mount Suswa, however, is an extensive series of lava flows (many of them of the coulée-type) that were erupted from the fissures of a complete ring-fracture complex which lies concentric with, but encircles, the caldera escarpment. These flows outcrop on the upper outer slopes of the volcano and were erupted in alternation with the pumice that constitutes the western flank. At the caldera escarpment the pumice beds mantle the caldera rim and occur as a banking inclined against the caldera wall. The intercalated ring-fracture flows are at all times concordant with the pyroclastic deposits and at the caldera rim are therefore seen to have flowed from the vents of the ring-fracture complex down the inwardly-inclined fault escarpment to occur in alternation with the beds of the inclined pumice banking. The eruption of the lava flows and the deposition of the pumice therefore post-date the formation of the caldera escarpment.

McCall in his earlier publication[5] clearly infers the post-caldera age of the Menengai pumice mantle from his accompanying sketch map and later referred[4] to the pyroclastic material as having originated by sudden pressure release caused by the initial downward movement of the cauldron block. This pumice is therefore the eruptive expression of the "weakly 'Krakatoan' characteristics" which are "a complication of the 'Glen Coe' mechanism".

Two main criticisms can be levelled at McCall's conclusions. The first is the use of the term 'Krakatoan'. Williams's hypothesis[1] clearly states that the ejection of the pumice pre-dates the subsidence and that therefore the evacuation of the magma chamber and the subsequent collapse are direct consequences of these eruptions. The demonstrable post-subsidence age of the Mount Suswa and Menengai pumice deposits obviously rules out the possibility that their eruption was the cause of the cauldron subsidence. The two calderas have apparently originated by collapse into a void caused by magmatic withdrawal, or as MacDonald has suggested[6], for the Hawaiian calderas, by the foundering of heavier crustal rocks into an underlying less-dense liquid magma. The

pumice eruptions associated with the formation of the Kenyan calderas appear to be consequences of this cauldron collapse and therefore their mode of origin is quite distinct from the role of the pumice eruptions in Williams's 'Krakatoan' mechanism. I therefore believe that the use of the term 'Krakatoan' in the context of the Kenyan calderas is an unfortunate one.

Secondly, McCall discusses the space problem presented by calderas of the 'Krakatoan' type (*sensu strictu*). It will be recalled that the problem is that estimates of the volume of pyroclastic material erupted at the time of caldera formation are usually much less than the volume of the caldera depression itself. Since a close agreement between the two volumes would add considerable support to the theory of the 'Krakatoan' mechanism, the problem is one which the advocates of the theory attempt to solve by various means. McCall, while recognizing the post-cauldron subsidence nature of the pumice of Menengai, finds it necessary to explain the volume deficit of the 'Krakatoan' calderas, the Kenyan examples included. However, if the pumice has originated by eruptions which were initiated by the pressure release resulting from cauldron subsidence (as suggested by McCall[4]), then no 'space-problem' exists as clearly the cauldron subsidence owes its primary origin to mechanisms other than removal of support by the initial explosive ejection of pumice; in other words, the volume of the caldera depression is not a function of the volume of juvenile magma ejected at the time of cauldron collapse.

The observation that eruptions of pumice may be the consequence and not a prerequisite of caldera formation has obvious implications to the general subject of cauldron subsidence mechanisms. 'Krakatoan' type calderas are frequently referred to in the literature, notably by authors working in the Pacific orogenic belts (for example, Yokoyama and Tajima[2], and Yokoyama[3]). The term 'Krakatoan' has precise genetic and geological implications, and from a consideration of the evidence presented by the Kenyan calderas it is perhaps justifiable to call for some restraint before assigning that name to calderas which merely have extensive pumice deposits associated with them: the exact relationship of the pumice deposits to the caldera ring fault is obviously of the greatest importance.

The preservation of post-subsidence pumice beds inclined against the caldera escarpment and the mantling of the caldera rim are features that perhaps are only found in special circumstances. If, for example (as suggested by McCall), a small initial downward movement of the cauldron block is arrested as the vesiculating magma expands and erupts, and the block continues to sink only when the eruptions have terminated, then the pumice beds would be truncated by the cauldron fracture and no mantling or banking would occur. In this manner pyroclastic flows ('ash flows', 'pumice flows', etc.) may also be preserved in exposures of the caldera wall. Similarly, if the upper surface of the subsided block is at such a depth that the top of any pumice banking fails to reach the higher levels of the caldera escarpment then the inclined pumice beds may consequently be obscured from view by post-caldera infilling. Backward erosion of the steep caldera escarpments would also remove any pumice beds that once were inclined against them.

McCall's recognition of the mechanism of eruption of the Menengai pumice beds (a mechanism confirmed by the depositional features on Mount Suswa) is therefore of considerable importance to the problem of the origin of calderas. It suggests that the primary mode of origin for certain collapse depressions which resemble the so-called 'Krakatoan' calderas may be by a mechanism that does not entail the initial evisceration of a magma chamber by the explosive ejection of pumice. I would like to emphasize, however, that the application of Williams's term 'Krakatoan' to the Kenyan calderas is misleading since (as already envisaged by McCall) the pumice in these examples has probably been ejected by eruptions which were inherent on the pressure release caused by the cauldron collapse, and not by primary eruptions occurring before the collapse.

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Occurrence of Pollen and Spores in the Roraima Formation of Venezuela and British Guiana

THE discovery of pollen and spores in beds considered Precambrian (Proterozoic) has received brief notice in geological journals and the press[1–3]. Individual authors will doubtless publish detailed stratigraphic and palynologic accounts of the occurrence in due course. Meanwhile it is considered desirable to give an outline of the facts of the case before distorted interpretations develop from inadequate data. The following summary statement has been prepared jointly by several members of the Asociación Venezolana de Geología, Minería y Petróleo. A single author is nominated to simplify bibliographic references.

Late in 1963 G. C. K. Dunsterville made an expedition to collect orchids around Cerro Venamo, at the westernmost point on the frontier between Venezuela and British Guiana (where this mountain is known as Wenamu Head). He noted some shale-like beds at the base of a towering cliff of Roraima sandstone and collected samples for their possible paleontological interest.

G. Fournier, palynologist of the Mene Grande Oil Company, processed the samples and recovered well-preserved pollen and spores. Subsequently, L. Nijssen and J. A. Sulek, palynologists of Compañía Shell de Venezuela and Creole Petroleum Corporation, respectively, processed other pieces and recovered identical plant microfossils.

This discovery of pollen and spores in a formation of supposed Precambrian age was so remarkable that a reconnaissance expedition of qualified geologists was organized to verify the facts of the case. During April, 1964, with the assistance of personnel and vehicles of the Ministerio de Minas e Hidrocarburos, the locality was visited by a party which included N. Benain, P. J. Bermúdez, A. Espejo, G. Fournier, A. Menéndez, J. A. Sulek and F. Wright. They confirmed the salient facts as recorded by Dunsterville. The shale-like beds, being less competent, had eroded away below the massive Roraima sandstone, leaving an undercut extending 10–12 ft. inwards at the base of the cliff. The original samples were loose, weathered fragments from the talus slope below. New samples of unweathered rock were collected from the face of the undercut.

On their return to Caracas, the three palynologists made independent investigations of the new samples. Utmost care was taken to avoid any possibility of superficial contamination. The rock cleaves along finely laminated bedding planes which are coated with limonite. Every effort was made to avoid these planes and some of the pieces processed were the central nubs left after chipping away the external parts of large blocks of the rock, which was dense enough to sound when struck with a hammer. Nevertheless, microfossils of the same type as before were recovered.

Dr. P. H. A. Martin-Kaye, director of the British Guiana Geological Survey, was told of these discoveries



Fig. 1. Hand specimen of 'shale' from Cerro Venamo locality, showing bedding plane parallel to bottom edge, and diagonal cleavage. Natural size

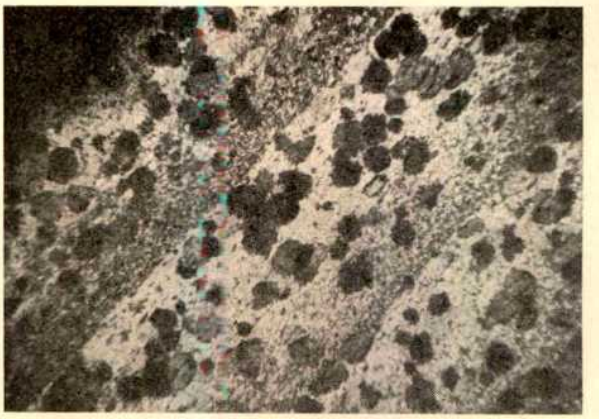


Fig. 2. Paruima hornfels (crossed nicols); large crystals are all cordierite in micaceous ground, with original fine quartz banding still preserved; biotite may be seen to right of centre of field. (×150)

in view of his group's sponsorship of radiometric dating of the Roraima Formation. He collaborated by sending samples of beds described as 'hornfelsized sediments' from a seemingly correlative level near Paruima on the Kamarang River, some 25 miles east of the original locality. As before, independent investigations were made of this material, and the same flora was recorded. Still more samples were submitted by the Survey, this time from the Kopinang area, about 110 miles south-east of Paruima. In this case, however, only indeterminate plant microfossils were obtained, not the distinctive suite recorded at Cerro Venamo and Paruima.

It needs to be stated that petrological investigation of the rocks which yielded pollen and spores showed them to be highly metamorphosed, even though in hand specimens they somewhat resembled indurated shales (Fig. 1). J. M. Bowen (Cia. Shell de Venezuela) described the samples from both Cerro Venamo and Paruima as true hornfelses in the cordierite-andalusite range, based on thin-section investigations (Fig. 2). Prof. H. H. Hess (Princeton University) confirmed this on the basis of X-ray diffraction investigations, and described the rock as a fairly typical hornfels, largely muscovite plus a little quartz, clay minerals apparently absent, and no chlorite noted. The samples from Paruima were rather more highly metamorphosed, as shown by the presence of biotite.

Fig. 3 shows a highly generalized regional cross-section based on the current geological map of British Guiana. The remarkably horizontal attitude of the Roraima Formation and the persistence of dolerite sills[4] over vast distances appear to justify the long-range correlations indicated. Relative positions are shown of the polliniferous samples and of samples dated Precambrian by radiometric techniques.

The palynological assemblage has not yet been matched conclusively against any known suite, a fact which is not surprising in view of the distance from control sections of known age and the highly endemic character of the flora of to-day, and probably the geological past, on the isolated Roraima Plateau. G. Fournier, by utilizing the herbarium of J. Steyermark, was able to compare the recent pollen of the area with the assemblage in the rocks, and has stated that they are not the same. The first opinion of Fournier was that the pollen showed Cretaceous affinities, but closer study has led to preference for a Tertiary age. L. Nijssen regards the best assemblage, from a Paruima sample, as not older than Eocene and quite possibly post-Eocene. J. W. Funkhouser (Bogotá) considers the pollen indigenous to the samples he processed but claims an age no older than Miocene, and probably younger; he notes similarities to the flora of the Mesa Formation (Pliocene-?Pleistocene) of Colombia, presence of pollen of the Compositae, and an uncompressed preservation highly unusual except in young sediments. T. van der Hammen (Leiden) recognizes a mixture of Mesozoic and Cenozoic elements, but suspects that they represent foreign material concentrated along cleavage

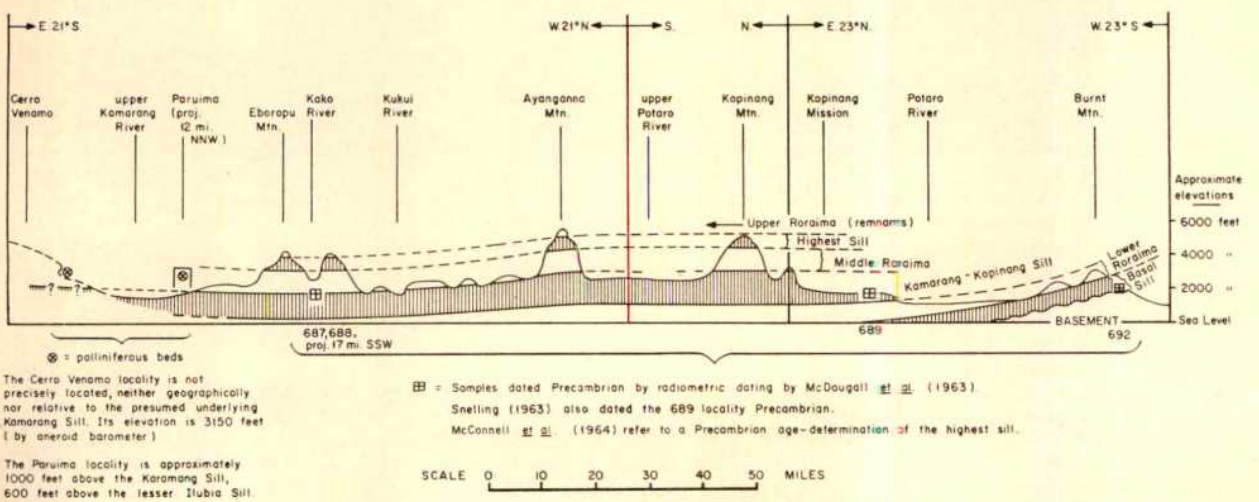


Fig. 3. Regional cross-section through the Roraima Formation in western British Guiana showing positions of polliniferous beds and of samples determined radiometrically as Precambrian

planes as, after cleaning fragments ultrasonically, he found the matrix practically barren.

As to interpretation of the significance of the fossil pollen and spores, two sharply divided opinions have been expressed. The writers make no attempt to adjudicate, but state the two concepts impartially.

One group adopts the attitude that the radiometric dating[5–7] of dolerites and a hornfels[6] within the Roraima Formation as Precambrian is beyond dispute, hence the pollen (and spores) must have entered as secondary contamination. The improbability that pollen could withstand the baking process, which converted shale to hornfels, is adduced as further evidence that the pollen must be allochthonous. The absence of macroscopic plant remains in the Roraima Formation is also noted, despite its assumed continental (? fluviatile) origin. It is admitted that entry of the pollen into its present site defies simple explanation, though some form of washing in by meteoric waters in the geological past via joints in the overlying sandstone seems the most probable cause.

The second group holds that by no conceivable physical means could the pollen (and spores) have entered the metamorphosed sediments from the outside. They are dense impermeable rocks compressed by an overburden of hundreds of feet of the overlying Roraima sandstones. The undercutting at Cerro Venamo suggests that the cliff has been steadily retreating, hence the face which was sampled must have been deep within the formation until quite recent times. The Roraima sandstones are quartzitic, of low permeability, hence carriage of extraneous pollen through them by percolating water seems highly improbable. Even if this process could occur, entry of such pollen and spores into the non-porous hornfels lacks an explanation. Furthermore, if plausibility of this process be granted, it would have been operative for a long period, and a mixed suite of spores and pollen should be expected.

In counter-argument against the first group, it is claimed that the assertion that pollen and spores cannot withstand anaerobic baking of their parent shales has never been tested experimentally. As regards the radiometric dating, there is a disquieting overlap between stated ages of the Roraima Formation[5,6] and the underlying basement rocks[7–9]. The latter suffered complex deformation and vulcanism, and were then deeply peneplaned before being covered by thousands of feet of Roraima sandstones, and only after these prolonged events were the dolerites intruded, on which age-determination of the Roraima has been based. If the radiometric technique is valid there should be a long and clear-cut time-gap between ages assigned to the basement rocks and to the Roraima beds. Such a gap does not exist in the experimental results published, but this discrepancy is glossed over in the latest summary of radiometric dating in British Guiana[7].

As stated, we offer no solution to the paradox. It is clear, however, that botanist Dunsterville in his hunt for rare orchids stumbled on a highly intriguing geological problem.

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CRYSTALLOGRAPHY

Structure of Ferrierite

AN investigation into the rare aluminosilicate mineral ferrierite[1], $(Na, K)_4MgAl_6Si_{30}O_{72}2OH_2O$, has led to its crystal structure being proposed, which is based on the space group *Immm* and agrees reasonably with our X-ray powder data of 62 reflexions. After several cycles of least-squares refinement in which reflexions of zero intensity were included, an $R(Fo)$ factor of 16·5 per cent was obtained corresponding to the co-ordinates in Table 1.

Table 1

Atom	x	y	z	B (Å^2)
Si_1	0·174	0·290	0·279	1·3
Si_2	0·085	0·202	0	3·8
Si_3	0·270	0	0·289	7·2
Si_4	0·161	0	0	2·2
O_1	0·111	0·267	0·175	3·2
O_2	0·306	0·104	0·250	3·2
O_3	0·114	0·095	0	3·2
O_4	0·668	0·790	0	3·2
O_5	0·208	0	0·175	3·2
O_6	0·25	0·25	0·25	3·2
O_7	0	0·193	0	3·2
O_8	0·772	0·5	0	3·2

The framework, which is shown schematically in Fig. 1, is composed from rings of 10, 8, 6 and 5 linked Si/AlO_4 tetrahedra, and is closely related to the structures of mordenite[2], dachiardite[3] and epistilbite[4]. There are two systems of intersecting channels: (1) bounded by ten-membered rings; running parallel to c and approximately circular and 5·2 Å free diameter; (2) bounded by eight-membered rings; parallel to b; 4·7 by 3·7 Å in free cross-section and leading into cavities about 6·2 Å free diameter.

Although it has not been possible to position the cations and water molecules from X-ray data, sorption experiments suggest the cations may be situated in the wide channels and cause their partial blocking. It is hoped to

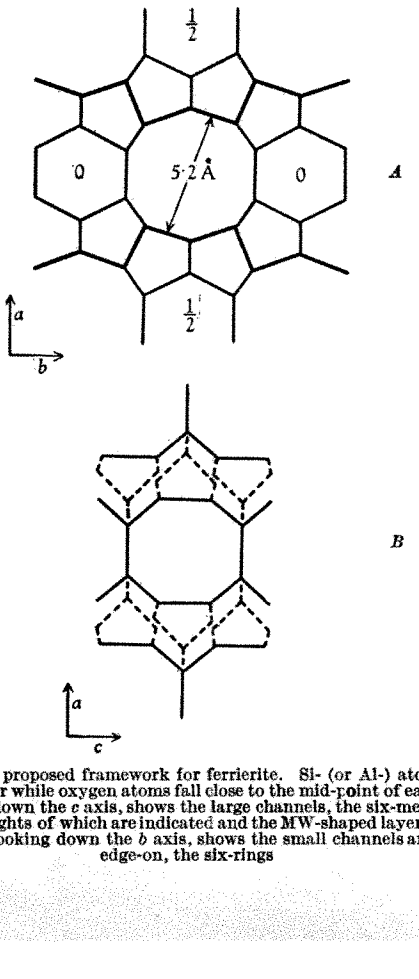


Fig. 1. The proposed framework for ferrierite. Si- (or Al-) atoms fall at each corner while oxygen atoms fall close to the mid-point of each line. *A*, Looking down the *c* axis, shows the large channels, the six-membered rings the heights of which are indicated and the MW-shaped layer (heavy lines). *B*, Looking down the *b* axis, shows the small channels and, seen edge-on, the six-rings

prepare a hydrogen-exchanged ferrierite for sorption experiments.

Since this work began, an incomplete description of a structure for ferrierite has been published[5]. While some features are similar to those described here, there are also marked differences, so that until more details of this other structure are made available, a measure of doubt will exist as to whether the two frameworks are identical or not. As part of the investigation the unit cell of our specimen was determined as orthorhombic I with $a = 19{\cdot}15$ Å, $b = 14{\cdot}12$ Å and $c = 7{\cdot}47$ Å. This differs from the heulandite cell by a small amount. It is, however, unlikely that there is a close connexion between the two structures, since the symmetry of heulandite is lower.

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CHEMISTRY

Electrical Conductivity and Photoconductivity of Pure Benzene

IN addition to reporting some results of measurements of electrical conductivity and photoconductivity of benzene, we wish to direct attention to the effect of conventional out-gassing and other techniques on the purity of solutions. The effect of contamination introduced by such procedures is of considerable practical importance and has also been noted by Dubois and Wilkinson[1] in another connexion. The conductivity measurements were incidental to a study which we undertook of photoconduction in organic solutions.

Recently, Forster[2] has published results of measurements of electrical conductivity of purified benzene. Typical values after polarization for 24 h at 25° C in a cell with fields up to a few hundred V/cm were $0{\cdot}61 \times 10^{-15}$ $(\Omega\ \text{cm})^{-1}$, or $6{\cdot}9 \times 10^{-15}$ $(\Omega\ \text{cm})^{-1}$ when the benzene was saturated with dry air. He found that close to the electrodes there was a rapid fall in potential, so that the effective field in the bulk of the solution was reduced. After correcting for this, the resulting conductivity was $1{\cdot}1 \times 10^{-14}$ $(\Omega\ \text{cm})^{-1}$. Forster also quotes work by Kraus and Fuoss[3] and Kortüm and Bockris[4] in which values of about 3×10^{-15} and 5×10^{-17} $(\Omega\ \text{cm})^{-1}$ respectively are stated. A recent paper by Sano and Akamatsu[5] gives a value of $6{\cdot}4 \times 10^{-14}$ $(\Omega\ \text{cm})^{-1}$.

Among these values, those given by Kortum and Bockris, which are from experiments by Jaffé, in 1908, are outstandingly low. Our work fully supports this value as the following account will show.

At first our experiments were carried out in a cell with platinum electrodes about 1 cm² in area with a 1 cm separation. Electrical measurements were made by means of a vibrating reed electrometer (E. K. Cole, Ltd., type 1341*A*).

After vacuum distillation and transfer of the benzene under dry oxygen-free nitrogen in a closed system to the conductivity cell, and polarization for 24 h, the conductivity was found to be $0{\cdot}33 \times 10^{-14}$ $(\Omega\ \text{cm})^{-1}$ in a field of roughly 240 cm^{-1}, which agrees with Forster's values. The measurements were then repeated in a special cell with a 0·0125 cm gap between transparent conducting quartz electrodes coated with stannic oxide, so that field strengths of 15,000 V/cm were readily attainable. After polarization for 15 min, the current was steady and the conductivity was $0{\cdot}7 \times 10^{-14}$ $(\Omega\ \text{cm})^{-1}$, which agreed with the earlier determination within the variation expected from different batches of vacuum-distilled benzene. If Forster's potential variation had applied to our system we should have expected the narrow gap cell to give considerably lower conductivity values (see a discussion of Forster's results by Silver[6], who has applied the classical Thomson treatment of conductivity in gases).

We then abandoned the conventional purification techniques, and used benzene which had first been crudely distilled, then very carefully distilled by means of a 25-stage bubble-cap fractionating column, with transfer to the conductivity cell by syringe. After 15 min polarization in the cell, conductivity values for different batches varied between 2×10^{-18} and 1×10^{-17} $(\Omega\ \text{cm})^{-1}$, similar to the values found by Jaffé. The success of this distillation procedure depends on careful control of conditions in the distillation column. Starting with 4 l. of sulphur-free benzene (May and Baker, Ltd.) about 400 ml. of purified benzene were obtained after distillation for 24 h. The purified benzene was stored before use in glass containers from which moisture was excluded by means of 3/16 in. 'Hi-drite' granules (Hi-drite, Ltd.) held in the hollow glass stoppers. Other than this, no special storage precautions were taken.

These observations suggested a further investigation of the effects of vacuum distillation. This was carried out in a system constructed of glass with 'Teflon' seals and 'Teflon' vacuum taps, using a glass freeze trap and an Edwards rotary pump containing type 8*A* oil. Degassing a sample of purified benzene with solid carbon dioxide in the trap caused a three-fold increase in conductivity. A complete vacuum transfer under the same conditions caused a hundred-fold increase in conductivity, which represents a return to values similar to those reported by Forster and others. Very similar results were obtained after thorough cleaning of the apparatus and use of a liquid nitrogen trap. Evidently such contamination is of major importance in conductivity work and may also be important in any other work where degassing cycles are used.

It is of interest to note the effect of oxygen on the electrical conductivity and photoconductivity of the very pure benzene. The conductivity of a control sample in a typical experiment was 4×10^{-18} $(\Omega\ \text{cm})^{-1}$ after 15 min polarization. After 5 sec exposure to a high-pressure mercury arc (Philips 125*W*) through a Corning 0–53 filter (less than 5 per cent transmission at 290 mμ; negligible at 280 mμ) the conductivity rose to 60×10^{-18} $(\Omega\ \text{cm})^{-1}$, and fell quickly to the original value when the radiation was cut off. When the sample was saturated with dry oxygen by bubbling for 30 min the initial conductivity was 14×10^{-18} $(\Omega\ \text{cm})^{-1}$ after 15 min polarization, which after the same 5 sec exposure rose to 100×10^{-18} $(\Omega\ \text{cm})^{-1}$. In both cases, prolonging the exposure beyond 5 sec caused a reduction in the photocurrent.

The results are qualitatively similar to Forster's, except that all processes are faster, presumably because the path lengths between the electrodes are shorter and the field strengths much higher. It will be seen that the conductivities in our experiments are more than two orders of magnitude less.

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Red Forms of Methylene Blue

METHYLENE blue dissolves in water and common organic solvents to give blue solutions absorbing light of wavelength near 660 mμ, for example 657 mμ in *n*-butanol (Fig. 1, curve *a*). A faint band at 480 mμ is just detectable, but can be seen more readily if the molar extinction coefficients are plotted on a logarithmic scale (ref. 1). Wine-red solutions have been obtained here when the dye is dissolved in simple aliphatic amines dried over potassium hydroxide pellets for several days and redistilled more than once if necessary. The blue colour of the dye could be restored by adding either water or ethanol; standing for several days in the amine, however, appeared to bring about some chemical change in the dye. Although the dye dissolved immediately in the hot amines the use of elevated temperatures was considered undesirable. Instead the dye was dispersed by ultrasonic vibration in the cold amine and then allowed to stand for several hours (usually overnight) to pass into solution. A typical spectrum is that in *n*-hexylamine shown in Fig. 1, curve *b*, and the special data for other systems are given in Table 1. The solutions were quite red, even when saturated; at the boiling point of the amine there was no trace of the blue form. This latter fact appears to eliminate dye–dye association (see ref. 2) and interaction with chloride counter-ions (see ref. 3) as important factors. The existence of the red form clearly depends on the strongly basic character of the amine. Thus the dye is red in primary, secondary (open-chain and cyclic), and tertiary aliphatic amines, all of which are strong bases with pK_b values in the range 2·8–3·4, and in the araliphatic amine benzylamine ($pK_b = 4·7$); aniline, pyridine, quinoline, and indeed all the other common organic solvents in which the dye is soluble are only weakly basic ($pK_b \geqslant 8·9$) and in them it is blue.

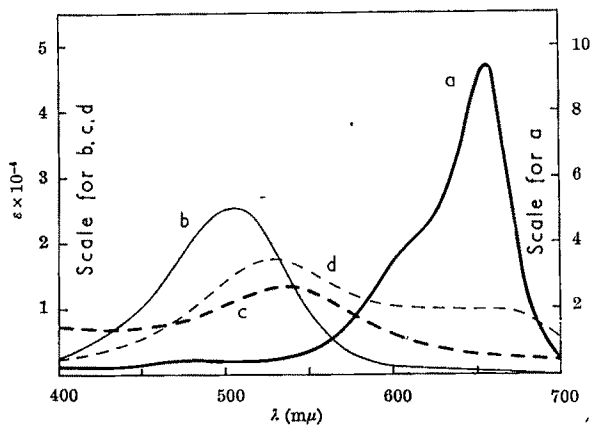


Fig. 1. Spectra of methylene blue in various solvents

It is of interest to note that in isopropanolamine, comparable in basic strength to benzylamine, yet partly an alcohol, the solution appeared blue if the light path were long and red if short. Indeed, examination of the spectrum (Fig. 1, curve *d*) shows that both forms are present.

Dahne and Stanko[4] reported a red form of the dye absorbing at 523 mμ in dilute solutions ($5 \times 10^{-4} - 10^{-6}$ M) in 'anhydrous' pyridine. Tests made here showed a similar type of effect though poorly reproducible quantitatively in pyridine dried over potassium hydroxide pellets, but not redistilled. The red form became increasingly important on dilution, but vanished in more concentrated solutions. If the dry pyridine were redistilled, however, with or without a fractionating column the effect vanished and the dye was blue even at 10^{-6} M (see Table 1).

A red solution was obtained when the dye was dissolved in an ethanolic solution of sodium (Table 1; Fig. 1, curve *c*). The red colour did not appear to be due merely to decomposition of the dye, for the blue colour could be restored by adding water.

Table 1. EXPERIMENTAL DATA FOR SOLUTIONS OF METHYLENE BLUE

Solvent	pK_b	Dye conc. (mole/l.)	λ(mμ)	ε × 10⁻⁴*
Pyridine	8·9	$0·87 \times 10^{-4}$	664	8·32
		$0·87 \times 10^{-5}$	664	8·03
		$0·87 \times 10^{-6}$	664	7·62
Quinoline	9 2	10^{-3}–10^{-4}	669	—
Aniline	9·3	0–10^{-4}	674	—
Benzylamine	4·7	$2·76 \times 10^{-4}$	519	1·92
n-Butylamine	3 4	$2 81 \times 10^{-4}$	508	2 48
n-Amylamine		$2·75 \times 10^{-4}$	504	2·69
n-Hexylamine		$2·87 \times 10^{-4}$	505	2 60
n-Heptylamine		$2·92 \times 10^{-4}$	508	2 44
n-Octylamine		$3·00 \times 10^{-4}$	505	2 61
Di-*n*-butylamine	*ca.* 3 4	+	499	—
Piperidine	2 8	$2·81 \times 10^{-4}$	510	0·51
Triethylamine	3·3	+	496	—
Isopropanolamine	*ca.* 4 5	$2 95 \times 10^{-4}$	530	1·77
Sodium hydroxide in pyridine		$0·96 \times 10^{-6}$	521	< 1
1 per cent NaOEt in ethanol		$0 95 \times 10^{-3}$	538	1 32

* Note: the molar extinction coefficients, ε, are only accurate to ± 4 per cent.

+, Dye sparingly soluble.

Red forms of methylene blue have also been observed in the crystalline state[1,5], which is dichroic, and in solutions of the dye in solvent mixtures of very low dielectric constant[3,6]. In the latter case the red form was rapidly precipitated and was attributed to undissociated dye in a colloidal state.

In the blue form of the dye the positive charge is an integral part of the chromophoric system and is distributed symmetrically throughout the system by resonance[6]. If this were not so, as in the colourless non-ionic *leuco* form, then the dye would absorb at much shorter wavelengths. The close approach of an anion will repel the electrons of the chromophoric system, thereby altering the distribution of the positive charge. Interaction of the anion with a terminal position in the dye ion destroys the energetic symmetry of the dye ion; loss of energetic symmetry has been shown experimentally to shift the visible absorption of a dye to shorter wave-lengths[7–9]. Interaction of the anion with a central position in the dye ion reduces the fraction of the positive charge shared by the terminal positions and this too has been shown to shift the visible absorption of a dye to shorter wave-lengths[10]. In effect, interaction of an anion with any position in the dye ion will induce a hypsochromic spectral shift. The red forms in the undistilled anhydrous pyridine and in the ethanolic solution of sodium may therefore be attributed to the interaction of the dye ions with strongly basic anions—ethoxyl ions in the latter case. This mechanism is analogous to the formation of undissociated dye molecules and indeed these two red forms absorb at wave-lengths close to that of the undissociated dye in solvents of low dielectric constant (λ = 521, 538 mμ; compare 530 mμ in ref. 3). Furthermore, the absorption bands of the red forms all have low molar extinction coefficients. This is consistent with interaction of the anion at a terminal group in the dye ion, thereby stabilizing one resonant structure of high dipole moment at the expense of another equivalent structure[11]. Indeed, Lewis considered that with methylene blue the positive charge was almost entirely shared by the terminal $-NMe_2$ groups[12].

The mechanism by which the charge distribution within the dye ion is reduced in simple aliphatic amines cannot be ascertained from the present data. Nevertheless, since the dye is completely in the red form in these solvents one important possibility that may be worthy of further investigation is complex-formation between the dye and the amine, acting as a Lewis base (see ref. 13). Water, however, cannot be completely eliminated from the amine, and although the presence of hydroxyl ions alone is no criterion for the production of the red form because the dye is blue in aqueous alkali, the possibility that hydroxyl ions, produced by the interaction of water and the amine, may have some effect cannot be ignored altogether.

The source, purification and analysis of methylene blue have already been described[3].

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Metachromasy

MANY dyes when used to colour certain tissue constituents (chromotropes) absorb light of wave-length shorter than that absorbed by the dyes in solution[1]. Similar metachromatic behaviour is exhibited in solution when the dyes interact with soluble chromotropes, for example nucleic acids[2], polyphosphates[3] and gelatine[4]. Metachromasy may also be induced in solution in the absence of chromotropic substances in three different ways[5]: by increasing the dye concentration (dye-induced), by adding salt (salt-induced) and by lowering the dielectric constant of the solvent from medium (about 20) to low (about 3) values (solvent-induced).

The phenomenon is exhibited only by dyes in which the characteristic ionic charge is an integral part of the chromopheric system and is distributed throughout the system by resonance, that is, most common basic dyes and a few acid dyes. The following discussion is therefore restricted to basic dyes, but the same arguments apply *mutatis mutandis* to dyes, such as fluorescein, with a distributed negative charge.

Metachromasy in solution has recently been attributed primarily to interaction of the dye ions and counter-ions[5], although in the past interaction of dye ions themselves was generally considered to be responsible. Usually the colour change is small and the hypsochromic ($H-$) band in the visible spectrum is removed by only about 50 mμ from the normal ($M-$) band; the interacting dye ion and counter-ion are considered to be separated by solvent molecules. In solvents of low dielectric constant[5] (and certain strongly basic solvents[6]) strong interaction occurs to form an undissociated species in which the ions are in contact and not separated by solvent molecules. In this case the colour change is large, for example, solutions of methylene blue become red and, indeed, a red form exists in crystals of the dye[7].

In organic solvents of moderate dielectric constant, for example simple alcohols, dye-induced metachromasy (at room temperature) only occurs at extremely high dye concentrations, but occurs at increasingly low concentrations as the dielectric constant is lowered (see ref. 5 and other references quoted therein). This is in keeping with electrolytic dissociation theory. In solvents of high dielectric constant, long-range electrostatic forces are smaller and solvation effects are relatively more important[8]. The marked metachromatic effects observed in water are attributed to the inability of water to solvate the large organic residues in the dye ions. The dye ions therefore tend to aggregate and the aggregation of organic ions is known to be associated with the uptake of counter-ions[9,10].

The foregoing concepts can also explain metachromasy induced by interaction with chromotropic substances either in tissues or in solution. Basic dyes exhibit metachromasy when they interact with negatively-charged substrates; the colour change is more marked the more strongly acidic the active groups (sulphate > phosphate > carboxylate)[1]. In solution, metachromasy of cyanine dyes induced by interaction with gelatine is only observed at a *p*H above the iso-ionic point of gelatine[4]. Ionic interaction of the dye ion with an ionic group in the substrate must clearly alter the charge distribution in the dye ion and, as described previously[5], this produces a hypsochromic spectral shift. We therefore believe that metachromasy induced by chromotropes should be attributed primarily to ionic interaction with the substrates, even though there is evidence[11] that basic dyes tend to aggregate when they interact with biological substrates. The aggregation requires uptake of counter-ions to reduce electrostatic repulsion between the dye ions and this will have an additional effect.

The interaction of a metachromatic dye and a chromotrope in solution often exhibits a curious phenomenon. As increasing amounts of the chromotrope, for example polyphosphate[3] and gelatine[4], are added to a solution of dye, the M-band of the dye is gradually replaced by the H-band. However, if still more chromotrope is added, until it is well in excess of the dye, the H-band gradually disappears and is replaced by a band at the same or slightly longer wave-length than the original M-band. This phenomenon is probably related to the behaviour of 1,1′-diethyl-2,2′-carbocyanine chloride in the presence of soaps, for example potassium laurate or oleate[12]. At the dye concentration used the dye absorbed light mainly in the H-band ($\lambda = 553$ mμ) and partly in the M-band ($\lambda = 610$ mμ). Both these bands diminished as soap was added and were replaced by a new band at much shorter wave-lengths ($\lambda = 480$ mμ), comparable in fact with those associated with absorption by the undissociated species of the dye insolvents of low dielectric constant[5]. When more soap was added, however, this band disappeared sharply and was replaced by a band at 610 mμ, in fact the normal M-band; the sharp spectral change occurred at the critical micelle concentration of the soap[12]. It is well known that the solubility of dyes is increased in the presence of soap micelles[13] and indeed there is evidence from diffusion data that sulphonated anthraquinone dyes form mixed micelles with surface-active agents[14]. We suggest that the basic dye ions are incorporated in the micelles of the soap and the aggregates of the chromotrope in such a way that they are effectively in a Faraday cage and are therefore subjected to a uniform electrical field. The charge distribution and thus the electronic configuration of the dye ion is therefore the same as that of the unperturbed dye ion, which absorbs in the M-band.

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High-spin Five-coordinate Cobalt(II)

THE tridentate ligand 1,3-*bis*(2′-pyridyl)-2,3-diaza-1-propene, (paphy), when reacted with cobalt(II) chloride in ethanolic solution, yields several products. Two of these complexes are formulated as $Co(paphy)Cl_2$: one (designated α-) forms red plates and the other (β-) dark green needles. The β-isomer may readily be converted to the α-compound by heating in ethanolic medium.

The magnetic properties over a temperature range and the visible and near infra-red spectra have been suggested as a method of differentiating between octahedral and tetrahedral stereochemistries for Co(II) complexes. It is important to emphasize that this procedure allows a differentiation between these two stereochemistries, but may not finally establish either of them. The compounds discussed here may be used to elaborate this point.

The magnetic moment (5·04 B.M. at 293° K, $\theta = 13°$) and diffuse reflectance spectrum [absorption at 7,900 ($^4T_2$); 17,300 ($^4A_2$); 19,000, 20,500 cm^{-1} ($^4T_1$)] of the α-isomer lead us to believe that it contains Co(II) in octahedral environment, probably with chloride bridging of adjacent octahedra to attain CoN_3Cl_3 co-ordination. It is isomorphous with the corresponding compound $Ni(paphy)Cl_2$. However, the co-ordination stereochemistry for β-$Co(paphy)Cl_2$ is not clearly revealed by the spectral and magnetic data. The most intense absorptions in the reflectance spectrum occur at 4,500, 7,700 and 15,750 cm^{-1} and would most closely indicate strong field tetrahedral co-ordination (with parameters $D_q = 450$ cm^{-1}, $B' = 670$ cm^{-1}). But there are weaker bands at 12,200 and 20,000 cm^{-1} and the magnetic moment (4·84 B.M. at 294° K, falling to 4·56 B.M. at 80° K) is about 0·25 B.M. greater than that predicted for these parameters. We have therefore determined the crystal structure of this compound.

The complex, β-$Co(paphy)Cl_2$, crystallizes in the monoclinic system with cell constants: $a = 8·72$; $b = 11·93$; $c = 12·64$ Å; $\beta = 98° 2'$ and $Z = 4$. From systematic absences, the space group was uniquely determined as $P2_1/n$. Visual estimation of the intensity data collected by the Weissenberg multiple-film technique yielded 1,257 independent reflexions. The phase problem was solved by the 'heavy-atom' procedure using three-dimensional Patterson and Fourier syntheses. Isotropic least-squares refinement of the atomic parameters led to a value for R of 0·12, while the subsequent inclusion of anisotropic temperature factors has reduced this index further, to 0·095.

The stereochemistry of the molecule is illustrated in Fig. 1, from which the essentially square pyramidal geometry around the cobalt is apparent. The metal atom is 0·39 Å above the basal plane which contains all other atoms except the chlorine atom Cl(2). The average Co—N bond length is 2·12 Å and the angles N(1)CoN(2) and N(3)CoN(2) are both 74°. Angle N(2)CoCl(1) is 157°; N(2)CoCl(2) is 94°. There are no close inter-molecular interactions and the cobalt–cobalt vector is 4·62 Å.

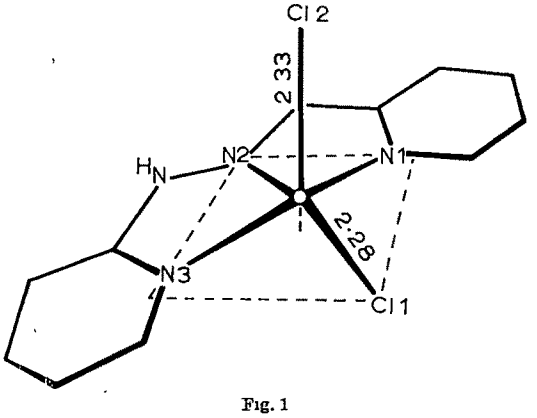


Fig. 1

Two other ML_3X_2 complexes with this stereochemistry, $Ni[CH_3As(CH_2CH_2CH_2As(CH_3)_2)_2]Br_2$ (ref. 1) and $(C_{14}H_{13}P)_3PdBr_2$ (ref. 2), have apical $M—X$ bonds considerably longer than the basal $M—X$ bonds, but in the present compound this difference is statistically just significant.

It is interesting to note that the ligand 2,2′,2″-terpyridyl, (terpy), which presents a tridentate nitrogen donor sequence closely corresponding to that of paphy, also forms a crystalline complex $Co(terpy)Cl_2$ (ref. 3). This is high-spin with a near infra-red and visible reflectance spectrum similar to that of β-$Co(paphy)Cl_2$. A comparison of the unit cell parameters and about 80 X-ray intensities from crystals of $Co(terpy)Cl_2$ and of $Zn(terpy)Cl_2$, in which there is trigonal bipyramidal stereochemistry[4], indicates that these two compounds are isostructural. A full three-dimensional refinement of both these terpy structures is in progress[5]. Trigonal bipyramidal geometry for $Co(terpy)Cl_2$ would be in contrast to the square pyramidal geometry for β-$Co(paphy)Cl_2$, but we believe that intermolecular crystal forces are a major factor influencing the co-ordination stereochemistry in these two compounds.

It is important to emphasize that a differentiation between a trigonal bipyramidal and a tetragonal pyramidal arrangement in molecules of this type is very often difficult and a reassessment of the geometry of $Zn(terpy)Cl_2$ implies a very close relationship with the paphy derivative reported here.

These complexes also provide further examples of high-spin five-co-ordinate cobalt(II), in addition to the compounds $[Co(Ph_2MeAsO)_4ClO_4]ClO_4.H_2O$ (ref. 6), *bis*(N-methylsalicylaldimato)Co(II) (ref. 7) and *bis*(N-β-diethylamineethyl-5-chlorosalicylaldimato)Co(II) (ref. 8).

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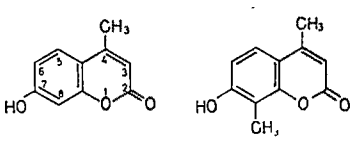
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Estimation of Nitrate by Nitration of 7-Hydroxy-4,8-dimethyl Coumarin

SKUJINS[1] proposed a colorimetric method of nitrate estimation using 7-hydroxy-4-methyl coumarin (I) as the reagent for nitration. The 3, 6 and 8 positions are activated for electrophilic substitution, but there is steric restriction to the entry of NO_2^+ to the 3 position.



(I) (II)

A mixture of products using Skujins's reagent and nitrate in a molar ratio of 4·5 : 1, and following the analytical procedure to be outlined later, was extracted with chloroform, and the resultant nitro derivatives subjected to paper chromatography using *n*-butanol–ammonia–water (8 : 30 : 62 v/v) as developing solvent. Three spots were observed with R_F values in agreement with 6-nitro,

Table 1. WAVE-LENGTHS OF MAXIMUM ABSORPTION, AND APPARENT MAXIMUM EXTINCTION COEFFICIENTS FOR NITRO-HYDROXY COUMARINS IN ETHYL ACETATE AND IN AMMONIA/AMMONIUM SULPHATE SOLUTIONS

Compound	M.p. ° C	Ethyl acetate			Ammonia/ammonium sulphate		
		Concentration (% w/v)	Wave-length maximum abs. (mμ)	ε_{max} (l. mole^{-1} cm^{-1})	Concentration (% w/v)	Wave-length maximum abs. (mμ)	ε^*_{max} (l. mole^{-1} cm^{-1})
6-Nitro-7-hydroxy-4-methyl coumarin	263–5	0·015	353	6,350	0 0015	408	9,300
8-Nitro-7-hydroxy-4-methyl coumarin	257‡	0 002	310	13,300	0·0015	353 420†	17,000
6,8-Dinitro-7-hydroxy-4-methyl coumarin	300‡	—	—	—	0·001	402	14,500
6-Nitro-7-hydroxy-4,8-dimethyl coumarin	226–7	0 002	360	5,690	0 006	420	8,000

* Kept for 4 h before reading. Absorbance increases slowly with time.
† Shoulder.
‡ Decomposes on melting.

8-nitro and 6,8-dinitro derivatives of the coumarin. These were synthesized by the methods of Shah and Mehta[2]. It was observed that the proportion of the dinitro compound in the mixture of products increased with reaction temperature.

7-Hydroxy-4,8-dimethyl coumarin (II) was synthesized from ethyl acetoacetate and 2-methyl resorcinol[3]. Only the 6 position of this reagent is available for nitration. Extraction and chromatography (as already outlined) of the products of nitration gave only one spot corresponding to 6-nitro-7-hydroxy-4,8-dimethyl coumarin. The formation of a dinitro derivative was avoided, thereby improving reproducibility and allowing the use of lower molar ratios of reagent to nitrate than proposed by Skujins[1]. Data from the absorption spectra of the foregoing nitro coumarins are given in Table 1.

The absorption peak at 420 mμ for the 6-nitro-7-hydroxy-4,8-dimethyl coumarin in ammonia/ammonium sulphate solution made it possible to use a simple colorimeter, equipped with a violet filter, in the analytical procedure.

The recommended procedure for estimating nitrate in soils is: (1) shake 5 g of the soil with 15 ml. of extraction solution (silver sulphate (5 g), anhydrous sodium sulphate (71 g), copper sulphate (8·3 g) and sulphuric acid (5·6 ml.) per l.) for 45 min. (2) Separate the solution by centrifugation or by standing overnight. (3) Mix supernatant (5 ml.) with 10 M sodium hydroxide (0·3 ml.) and filter. (4) Transfer cleared filtrate (0·5 ml.) to a 6 in. × 1 in. test-tube and cool in an ice bath. (5) Add 1·0 ml. reagent solution (0·03 per cent 7-hydroxy-4,8-dimethyl coumarin in sulphuric acid) and transfer to a boiling water bath for 10 min. (6) Cool again in ice and rapidly add 6 M ammonia solution (10·0 ml.). (7) Read absorption after 20 min at 420 mμ or with an Evans Electroselenium, Ltd., colorimeter equipped with an *OB*10 filter.

A linear calibration curve from 0 to 35 p.p.m. nitrate nitrogen was obtained using the aforementioned colorimeter and filter. The absorbance in ammonia/ammonium sulphate varies with ammonia concentration, and the calibration of the colorimeter should be re-determined for each batch of 6 M ammonia used.

Chloride interferes above 50 μg/ml. which necessitates the inclusion of silver sulphate in the extraction solution. The interference was found to be the chlorination of the coumarin by nitrosyl chloride. The problem of chloride interference and other chemical aspects of the estimation of nitrate by the nitration reaction will be discussed more fully elsewhere.

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Elution Volume versus Reciprocal Elution Volume in the Interpretation of Gel-filtration Experiments

RECENTLY, Winzor and Nichol have suggested[1,2] that if velocities in the conventional moving boundary equation[3–5] for free electrophoresis are replaced by the reciprocals of corresponding elution volumes, the equation is then applicable to gel-filtration experiments.

At first sight this is a reasonable transformation since the velocity of a boundary within a gel-filtration column is inversely proportional to the volume of effluent that leaves the column during the time of passage of the boundary down the column. Nevertheless, further consideration leads to the different conclusion that it is not the reciprocal of the elution volume but the elution volume itself which must replace the corresponding velocity in the moving boundary equation. This can be shown as follows.

Let $\bar{V}$ be the constituent elution volume of a substance at constituent concentration w, and let $\bar{V} + d\bar{V}$ be the corresponding volume for a concentration $w + dw$. Then if a solution of concentration w is poured into a column that initially contains only solvent, the substance appears in the effluent, at a concentration w, when a volume $\bar{V}$ has passed through the column, volumes being estimated by constructing the hypothetical equivalent sharp boundary[6] on the recorded concentration profile of the substance in the effluent. At the moment when the substance first appears in the effluent, the column contains a quantity $w\bar{V}$ of substance. At this moment let the concentration of the inflowing solution be raised to $w + dw$ and the influx continued until the differential boundary so formed (estimated again as the hypothetical equivalent sharp boundary) appears in the effluent. The further volume that has been added to achieve this, say, V, represents the elution volume of the differential boundary. The whole column now contains a quantity $(w + dw)(\bar{V} + d\bar{V})$ of substance and the effluent a quantity wV. The sum of these two quantities must equal the total quantity poured into the column. Therefore:

$$(w + dw)(\bar{V} + d\bar{V}) + wV = w\bar{V} + (w + dw)V \qquad (1)$$

which simplifies to:

$$V = \bar{V} + w\frac{d\bar{V}}{dw} = \frac{d(w\bar{V})}{dw} \qquad (2)$$

when second-order differentials are neglected. A comparison of equation (2) with the conventional moving boundary equation[4,5,7]:

$$v = \frac{d(w\bar{v})}{dw} \qquad (3)$$

where v is the velocity of a differential boundary between solutions of concentration w and $w + dw$, and $\bar{v}$ is the constituent velocity of the substance, shows that elution volumes have replaced velocities, but that in other respects the equations are mathematically identical. This has

the convenient consequence that all the procedures, for example integration to determine concentration profiles[8], that have been worked out for homogeneous solutions with equation (3) can be duplicated for chromatography and gel-filtration with equation (2) simply by a change of symbol.

It is important to realize that equation (3) is not the moving boundary equation for the boundary within the column. The equation for the column must take into account the substance in the stationary as well as the mobile phase, and as is well known[9,10], can be written in the form:

$$v = \frac{\mathrm{d}(W\bar{v})}{\mathrm{d}(W)} \qquad (4)$$

where W is the total quantity of substance per unit length of column. Naturally equation (4) should transform to equation (2) since the elution record is a consequence of the flow within the column. That it actually does so follows from the fact that besides v and $\bar{v}$ being inversely proportional to V and $\bar{V}$ respectively, W is directly proportional to the product $w\bar{V}$. Substitution for v, $\bar{v}$ and W in (4) and cancellation of the coefficients of proportionality lead to:

$$\frac{1}{V} = \frac{\mathrm{d}\left(w\bar{V}\frac{1}{\bar{V}}\right)}{\mathrm{d}(w\bar{V})} = \frac{\mathrm{d}w}{\mathrm{d}(w\bar{V})} \qquad (5)$$

which is identical with (2). The paradox of velocities being replaceable by elution volumes has disappeared because both sides of the equation are inverted when the parameters for the column are replaced by their equivalents from the elution record.

Table 1. DISSOCIATION CONSTANT K FOR THE REVERSIBLE REACTION BETWEEN TRYPSIN AND SOYBEAN TRYPSIN INHIBITOR

pH	$10^7 \times K$ (mole l.$^{-1}$) From gel-filtration by equation (34), ref. 12, using (a) Reciprocal volumes	(b) Volumes	By potentiometric technique[11]
4·25	15	10·8	10·5
4·50	4·2	2·8	2·3
4·75	0·9	0·5	0 7

Finally, in Table 1, three sets of values for the equilibrium constant of the reaction between trypsin and soybean trypsin inhibitor are compared. The first set is taken from Winzor and Nichol's paper[2], and is calculated from the results of their gel-filtration experiments by substituting the reciprocals of elution volumes for velocities in a particular form of the moving boundary equation (equation (34) in ref. 12). The second set is calculated in the same way except that elution volumes themselves, instead of their reciprocals, are used to replace velocities. The third set derives from the potentiometric experiments of Lebowitz and Laskowski[11]. Clearly, the use of elution volumes rather than their reciprocals is supported by the closeness of the values of the second set to those of the third, and in fact the agreement is then to within the accuracy of measurement (0·1 ml.) of the elution volumes.

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BIOPHYSICS

Properties and Structures of Lecithin Monolayers containing Potassium Ions or Acetylcholine

MANY experiments have been carried out with monolayer films of lecithin[1,2], but reports of them do not fully describe the details of phospholipid preparations. On the other hand, three-dimensional liquid crystal phases of mixtures of phospholipids and water have been studied in detail[3,4], and it has been shown that the phases are greatly influenced by the freshness of lecithin and also of the phospholipids. Aged phospholipids are not able to form liquid crystals. The electrical resistance of the fresh phospholipid bilayer at the water–water interface decreases rapidly 30 min after spreading the films in the presence of oxygen[5]. Our monolayer films of lecithin were also influenced by the degree of freshness of the lecithin.

Monolayer films of lecithin were spread on the surface of water in which salts had been dissolved. Lecithin was extracted from egg yolk[6] and dissolved in purified benzene. Water, salts and also cholesterol were purified as much as possible, particularly to eliminate the surface active substances. Surface pressures were measured with the vertical plate method and the flotation method. The curves produced by plotting surface pressures against surface areas are analogous to ones which have been produced by other workers (Fig. 1)[1,2]. If, however, the substrates on which the films are spread contain potassium ions or acetylcholine, regardless of the existence of the other ions, the surface pressure measured with the vertical plate method for fresh lecithin exhibits a plateau at large surface areas. On the other hand, plateaux were not observed in the force-area curves for substrates containing the same potassium ions or acetylcholine when the surface pressure was measured by the flotation method.

To find out whether the vertical plate was contaminated during the spreading of the samples, the surface pressure was measured by the vertical plate method, and with the lecithin spread on distilled water. The surface pressure was naturally almost zero at the larger surface

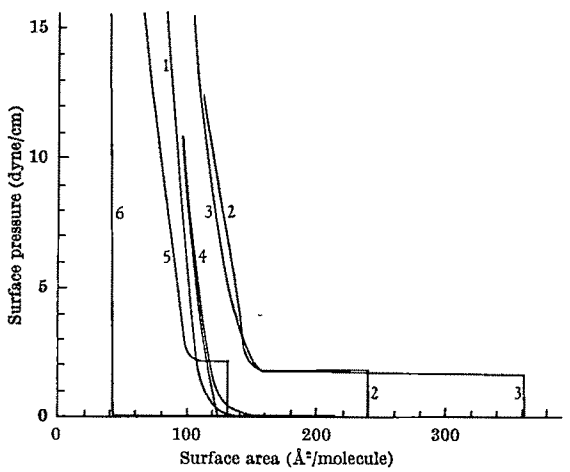


Fig. 1. Curves of surface pressure versus surface area for lecithin and other substances The abscissa unit is Å² per molecule of the substances, but for the mixture of lecithin and cholesterol, total surface area (Å²) per number of total molecules of the mixture. (1) Fresh lecithin monolayer on the distilled water. If the substrate contained sodium chloride, calcium chloride, or barium chloride, the curves were displaced over 20 Å²/molecule to the right from this curve, but they had the similar shapes to this curve. (2) Fresh lecithin monolayer on the potassium chloride aqueous solution (8 mmoles/l.). (3) Fresh lecithin monolayer on the acetylcholine aqueous solution (1 mmole/l.). (4) Fresh lecithin monolayer on the distilled water measured reversibly with the floating method. (5) Monolayer of the mixture of fresh lecithin and cholesterol (mol. ratio, 3 : 1) on the aqueous solution of potassium chloride (6 mmoles/l.). The mean molecular weight of this mixture is less than the lecithin one, then the curve displaced to the left. (6) Cholesterol monolayer on the distilled water. All curves except curve 4 were measured with vertical plate method. The plateaux were seen only on the curves 2, 3 and 5

area; however, keeping this surface area of film constant, a few ml. of aqueous potassium chloride solution (about 5 weight per cent) or aqueous acetylcholine solution (about 10 weight per cent) were gradually added to the substrate at that part of the surface kept free from lecithin by a movable barrier. The vertical plate was then raised a few mm, and there was a corresponding increase of the surface pressure of the lecithin film from zero to a few dynes per centimetre. On the other hand, with sodium chloride, calcium chloride or barium chloride (in aqueous solutions) added in this same way, the vertical plate was not raised up at all. When the surface pressure was measured with the flotation method, this phenomenon was not seen. It is therefore natural to suppose that the plateau of the force-area curves originates in contamination of the vertical plate with the benzene solution of lecithin during the initial spreading process, although this is not certain. The mixture of lecithin and cholesterol (mol. ratio, 3 : 1) produced results the same as with lecithin alone, but other mixtures of these (mol. ratio 1:3) did not show the plateaux[7].

What is the reason for the appearance of the plateaux? Supposing that the vertical plate is not contaminated, there are two possibilities. One is that the surface pressure of the lecithin film near the vertical plate, where the surface and also the monolayer film are curved, may be larger than the surface pressure of the same film at the other main flat surface. This means that the structures of the curved monolayer films are different from those of the flat monolayer[8]. If this is so, it may be thought that there are a few structures which differ from one another according to the sizes and the signs (upward or downward) of the radius of the lecithin films. Potassium ions or acetylcholine would make the lecithin film curve so that its centre of radius would exist on the hydrocarbon sides of the film under conditions similar to those in biological cells. As previously mentioned, mixtures of water and lecithin form the liquid crystal phases of three dimensions[3,4]. In the same way, lecithin may form similar two-dimensional phases with water at water surfaces, and they are easily changed into one another by ions and acetylcholine[9] and also by other surface-active substances.

The second reason for the appearance of the plateaux may lie in the two-dimensional rheological properties of the lecithin monolayer. The phases of this two-dimensional structure were usually disturbed during the measurement of surface pressure with the flotation method and the phases may have been changed into one another much as in thixotropy; but once changed, they would not be able to return to the initial phases. We have experienced similar thixotropic phenomena in the measurements of surface pressure of rhodopsin monolayers[10], although this has not been reported. Of the two possible explanations, we prefer the former.

The structures of fresh phospholipid monolayers are thus thought to be very different from those of other monolayers—for example, those of stearic acid. In the presence of K^+ or of acetylcholine, the structure would have a great many pores, each full of water molecules. We can now construct a membrane model for a biological cell. The structure of the outer side of the membrane may differ from that of the inner side, and the membrane will have a great many hydrated pores. Hydrated ions pass through a pore, but with difficulty, because of the mutual steric hindrance of the hydration of the pore and the hydration of the ions. For this reason K^+ can more easily pass through a pore than Na^+ (refs. 11–13). Water molecules can pass through the pore as easily as they travel in water or in ice in three dimensions[5,14].

It is certain that there are many types of biological membranes, but each structure would be one of the two-dimensional liquid crystal phases, some of which are easily changed by some ions or molecules and, of course, by surface-active substances. This model will make clear the other important phenomena which are related not only to the more familiar membranes but also to the surfaces of large biological molecules, especially lipoproteins, bacteriophages and so on.

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Determination of the Diffusivity of Water in Biolcgical Tissue

THE problem of dehydrating vegetable tissue has been investigated for many years. We have attempted to establish a method for calculating the diffusion coefficient of water during dehydration of several systems. From the work of Carslaw and Jaeger[1], Crank[2] has developed a series of equations for diffusion with surface evaporation from porous membranes of different geometrical shapes. We have considered the cell structure of vegetables to approximate to a porous membrane and have applied Crank's equations for a plane sheet and a cylinder to vegetable dehydration.

Several attempts have been made to calculate diffusion coefficients in tissue. Sherwood[3] used a degenerate form of the equation we use, Benkar and Salkins[4] used a similar type of equation and Haque and Eubank[5] also used an equation developed from Crank's work. Fish[6] has investigated the diffusion of water through starch gel and blanched potato.

Crank[2] has developed equations for surface evaporation from (*a*) a plane sheet and (*b*) a cylinder. For a sheet the equation is:

$$\left(\frac{M_t}{M_\infty}\right) = 1 - \sum_{n=1}^{\infty} \frac{2L^2 \exp\left(-\beta_n^2\, Dt/l^2\right)}{\beta_n^2\left(\beta_n^2 + L^2 + L\right)} \qquad (1)$$

where β_n are positive roots of $\beta \tan \beta = L$, $L = l\alpha/D$ and $l = \frac{1}{2}$ sheet thickness, M_t = mass lost at time t, M_∞ = total mass lost, t = time, D = diffusion coefficient, α = evaporation constant.

For a cylinder:

$$\left(\frac{M_t}{M_\infty}\right) = 1 - \sum_{n=1}^{\infty} \frac{4L^2 \exp\left(-\beta_n^2\, Dt/a^2\right)}{\beta_n^2\left(\beta_n^2 + L^2\right)} \qquad (2)$$

where β_n are roots of $\beta J_1(\beta) - LJ_0(\beta) = 0$ and $L = a\alpha/D$, a = radius of the cylinder and other variables have been defined here.

Equations (1) and (2) are true for one-component diffusion at constant temperature. These equations were used to evaluate Dt/l^2 and Dt/a^2 in terms of a range of values of L ($0 - \infty$) for each of a series of values of M_t/M_∞ (0–1·0).

Before use could be made of the equations, the evaporation constant had to be determined. α, here defined as mass transfer per unit area per unit time per concentration difference, was determined experimentally by observing

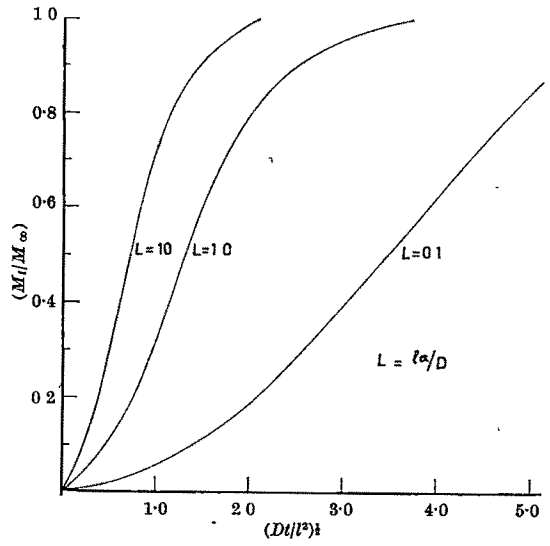


Fig. 1. The solution of equation (1) given as M_t/M_∞ against $(Dt/l^2)^{\frac{1}{2}}$ for a number of values of $L = l\alpha/D$

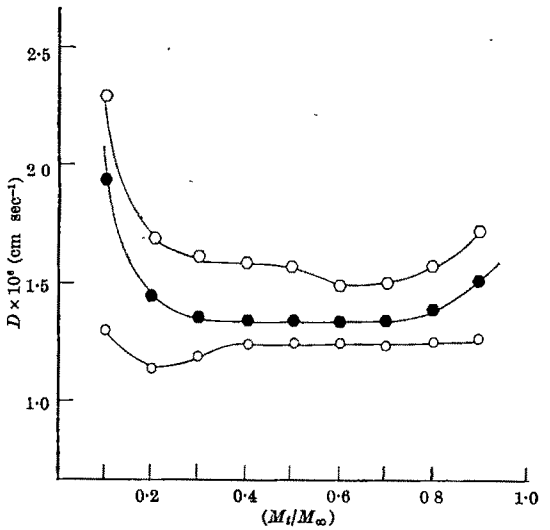


Fig. 2. The variation of D with M_t/M_∞ for a group of cylinders o Pentland Crown potato dehydrated at 31° C and zero humidity

the evaporation of water from filter paper. A value of $\alpha = 1{\cdot}80 \times 10^{-5}$ cm sec^{-1} was obtained. α was also determined from the dehydration curves by eliminating L and D from equations (1) and (2). The value thus obtained varied from $\alpha = 1{\cdot}20 \times 10^{-5}$ cm sec^{-1} to $\alpha = 2{\cdot}20 \times 10^{-5}$ cm sec^{-1} depending on the age and variety of potato.

As there is an infinite family of curves relating M_t/M_∞ to time (Fig. 1), it is important to select the curve relevant to the experimental data. To do this, the following procedure was developed: $L = \alpha R/D$ and let $X = Dt/R^2$ where $R = l$ or a; then $LX = \alpha t/R$.

The theoretical results determined allow us to plot L against LX for any value of M_t/M_∞. LX can be determined from the experimental results since α, t and R are known. We can thus, using the experimental values of LX on the theoretical L against LX curve, determine L and, from the definition of L, determine D.

Disks (0·85 cm diameter; 0·8–1·5 mm thick) and cylinders (0·4 cm diameter; 1·0–1·6 cm high) were obtained from potato using cork borers and a section razor. The cork borer 0·85 cm in diameter was fitted with a plunger on a screw thread so that known thicknesses of disks could readily be cut. The sections were suspended (in an oven at 30°–31° C and zero humidity) from a Stanton recording balance, which continually traced the weight loss. The oven fitted below the balance and could be raised and lowered to allow exchange of samples. The heating coil of the oven, composed of alternate coils of copper and manganin wire, also acted as the sensing element of the oven and was wound on an aluminium former. Inside the oven was a large gauge gauze cylinder, 2 cm in diameter, concentric with the former. Between gauze and former was placed silica gel.

The varieties of potato used were Arran Banner, Binjie, King Edward and Pentland Crown. Samples were taken from the medulla of each variety where a uniform cell structure is found.

It was found that the sample shrank in direct proportion to the amount of water lost up to 95–100 per cent loss. This was taken as 100 per cent for the purposes of calculation. The dimensions of the samples used were also such that we neglected edge effects and considered diffusion as taking place in one direction only.

The variation of D with M_t/M_∞ is shown in Fig. 2 for a group of cylindrical sections of Pentland Crown. It is obvious that there is little variation between members of a group of dehydrations carried out successively. Fig. 3 shows D against M_t/M_∞ for cylinder and disk of Arran Banner and Pentland Crown. Each curve shown is the average of a group of dehydrations. The agreement between the two cylindrical systems is exceedingly good and in the range $M_t/M_\infty = 0{\cdot}2$–$0{\cdot}9$, the agreement between cylinders and disks is within a factor of 2. Table 1 demonstrates the variation of D between varieties and within groups for a number of potatoes.

Table 1. VARIATION OF DIFFUSIVITY WITH VARIETY AND GEOMETRIC SHAPE FOR A NUMBER OF SAMPLES OF POTATO DEHYDRATED AT 31° C AND ZERO HUMIDITY

Variety	Shape	$D \times 10^6$ cm^2 sec^{-1}
King Edward	Cylinder	1·36
,, ,,	,,	1·57
,, ,,	,,	1·91
,, ,,	,,	1·36
Binjie	Disk	0·94
,,	,,	0·49
,,	,,	0·57
,,	,,	0·47
,,	,,	0·64
Arran Banner	Cylinder	1·93
,, ,,	,,	1·34
,, ,,	,,	1·20
,, ,,	Disk	1·00
,, ,,	,,	0·91
,, ,,	,,	1·10
Pentland Crown	Cylinder	1·54
,, ,,	,,	1·34
,, ,,	,,	1·26
,, ,,	Disk	1·16
,, ,,	,,	0·65
,, ,,	,,	0·83

To obtain a direct comparison of theory and experiment, a correlation curve was constructed. The experimental curve of M_t/M_∞ against $(t/R^2)^{\frac{1}{2}}$ was first drawn. This has the typical sigmoid shape of the family of curves (Fig. 1). The theoretical curve of M_t/M_∞ against $(Dt/R^2)^{\frac{1}{2}}$ requires several assumptions in its construction. The major assumption is that as M_t/M_∞ increases, we can change L by an amount proportional to the change in R. Taking small intervals of M_t/M_∞, a value of L can be estimated and the increment in Dt/R^2 over the interval thus found. Combining the increments in Dt/R^2, a curve of M_t/M_∞ against Dt/R^2 and $(Dt/R^2)^{\frac{1}{2}}$ can be drawn. Assuming that theory and experiment agree at one point (say, $M_t/M_\infty = 0{\cdot}2$), a comparison of $(M_t/M_\infty)_T$ obtained from $(Dt/R^2)^{\frac{1}{2}}$ can be made with $(M_t/M_\infty)_E$ obtained from $(t/R^2)^{\frac{1}{2}}$. The curve obtained of $(M_t/M_\infty)_T$ against $(M_t/M_\infty)_E$ should be a straight line of slope 45°. It will be seen from Fig. 4 that close agreement is obtained with theoretical prediction, suggesting that the calculati[illegible] procedures are valid.

In view of the fact that samples were taken from different tubers of differing maturities, the results shown here are in good internal agreement for a biological system.

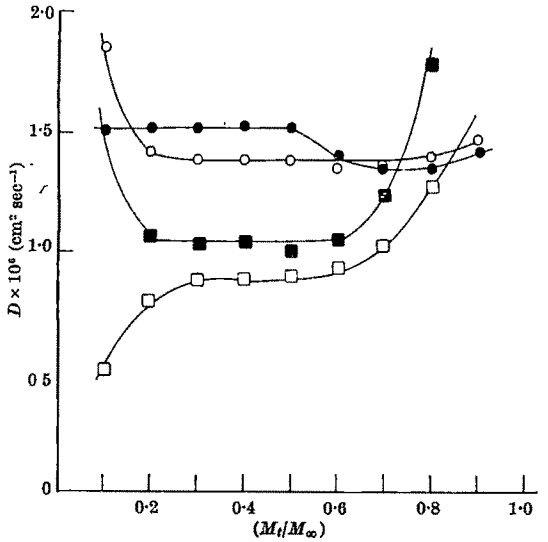


Fig. 3 The variation of D with M_t/M_∞ for different geometric shapes of samples. The samples are taken from Pentland Crown (○, cylinder; □, disk) and Arran Banner (●, cylinder; ■, disk) potatoes and each curve is the average of several dehydrations at 31° C and zero humidity

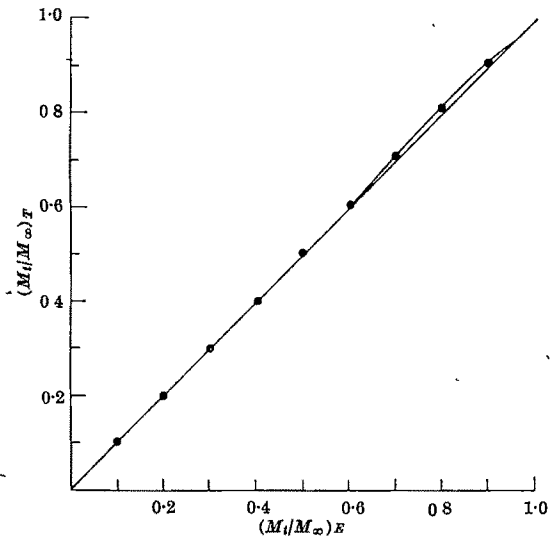


Fig. 4. Correlation curve to compare theoretical and experimental results as functions of M_t/M_∞

The value of D obtained is of the order of 10^{-6} cm² sec⁻¹, whereas the coefficient of self-diffusion for water obtained by Graupner and Winter[7] is $2{\cdot}04 \times 10^{-5}$ cm² sec⁻¹ at 25° C. It would thus appear that diffusion of water in potato is more than one order less than diffusion of water in water. There thus appears to be a greater resistance to water transport in potato than in water.

There is much greater scatter of results at 10 per cent dehydration than later in the dehydration. This scatter is possibly due to surface water obtained in cutting the samples and also to errors in measurement of the time of dehydration. Errors in timing decrease as the dehydration proceeds and the sample surface rapidly reaches equilibrium with the surrounding atmosphere, so that as dehydration proceeds the results increase in accuracy. It should thus be possible to get very accurate results over the last 10 per cent of dehydration. Before doing so, a number of equations must be solved and this programme has not yet been carried out. The programme is an extension of that described previously involving evaluation of equations (1) and (2) at small intervals between $M_t/M_\infty = 0{\cdot}9$ and $M_t/M_\infty = 1{\cdot}0$.

Fish[6] has obtained a value for D of $2{\cdot}4 \times 10^{-7}$ cm² sec⁻¹ at 25° C for the coefficient of diffusion of water in starch gel and a similar value for blanched potato cells of high starch content. This value decreases rapidly at below 30 per cent moisture content on a dry weight basis. This is in reasonable agreement with the value which we obtained in potato at a slightly higher temperature. The difference between the values may be explained by the different starch contents.

The advantages of the method are that it is more rapid, can be carried out on the tissue immediately after cutting before enzymatic attack occurs and it requires only a few simple measurements.

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BIOCHEMISTRY

Substrate Specificity of Mycodextranase

Mycodextran (nigeran) is an unbranched polysaccharide composed of a regular alternating sequence of α-(1→3) and α-(1→4) linked glucopyranosyl units. It is produced in the mycelia of various species of moulds[1]. Reese and Mandels have recently reported[2] the isolation and partial purification of an inducible glucanase (mycodextranase) produced in the culture filtrates of selected species of fungi grown on a medium containing mycodextran as a carbon source. The enzyme is an endoglucanase that hydrolyses nigeran to produce hexameric and tetrameric units which are subsequently hydrolysed to produce the disaccharide nigerose (*O*-α-D-glucopyranosyl-(1→3)-D-glucose) as the final product[2].

On the basis of their experimental results, Reese and Mandels suggest that this α-glucanase is specific for the nigerosyl moiety in the polymeric chain, binding to this group and then splitting the glycosidic bond immediately to the right of the α-(1→3) linkage. Further, they suggest that this enzyme is not specific for α-(1→4) linkages (which is the one cleaved in nigeran by this enzyme). Other research[3-5] has suggested that certain β glucanases (such as laminarase and cellulase) are not specific for the linkage they attack but rather are specific for the substitution on the glycosyl unit that becomes the reducing group of the unit liberated[4].

We wish to present evidence that the enzyme mycodextranase does require α-(1→4) linkages for its action and that these linkages must be located in specific positions in the repeating sequence(s) relative to the α-(1→3) linkages also present.

Growth of *Aspergillus niger NRRL* 326 on glucose or sucrose results in the formation of nigeran. However, growth of this organism on starch results in the formation of a new linear intracellular polysaccharide ($[\alpha]_D^{20} + 242°$ (c 0·6, NaOH)) which contains 87 per cent α-(1→3) and 13 per cent α-(1→4) linkages[6] and differs from nigeran in that it is insoluble in hot water. Treatment of this 'pseudonigeran' with periodate followed by reduction with sodium borohydride and mild acid hydrolysis gives polymer[6] which is insoluble in water, consumes no periodate and has a high positive optical rotation ($[\alpha]_D^{20} + 135°$

(*c* 0·3, dimethyl sulphoxide)). It yields only glucose on acid hydrolysis. It is evident that this polysaccharide is a linear α-(1→3) glucan. This novel substrate and 'pseudonigeran' were used to gain more information on the specificity of mycodextranase.

Mycodextranase was isolated from culture filtrates of *Penicillium melanii* and purified by the method outlined by Reese and Mandels[2]. The final product has a protein concentration of 34 μg/ml.[7]. Assay mixtures were set up as follows: 60 μmoles sodium citrate buffer, *p*H 4·5; 2·0 mg polysaccharide and enzyme were incubated together in a total volume of 1·2 ml., at 43° C. The high assay temperature is permitted by the thermal stability of this enzyme. Aliquots of the reaction mixture were withdrawn and assayed for reducing power[8]. Liberation of reducing power was proportional to enzyme concentration over at least a four-fold increase in enzyme concentration. A unit of enzyme activity is defined as that amount causing the liberation of 1·0 micromole of glucose equivalent from nigeran in 10 min under the above conditions.

The purified mycodextranase readily hydrolysed nigeran but had no activity toward starch or maltose. These activities (amylase and maltase) are well separated from the mycodextranase by DEAE-'Sephadex' chromatography. Reese and Mandels have shown the enzyme to be highly specific when tested with a wide variety of polysaccharides[2]. Incubation of the α-(1→3) glucan or 'pseudonigeran' with 1·2 units of the enzyme for very long periods (up to 24 h) and at polymer concentrations up to 0·4 per cent failed to yield any detectable reducing power while nigeran was rapidly hydrolysed under the same conditions. Enzymatically liberated reducing groups can be readily detected by their reduction with tritiated sodium borohydride to labelled alcohol groups, and this principle was used to develop a more sensitive test of enzymatic cleavage. 2·5 mg samples of α-(1→3) glucan and 'pseudonigeran' were reduced with sodium borohydride washed and incubated as above for 17 h in the presence and absence of mycodextranase. Twenty μg of glucose were also incubated without enzyme. These reaction mixtures were then made alkaline and treated with excess tritiated sodium borohydride (20×10^6 DPM). After 4 h the *p*H was adjusted to 2 with hydrochloric acid and the excess tritium removed by exchange with water and repeated evaporation of the solution to dryness at 40° C. The residues were then suspended in 1·0 ml of water and 10 μl. withdrawn for counting. No radioactivity above control level was incorporated into polysaccharide samples incubated with mycodextranase. (A small amount of activity above background was present in both control and reaction samples.) The sample of glucose which had been reduced with tritiated sodium borohydride had approximately 2·5 per cent of the added radioactivity ($5{\cdot}1 \times 10^5$ DPM) incorporated. From this amount of tritium incorporated in 20 μg glucose it may be calculated that if one glycosidic bond in 250 were split by the mycodextranase in either polysaccharide preparation, 2,500 DPM would be present in the aliquot taken for counting.

Nigeran polyalcohol was prepared by periodate oxidation and sodium borohydride reduction of nigeran[9]. The parent nigeran consumed 0·51 mole of iodate per mole of anhydrohexose unit. The polyalcohol was desalted on 'Sephadex *G*-25' and a portion subjected to acid hydrolysis. Only glucose, erythritol and glycerol were detected after paper chromatography of the neutralized hydrolysate in two solvent systems. When a 5-mg sample of the polyalcohol was tested for hydrolysis with 1·2 units of mycodextranase no reducing power was liberated, even after 4 h of incubation. This evidence indicates that for demonstrable activity the enzyme requires an intact glucosyl residue immediately to the right or left (or both) of the glucosyl unit which is substituted in its 1 and 3 positions.

It appears, therefore, that this enzyme is specific for more than just nigerosyl moieties to allow enzyme binding since the α-(1→3) glucan and the 'pseudonigeran' provide large areas of uninterrupted α-(1→3) linkages for this purpose. Specificity for the bond being cleaved certainly appears to play a part in the mechanism of action. The failure of the enzyme to liberate detectable reducing groups from the 'pseudonigeran' poses some interesting questions. Since the polymer contains 13 per cent α-(1→4) linkages and is linear, this means slightly more than one unit in eight is linked α-(1→4). If the (1→4) links occur in a regular fashion in the chain, the largest α-(1→3) glucan that could be liberated on periodate oxidation, borohydride reduction and mild acid hydrolysis would be seven glucose units in length and would be water soluble. Since the glucan formed is not soluble this indicates the α-(1→4) groups must be in close proximity to one another in the 'pseudonigeran', possibly in one or two continuous segments. If this is the case and the enzyme cleaved the one or two α-(1→4) linkages in this polymer which would be adjacent to an α-(1→3) linkage, then the radioisotope technique employed in the present investigation should have detected this. Perhaps 'pseudonigeran' is not attacked because the relationship of two α-(1→4) linkages to one another is not that required for the binding and(or) release of a hexameric or tetrameric unit as is the case with nigeran. Another explanation that would account for both the failure of the enzyme to attack and the water insolubility of the periodate-treated material is that 'pseudonigeran' is a mixture of a α-(1→3) linked glucan and a periodate susceptible contaminant. Further work is in progress to elucidate the structure of 'pseudonigeran'. It remains clear, however, that at least part of the specificity of mycodextranase must involve the bond being hydrolysed in addition to the moiety being bound and that the Perlin mechanism[4] will not explain the action of this enzyme.

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Chemical Phenotypes of Pteridine Colour Forms in *Pieris* Butterflies

THE biochemical analysis of genetic variation has only rarely been extended to hereditary variation isolated from wild populations. This communication deals with a series of colour forms, whose genetics has been studied[1–3], and which were originally isolated from wild populations of the *Pieris napi-bryoniae* species complex. These genotypes govern changes in pteridine content of the scales of the wings, and have here been analysed by methods developed for investigations on pteridines of the related *Colias* butterflies[4,5].

The wild-type phenotype of the *P. napi-bryoniae* complex has yellow ground colour on the underside of the hindwing and on the tip of the forewing, and white ground

Table 1. CHEMICAL PROPERTIES OF PTERIDINES

Compound	R_F value*	Electrophoretic migration. cm toward anode†	Assay absorption peak (mμ)‡	Extinction coefficient‡
2-amino-4-hydroxy-6-carboxyl-pteridine	0·18§	23	262	16460[10]
Xanthopterin	0·36	22§	392	6500
Isoxanthopterin	0·24§	17	339	14200[9]
Leucopterin	0·09	16·5§	345	10250
Erythropterin	0·14§	14	470	13400
Sepiapterin	0·36	3·5§	Assayed after conversion to 6-COOH derivative, see text	

* Whatman No. 3*MM* paper; solvent: organic layer of 4 : 1 : 5 *n*-butanol : acetic acid : water.
† At *p*H 10, 2 h at 1,500 V.
‡ In 0·1 *N* sodium hydroxide.
§ Separation method used in quantitative analysis of this fraction. Extinction coefficients determined on synthetic pteridines, save for isoxanthopterin and the 6-carboxylpteridine, the coefficients of which were taken from Albert and Wood[9] and Levy[10].

colour on other parts of the wings. Black markings, composed of melanin-bearing scales, occur on both sides of the wings; the appearance of 'green' markings on the hindwing underside results from the distribution of black scales among pteridine-bearing scales, and no green pigment is actually present. In the colour form 'subtalba', all normally yellow ground colour is turned white. Two 'sulphurea' colour forms are known: one ('bright yellow') shows pronounced yellow ground colour on all wing surfaces, the other ('pale yellow') has a very pale yellow colour in those areas usually white, while preserving the normal yellow areas. (In certain populations some or all of the females, but never the males, have the white upperside colour replaced by an ochreous yellow; this sex-limited variation is not considered here.) None of these variants affects the black markings. Wild-type and all three variant colour forms are maintained in culture by one of us (S. R. B.) and specimens for analysis are shipped to the other (W. B. W.) to be stored frozen until analysed.

For reasons explained later, wild-type specimens of *Pieris brassicae* L. were also analysed. These had been obtained commercially, and were labelled "All bred Cambridge (England) 1963" by the dealer.

The methods used for pteridine identification have been described previously[4]. Quantitative analysis is performed as follows: Extraction of the pteridines is accomplished by heating one fore and one hindwing of a specimen in 0·5 ml. *p*H 10 carbonate–bicarbonate buffer[6] on a boiling-water bath for 1 min. After removal of the wing residues, 50 μl. (for electrophoresis) or 100 μl. (for chromatography) of the solution are streaked on to strips of Whatman No. 3*MM* chromatography paper with calibrated micropipettes. Electrophoresis is performed at *p*H 10 in a Markham–Smith apparatus[7]; descending chromatography is performed with the organic layer of 4 : 1 : 5 *n*-butanol : acetic acid : water as solvent. Separated fractions are located by fluorescence under 254 mμ ultra-violet light and eluted from the paper with 2 ml. 0·1 N sodium hydroxide for 1·5 min at 100° C; absorbances are then determined against eluates of blank strips in a Zeiss *PMQ* II spectrophotometer. Under these conditions, sepiapterin decomposes (after separation, during elution) to 2-amino-4-hydroxy-6-carboxylpteridine; while the decomposition can be avoided, in this work it was allowed to proceed to completion and sepiapterin was assayed as the carboxyl derivative. Relevant R_F values, migration distances, absorption peaks, and extinction coefficients are presented in Table 1. Overall reproducibility of the technique is about ±3 per cent. Data are converted to total μmoles per insect.

In one brood, it was necessary to examine the content of only those areas which are yellow in wild-type (see following explanation). Scales of those areas normally white were removed from one fore and one hindwing of the specimen with a camel-hair brush moistened with glycerol, leaving the scales required for analysis on the wings. The wings of the insect's opposite side were analysed whole.

The following pteridines were found in *Pieris*: xanthopterin, isoxanthopterin, leucopterin, erythropterin, sepiapterin, 2-amino-4-hydroxy-6-carboxylpteridine. The carboxylpteridine is perhaps not a natural product but may result solely from decomposition of sepiapterin *in situ* or during extraction (not to be confused with the intentional conversion of sepiapterin to the carboxylpteridine used in the assay procedure). Its concentration is less than 10 per cent that of sepiapterin, and thus it is in measurable amounts only in 'bright yellow' specimens. Since the changes in its amount exactly parallel those of sepiapterin, it has not been tabled here. Erythropterin was found only in *P. brassicae*. Uric acid, present in all the *Pieris*, is found with isoxanthopterin on the chromatograms, but has no absorbance at 339 mμ so that it does not interfere with the isoxanthopterin assay. It does not correlate with any of the genetic forms.

From the quantitative results, presented in Table 2, it may be seen that sepiapterin is responsible for the yellow pigmentation of *Pieris napi* L. The action of the 'subtalba' allele is to eliminate sepiapterin; the action of the 'sulphurea' alleles is to raise sepiapterin above normal levels. In brood 1963-*r*, areas yellow in wild-type were analysed separately from whole wings in wild-type and 'bright yellow' phenotypes, to see whether in the latter form sepiapterin increased in normally yellow areas as well as normally white areas; in fact, it does, though not to such an extent. In the 'bright yellow' form, the colourless isoxanthopterin and leucopterin are significantly reduced

Table 2 QUANTITATIVE ANALYSES OF *Pieris* WING PTERIDINES

Brood	Genotype	Phenotype	Sex	*N*	Sepiapterin	Xanthopterin	Leucopterin	Isoxanthopterin	Erythropterin
1963-*r*	$+^s/s^h$	Wild-type	♂	5	0·04 ± 0·01	0·01 ± 0·003	3·10 ± 0·24	0 36 ± 0 05	0 00
			♀	5	0 06 ± 0·015	0·01 ± 0·003	1·94 ± 0·28	0 26 ± 0·07	0 00
	s^h/s^h	Bright yellow	♂	5	0 16 ± 0·02	0·01 ± 0·003	2·68 ± 0·40	0·25 ± 0 02	0·00
			♀	5	0 23 ± 0·04	0·01 ± 0 003	1·60 ± 0·22	0·16 ± 0 05	0·00
1963-*r*	$+^s/s^h$	Wild-type	♂	5	0 04 ± 0·01	<0·01	0·76 ± 0·18	0 13 ± 0·03	0·00
			♀	5	0·06 ± 0·01	<0·01	0·65 ± 0·12	0·11 ± 0·02	0·00
Areas normally yellow only	s^h/s^h	Bright yellow	♂	5	0·09 ± 0·01	<0·01	0·55 ± 0·13	0·08 ± 0·01	0 00
			♀	5	0·12 ± 0·03	<0·01	0·51 ± 0·12	0·09 ± 0·02	0·00
1963-*o*	s^p/s^h	Pale yellow	♂	5	0 06 ± 0 015	0 01 ± 0·003	2·46 ± 0·17	0·23 ± 0·03	0 00
			♀	5	0 08 ± 0 02	0 01 ± 0 003	1·53 ± 0·33	0·18 ± 0·03	0·00
	s^h/s^h	Bright yellow	♂	5	0 23 ± 0·04	0 01 ± 0·003	1·61 ± 0·08	0·14 ± 0·01	0·00
			♀	5	0·24 ± 0·04	0 01 ± 0 003	1·40 ± 0·16	0·14 ± 0·01	0 00
1964-*u*	$+^s/+^s$	Wild-type	♂	3	0·06 ± 0·01	0·01 ± 0·005	2·16 ± 0·23	0·22 ± 0 01	0·00
	$S/+^s$	Subtalba	♂	3	0·00	0 01 ± 0 005	2·42 ± 0·20	0·22 ± 0 01	0·00
P brassicae		Wild-type	♂	3	<0·01	0·04 ± 0·006	2·16 ± 0·18	0·34 ± 0·09	<0·01
			♀	3	<0·01	0·07 ± 0·01	1·86 ± 0·33	0·16 ± 0·02	<0 01

Means and standard deviations in μmoles per whole set of wings (or per whole set of wild-type yellow areas, in one case) <0·001 Means that the amount present is detectable by fluorescence but below the quantity (0·01 μmole/insect) accurately assayable by our technique. *N* = No. of animals. Genetic symbols: $+^s$ = wild-type allele; *S* = 'subtalba' allele, s^p = 'pale yellow' allele; s^h = 'bright yellow' allele.

in comparison to wild-type. We appear to be dealing here with changes in kinetic balance throughout the pteridine metabolic pathway, resulting probably from a rate change at some individual reaction step. It is of interest that the colour forms appear to be allelic (or very closely linked)[1,2], and that a dominance order exists among them such that, in any pairwise combination of the three variant alleles and wild-type, that allele yielding less yellow when homozygous is dominant in the heterozygote. Further investigations of this phenomenon are planned.

While the work on *P. napi* was in progress, Harmsen[8] reported finding xanthopterin, leucopterin, isoxanthopterin and erythropterin in wild-type *P. brassicae*, and reported that a yellow form ('jauni'), of that species had increased amounts of xanthopterin and erythropterin. His failure to find sepiapterin stimulated us to examine wild-type *P. brassicae*, which as shown in Table 2 does contain small amounts of sepiapterin, similar in quantity to erythropterin. We are not able to explain the discrepancy, since Harmsen did not give sufficient account of his separation methods and gave no criteria for pteridine identification. The *P. brassicae* 'jauni' form cannot be truly analogous to the *P. napi* 'sulphurea' forms in any case, since the latter contain no erythropterin and show no increase of xanthopterin. It would be of interest to know whether sepiapterin increases in the *P. brassicae* 'jauni' form.

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Effect of Vitamin A Deficiency on the Incorporation of ^{14}C-Leucine into Tissue Proteins of Rats

THOUGH vitamin A is known to be essential for growth and to be involved in specific functions in the body such as the visual process and mucopolysaccharide synthesis[1], direct involvement of vitamin A in the regulation of protein metabolism has not been clearly established. There is, however, some indirect evidence pointing to a relationship between vitamin A and protein metabolism. Thus the level of protein in the diet has been shown to influence the storage of vitamin A in the liver and its utilization[2]. Serum proteins, especially serum albumin, have been shown to be lowered in vitamin A deficiency in rats[3] and swine[4]. A study of the capacity of tissue proteins to incorporate amino-acids in rats deficient in vitamin A may throw light on the possible direct role of vitamin A in protein utilization. In such a study we have used ^{14}C-amino-acids.

Thirty-four weanling male rats were divided into two groups of seventeen animals each. One group received 18 per cent protein (vitamin-free casein) diet without vitamin A, while the second group was fed the diet with vitamin A. The composition of the diet was similar to the one described by Jagannathan and Patwardhan[2]. After 6 weeks of *ad lib.* feeding, by which time growth had ceased and signs of vitamin A deficiency had appeared in the deficient group, a study of the incorporation of (1-^{14}C) DL-leucine (obtained from Atomic Energy Establishment, Trombay, India) was carried out by means of both *in vivo* and *in vitro* experiments.

For the *in vivo* experiment, four animals from the control group (group II), four animals from the deficient group (group I) and four vitamin A deficient animals which were fed 1,200 I.U. vitamin A the previous day (group I*a*), fasted overnight, and then were injected intraperitoneally with ^{14}C-leucine solution. The dose administered was about 1 μc./100 g body-weight. The exact quantity of solution injected was determined by weighing the syringe before and after. Five hours after the injections the animals were killed and liver, kidney, thigh muscle, small intestine and pancreas were removed. ^{14}C-activities of the isolated proteins of these tissues were assayed as previously described[5].

For the *in vitro* study, six rats each from the control and vitamin A deficient groups were killed with a sharp blow, their livers were removed rapidly and thin slices were prepared by the free-hand technique. Two slices from each rat were incubated in 2 ml. of buffer[6] containing 3 μc. ^{14}C-leucine, 2 mg glucose and 0·3 mg tryptophan-cystine fortified acid hydrolysate of casein for 3 h at 37·5° C under an atmosphere of 95 per cent oxygen and 5 per cent carbon dioxide. After the incubation, slices were thoroughly washed with buffer and dropped into 5 ml. of 10 per cent trichloroacetic acid containing 50 mg cold leucine. The carbon-14 activity in the isolated protein was measured.

The results of the *in vivo* study (Table 1) indicated that the specific activities (c.p.m./g of dry protein expressed as a percentage of the injected dose) in liver ($P < 0·01$), intestine ($P < 0·02$) and pancreas ($P < 0·001$) were significantly higher in vitamin A deficient animals than in vitamin A fed controls. The carbon-14 specific activity of

Table 1. EFFECT OF VITAMIN A DEFICIENCY ON ^{14}C-LEUCINE INCORPORATION INTO TISSUE PROTEINS OF RATS

Groups	*In vivo* (4 animals)									*In vitro* (6 animals)
	Liver		Kidney		Pancreas		Intestine		Muscle	Liver slices
	Spec. act.*	Total act.†	Spec. act.*	Total act.†	Spec. act.*	Total act.†	Spec. act.*	Total act.†	Spec. act.*	Spec. act.‡
I. Vitamin A deficient	4·05 ±0·440§	13·98 ±1·719	5·64 ±0·705	5·96 ±1·080	15·89 ±1·100	3·89 ±0·309	9·78 ±0·881	43·06 ±5 080	0·87 ±0·139	1,679 ± 74·3¶
I*a*. Vitamin A deficient pre-fed with vitamin A	4 05 ±0·428	16·46 ±1·175	5·13 ±0·220	5·22 ±0 265	14·24 ±1·076	3·94 ±0·349	8·52 ±0·678	32 30 ±0·996	1·04 ±0·173	—
II. Vitamin A fed control	2·65 ±0·246	14·55 ±1 643	4·15 ±0·149	5·34 ±0·340	8·71 ±0·417	2·08 ±0·132	5·92 ±0·785	26 73 ±4·065	0·72 ±0·118	1,389 ± 93 6

* C.p.m./g dry protein, as per cent of injected dose.

† Specific activity × organ weight.

‡ C.p.m./mg dry protein.

§ Mean ± standard error.

¶ Mean of five values only—one abnormally high value was rejected.

the protein of muscle was not affected by vitamin A deficiency. Pre-treatment of deficient animals with vitamin A had no demonstrable effect on ^{14}C-leucine uptake by the tissues studied. The total ^{14}C-activity (specific activity × organ weight), which may be a more reliable index of incorporating capacity[5] of an organ, was significantly increased in intestine ($P < 0.02$) and pancreas ($P < 0.01$) of the vitamin A deficient animals. But the total ^{14}C-activity of livers of vitamin A deficient rats in the three groups did not significantly differ one from another. The *in vitro* results in Table 1 show that the carbon-14 specific activity (c.p.m./mg of dry protein) of the liver slice of vitamin A deficient animals was significantly ($P < 0.05$) higher than that of the control animals.

These results indicate that vitamin A deficiency in rats does not adversely influence protein synthesis as judged by the *in vivo* ^{14}C-amino-acid incorporation into tissue proteins. On the other hand, there was increased incorporation of ^{14}C-amino-acid, both *in vivo* and *in vitro*, associated with vitamin A deficiency. This increased incorporation (*in vivo*) was observed in pancreas and intestine (both specific activity and total activity) and in liver (specific activity only). Increased *in vitro* incorporation was also observed in liver. Thus the increased incorporation appears to be confined to organs which have a high protein turnover such as the pancreas, intestine and liver. Increased incorporation of amino-acid in vitamin A deficiency has also been observed by Roels *et al.*[7], who have shown that the *in vitro* incorporation of (methyl ^{14}C)-methionine and (U-^{14}C)-phenylalanine by diaphragm muscle of rats increases in vitamin A deficiency. One possible reason for increased incorporation may be an increased mobilization of amino-acid into the cells of these tissues in vitamin A deficiency, perhaps because of alterations in cellular permeability[7]. But the free amino-acid concentrations of tissues are not increased except for tyrosine and phenylalanine in blood, liver and kidney of vitamin A-deficient rats[8]. An apparent increase of amino-acid incorporation could also be due to an inhibition of the release of synthesized protein from the tissue in vitamin A deficiency. In fact, it has been shown that one of the functions of vitamin A is the release of proteolytic enzymes from intracellular particles[9]. This kind of inhibition may be particularly important in pancreas. By analogy, the observed low serum albumin in the face of an increased incorporation of amino-acid by liver in vitamin A deficiency may be due to an inhibition of release of albumin from liver cells. Whether particular enzyme systems involved in protein synthesis are stimulated in vitamin A deficiency has also to be considered in this connexion. Stimulation of amino-acid activating enzymes of the liver in protein deficiency, which also causes an increased incorporation of ^{14}C-amino-acids into liver protein[5], has been described[10].

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Metabolism and Sodium Transport in the Isolated Rat Intestine

IT is well known that sodium is actively transported through many biological membranes[1–7] and several authors have also determined the amount of energy necessary for this[8–18]. The ratio between energy and transported sodium has usually been calculated by determining the increase of oxygen consumption (or lactic acid production) and the associated increase of sodium transport. These increases were obtained by modifying the quantity of available sodium[8,10,11,15,16], or by stimulating the sodium pump (posthypophyseal hormones)[8,9] or by modifying the quantity of chemical energy at the disposal of the membrane[13,26]. In this work the third procedure has been followed, and results are reported concerning the relationship between the sodium transport, the oxygen consumption and the lactic acid production in the isolated rat intestine perfused *in vitro* with different concentrations of glucose.

The jejunal tract of the albino male rat (Wistar strain) was used. The isolated intestine was everted according to the Wilson and Wiseman technique[19] and incubated *in vitro* for 1 h at a temperature of 28° C in 50 ml. of Krebs–Henseleit solution[20]. To this mucosal incubation fluid, glucose at a concentration of 1·39 mmoles/l. or 2·78 mmoles/l. or 13·9 mmoles/l. was added. Before tying off the everted sac, 2 ml. of Krebs–Henseleit solution was introduced. The mucosal incubation fluid was saturated by a gas mixture of 95 per cent oxygen and 5 per cent carbon dioxide; 20 ml. of this mixture, at a constant rate flow of 20 ml. per min, were made to recirculate continuously within the system (glass container and 'Tygon' tubes), without connexion with the outside. The oxygen pressures were recorded during the whole experimental period by means of an oxygen cathode[21] in the gas phase, transduced by a 'Combi-Analyser' (Eschweiler, Kerl) and at the same time recorded on a Metrawatt registering device[26].

At the end of the experiment the sodium concentration was determined in the serosal fluid by means of an 'Optica C F_4' flame spectrophotometer ($\lambda = 589$ mμ). The amount of the net sodium transport across the intestinal epithelium was calculated on the assumption that the concentrations of sodium are the same in the serosal liquid, and in 20 per cent of the fresh tissue. Lactic acid and glucose were determined both in the mucosal and serosal fluids according to enzymatic methods[22,27]. The amount of lactic acid present in the serosal space was calculated by assuming an equal distribution in the serosal fluid and in 80 per cent of the fresh tissue. The volume of the solution transported across the intestinal wall was determined by weighing the intestinal sac before and at the end of the incubation period.

Fig. 1 clearly shows a correlation between sodium transport and oxygen consumption (*A*) [O_2 (μmoles g^{-1} h^{-1}) = 37·6 + 0·088 sodium (μequiv. g^{-1} h^{-1}); *S.D.* ± 8·2], as well as between sodium transport and lactic acid production (*B*) [lactic acid (μmoles g^{-1} h^{-1}) = 46·7 + 0·471 sodium (μequiv. g^{-1} h^{-1}); *S.D.* ± 37·5]. These regression lines were calculated by using all the experimental values, because there exists a regression within the single groups, as well as a regression among the mean values of the three groups.

In order to compare the total available energy with the sodium transport, the total amount of ATP was calculated. Assuming a 1 : 1 molar ratio between the synthesized ATP and the lactic acid arising in the glycolytic process from glucose, and a molar ratio of 38 : 6 between the synthesized ATP and the oxygen consumed in the glucose oxidation[23], it was possible to draw the regression line reported in Fig. 1*C*. Like the others (Fig. 1*A* and *B*), this regression line [ATP (μmoles g^{-1} h^{-1}) = 287·0 + 1·017 sodium (μequiv g^{-1} h^{-1}); *S.D.* ± 70·4] has been

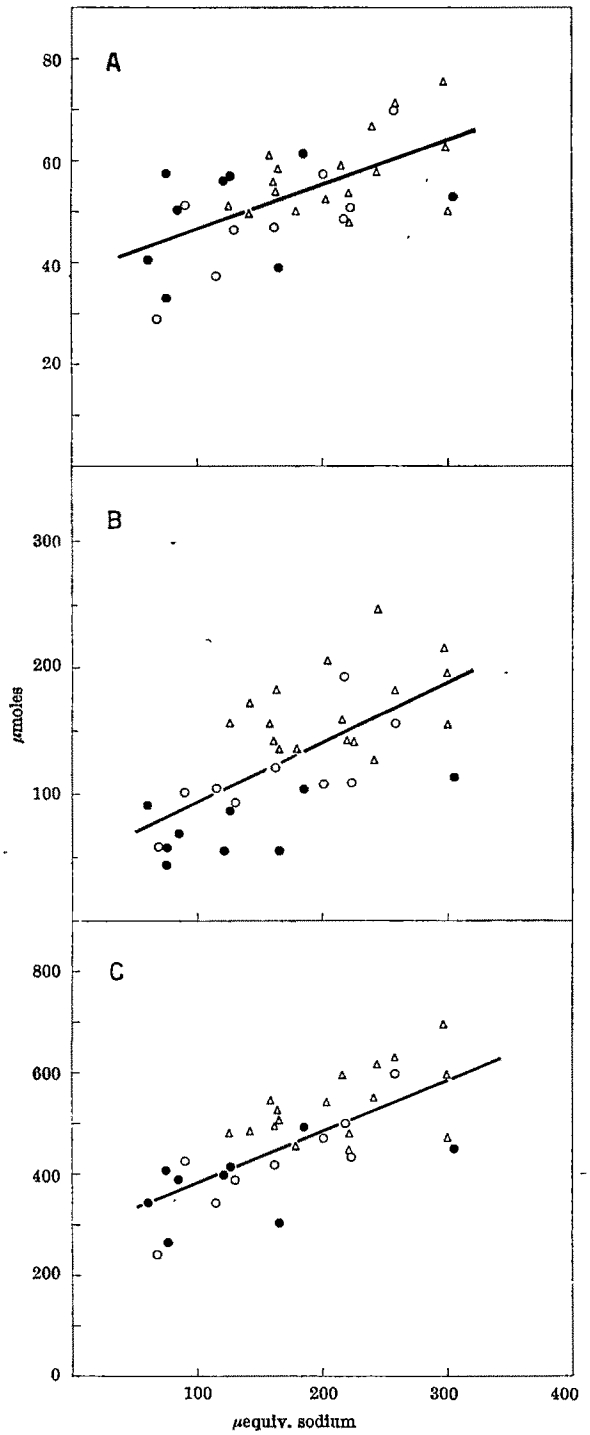


Fig. 1. Abscissa, sodium net transport μequiv. g^{-1} h^{-1}. Ordinate, *A*—oxygen consumption μM g^{-1} h^{-1}; *B*—lactic acid production μM g^{-1} h^{-1}; *C*—ATP produced μM g^{-1} h^{-1}. Glucose concentrations of the mucosal fluid: ●, 1·39 mM; ○, 2·78 mM; △, 13·9 mM. The values and standard deviation of regression lines (according to Croxton[24]) are reported in the text

calculated by utilizing all the experimental values obtained at different glucose concentrations and it is statistically significant ($P \ll 0.01$). The ratio ΔATP/Δsodium turned out to be nearly unity.

Another regression line was calculated by plotting the values of ATP against the sum of the sodium and the glucose transported at the same time through the intestine [ATP (μmoles g^{-1} h^{-1}) = 288·7 + 0·934 (sodium + glucose) (μequiv. + μmoles g^{-1} h^{-1})]. This regression was also highly significant, with a P lower than that for the regression of Fig. 1*C*. This seems to suggest that the energy required for the transport of glucose does not vary much from that required for the transport of sodium.

The values of basic metabolism which can be extrapolated from two regression lines are about the same—in other words, 287 and 289 μmoles of ATP g^{-1} h^{-1}, respectively. The ratio ΔATP/Δsodium obtained is much higher than those calculated from the data obtained from the bladder of *Bufo marinus*[10], in the mammalian kidney[11,16], and in the stimulated skin of *Rana temporaria*[8,9]. On the contrary, our ratio is similar to those obtained in the *Loligo* giant axon[13], in the frog muscle[17] and in the unstimulated frog skin (in which the basic metabolism had not been subtracted[8]).

Some of the remarkable differences in the ratios reported for ΔATP/Δsodium may possibly be accounted for by the procedure followed in the determination of the basic metabolism.

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Purification and Properties of Lipase from *Torulopsis*

The production of lipolytic enzymes by micro-organisms is widely recognized, and several have been partially purified from certain bacteria[1–4] and fungi[5–8]. Recently, Baskys *et al.*[9] have obtained pancreatic lipase purified with calcium phosphate, which was homogeneous on ultracentrifugation and free-boundary electrophoresis at *p*H 8·6, but they observed at least two components on starch-gel electrophoresis at *p*H 8·0 and DEAE-cellulose chromatography. The milk lipase prepared by 'Sephadex *G*-50' was found to be homogeneous on ultracentrifugation and starch-gel electrophoresis at *p*H 8·5 by Chanden *et al.*[10]. We have isolated from soil a strain of *Torulopsis* which produces high lipolytic activity in the culture

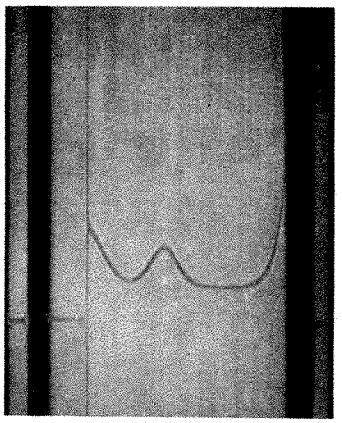
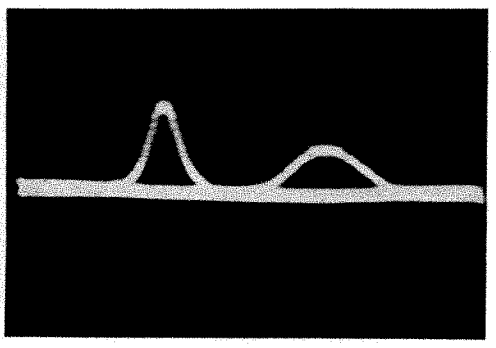


Fig. 1. Ultracentrifuge pattern of *Torulopsis* lipase. Picture taken 120 min after 60,000 r.p.m. was reached. The computed S_{20w} is 3·4 *S*



Descending Ascending

Fig. 2. Electrophoretic pattern of *Torulopsis* lipase at *p*H 6·9 (6 m.amp, 4 h)

broth. The extracellular lipase was also obtained in a highly purified state from the culture broth by the use of ammonium sulphate fractionation, DEAE-cellulose chromatography and 'Sephadex *G*-100' gel filtration. The highly purified enzyme preparation was found to be essentially homogeneous by such criteria as sedimentation in the ultracentrifuge and free electrophoresis.

The culture grown overnight on peptone medium was intensively aerated for 40 h in a soybean medium at 30° C. The lipase activity was assayed at *p*H 6·5 by the method of Yamada *et al.*[11]. The culture supernatant was precipitated at 60 per cent saturated ammonium sulphate, and precipitate was centrifuged at 4,000 r.p.m. for 30 min. The precipitate was then dissolved in 0·01 M phosphate buffer, *p*H 5·9, and dialysed against the same buffer for 2 days at 5° C. The dialysate was absorbed on DEAE-cellulose at *p*H 5·9. After washing with 0·1 M sodium chloride in 0·01 M phosphate buffer, *p*H 5·9, the DEAE-cellulose bed was extracted with 0·2 M sodium chloride in the same buffer. This fraction was dialysed against 0·01 M phosphate buffer, *p*H 5·9, and subjected to chromatography on DEAE-cellulose (linear salt gradient, 0–0·15 M sodium chloride in 0·01 M phosphate buffer, *p*H 5·9, column dimension 2 × 60 cm, flow rate 30 ml./h). Chromatography of the dialysed lipase fraction on DEAE-cellulose was then performed again, under the same conditions. The effluent fraction, partially purified with DEAE-cellulose, was dialysed against 0·01 M ammonium acetate buffer, *p*H 4·9, and lyophilized. Further purification of this partially purified enzyme preparation was attempted using a 'Sephadex *G*-100' column (3 × 70 cm) buffered with 0·01 M ammonium acetate buffer, *p*H 4·9, at a flow rate of 5 ml./h. The yield of the lipase through this purification procedure was more than 30 per cent and the specific activity was about 230 times that of the initial solution.

Analysis of the purified lipase performed on the ultracentrifuge at 60,000 r.p.m. in acetate buffer, *p*H 5·05, at a concentration of 2 per cent showed a single component with an S_{20w} of 3·4 *S* (Fig. 1).

Electrophoretic analysis of the purified lipase showed apparent homogeneity at *p*H 2·0, 3·0, 5·1 and 6·9. Fig. 2 shows the electrophoretic pattern at *p*H 6·9. The isoelectric point has been found to be below *p*H 2·0 in acetate buffer.

These experiments with purified lipase indicate that the enzyme has the following properties and specificity: the *p*H optimum for hydrolysis of some substrates (tributyrin, triolein, olive oil) is 6·5; the lipase is quite stable at *p*H 3–8 at 37° C for 1 h; temperature optimum is 45° C; the lipase is stable at temperatures of up to about 65° C at *p*H 5·0 for 10 min, but rapid inactivation occurs at temperatures above 70° C. The order of hydrolysis rate of some triglycerides by highly purified lipase is as follows: tributyrin > triolein > tristearin > tripalmitin > triacetin, trilaurin. Triacetin and trilaurin were not hydrolysed at all. The lipase activity can be inhibited by such metal ions as Fe^{++}, Fe^{+++}, Cu^{++}, Cd^{++}, Ag^{+} (0·005 M, *p*H 5·0). Meanwhile, the enzyme activity can be inhibited by cyanogen bromide, *N*-bromosuccinimide, iodoacetic acid, bromoacetic acid, diazobenzene sulphonic acid and sodium lauryl sulphate. Photo-oxidation of lipase with ultra-violet light in the presence or absence of a catalyst (such as methylene blue or riboflavin) resulted in the rapid inactivation of the enzyme. It seems that the activity of lipase requires methionine and histidine as essential groups.

A detailed account of the isolation and properties of this unique protein will appear elsewhere.

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PHYSIOLOGY

Muscle Glycogen Synthesis after Exercise: an Enhancing Factor localized to the Muscle Cells in Man

IT is well known that glycogen is utilized during muscular work, but there is very little information available about the resynthesis of glycogen after exhaustive exercise. Goldstein[1] has shown that a humoral factor, which decreases the blood glucose concentration, is released during exercise. Furthermore, it is known that the insulin requirement decreases in diabetic subjects during exercise.

In a recent study of the resynthesis of muscle glycogen after exercise (glycogen values down to zero) we found that a carbohydrate-rich diet increased the muscle-glycogen content far above the basal values (1·0–2·0/100 g fresh muscle). In order to clarify whether a humoral factor similar to the Goldstein factor or a local factor in the muscle cells is in operation, we performed the following experiment.

Two normal subjects (J. B. and E. H.) were placed on either side of a bicycle ergometer. Exercise was performed with one leg while the other leg was in complete rest. Thus J. B. worked with his left leg and E. H. worked simultaneously with his right leg. The load was 1,200 kpm/min. Periods of exercise from 5 to 20 min were interrupted by rest periods of the same duration. The exercise was continued until both subjects were completely exhausted (that is, unable to work continuously for more than 1 min). After this procedure, biopsies of the quadriceps femoris muscles of both legs were performed, and the glycogen content was determined in the specimens[2–4]. During the rest of the day and on the following two days both subjects consumed a diet which consisted almost exclusively of carbohydrates. J. B.'s caloric intake was 2,200 kcal per day (35 kcal/kg body-weight), and E. H.'s was 2,600 kcal per day (33 kcal/kg).

Muscle biopsies on both legs were performed in the morning after 1, 2 and 3 days on a carbohydrate diet, and glycogen was determined in the muscle specimens. A comparison of the exercised leg with the 'resting' leg should disclose any local enhancement of the glycogen resynthesis.

The results are shown in Fig. 1.

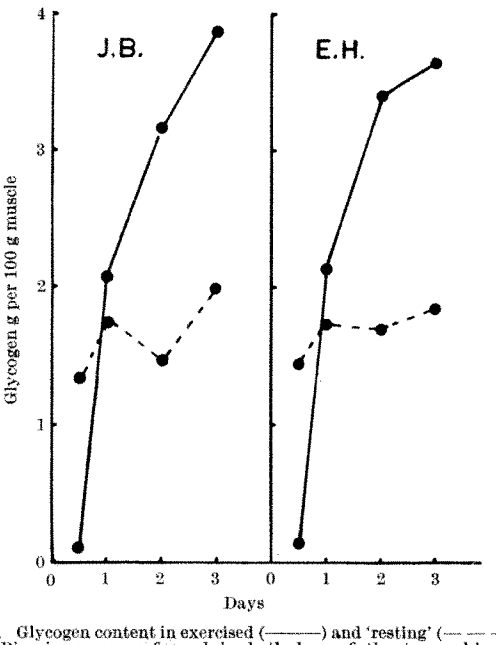


Fig. 1. Glycogen content in exercised (———) and 'resting' (— — — —) leg. Biopsies were performed in both legs of the two subjects at approximately the same time (within 15 min) on each occasion

The muscle glycogen content in the exercised leg was very low immediately after exercise, while the resting leg had a normal glycogen content. The effect of the carbohydrate diet was drastic. After 1 day the glycogen content in the exercised leg had risen from practically zero to higher values than in the resting leg, where only a small increase of the glycogen content was found. The glycogen content of the exercised leg continued to rise dramatically during the following 2 days on the carbohydrate diet, until the values were about twice as high as those in the other leg. The glycogen changes showed the same pattern and were of the same magnitude in both subjects.

From this experiment it can be concluded that exercise with glycogen depletion enhances the resynthesis of glycogen. The factor operates locally in the exercised muscle and the effect persists for at least 3 days.

The nature of the mechanisms involved is unknown. It could be that a stimulation of one or more of the factors directly involved in glycogen synthesis takes place or that an effect is provided on the cell membrane, stimulating glucose uptake. It is possible that some of the beneficial effects of exercise in normal as well as in diabetic subjects are mediated by this factor, thus promoting the storage of carbohydrate as glycogen instead of fat.

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Soluble Saccharin Preference: a Nutritive Basis for Persistence

ANIMALS will drink more of a saccharin solution than water, and will run a maze or bar-press for a saccharin reward. It has been suggested that since saccharin is non-nutritive, such behaviour may be hedonic rather than drive reducing[1]. Sheffield and Roby, and Carper and Polliard maintain that the taste of saccharin is a primary re-inforcer, because hungry animals do not extinguish their response to it as would be expected for an acquired reward related to drive or need reduction[2–4].

Since it is the sodium salt of saccharin ($C_6H_4CONSO_2Na$, 'soluble' saccharin) which is regularly used, the validity of ignoring nutritive effects is questionable. The Na^+ ion is an essential food item, and rats and mice on the usual laboratory chow show a preference for weak sodium chloride solutions to water[5,6]. Thus the persistence in preferring sodium saccharin (Na-saccharin) to water might depend on the re-inforcing value of sodium.

The 'insoluble' saccharin ($C_6H_4CONHSO_2$) is sufficiently soluble to sweeten water to the extent used in motivational studies[7]. It was expected that if the Na^+ ion is indeed a factor in preference, animals should respond differentially to equimolar Na-saccharin and saccharin. Accordingly, eighteen *NIH/N* albino male mice were tested with one or the other of these solutions. Preference was determined by the two-bottle choice technique, the experimental solution paired with water. Consumption was measured to the nearest 0·1 ml. daily. Sides were alternated every other day and stainless steel drinking tubes were alternated daily. In this way the results were controlled for side preference and/or tube preference. Animals had 'Purina' laboratory chow constantly available.

The results are summarized in Figs. 1 and 2. Equimolar concentrations ($8{\cdot}2 \times 10^{-3}$ M) of saccharin and Na-saccharin are each preferred to water ($P < 0{\cdot}001$). The Na-saccharin group maintained a high level of preference over a three-week interval (Fig. 1) and maintained a significantly increased ($P < 0{\cdot}001$) daily fluid intake (Fig. 2). On the other hand, mice on saccharin showed a decreasing preference, and total fluid consumption (which initially rose) was soon equal to the volume taken when water was in both drinkers. Following this period, all the mice received water from both drinkers for three days. The the total fluid consumed by both groups was the same (Fig. 2).

On the 23rd day concentrations were doubled ($1{\cdot}6 \times 10^{-2}$ M) for both the saccharin and Na-saccharin animals. The next day both groups showed preference for the

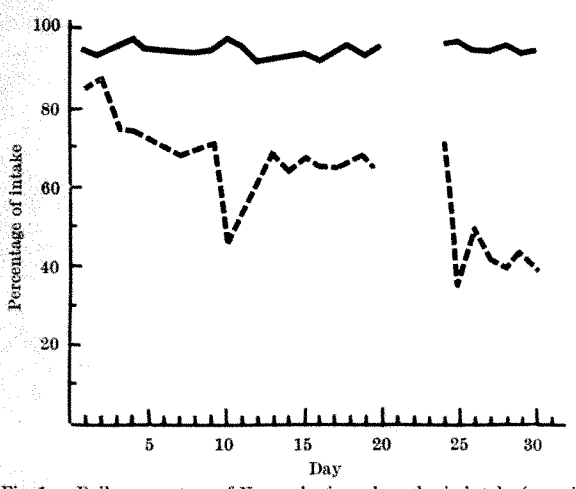


Fig. 1. Daily percentage of Na-saccharin and saccharin intake (experimental solution/experimental solution + water) (× 100). Solid line is Na-saccharin; broken line is saccharin

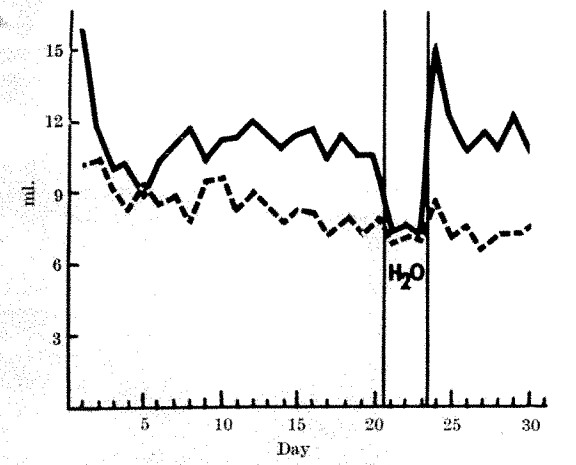


Fig. 2. Mean daily fluid intake. Solid line is Na-saccharin + water; broken line is saccharin + water

'sweet' flavoured water. But the second day on this increased concentration the saccharin group reversed its preference and was drinking considerably more water than saccharin and this persisted throughout the testing period. In order to determine whether the initial saccharin preference at this higher concentration resulted from prior experience with the weaker concentration, a group of six 'naïve' animals was tested with this concentration. The initial preference followed by aversion was not significantly different from that shown by experienced animals.

Doubling the concentration for the Na-saccharin group produced none of the marked changes observed for saccharin. These mice continued to show the same preference and increased fluid intake as with the weaker concentration.

The results cast doubt on the validity of considering the sweet taste as a primary re-inforcer, in the sense in which it is usually used, that is, "sweet for sweet's sake"[8]. Although mice initially preferred both concentrations of saccharin, preference declined with time. This is in keeping with behaviour towards a secondary re-inforcer no longer associated with a reward. The persistence of Na-saccharin preference, without diminution over extended periods, may be explained by the reward value of the sodium ion. In apparent contradiction to this thesis are the observations that animals will bar-press or run mazes for amounts of Na-saccharin of negligible physiological consequence[2–4]. However, Smith and Capretta[9], and Capretta[10], have shown that it is possible to consider that re-inforcement may be associated with juxtaposition of feeding schedules and experimental periods. These investigators have demonstrated that the taste of an Na-saccharin 'reward' gained in re-inforcing strength the more frequently it had been associated with food already in the stomach. Further, some of the initial motivating value of Na-saccharin was lost when there was no association with need reduction in the food-deprived animal. The problem of why an animal shows a preference for a 'sweet' substance in the first place is still not resolved.

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Recovery from the Increase of the Stiles–Crawford Effect after Bleaching

THE Stiles–Crawford effect[1,2], or the dependence of luminous efficiency on the eccentricity of the point of entry of the light in the pupil, is a function of the wavelength of the light. In Fig. 1 is given the inverse $1/\eta$ of the luminous efficiency as a function of wave-length at a distance of 3·5 mm from the point of maximum efficiency, according to the measurements of Stiles[3]. In the same graph is a theoretical curve according with the explanation by Walraven and Bouman[4]. The dip in the curve in the yellow-green is due to the high absorption (about 80 per cent) by the visual pigments of perpendicularly incident light in that region of the spectrum. Perpendicularly, light traverses the whole amount of pigment, while obliquely incident light leaks out of the receptor and therefore has a much shorter absorbent pathway to travel. But shortening of the pathway through a solution of high optical density is accompanied by a narrowing of the spectral absorption curves of the pigments. In other words, when the pigment has a high absorption, there is only a small chance that light will leak out of the receptor obliquely, so that nearly all of it will be absorbed. With low density, however there is more opportunity for leakage, which is why the Stiles–Crawford effect is larger at both ends of the spectrum than in the middle.

A consequence of this theory is that the dip in Fig. 1 disappears when the absorption of the pigment is low throughout the spectrum, which can be achieved by bleaching the pigments. Stiles[5] indeed found an increase of the directional effect in the middle of the spectrum with a retinal illumination of 2×10^4 trolands. Miller[6] found recently the same effect with a retinal illumination of 10^4 trolands. In her experiments, the test patch was superimposed on the white background. In our experiments, the Stiles–Crawford effect is measured after presentation of a bright (10^5 trolands) adaptation field of 10 degrees diameter with monochromatic light of 650 nm. The eye was adapted to these conditions during a period of 2 min.

Changes of the Stiles–Crawford effect could be detected by brightness-matching two half-fields of equal wavelength together subtending one degree. One half-field was focused on the retina by light through the centre of

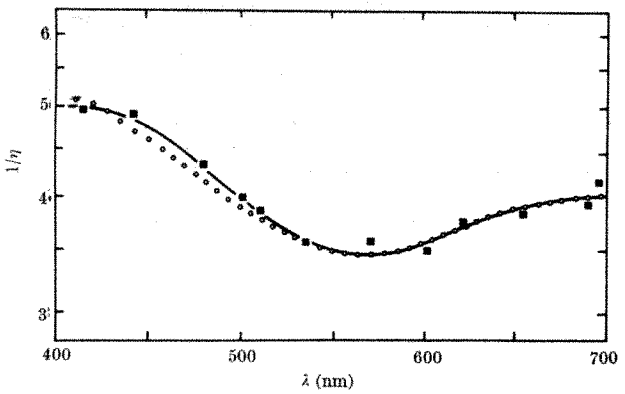


Fig. 1. Reciprocal of the luminous efficiency as a function of wave-length according to Stiles (ref. 3) (■) and according to the theory after Walraven and Bouman (ref. 4) (○). Eccentricity, 3·5 mm

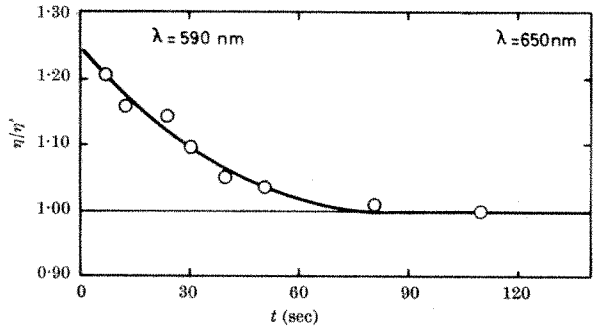


Fig. 2. Recovery of the increase of the Stiles–Crawford effect after bleaching at a wave-length corresponding with the dip in Fig. 1. Pre-adaptation time, 2 min; eccentricity, 3·5 mm; retinal illumination, 10^5 trolands

the pupil, and the other by light through a point 3·5 mm eccentric in the pupil. A match was first established before presenting the adaptation field. Any change in $1/\eta$ due to adaptation would upset this match. At a wave-length of 630 nm no significant disturbance could be measured, as might be expected.

Miller[6] found some increase even at the ends of the spectrum, and this may be obscured in our measurements because the accuracy is not better than a few per cent. At 590 nm, however, a large increase could be detected (Fig. 2) and the recovery of this increase as a function of time after the offset of the adaptation field was followed. One can see in the graph that the reciprocal $1/\eta$ is changed by about 20 per cent to the value of $1/\eta'$ after the adaptation and that the time constant of recovery is about 30 sec. The curve drawn is the best-fitting exponential curve. This same time constant has been found in the recovery of the change in the anomaloscope setting after adaptation to bright light, which suggests that we can interpret the change in $1/\eta'$ as a photochemical reaction in the receptors[7].

The characteristics, the time constant of the recovery from the increase of the Stiles–Crawford effect after bleaching, and the size of the effect, add evidence in support of a high value for the absorption of the visual pigments, which has been derived from other data[4,8].

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Physiological Competition between Host and Implanted Ganglia in an Insect (*Periplaneta americana*)

BODENSTEIN[1] found evidence that ganglia from the central nervous system implanted into coxae which had been denervated were, at least to a small degree, able to mediate local reflexes. This observation suggests possible ways of studying the competition for motor and sensory functions of a limb by host and donor ganglia.

Larval cockroaches were selected in the middle and late stages of growth, that is to say, between the sixth and ninth instars, and donor abdominal or thoracic ganglia derived from a similar insect were implanted near the base of the coxa. The fifth nerve on that side was cut through near the point of ganglion implantation.

After 5 or more weeks the operated limb showed signs of recovery, although anomalous postures occurred, especially those involving hypercontraction of the tibial depressor muscles. Anatomical investigations of the graft area involved dissections, and the preparation of sections stained by a modification of the Holmes's silver technique. Some material was examined under the electron-microscope. The electrical activity of axons in the graft area was studied in partially dissected preparations, and recordings were also made from the tibial depressor muscles, which are normally innervated solely by the fifth nerve. A few simultaneous records from operated and unoperated parts of the insect were made using five channels of a Grass *P7* polygraph.

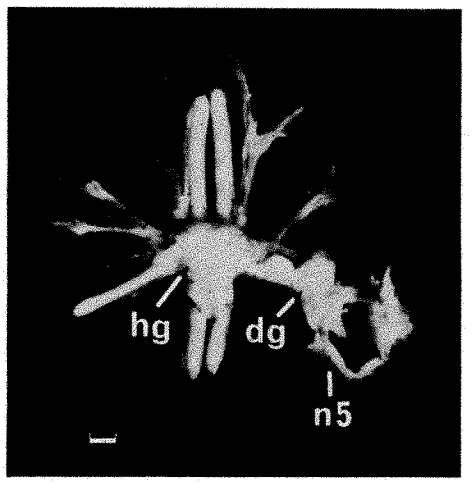


Fig. 1. Isolated host and graft ganglion. Scale: 0·5 mm. *Hg*, Host ganglion; *Dg*, donor ganglion; *n5*, fifth (podial) nerve



Fig. 2. Response of graft motor axons in the tibial depressor muscle to the repetitive stimulation of a tibial spine. Scale, 20 msec

Following implantation, the donor ganglion developed a new growth of axons so that a fine feltwork of processes eventually united the donor to the host ganglion (shown in Fig. 1). In some specimens considerable rearrangement of the internal ganglionic tracts occurred, in others the normal appearance was fairly well preserved. In rare examples the donor ganglia exhibited a complete absence of transverse tracts, and in some instances the normal peripheral cell bodies had wandered out into the central neuropile. The dictyosomes and the main cell process of the neurones often appeared particularly dark as if some argyrophil substance was localized at these points. Sections of the synaptic zones of donor ganglia examined with the electron-microscope have so far shown a fairly normal structure.

Early investigations of the physiology of these limbs, mostly after implantation of abdominal ganglia, were disappointing. Section of the donor–host connexion usually abolished contractions in muscles innervated by the fifth nerve, which suggested that the donor ganglion was functioning merely as a conduit for host ganglion motor neurones; on the other hand, the donor ganglion was clearly capturing most of the regenerating sensory fibres. Recording from the graft area was hampered by the large amount of connective tissue that had developed around the axons, and by the delicacy of donor cells following exposure by dissection. Nevertheless, it was possible to demonstrate spontaneous activity, often of great regularity. This is sometimes characteristic of autonomous neurones which are not part of a hierarchical system.

Implantation of thoracic rather than abdominal ganglia resulted in the leg muscles, especially the tibial depressors, retaining much more of their function after the host ganglion had been removed. Recordings from the leg muscles themselves, after this treatment, indicated a high level of tonic activity, and stimulation of spine sense organs produced well-defined reflex discharges accompanied by muscular contractions.

Repetitive stimulation of this kind was sometimes accompanied by a regular augmentation of the response that may have been due to the activation of additional elements within the donor ganglion. The latter is therefore capable of a complex response (see Fig. 2).

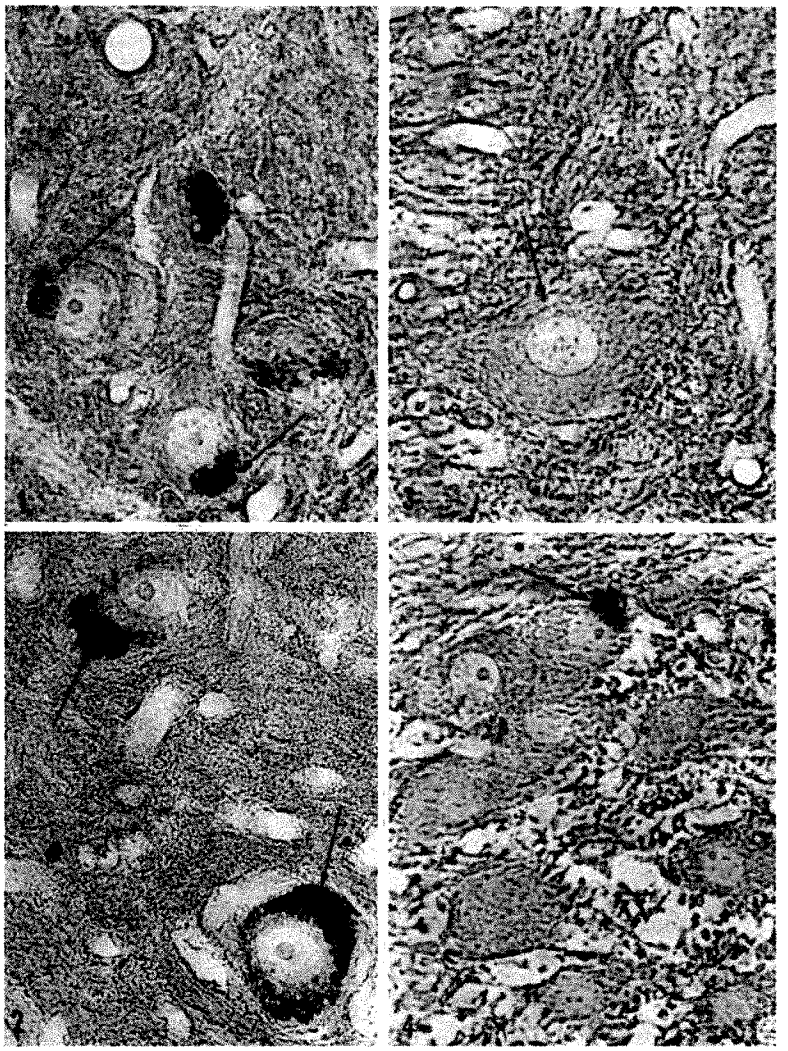
The functional interaction between donor and host ganglia is somewhat obscure, but removal of the host ganglion often results in increased activity and responsiveness in the graft.

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Figs. 1–4. Figs. 1 and 2, Neurones of the brain-stem reticular nuclei from senile guinea-pig (5 years). Control animal. Hueck's stain. Note that the cytoplasms of the neurones are heavily laden with lipofuscin pigments (arrows). Figs. 3 and 4, Neurones of the brain-stem reticular nuclei from senile guinea-pig (5 years). Animal treated with ANP–235 for 8 weeks. Note that the nerve cell (arrow) is completely free of pigments. In Fig. 4 one neurone shows a small amount of the pigment (arrow)

PHARMACOLOGY

Effect of Centrophenoxine on the Lipofuscin Pigments in the Neurones of Senile Guinea-pigs

CLINICAL and psychological studies indicate that a decline in the functions of the nervous system occurs in old age. Although it is difficult to evaluate the physiopathological changes in the neurones in senescence, the cells nevertheless show changes which they have acquired during functional activities in the past. Neurones are particularly suitable for the study of the effects of time on living cells as these are not generally replaced during life.

The accumulation of lipofuscin (old-age) pigments in the cytoplasm is possibly the constant cytological evidence of ageing in neurones, although the nature of the former and their mode of appearance are not definitely known. The present report deals with the effect of a new drug, ANP-235 (centrophenoxine), on these old-age pigments in the neurones of the central nervous system in guinea-pigs.

ANP-235 is a new drug synthesized by Thuillier[1] and does not belong to any of the pharmacological classifications. Numerous clinical experiments have shown rather striking results in the treatment of confusional states, apathy, disturbance of memory and intellectual concentration, states of agitation and fatigue in senescence[2–11].

In a histochemical study of the effects of this drug on the enzyme systems of guinea-pig neurones a striking decrease was observed of lipofuscin granules in the neurones of the treated animals.

About 100 guinea-pigs belonging to the age groups of 4–6 years were used in this project. The animals were injected intramuscularly or intraperitoneally (80 mg/kg of body-weight) daily for 4–8 weeks and the untreated animals of the same age groups were studied as controls. The animals were perfused with Lillie's 10 per cent buffered neutral formalin and the cerebrum, cerebellum and brain-stems were dissected out and fixed in the same solution for 3 days or more. Similar tissues from control animals of the same age groups were taken as controls. Paraffin blocks were prepared and sections (10μ) were stained for lipofuscin pigments by the following methods: periodic acid–Schiff (PAS) stain with diastase digestion as control, Schmorl's ferric–ferricyanide method, Hueck's

Nile blue stain with hydrogen peroxide bleaching to eliminate melanin, Lillie's Nile blue stain with and without acetone extraction to distinguish from melanin, Gomori's chrome alum–haematoxylin method, Long Ziel-Neelsen's stain for acid-fast lipofuscin and Sudan black *B* stain. Apart from the stains for lipofuscin pigments, the tissues of both control and drug-treated animals were stained with toluidine blue for Nissl's substance. Six untreated young guinea-pigs (less than 2 years) were studied as additional controls.

As no stain is at present available which specifically demonstrates the lipofuscin pigments, various tests have been used to eliminate possible sources of errors in its demonstration. The pigments were stained pinkish with PAS method and the colour was resistant to diastase digestion. These were stained dark blue with Schmorl's method and blue with Nile blue even after treatment with 10 per cent hydrogen peroxide for 48 h[12], but quickly disappeared after treatment with acetone[13]. In the chrome–alum haematoxylin method the pigments were stained dark-blue and with carbol fuchsin they were coloured bright red.

Lipofuscin pigments are present in most neurones of both young and old animals. They become more prominent at about the age of four years and thereafter increase steadily until they occupy a considerable part of the cytoplasm at 6 years. The pigments appeared rather scattered in younger animals but tended to accumulate in one part of the cytoplasm near the nucleus after the age of 5 years.

Hermann[14] observed progressive accumulation of lipofuscin pigments in the increasing age groups in the neurones of vagal and sympathetic ganglia.

A difference in the regional distribution of the lipofuscin pigments has been observed in the neurones of the central nervous system of these animals. While the largest accumulation of the pigments occurred in the neurones of the reticular formation of the brain stem (Figs. 1 and 2) and olivary nuclei, a lesser amount was observed in the neurones of the cerebral cortex and hypothalamic nuclei. It was interesting to note that the Purkinje cells of the cerebellum show very little of these pigments and this is in contrast to the large neurones of other regions of the central nervous system which show considerable pigment accumulation. The regional difference in the distribution of the pigment in man has been also reported by Vogt and Vogt[15], Sanides[16] in the amygdala, Balthasar[17] in cortical pyramidal cells, Hopker[18] in the dentate nucleus and Von Buttlar-Brentano[19] in the hypothalamic nuclei. We have also investigated[20] the difference in the distribution of the pigments in various parts of the central nervous system of the guinea-pigs of various age groups.

The nuclei and the Nissl substance also showed certain changes in the older neurones compared with the younger ones with the toluidine blue stain. While the Nissl bodies showed an increased staining with a clumpy appearance, the nuclei were more variable in shape and size with increased basophilia in the nucleolus. The underlying causes for such changes is by no means clear, but they may be a compensatory reaction prior to nuclear and cytoplasmic hypochromidia.

In animals treated with the drug, the neurones showed a notable diminution of the lipofuscin pigments in most parts of the central nervous system. The intensity of the effect of the drug was largely dependent on the duration of the treatment and a marked effect was observed after 8 weeks. Observation after varying periods of treatment with the drug showed the pigments to occupy a smaller and smaller area in the cytoplasm as if gradually shrinking away (Figs. 3 and 4).

It is generally believed that the gradual accumulation of these pigments was the constant sign of ageing and prolonged functional stress and strain in the neurones[21]; no drug, to our knowledge, has hitherto been reported which has exerted any influence on their gradual and progressive accumulation. Clinical data have indicated a marked improvement in the treatment of functional activities of senescence as a result of the drug, and the reduction of the lipofuscin pigments may be at least one of the important ways by which the drug ANP-235 renders its beneficial effects.

This work was carried out with the assistance of grants from the Animal Resources Branch, from the National Heart Institute, and from the Division of Research Facilities and Resources, National Institutes of Health.

We thank Dr. J. B. Rogers, of the Institute of Animal Geriatrics, Louisville, Kentucky, for the supply of senescent guinea-pigs used in this work.

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HAEMATOLOGY

Role of Plasma Factors in the Transformation of Peripheral Blood Lymphocytes into Lymphoblasts

HUMAN peripheral blood lymphocytes maintained in tissue culture can be stimulated to transform into lymphoblasts and to undergo mitosis by phytohaemagglutinin (PHA)[1] and by antigens to which the donor of the lymphocytes is allergic[2,3]. In the previous reports describing the transformation of lymphocytes *in vitro* the culture medium has always contained plasma from the lymphocyte donor.

The present investigation was designed to assess the importance of plasma from the lymphocyte donor Three subjects were used as plasma and lymphocyte donors. The first was a man aged 32 who gave a positive reaction (erythema and induration of 2 cm. diameter) on intradermal testing with 1/1,000 old tuberculin and who had been vaccinated several times against smallpox. An accelerated scratch test reaction to vaccinia virus occurred at the most recent smallpox vaccination. The second was a 24-year old woman who had never been vaccinated against smallpox. The third was a 9-year old girl who gave a negative reaction when tested intradermally with 1/100 old tuberculin. Ten ml. of venous blood from each subject was taken in a sterile polystyrene tube containing 200 U. of lithium heparin. The tube was sealed, shaken gently and stood upright at 37° C for 1 h. The supernatant leucocyte-rich plasma and a small volume of erythrocytes were removed and centrifuged at 250*g* for 3 min. The plasma was then centrifuged at 20 min three times at 1,000*g* in order that all blood cells be removed completely. The original

Table 1. LYMPHOBLAST TRANSFORMATION AFTER 6 DAYS' CULTURE OF LYMPHOCYTES FROM A SMALLPOX-VACCINATED (*B*) AND A NON-VACCINATED (*A*) DONOR

Total cells counted on each slide were from 2 to 6×10^3

Lympho-cytes from donor	Plasma from donor	Vaccinia virus	PHA*	Lympho-blasts (%)	Mitoses (%)	Tritiated cells (%)
B	*B*	+	−	3·67	0·29	1·34
B	*A*	+	−	3 68	0·42	1·40
B	*B*	−	−	0 03	0	0
B	*A*	−	−	0	0	0
A	*A*	+	−	0	0	0
A	*B*	+	−	0	0	0
A	*A*	−	−	0 03	0	0
A	*B*	−	−	0·05	0	0·02
A	*A*	−	+	70	+	Not done

Subject *B*, male, 32, vaccinated against smallpox.
Subject *A*, female, 24, never vaccinated against smallpox.
+, Large numbers of mitoses present.
* PHA cultures were harvested after three days.

Table 2. LYMPHOBLAST TRANSFORMATION AFTER 6 DAYS' CULTURE OF LYMPHOCYTES FROM MANTOUX POSITIVE (*B*) AND MANTOUX NEGATIVE (*C*) DONORS

Total cells counted on each slide were 2 to 6×10^3

Lympho-cytes from donor	Plasma from donor	Old tuber-culin	PHA*	Lympho-blasts (%)	Mitoses (%)	Tritiated labelled cells (%)
B	*B*	+	−	11 8	0·33	8·43
B	*C*	+	−	10 7	0·26	3·49
B	*B*	−	−	0 03	0	0 02
B	*C*	−	−	0·05	0	0 02
B	*B*	−	+	70	+	Not done
C	*B*	+	−	0	0	0
C	*C*	+	−	0 05	0	0 01
C	*B*	−	−	0 02	0	0·02
C	*C*	−	−	0	0	0 016
C	*C*	−	+	70	+	Not done

Subject *B*, male, 32, Mantoux positive with 1/1,000 old tuberculin.
Subject *C*, child aged 9, Mantoux negative with 1/100 old tuberculin.
+, Large numbers of mitoses present.
* PHA cultures were harvested after 3 days.

cellular deposit from the leucocyte rich plasma was washed four times with 0·15 M sodium chloride, pre-heated to 37° C, the cells being deposited after each washing by centrifuging at 250*g* for 5 min. A total white cell count was made after the final washing. The tissue culture medium was made by adding 0·4 ml. of the subject's plasma to 1·6 ml. of tissue culture 199 (ref. 4) containing 200 u penicillin per ml. and 100 µg streptomycin per ml. in 1 × 8 cm capped polystyrene tube[illegible] the appropriate subject[illegible] of $1{\cdot}2 \times 10^6$ cells per[illegible] culin to a final c[illegible] one vial of h[illegible] light blue cytoplasm of 'frosted-glass' appearance often containing several vacuoles. Mitotic figures arrested in metaphase were seen in cells of the same order of size as lymphoblasts and with similar cytoplasmic features. In the autoradiographs, cells were accepted as being labelled if they contained more than twenty silver grains over the nucleus.

The degree of lymphoblast transformation, mitotic activity and uptake of tritiated thymidine is shown in Tables 1 and 2.

These results show that old tuberculin and heat-killed vaccinia virus induce lymphoblast transformation of peripheral blood lymphocytes from an immune donor in the absence of autologous plasma. They also demonstrate that the plasma from an immune individual does not confer the potential for lymphoblastic transformation *in vitro* in the presence of antigen to lymphocytes from a non-immune individual. This finding is consistent with the ability to transfer delayed-type tuberculin sensitivity *in vivo* with lymphocyte suspensions and not with plasma[5].

In these experiments, PHA stimulated lymphoblast transformation in the absence of autologous plasma. The considerable quantitative differences between the dynamics of lymphoblast transformation caused by antigens and that caused by PHA suggest that PHA acts as a direct cellular stimulant and not as an antigen[6]. This is supported by our recent finding that PHA stimulates mitosis in epithelial cells of adult human skin grown in an organ culture system *in vitro*[7]. The fact that the plasma from sensitized individuals does not contain any component which can induce the transformation of lymphocytes from unsensitized individuals into lymphoblasts in the presence of specific antigen would indicate the probability that cytophilic antibodies[8] were not involved in this reaction.

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Table 1. DISTRIBUTION OF HAPTOGLOBIN TYPES IN TURKISH PEOPLE

No. tested	Distribution of haptoglobin types (per cent)					Gene frequency	
	Type 1–1	Type 2–1	Type 2–2	Type 2–1M	Type Johnson	Hp^1	Hp^2
300	8	46	45·1/3	1/3	1/3	0·31	0·68

haptoglobin types were determined by the vertical starch-gel electrophoretic method of Smithies[2]. The position of the haptoglobin bands was determined by staining with orthodianisidine[3]. The results of these investigations are presented in Table 1.

The distribution of the major haptoglobin types is quite uniform in populations living in different European countries : Hp 1-1, 14–17 per cent ; Hp 2-1, 47–49 per cent ; and Hp 2-2, 36–40 per cent[4–12]. Gene frequencies for Hp^1 and Hp^2 are also very similar : 0·36–0·40 and 0·60–0·66 respectively[4–12]. On the other hand, in Africa, Hp 1-1 is much more frequent (49–54 per cent), and the gene frequency of Hp^1 is much higher (0·60–0·72) (refs. 13–15). In Asia, on the contrary, the percentage of type 1-1 is lower (5–10 per cent) and so is the gene frequency of Hp^1 (0·21–0·31) (refs. 16–19).

As can be seen from Table 1, the distribution of major types in Turkish people is similar to that seen in Asiatic countries. This is not surprising, considering the historical fact that the Turks emigrated for several centuries from Central Asia to Asia Minor. On the other hand, a survey of different blood-group frequencies (ABO–MNS, Rh, Lutheran, Kell and Duffy) in Turkish people[20] showed that they did not differ greatly from the values commonly found in Europe. Such a discrepancy also occurred in the case of the Bushmen of Southern Africa. Although their physical appearance suggested a Mongoloid ancestry, blood-group studies performed by Weiner *et al.*[21] enabled them to conclude that the blood group frequencies of Bushmen showed a negroid pattern. But the distribution of haptoglobin types in Bushmen was different from other Africans and similar to the distribution of haptoglobin types in Chinese[19,22]. These two examples of discrepancy between blood group frequencies and the distribution of haptoglobin ty[illegible]

Microsomal Incorporation of *N*-Acetyl-D-galactosamine into Blood Group Substance

SOLUBLE blood group A and H substances from hog gastric mucosa consist of a protein 'backbone' to which numerous oligosaccharide units are attached[1,2]. These oligosaccharide units are supposed to consist of a chain of D-galactose and *N*-acetyl-D-hexosamines to which L-fucose residues are laterally linked[3–5]. Group A substance is considered to differ from group H substance only by an additional α-*N*-acetylgalactosamine residue at the non-reducing end of the sugar chains. In fact, substances showing H specificity can be obtained from A substances by treatment with α-*N*-acetylgalactosaminidases[6,7].

The mechanism of the biosynthesis of blood group substances is unknown. *In vivo* labelling experiments in which [^{14}C]-glycerol was injected into the gastric artery of hogs indicated that blood group substances are synthesized on the microsomes of the gastric mucosa[8]. This communication describes the transfer of ^{14}C-labelled *N*-acetylgalactosamine from uridine diphosphate (UDP) [^{14}C]-*N*-acetylgalactosamine to terminal positions of blood group H substance in the presence of microsomes from hog gastric mucosa.

A mixture of UDP-[^{14}C]-*N*-acetylglucosamine and UDP-[^{14}C]-*N*-acetylgalactosamine was prepared by injection of glucosamine-1-^{14}C (10·0 mc./m mole, obtained from New England Nuclear Corporation) into the tail vein of rats, and the UDP-*N*-acetylhexosamines isolated from the livers according to the method of O'Brien and Neufeld[9].

Microsomes were prepared from hog stomachs freshly obtained from the slaughter-house. The mucosal linings were stripped off, separated from the underlying muscularis mucosa, and homogenized in a Waring blender in 0·35 M sucrose, 0·025 M potassium chloride, 0·01 M magnesium chloride, 0·001 M ethylenediamine tetraacetic acid, 0·05 M *tris* pH 7·6 (3 ml. per g of mucosa). The homogenates were centrifuged at 15,000*g* for 20 min and the [illegible] assayed for blood group activity by haem- [illegible] further centrifuged at [illegible]llet from 5 g mucosa [illegible]ernatant. To the [illegible]oles adenosine

Table 1. *In vitro* INCORPORATION OF [^{14}C]-*N*-ACETYLHEXOSAMINES INTO BLOOD GROUP SUBSTANCE BY MICROSOMES OF A- AND O(H)-ACTIVE HOG GASTRIC MUCOSA

Blood group specificity of gastric mucosa	Time of incubation (min) of microsomes with UDP-[^{14}C]-*N*-acetylhexosamines	C p.m. incorporated into blood group substance
A	60	900
A	120	1,750
O (H)	120	70

In the following experiments, microsomal preparations from gastric mucosa showing a strong A activity were used and the incubation was stopped after 2 h. As shown in Table 2, the amount of incorporated radioactivity could be increased nearly three-fold by the addition of soluble blood group H substance. Sonication of the microsomal suspension prior to incubation resulted in a further two-fold increase in incorporating ability. This treatment obviously facilitated the approach of UDP-*N*-acetylhexosamines to the sites of synthesis on the microsomes.

Table 2. INCORPORATION OF [^{14}C]-*N*-ACETYLHEXOSAMINES INTO BLOOD GROUP SUBSTANCE BY MICROSOMES OF A-ACTIVE MUCOSA

Stimulation by the addition of extraneous H substance and by sonic treatment of the microsomal preparations

Sonic treatment of microsomes	Addition of extraneous H substance	C.p.m. incorporated into blood group substance
Not sonicated	No addition	500
Not sonicated	H substance added	1,400
Sonicated	H substance added	2,950

More than 90 per cent of the incorporated label (1,850 c.p.m. out of a total activity of 1,975 c.p.m.) could be removed from the blood group substance present in the incubation mixture by treatment with an α-*N*-acetylgalactosaminidase, which had been purified from the hepatopancreas of the snail, *Helix pomatia*. This enzyme had previously been shown to release terminal *N*-acetylgalactosamine from blood group A substance[7]. This result indicated that the incorporation of radioactivity into blood group substance was mainly due to the attachment of terminal *N*-acetylgalactosamine residues to oligosaccharide units of blood group A precursor substances present in the microsomal fraction and of H substance added to the incubation mixture.

The experiments reported here suggest that the microsomal preparations obtained from hog gastric mucosa showing blood group A activity contain an enzyme which catalyses the transfer of *N*-acetylgalactosamine from UDP-*N*-acetylgalactosamine to blood group H substance. This enzyme is formed under the control of the *A* gene and is absent or inactive in individuals of blood group O(H). The results of the present investigation are consistent with the scheme of Watkins and Morgan[11], which postulates the conversion of pre-formed blood group H substance into A substance by the attachment of non-reducing α-*N*-acetylgalactosamine residues.

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GENETICS

Serum Transferrins of some Gadoid Fishes

SERUM transferrin polymorphism has been demonstrated in carp (*Cyprinus carpio*)[1] and cod (*Gadus morhua*)[2]. Investigations undertaken at this Institute indicate that serum protein polymorphism, and especially transferrin polymorphism, is a common feature among fishes, and we hope to use these characteristics in population studies of several species of commercial value. This report deals with preliminary results from studies of coalfish (*Gadus virens*), pollack (*G. pollachius*), and whiting (*G. merlangus*).

The blood was collected by cardiac puncture. Blood was obtained from small fish by cutting the tail. After clotting, the blood was centrifuged and the sera pipetted off. Most sera were stored for some time in deep freezer until the analyses were carried out.

Most coalfish sera were taken from immature fish caught by purse seine on the west coast of Norway. Thirteen specimens were taken from larvae from Vestfjorden, Northern Norway. The pollack sera also were taken from fishes caught by purse seine. The whitings were caught by purse seine or otter trawl, and several year groups were represented. Numbers in samples, month of sampling and catching areas are shown in Table 1 (coalfish), Table 2 (pollack), and Table 3 (whiting).

Table 1. TRANSFERRIN GROUPS IN COALFISH. DISTRIBUTIONS AND GENE FREQUENCIES

		Transferrin groups AA	AB	BB (exp.)	Total	Gene frequencies q_A	q_B
Rogaland	Sept. 1965	57	—	—	57	1 000	—
Hordaland	Aug. 1965	97	3	—	100	0·9850	0·0150
Hordaland	Dec. 1965	108	4	—	112	0·9821	0 0179
Smøla, Nordmøre	Sept. 1965	108	3	—	111	0·9865	0 0135
Sandøy, Romsdal	Dec. 1965	86	4	—	90	0·9778	0·0222
Veidholmen, Nordmøre	Dec. 1965	38	—	—	38	1·000	—
Gamsvik, Vestfjorden	Oct. 1965	13	—	—	13	1 000	—
Total	n_{obs}	507	14	—	521	0·9836	0 0134
	n_{exp}	507·1	13·8	0 1	521·0		

Table 2. TRANSFERRIN GROUPS IN POLLACK. DISTRIBUTION AND GENE FREQUENCIES

		Transferrin groups AA	AB	BB	Total	Gene frequencies q_A	q_B
Hordaland Aug. 1965	n_{obs}	2	18	55	75	0·1467	0·8533
	n_{exp}	1·6	18·8	54·6	75·0		

Table 3. TRANSFERRIN GROUPS IN WHITING. DISTRIBUTIONS AND GENE FREQUENCIES

		Transferrin groups AA	AB	BB	Total	Gene frequencies q_A	q_B
Hordaland	n_{obs}	1	8	35	44	0·1136	0·8864
Sept.–Oct. 1965	n_{exp}	0 6	8·9	34·6	44·1		
Altafjorden	n_{obs}	1	13	58	72	0 1042	0·8958
Oct. 1965	n_{exp}	0 8	13·4	57 8	72·0		

The sera were examined by the combined starch/agar-gel electrophoresis described by Sick[3] and Møller[2]. Autoradiography has been carried out as for cod sera[2] based on the methods of Giblett, Hickman and Smithies[4].

An outline of the transferrin bands of the electrophoretograms, identified by autoradiography, is shown in Fig. 1 together with the general transferrin pattern in cod[2]. Regarding pollack and whiting three transferrin groups (phenotypes) were found: two with one single transferrin band each, and a third with both these bands. In coalfish only two groups were detected, one with a single transferrin band, the other with a double band.

For each species the fastest moving component is called Tf A, and the slowest moving component Tf B. The phenotype possessing only Tf A is labelled AA, that containing only Tf B is labelled BB, and when possessing both transferrins the phenotype is labelled AB.

None of the four species seems to have any transferrins in common, and the electrophoretic mobilities of transferrin molecules differ widely despite the fact that the species are closely related. The transferrin molecules of pollack move fastest towards the anode at *p*H 9·0, and the mobilities of the five cod transferrins are intermediate

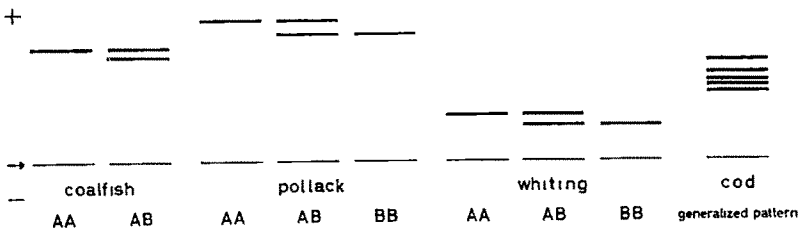


Fig. 1. Transferrin pattern of three species of gadoid fishes revealed by starch-/agar-gel-electrophoresis at *p*H 9·0. Duration of run, 90 min. Arrow indicates the point of application. The general transferrin pattern of cod is given for comparison

between the transferrins of coalfish and of whiting, which possess by far the slowest moving transferrin molecules of the four species yet investigated.

The observed distributions of the phenotypes in the collected samples of coalfish, pollack, and whiting are shown in Tables 1–3.

A hypothesis of hereditary control involving two co-dominant alleles (called Tf^A and Tf^B) has been adopted to explain the observed heterogeneity. Corresponding gene frequencies have been calculated and are shown in the last columns of the tables, and expected distributions according to the Hardy–Weinberg law are given in the lines signed 'n_{exp}' in Tables 1–3. The phenotype BB is not found in coalfish sera, but it follows from Table 1 that, due to the rareness of the gene Tf^B, the expectance of this gene in a homozygous state is low in this material.

It is seen from Tables 1–3 that the accordance between observed and expected distributions is very good, and this strongly supports the hypothesis that the transferrins in these species are controlled by two co-dominant alleles.

Individual differences were indicated also in other serum proteins, but with the technique used here, these differences were not clear enough for proper classification of the specimens. Similar investigations of haddock (*G. aeglefinus*) sera have also revealed individual differences, but the genetic basis of these differences seems to be complicated and has not yet been worked out. Investigations are now being carried out on herring (*Clupea harengus*) and sprat (*C. sprattus*) and have so far revealed individual variations in serum proteins in both species.

No differences were found when results from fresh and frozen sera were compared; moreover, sera can be stored in the refrigerator (0°–4° C) for 4–6 days before any decomposition of proteins occurs.

These preliminary results suggest that polymorphism in serum proteins are common in fishes and may become useful in fishery biology for identification of populations.

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HISTOLOGY

Free Aldehydic Groups in Collagen and Other Tissue Components

THE reaction of free carbonyl groups with *N*-methyl benzothiazolone hydrazone (MBTH) is a very sensitive and specific method for the quantitative determination of free aldehydes[1,2]. Carbonyl compounds react with 1 mole of MBTH to form an azine derivative. Azines of aldehydes react sequentially with a second mole of MBTH, oxidized by ferric ion under acid conditions, to form blue-green tetraazopentamethine cyanine dyes[3]. These products can be specifically identified for aldehydes by their absorption spectra[2,4–6]. While a variety of compounds will form azines with 1 mole of MBTH, the second stage of the reaction is specific for aldehydes and can be carried on in the presence of other carbonyl compounds. Carbohydrates having pyranose structures do not react with MBTH, nor do they interfere with the reaction of aldehydes with MBTH. The reaction is at least 100 times more sensitive than the Schiff reaction[7]. It differs from the periodic-acid–Schiff (PAS) reaction[8] in that the MBTH reaction detects 'natively' free aldehydic groups while the PAS reaction uncovers the aldehydic group through oxidative reactions prior to reaction with Schiff's reagent.

Recently, Gallop *et al.*[4–6,9] have demonstrated the presence of small amounts of an unusual free aldehydic group in collagen, covalently attached to the protein and possibly involved in intramolecular cross-linking. This aldehyde does not seem to be one of a variety of simple aldehydes nor one of various commonly encountered carbohydrate substances[4]. On the basis of their demonstration of the great sensitivity of MBTH in detecting minute quantities of aldehyde coupled to protein molecules, we postulated that the reaction might be a useful test for free aldehydic groups when carried out directly with tissue sections. It might, furthermore, provide helpful chemical information about collagen in tissue, since the MBTH reaction permits the specific examination of an aldehydic function which may be important to maturation phenomena of collagen or disorders of collagen structure.

The investigations were performed mostly with paraffin-embedded tissue which had been fixed in 10 per cent formalin buffered to *p*H 7·2 with phosphate. The excess formalin was removed by extensive tissue washing prior to reaction. Fixation of tissue in osmic acid buffered with phosphate at *p*H 7·2 gives results identical to those with formalin fixation; it, therefore, appears likely that formalin fixation does not introduce any artefact. Rat, toad and human tissues give comparable results.

Tissue sections of 2–3μ thickness on glass microscopic slides were deparaffinized through three changes for 5 min each in xylol, three changes for 3 min each in absolute alcohol, two changes for 3 min each in 95 per cent alcohol, and then washed well in distilled water. The staining reaction was adapted for development in conventional staining dishes. In the first dish was an aqueous solution of MBTH hydrochloride (Aldrich Chemical Co.), 2·0 g per 100 ml. In the second dish was an aqueous solution of MBTH hydrochloride, 2·0 g per 100 ml., plus $FeCl_3$, 2·5 g per 100 ml. Slight improvement in the reaction progress was obtained by including in each tray 0·1 M glycine buffer, *p*H 4. The reaction was usually carried on at room temperature, but it is slightly improved at 0° C. The tissue sections, in a rack, were placed in the first dish for 15 min, then transferred to the second dish for 45 min. The sections were then rinsed briefly with distilled water and subjected to a modified, abbreviated dehydration procedure since it had been noted that conventional dehydration caused significant loss of specific staining. From distilled water the sections were dipped three times in two changes of 95 per cent alcohol, three times in three changes of absolute alcohol, twice in xylol, and were then allowed to remain in a second dish of xylol for 5 min before mounting glass cover slips with 'Permount'. Blue-green staining of the sections was grossly evident. For best results the sections should be examined on the first day or second day after preparation, as fading of the reaction ensues thereafter and is quite evident within 3 or 4 days.

Collagen is consistently and apparently specifically stained with MBTH (Fig. 1). This is equally true in all species studied. The collagen fibrils were stained deeply

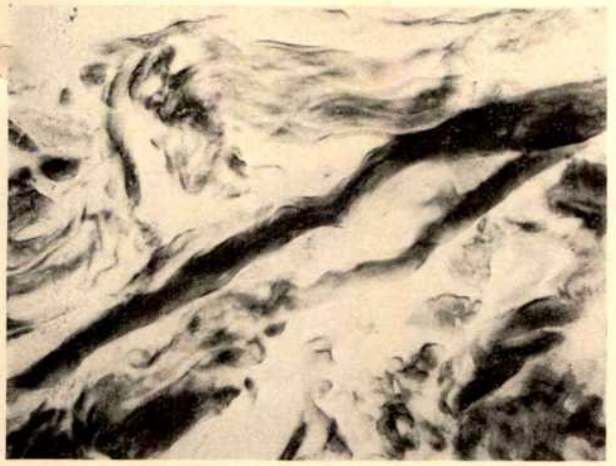


Fig. 1. Section of human skin. Dermal collagen stained by reaction with MBTH followed by reaction with oxidized MBTH, a method specific for free aldehydic groups. Collagen is stained blue-green. (×342)

blue-green and very fine strands can be resolved readily at 200–300 × magnification. Epithelial cells usually are stained a faint pea-green colour, readily distinguishable from the colour of collagen. It seemed possible that the pea-green colour arose from azo compounds formed by the reaction of a diazonium salt with epithelial cell components. MBTH oxidized by ferric ion can itself act similarly to diazonium salts in reacting with phenolic compounds like tyrosine; the products of these reactions are coloured, but have a different absorption spectrum from the tetraazopentamethine cyanine dyes which are formed only on the addition of 2 moles of MBTH with aldehydes. Separate fluorescence microscopic studies of skin stained with *p*-nitrobenzene-diazonium fluoborate, a fluorescent diazonium reagent, showed reaction of this compound primarily in epithelial cells. This distribution of fluorescence is identical with the distribution of the pea-green colour seen on staining with ferric chloride oxidized MBTH. Epithelial cells, then, probably contain some material which readily takes up one mole of MBTH in the presence of Fe^{+++} to form a pea-green-coloured azo product. On the other hand, collagen, containing free aldehydic groups, develops a blue-green colour on addition of two moles of MBTH in the presence of Fe^{+++}.

The only other tissue component which has so far been shown to react comparably to collagen is basement membrane material. Other tissues produce a faint blue colour different from that of collagen on reaction with MBTH. These include mucus and cartilage matrix; occasional cells in lacunae deep in the centre of a section of cartilage stain intensely blue. Presumably, glyco-proteins of basement membrane contain free aldehyde groups or reactive hemiacetals; this may also be true of mucus and cartilage although the nature of the blue reaction product is unknown. Amyloid and elastin do not react with MBTH whereas fibrinoid, as seen in rheumatoid nodule, sarcoid granulomata and the glomerular lesions of disseminated lupus erythematosus, produces a blue colour with MBTH.

Treatment of tissue with periodic acid followed by staining with MBTH intensifies the normal staining of collagen, as may be expected since periodate oxidation of hydroxylysine side-chains form additional protein-bound aldehyde[6]. There is also the appearance of new staining in regions where the periodic acid–Schiff reagent would conventionally stain. Treatment of the tissue with sodium borohydride, which reduces free aldehydic groups[9], prevents subsequent staining of collagen with MBTH.

Further studies with this method for demonstrating free aldehydic groups in collagen and other tissues are in progress. This method provides the advantage of permitting the direct visualization of a specific chemical functional group which may be important in the maturation and cross-linking of collagen and it enables study of this element of protein structure in diseases of collagen. The reaction may also permit the detection of transitory aldehydic intermediates in physiological and pathological reactions.

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IMMUNOLOGY

Absorption of Thyroid-stimulating Globulin from Thyrotoxic Sera by Organ Homogenates

RECENT experiments have shown that the long-acting thyroid stimulator present in the serum of patients with Graves's disease[1] has physicochemical and immunological characteristics indistinguishable from those of IgG (7*S* gamma-globulin)[2–6]. These findings and the frequent association of this disease with thyroiditis and other autoimmune disorders suggested that the long-acting thyroid stimulator might be an autoantibody directed against a constituent of the thyroid gland[7–9]. This communication reports the results of absorption experiments which provide further evidence for this hypothesis.

Homogenates were prepared from human organs by dispersion in an MSE top drive blender with 5 vol. of saline. They were filtered through muslin and centrifuged at 14,400*g* for 50 min at 4° C. The pellets were washed successively in 0·9 per cent sodium chloride and in distilled water to disrupt subcellular structures and remove substances which might prove toxic in the bioassay. The final pellets were suspended in small volumes of saline, dialysed for 2 days and recentrifuged as above. The sera of five thyrotoxic patients containing long-acting thyroid stimulatory activity were absorbed with varying weights of human thyroid, liver, kidney, stomach and muscle homogenate for 60–90 min, at 37° C and then overnight at 4° C. Following absorption, the sera were spun and dialysed against saline. This was not always effective in removing toxic products, and in recent experiments it has been found preferable to precipitate the globulin fraction with 14 per cent sodium sulphate. In some experiments the IgG fraction was isolated by chromatography on DEAE-cellulose. For bioassay, comparison was made with globulin fractions similarly prepared from the unabsorbed sera, the absorbed and unabsorbed fractions being tested at the same protein concentration.

The absorbed sera together with the unabsorbed controls were assayed for the long-acting thyroid stimulator by the following modification of Mackenzie's method[10]: Swiss mice weighing 15–20 g were given 6 μc. iodine-131 subcutaneously 3 days before the assay, followed 6 h later by an intraperitoneal injection of 10 μg thyroxine; further thyroxine was given in the drinking water. Twenty-four hours before the assay, the animals received a final injection of 10 μg thyroxine intraperitoneally. 0·5 ml. of each sample to be tested was injected intravenously into each of 6–10 mice. The radioactivity of blood obtained from the retro-orbital sinus was determined before and at 2, 7, and 24 h after injection. Long-acting thyroid stimulatory activity was measured by the radioactivity at 7 h (expressed as a mean percentage of the pre-injection value, ± the standard deviation). Two dose levels of thyroid-stimulating hormone were included to assess the sensitivity of each assay. The absorbed and unabsorbed sera were usually diluted in parallel so as to give a value of 300–400 per cent for the unabsorbed specimen. Under the conditions of assay used, twenty-five normal sera produced 7 h responses of 107 ± 11 per cent.

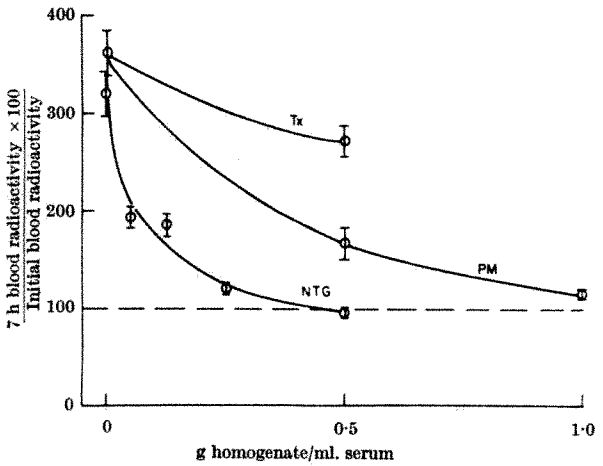


Fig. 1. Absorption of the activity of the long-acting thyroid stimulator by thyroid homogenates. *NTG*, Non-toxic goitre; *PM*, post-mortem; *Tx*, thyrotoxic thyroid. Results are expressed as the mean ± *S.D.*

Complete absorption of long-acting thyroid stimulatory activity was obtained with particulate homogenates of fresh non-toxic colloid goitre using 0·5 g (wet weight) of pellet equivalent to 5–10 g of fresh tissue per ml. of serum. An absorption curve using increasing amounts of homogenate is shown in Fig. 1. Normal thyroid gland obtained at post-mortem was somewhat less effective although 90 per cent absorption was attained using 1 g/ml. serum. Fresh thyrotoxic glands were also less effective than non-toxic goitre possibly due to partial saturation *in vivo* of the combining sites with the patient's own long-acting thyroid stimulator. Results of absorption with different thyroid preparations using 0·5 g homogenate/ml. serum are summarized in Table 1.

Table 1. ABSORPTION OF THE LONG-ACTING THYROID STIMULATOR BY 0·5 G OF DIFFERENT THYROID HOMOGENATES/ML. SERUM

Thyroid	No. of glands	Decrease in activity of the long-acting thyroid stimulator (%)
Non-toxic nodular goitre	2	92, 92*, 102*
Normal (post-mortem)	2	74, 57
Thyrotoxic	1	34
Toxic nodule	1	60

* Different sera absorbed with the same gland.

Of the other organs investigated, post-mortem liver had the least effect on the long-acting thyroid stimulator, absorption with 1 g of particulate preparation/ml. serum giving a 14 per cent reduction of activity. With post-mortem kidney, as little as 0·05 g of tissue preparation

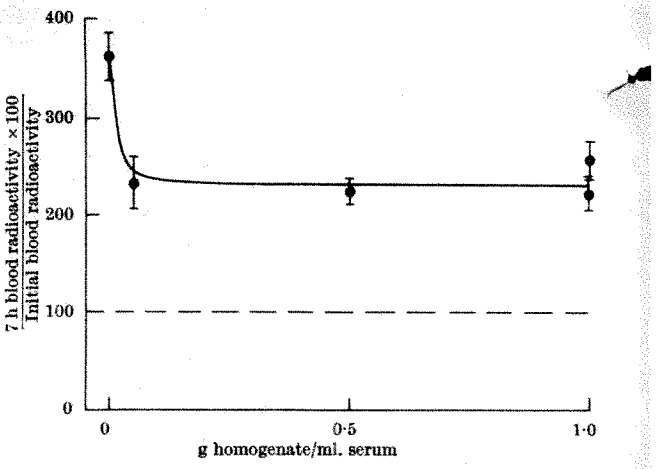


Fig. 2. Absorption of the activity of the long-acting thyroid stimulator by post-mortem kidney. The two absorptions carried out with 1 g/ml. were made with homogenates from different kidneys

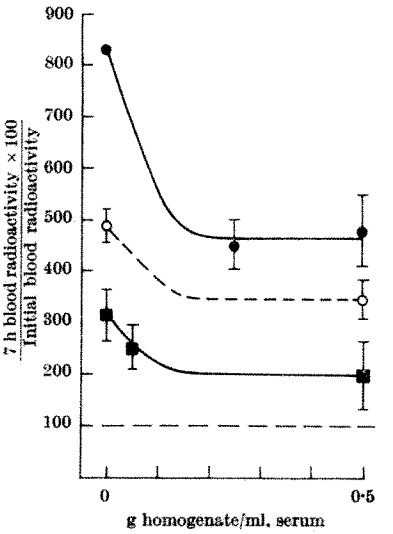


Fig. 3. Absorption of the activity of the long-acting thyroid stimulator by fresh muscle homogenate. ●, Serum from patient Wa.; ○, serum of Wa. diluted 2·5 times; ■, γ-globulin from patient Gi. (the concentration was adjusted to 5 mg/ml.)

reduced the activity of the long-acting thyroid stimulator by about half, but a plateau was reached and no further loss of activity could be demonstrated even when the sera were absorbed with as much as 1 g of tissue pellet/ml. (Fig. 2). A similar phenomenon was observed with fresh skeletal muscle (Fig. 3) and with human gastric mucosa obtained at operation.

Our findings are in accordance with the observations of Kriss *et al.*[5]. In their investigations, although 80 per cent absorption was obtained with human thyroid gland, other human organs were not tested. In experiments with canine tissues, thyroid was more effective than other organs on a weight basis. However, in our investigations partial removal of the activity of the long-acting thyroid stimulator was seen with small amounts of muscle, stomach and kidney, and the absorption with heterologous thyroid reported by Kriss *et al.* could have produced a similar effect without implying that an organ-specific antigen–antibody reaction had taken place. Pincherle *et al.*[11] were unable to demonstrate any absorption of the long-acting thyroid stimulator with slices or cell membranes from a thyrotoxic thyroid gland. On the other hand, Beall and Solomon[12] briefly reported the absorption of long-acting thyroid stimulator by thyroid subcellular fractions.

In the present experiments, the ability of thyroid tissue to remove virtually all the activity of the long-

acting thyroid stimulator from thyrotoxic sera contrasts with the partial absorption observed when other organs were used. Thus it would appear that the absorption of the activity of the long-acting thyroid stimulator depends on at least two components. One of these is thyroid specific and could represent an autoantigen derived from the gland. The non-organ-specific effect is not due to a physical adsorption of IgG to the particulate elements of the tissue preparation since increasing amounts of homogenate did not further reduce the activity of the long-acting thyroid stimulator, and a number of experiments were conducted using IgG concentrations identical with those present in the unabsorbed sera. The possibility cannot be excluded, however, that a proportion of the activity of the long-acting thyroid stimulator is present in an IgG sub-class with a particular tissue-binding affinity. The same arguments make it unlikely that the phenomenon represents an enzymatic inactivation of the long-acting thyroid stimulator, although again there might be a special susceptibility to enzymes of certain sub-classes of immunoglobulin. Recovery of activity by elution from the tissue preparation used for absorption would exclude enzymatic degradation. Loss of activity following absorption is unlikely to be due to a thyroid inhibitor eluted from the tissue homogenate since the decrease in the activity of the long-acting thyroid stimulator was also observed when the γ-globulin was purified by ion-exchange chromatography. The possibility remains that the hypothetical thyroid autoantigen may share some determinants with other organs and that the absorption with non-thyroid tissues is due to an immunological reaction. Whatever the true nature of this phenomenon, the results indicating the existence of a thyroid specific component support the concept that the long-acting thyroid stimulator is a thyroid autoantibody.

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Note added in proof. Since this work was submitted Dorrington *et al.* (*J. Endocrinol.*, 34, 135; 1966) have described the preferential absorption of long-acting thyroid stimulator by thyroid microsomes. We have now succeeded in recovering activity after absorption on thyroid microsomes, by elution at *p*H 3·6.

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RADIOBIOLOGY

Decreased Radiation Mortality in Dogs treated with Typhoid–Paratyphoid Vaccine

Bacterial endotoxins and bacterial vaccines have been shown to decrease X-radiation mortality in small laboratory rodents[1–4]. Typhoid–paratyphoid vaccine (TAB), for example, protects maximally if injected 24 h before irradiation, but, in the mid-lethal dose range, even post-irradiation injection significantly increases survival[2,4]. No such protection of bacterial endotoxins or vaccines, administered pre- or post-irradiation, has been reported for large animals. Byron *et al.*[5] evaluated the effect of post-irradiation treatment with TAB and antibiotics on survival of irradiated rhesus monkeys. A combination of TAB and antibiotic therapy resulted in no greater survival than was observed with antibiotics alone, so no protective effect was ascribed to TAB. The TAB did, however, affect the haematopoietic system; there was a delayed fall and earlier recovery in numbers of platelets and granulocytes. On the other hand, several years ago, Cronkite and Brecher[6] reported that survival was significantly increased and granulocyte recovery occurred earlier in dogs in which an abscess had been produced by a post-irradiation injection of sterile turpentine, bacterial cells, and bacterial components of this mixture.

The purpose of the present work was to determine if TAB injected either before or after irradiation would increase survival in dogs. The present data show that TAB, although quite toxic and pyrogenic, protects dogs when given 24 h before irradiation, but does not significantly increase survival when given 1 h after irradiation.

The dogs used were mongrels weighing 15–30 lb. They were injected intravenously (i.v.) with 1·0 ml. of TAB 24 h before or 1 h after bilateral exposure to 1 MVp. X-rays delivered at ~9 rads/min. No anaesthesia was used. The TAB was a commercial preparation (Parke-Davis) containing $1·5 \times 10^9$ killed organisms per ml. A further description of the experimental animals and of the radiation source may be found elsewhere[7]. Control animals were injected intravenously with 1·0 ml. sterile pyrogen-free saline. Blood samples were drawn aseptically from the forelegs before and following irradiation, and white blood cell and platelet counts were made within an hour of sampling. Standard haematological methods were used.

The intravenously administered TAB produced vomiting, diarrhoea and fever within 2 h, and killed 6 per cent of the animals within 48 h. By 3 h, the average rectal temperature reached a maximum of 104·5° F, and thereafter decreased toward the pre-injection temperature of 101·2° F. By 12 h normal body temperatures were reached, and most signs of acute toxicity had passed. Transitory nausea and vomiting were also produced by exposure to 360 rads.

The 30-day survival data for irradiated TAB- or saline-injected dogs are summarized in Table 1. The injection of TAB 24 h before exposure to 360 rads reduced mortality to 13 per cent as compared with 60 per cent among saline-injected controls. In animals given TAB 1 h after irradiation the mortality was 50 per cent, which is not a statistically significant reduction of mortality. Therefore, if any protective effect at all is produced by post-irradiation injection, it is much less than the protection afforded by TAB injection 24 h before irradiation.

The peripheral leucocyte counts for groups of treated and control dogs are shown in Table 2 and Fig. 1. In the

Table 1. Influence of TAB on 30-day Mortality in Dogs exposed to 360 Rads

Treatment	Mortality	Percentage
TAB 24 h before 360 rads	5/38*	13
TAB 1 h after 360 rads	11/22	50
Sodium chloride†	29/48	60

* Dead/total
† Ten injected after and the remainder injected before irradiation.

Table 2 POST-IRRADIATION GRANULOCYTE AND PLATELET COUNTS (PER mm³) IN DOGS GIVEN TAB OR SODIUM CHLORIDE

Days after irradiation	Granulocytes ×10³				Platelets ×10³	
	TAB pre 360 rads*		TAB post 360 rads		TAB pre 195 rads	
	TAB†	NaCl†	TAB‡	NaCl‡	TAB§	NaCl§
7	—	—	5·9 (4·0–7·8)	2·8 (2·1–3·4)	—	—
8	5·4 (3·5–7·3)‖	2·2 (1·5–2·9)	—	—	—	—
9	—	—	5·4 (3·4–7·5)	2·8 (1·9–3·7)	217 (142–292)	127 (81–173)
10	7·2 (4·5–9·9)	2·0 (1·5–2·6)	—	—	—	—
11	—	—	5·0 (2·9–7·1)	3·0 (1·5–4·5)	148 (127–169)	53 (30–76)
12	7·3 (4·1–10·5)	1·0 (0–2·0)	—	—	—	—
13	3·9 (2·1–5·3)	0·7 (0–1·4)	3·1 (0·8–5·5)	1·5 (0·5–2·4)	93 (0–218)	17 (0–47)
14	—	—	—	—	107 (36–176)	15 (2–28)

* 'Pre' indicates injection 24 h before and 'post' indicates injection 1 h after exposure
† 7/Group.
‡ 8 TAB and 10 sodium chloride.
§ Initially 6/group; 3/group on day 14.
‖ 95 per cent confidence intervals are in parentheses.

group given TAB 24 h before 360 rads there was an initial granulocytosis and the granulocyte count was still elevated 1 day after irradiation. At 3 and 5 days' post-irradiation, the counts in the two groups were similar, but between 8 and 13 days there was a significant ($P < 0.05$) elevation of the granulocyte count in the TAB-treated group (Table 2). In dogs given TAB 1 h after 360 rads no rise in granulocyte count was detected during the second post-irradiation week; however, the counts in treated animals were higher than in the controls, and on day 7 the difference was significant. Platelets have been enumerated for 14 days following exposure to 195 rads, a sub-lethal exposure. In animals given TAB 24 h before exposure, the decrease in numbers of platelets occurred somewhat more slowly than in the controls, and at 11 and 14 days the platelet counts in the TAB-treated animals were significantly higher ($P < 0.05$) than in the controls.

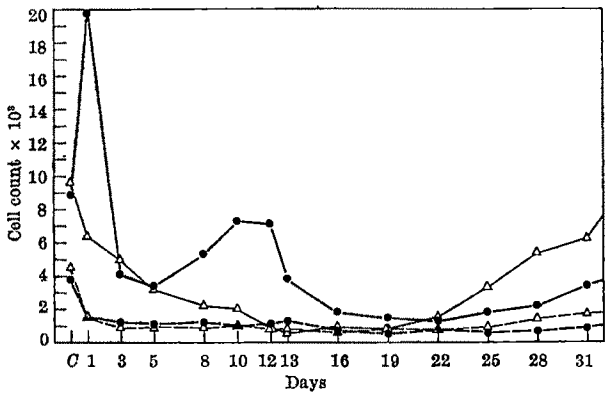


FIG. 1. Granulocyte (—— and ——) and agranulocyte (– – – and – – –) counts as a function of time after irradiation. Groups of seven dogs were given TAB or sodium chloride 24 h before 360 rads. The *C* on the *X* axis indicates the mean of three pre-irradiation cell counts ●, TAB 0/7, △, sodium chloride 4/7

The present data show that TAB significantly increases survival in dogs when given 24 h before mid-lethal exposure, whereas a post-irradiation injection at 1 h has little or no effect on survival. Thus, both in monkeys receiving supra-lethal exposures[5] and dogs receiving mid-lethal exposures, post-irradiation injection of TAB would not seem to increase 30-day survival. This may partially relate to combined toxicity responses to TAB and irradiation, because in animals treated after irradiation the toxic manifestations produced by TAB are superimposed on the nausea and vomiting which occur for several hours following exposure to 360 rads. No attempt has been made so far to mitigate the toxicity or to evaluate the protective effects of lower doses of TAB. Based on the reduction of mortality shown here and the radiation dose-response curve for our mongrel dogs exposed to 1 MVp. X-rays[7] a pre-irradiation injection of TAB probably increases the LD_{50} by a factor of 1·3–1·4. This is not so great a protective factor as is estimated for a combination of AET and PAPP in dogs[8], but TAB is one of the few substances which protects animals larger than laboratory rodents.

The mechanism by which TAB protects, and the mechanism by which endotoxin effects such marked chan[illegible] in the number of circulating granulocytes and platelets during the second post-irradiation week are not known. TAB given before exposure may produce population shifts in bone marrow compartments which result in an increased surviving fraction of precursor cells. Some evidence for an increased surviving fraction of colony-forming units (C.F.U.) has been obtained in mice given endotoxin 24 h before irradiation[11]. Endotoxin produced a twenty-fold increase in the number of C.F.U. detected in the spleen 8 days after irradiation. However, this increase does not necessarily indicate an increased surviving fraction alone, for the increased numbers may be influenced by the rate of migration of C.F.U. to the spleen[12]. Investigations of the extent to which the numbers of C.F.U. may be related to endotoxin protection are in progress. In dogs treated with TAB after irradiation, the diminished rate of fall in granulocytes during the second post-irradiation week may indicate an increased production and/or release of granulocytes into the circulation. Therefore, TAB given either before or after irradiation may somehow influence production, differentiation, and/or release of granulocytes.

The protective effect of TAB may be attributed to 'haematopoietic recovery' and the transitory rise in granulocytes and increased numbers of platelets. Since the mean survival time of saline-treated decedents is ~19 days, increased numbers of granulocytes and platelets during the second week might be propitious in combating infection and haemorrhage during a critical post-irradiation phase. However, the present results show that post-irradiation administration of TAB influences granulocyte-levels during the second week, although this treatment did not significantly reduce mortality. The question therefore arises as to whether the difference in granulocyte numbers resulting from pre- and post-irradiation injection is sufficient to account for the presence and absence, respectively, of protective effects of TAB. On the other hand, based on the granulocyte-levels in animals treated after irradiation, some beneficial effect of TAB on survival might be predicted; perhaps the early toxicity resulting from the combination of radiation and TAB may have some detrimental effect.

It is noteworthy that the effects of TAB on post-irradiation granulocyte-levels, that is, the rise or at least the slower rate of fall during the second week, coincides temporarily with the "abortive recovery or rise" in granulocytes which has been reported to occur spo[illegible]taneously in several species including man[13,14]. Thus, the TAB given either before or after irradiation may somehow influence the abortive recovery which has been attributed to haematopoietic precursor cells that complete only a

few divisions after irradiation[13,14]. Investigations of the interactions between TAB and the haematopoietic system are continuing.

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Influence of Aminoethylisothiouronium Bromide·hydrobromide and Hypoxia on Recovery from Radiation Injury in Mice

AMINOETHYLISOTHIOURONIUM bromide·hydrobromide (AET) and hypoxia appear to act as radiation dose reducers; that is, to obtain the same biological response to radiation, higher doses are required in the presence of these agents than in their absence. The ratio of the doses required to elicit the same response in the presence and in the absence of the protectant (protection ratio) is about the same for radiation-induced mortality, changes in the various elements of the haematopoietic system, and changes in spleen, thymus, and body-weights[1,2]. The constancy of the protection ratio and recent cell culture studies[3] imply that radiation injury produced under the two conditions is equivalent. Recent investigations in this laboratory, however, have suggested that the radiation injury may not be completely equivalent. In sheep, pentobarbital anaesthesia and assumed attendant hypoxia resulted in a mortality protection ratio of 1·4. However, recovery at 30 days as measured by the split-dose technique was significantly less in the anaesthetized group than in animals irradiated without anaesthesia[4,5]. The present experiments with AET and hypoxia were conducted to determine the influence of protectants on initial injury and recovery from this injury. The term 'recovery' is used here to indicate the gradual return to normal radiosensitivity following a sub-lethal dose.

The radiation source was a Westinghouse 250 kVp. machine operated at 15 m.amp; the filtration consisted of 0·5 mm copper and 1·0 mm aluminium; the half-value layer was 1·4 mm copper. *CF* No. 1 female mice, 90–120 days old, were placed on a rotating turntable in 'Lusteroid' tubes and exposed at ~30 rads/min at a distance of 92 cm from the focus. LAF_1 male mice, 5 weeks old, were placed in a specially constructed 11 × 17 × 28 cm gassing chamber divided into 6 × 6 cm compartments and exposed at 100 rads/min without rotation; the distance from the focus was 51 cm. Exposures were converted to rad doses using established factors of 1·2 and 1·5 for the turntable and gassing chamber configurations, respectively. After irradiation, all animals were maintained ten in a cage on pine shavings and permitted standard mouse chow and tap water *ad libitum*. The tap water in the drinking bottles was acidified to *p*H 2·6 with hydrochloric acid to retard microbial growth. The number of animals dying was checked daily after the irradiation treatment and the $LD_{50/30}$'s calculated.

The experimental method was that of the split-dose technique wherein the animals are first given a conditioning radiation dose and afterwards divided into groups and exposed to graded challenge doses for determination of the $LD_{50/30}$ at different times after the conditioning dose. The extent to which the conditioning dose and the $LD_{50/30}$ exceeds the single dose LD_{50} indicates the amount of recovery (in rads) from the conditioning dose. The difference between the conditioning dose and the recovery (in rads) is the remaining injury and is expressed as a percentage of the conditioning dose in this report.

In experiments with AET using *CF* No. 1 mice, the agent, buffered to *p*H 7·2, was administered intraperitoneally at a concentration of 275 mg/kg 15 min before the first exposure. Challenge doses were later given without AET.

In the hypoxia studies with LAF_1 mice, both the conditioning and challenging doses were given under hypoxic conditions. Hypoxia, at the time of irradiation, was produced by placing the animals in a gassing chamber in an atmosphere of 95·8 per cent nitrogen and 4·2 per cent oxygen. The oxygen-level was controlled by mixing air and nitrogen using flow meters and measuring the effluent air from the chamber with a calibrated Beckman *C*-2 oxygen analyser. For exposure under non-hypoxic conditions, LAF_1 mice were placed in the gassing chamber and exposed to the conditioning and challenging doses in an atmosphere of air.

The radiation protection ratio for 275 mg/kg of AET was 1·65 based on the $LD_{50/30}$ of 1,270 rads (95 per cent C. I. of 1,229–1,308) for protected CF No. 1 mice and 775 rads (95 per cent C. I. of 758–791) for non-protected mice. The protection ratio for the 4·2 per cent oxygen atmosphere was 2·03 based on the $LD_{50/30}$ of 1,710 rads (95 per cent C. I. of 1,692–1,745) for protected animals and 846 rads (95 per cent C. I. of 831–863) for non-protected animals. About 2 per cent of the mice died from acute AET toxicity and 5 per cent of the mice died during irradiation in the 4·2 per cent oxygen atmosphere. These animals were not included in the LD_{50} determinations. The slopes of the radiation dose-response curves for protected animals do not differ significantly from the slopes for non-protected animals.

The influence of AET on the pattern of recovery is shown in Fig. 1. The curves are for animals conditioned with 854 rads in the presence of AET or with 514 rads alone. These exposures represent approximately 2/3 of the LD_{50} for treated and control mice. Based on the protection ratio for AET, the initial injury in the AET-protected group should be approximately equivalent to that in the non-protected group, namely, 514 rads. Therefore, this value was used as the basis for the determination of the percentage of remaining injury for both groups. The 2- and 24-h points on the AET curve indicate greater injury, 114 per cent, than is assumed for 0 time. Whether or not these values are significantly different from 100 per cent injury is uncertain. Nevertheless, the curves show that the rate of return toward normal radiosensitivity is markedly different in the two groups.

Recovery was evaluated in animals given both the conditioning and challenging dose either in air or under hypoxic conditions. Mice were conditioned with 1148 rads in a 4·2 per cent oxygen atmosphere or with 563 rads in air (approximately 2/3 of the LD_{50} determined for the respective gas mixtures). Based on the protection ratio, the initial injury in the two groups should be equivalent, and the pattern of recovery should be comparable. The curves presented in Fig. 2 indicate a marked difference between the groups in the pattern of recovery during the first 3 days.

In non-protected mice, recovery from radiation injury, as measured by the split-dose technique, proceeds rapidly during the first few days. In contrast, mice protected with AET or hypoxia exhibit a different pattern of recovery,

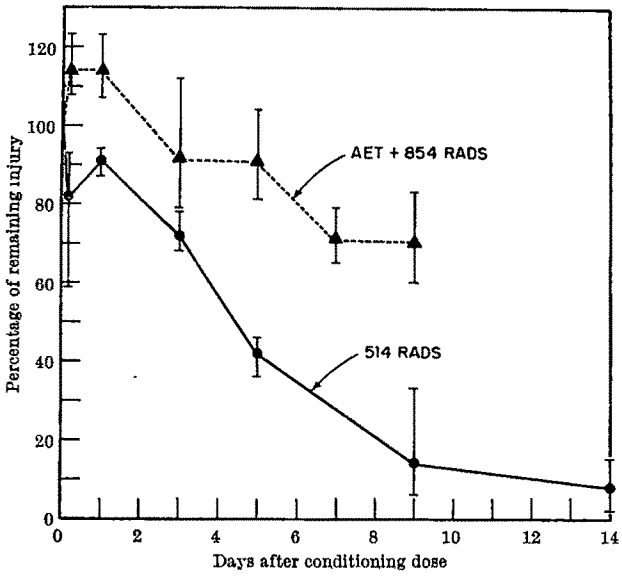


Fig. 1. Influence of AET on recovery in *CF* No. 1 mice

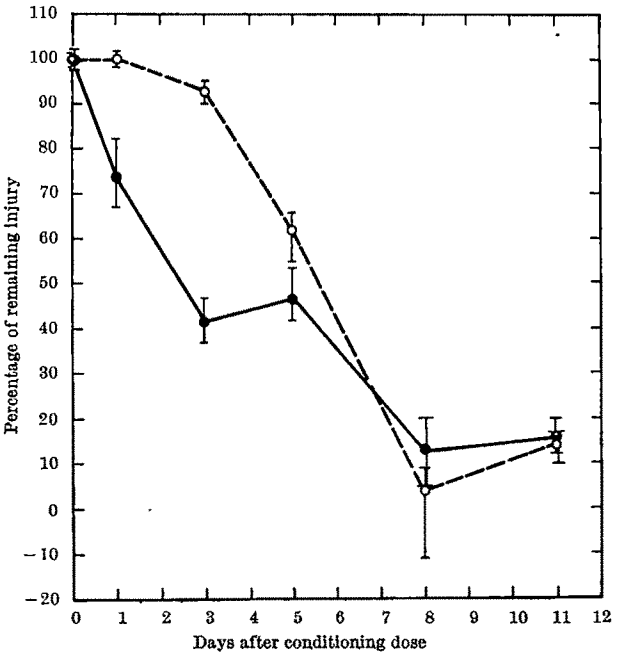


Fig. 2. Influence of hypoxia on recovery in *LAF* mice: ●, in air; ○, in hypoxic conditions

although the initial injury was theoretically equivalent to that in non-protected controls. In protected mice there is an initial delay of at least 24 h before the animals show significant recovery. Beginning at 3 days, recovery proceeds rapidly in the hypoxic group at a rate comparable with the controls, but in the AET group rapid recovery does not begin even by 7 days. Assuming that the pattern of recovery from quantitatively and qualitatively equivalent initial injury should be similar, the present results indicate that the initial injury in the protected and non-protected groups is not equivalent.

The differences in the patterns of recovery might be attributed to: (*a*) differential protection in various physiological systems[6]; (*b*) comparable injury but inherent differences in tissue repair potentials[7]; (*c*) qualitative differences in the injury sustained under different exposure conditions[8–11]; (*d*) an inhibitory effect of the protective procedures on recovery. We are at present attempting to determine which of these effects is responsible for the differences.

The influence of hypoxia on recovery, as shown both in cell cultures[9,11] and now in mice, is relevant to radiotherapy providing these findings are applicable to megavoltage radiations and to tumours. If the damage sustained in tumours under anoxic conditions is such that recovery is impaired, fractionated irradiation would result in greater additivity of injury in anoxic cells than in oxygenated cells. In effect, fractionation would reduce the anoxic protection ratio. This effect is shown by the present data when two fraction protection ratios are calculated by adding the conditioning dose and the LD_{50}'s. When fractions were separated by 1, 3, or 5 days, the protection ratios were 1·74, 1·54, and 1·88, respectively; the single dose protection ratio is 2·03.

In summary, the present data indicate that mice protected by AET or hypoxia show a greater sensitivity to a second exposure than would be expected on the basis of the protection ratio determined from acute lethality studies. Recovery is impaired when radiation injury is sustained in the presence of AET or under hypoxic conditions.

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Direct and Indirect Effects of Radiation: their Relation to Growth

Holsten, Sugii and Steward[1] have recently added the colony-forming ability of single, isolated carrot cells to the spectrum of biological systems in which heavy X- or γ-irradiation of the carbohydrate component of the growth medium results in the production of a cytotoxic, growth-inhibiting substance in that medium. Similar demonstrations of this effect were reported by Molin and Ehrenberg[2] for a bacterial species, and by Berry, Hills and Trillwood[3] for two strains of mammalian cells grown *in vitro*. From their own results, however, Steward *et al.* have implied a potential biological hazard to man, by the supposition that radiation effects can be transmitted indirectly to cells (perhaps at times long after the actual irradiation) by stable, toxic radiolysis products derived from irradiated sugars. This supposition seems untenable. Even in their own very radio-resistant biological system, Steward *et al.* found quantitative differences between the growth inhibition produced by direct irradiation of the biological material, and that produced by the cytotoxic radiolysis products in irradiated growth medium. In mammalian cells, this quantitative difference is dramatic; radiation doses of the order of thousands of rads delivered to the carbohydrate-containing part of the growth medium were necessary to reduce cell growth even marginally[3,4], while

direct X- or γ-irradiation of mammalian cells *in vitro* with doses of the order of only tens of rads is sufficient not only to reduce the rate of cell growth, but also to produce cell reproductive death (see, for example, Puck *et al.*[5]). There is, as yet, no evidence that the cytotoxic effects of irradiated carbohydrates, and the toxic effects of ionizing radiation are in any way similarly produced at the cellular level.

Secondly, in drawing "obvious implications for the radiation sterilization of food", Steward *et al.* overlook the difference between their *in vitro* situation, in which the biological material is grown in a heavily-irradiated medium, and the *in vivo* situation for the whole animal (or human being) where ingestion of any potentially toxic materials by no means assures their absorption from the gastro-intestinal tract, or their transportation in a toxic form to any vulnerable sites within the body[6]. The only relevant test for *in vivo* toxicity is the birth-to-death study of health, longevity and reproductive function in large numbers of animals fed throughout their lives on irradiated foodstuffs (see, for example, Radomski *et al.*[7]). The necessity for such tests has already been clearly recognized in many countries[8], including the United Kingdom[9].

While some of these comments may seem obvious, we feel that they are necessary in the light of the wide publicity which the summary conclusions from the work of Steward *et al.* have received in the popular Press in Britain, and in the United States.

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Breakdown of Ribonucleic Acid in *Escherichia coli B* after X-irradiation

The influence of ionizing radiation on the growth and metabolism of bacteria has been the subject of many reports. One of the most significant effects of X-irradiation is the degradation of a part of deoxyribonucleic acid (DNA) in bacterial cultures[1,2]. It was therefore interesting to examine whether the radiation damage of DNA is accompanied by an alteration of the synthesis of ribonucleic acid (RNA) in bacteria exposed to irradiation. Experiments of this kind by Frampton[3] have shown a depression of RNA synthesis in *Escherichia coli B/r* after exposure to X-irradiation. His analyses of cell-free extracts by ultracentrifugation in sucrose density gradients indicated a decline in the synthesis of 50 *S* ribosomes. In this paper we are going to describe experiments showing a breakdown of a part of RNA synthesized in a growing population of bacteria before or shortly after the irradiation.

We used *Escherichia coli B* in all our experiments. In a typical experiment the bacteria were grown in a mineral medium containing glucose at 37° C. Adenine-8-^{14}C (specific activity 53·5 mc./mmole) was added to an exponentially growing culture of about 3×10^8 cells per ml. to yield a final concentration of 4×10^{-7} M. The bacteria were able to incorporate more than 90 per cent of radioactivity in 15 min. Forty min after adding the isotope, the culture was cooled to +4° C; one half of it was irradiated with 16,000 r., and the other half was kept as the control. Such a dose of X-irradiation has always given a survival of about 1 per cent, and a marked degradation of DNA during subsequent incubation[2].

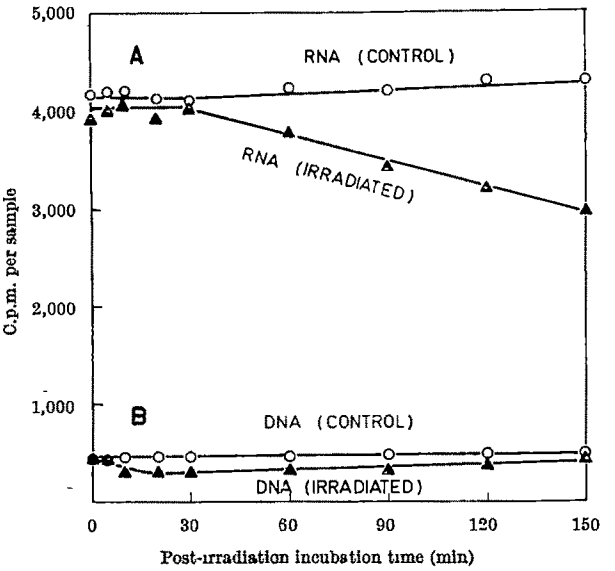


Fig. 1. Breakdown of ribonucleic acid (*A*) and deoxyribonucleic acid (*B*) in *Escherichia coli B* exposed to 16,000 r. of X-irradiation. The cells were labelled with adenine-8-^{14}C 40 min before irradiation

After the irradiation, both cultures were incubated at 37° C; 0·5 ml. samples were taken at various times and precipitated with 5 per cent trichloroacetic acid. The precipitates were collected on 'Millipore' filters, washed with 5 per cent trichloroacetic acid, and the radioactivity in both DNA and RNA measured in a window-less gas-flow counter. The activity of DNA was measured in parallel 0·5 ml. samples after hydrolysis of RNA with 0·3 N potassium hydroxide[4]. Radioactivity of RNA was computed from the difference of radioactivities of total nucleic acids and DNA. The results of such an experiment are presented in Fig. 1. It can be seen that a part of the RNA in an irradiated culture begins to break down after 30 min of incubation. The loss of radioactivity is linear during the following 120 min. At the end of this period the breakdown affects about 25 per cent of the radioactivity originally present in RNA.

It should be emphasized that this breakdown of a part of the RNA is accompanied by a significant, although somewhat depressed, net synthesis of RNA. This increase in the RNA content, demonstrated in similar experiments using the orcinol method[5], has always run parallel with the increase in turbidity of irradiated cultures.

Fig. 1 also shows that the loss of radioactivity from DNA during the first minutes after irradiation is 25 per cent, which is in good agreement with results on the degradation of DNA (ref. 2).

The breakdown of RNA depends on the dose of irradiation, as shown in Fig. 2. At doses up to 16,000 r., the breakdown is a linear function of the dose; at higher doses, a saturation can be observed. It is characteristic that, at all doses, the breakdown begins 30 min after irradiation and is linear during the next 120 min.

Since the breakdown of RNA described in the preceding paragraphs refers to the RNA synthesized before irradiation, it was of interest to find out whether the RNA

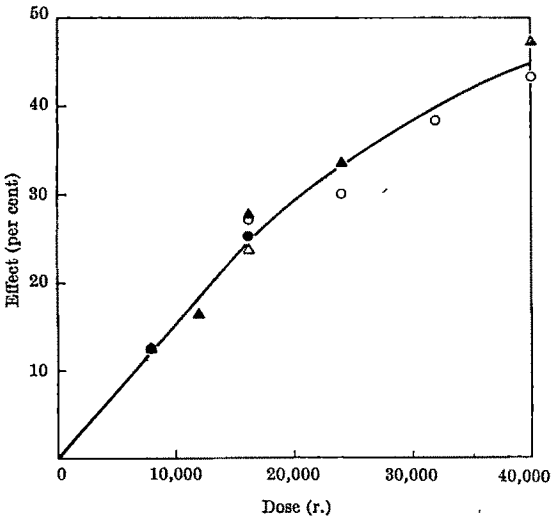


Fig. 2. Dose-effect curve for the breakdown of ribonucleic acid in *Escherichia coli B* exposed to X-rays. RNA broken down after 150 min of post-irradiation incubation is plotted against the dose of X-irradiation. RNA was labelled by adding adenine-8-^{14}C 40 min before irradiation. Various symbols are used for the values obtained in four independent experiments

synthesized after irradiation is similarly affected. In a series of experiments similar to that already described, adenine-8-^{14}C was added to the cultures at various times after irradiation with 16,000 r. The results show that the breakdown of RNA labelled immediately after irradiation begins at the same time, and at the same rate as in the experiment with the RNA labelled 40 min before irradiation. RNA labelled 40 min after irradiation showed a considerably lower rate of breakdown. We did not find any loss of radioactivity from RNA labelled 60 min after irradiation.

The radioactive products of RNA breakdown are found in the incubation medium as fragments of low molecular weight. This agrees to a great extent with the results of Billen[6], who found a release of the cellular constituents from irradiated *Escherichia coli B/r* incubated in phosphate buffer and glucose at 37° C. These were nucleic acid fragments, varying in size from free bases to oligo nucleotides. In our experiments we have shown that the material released originates from the labelled RNA. The cells were not able to re-incorporate these fragments into their nucleic acids. At the same time the irradiated cells retained their ability to incorporate added adenine-8-^{14}C. One hour after irradiation the rate of incorporation was only a little reduced compared with the control.

These experiments were performed with the aim of demonstrating a possible connexion between the degradation of DNA caused by X-irradiation, and presumable similar effects on RNA. The fact that the RNA synthesized before irradiation can be broken down, however, excludes a transmission (or copying) of the radiation-induced damage from DNA to RNA. On the other hand, RNA subject to post-irradiation breakdown does not necessarily have to be synthesized before irradiation, since RNA labelled shortly after irradiation shows the same effect.

To clarify the nature of the RNA broken down, we performed preliminary analyses of cell-free extracts on sucrose density gradients. Under our conditions 40 min after the addition of adenine-8-^{14}C 54 per cent of radioactivity was found in 50*S* ribosomes, 30 per cent in 30*S* ribosomes, and 16 per cent in 4*S* RNA. Taking into account that the loss of radioactivity from RNA is 25 per cent after the dose of 16,000 r., and about 50 per cent at 40,000 r., it can be concluded that the ribosomal RNA must represent a considerable fraction of the RNA which is degraded. More detailed experiments are still in progress.

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BIOLOGY

Visual Orientation and Feeding in Seals and Sea Lions

At least some, and perhaps most, pinnipeds of the families Phocidae and Otariidae are nocturnal feeders[1]. The eyes of these predators, being sensitive to very low light intensities[2], are suited for use at night and in deep water. During the day the visual acuity of the California sea lion (*Zalophus californianus*) allows this animal to discriminate a size difference ratio as small as 1·06 : 1 (ref. 3). A great deal of vocalizing occurs among pinnipeds, both above and below the surface of the water[1,4–6]. This fact, along with experimental work such as that of Mohl with the harbour seal (*Phoca vitulina*)[7], indicates that hearing is generally well developed. Poulter suggested that the California sea lion, and probably other species, is able to capture prey in darkness by utilizing an echo-ranging sense similar to that known to occur among cetaceans[4]. However, subsequent work[5,6] has failed to support Poulter's theory. Nevertheless, as "individuals totally blind but in a good state of health have been reported"[1], the possibility that such a sense may be developed when needed should not be dismissed lightly.

While observing behaviour in fishes during the past ten years, I have spent many thousands of hours underwater at all hours of both day and night. This experience has provided me with some insight into factors likely to influence the activity of mammals underwater which may have been overlooked by more terrestrially oriented students of mammalian behaviour.

Poulter was impressed by the ability of the sea lion to locate bait which he presented after it had become "so dark that the pieces of fish, and much of the time the animals, were not visible to the human eye". On picking up vocalizations by the sea lions in "a series of short pulses which are similar in many ways to the sonar pings of the porpoise", Poulter concluded, "Since the animals will pick up pieces of fish thrown into the tank just as quickly under the darkest conditions mentioned above as they do in daylight, I believe these sounds to be echo ranging signals of a complex and sophisticated nature".

The ability of the sea lion to locate bait under the conditions of darkness described by Poulter seems of questionable significance in an argument for an echo ranging ability in this animal. As sea lions possess an extensive tapetum lucidum[2], they presumably have a visual sense far more sensitive to low light intensities than that of humans. But quite apart from this fact, while it was too dark for a human looking down into the tank to distinguish the bait, and often the animal, the sea lion was below the surface of the water looking up at the bait from below. When located beneath the surface as light diminishes at dusk, I have found that I can readily distinguish an object in the water above me, silhouetted against the relatively light surface, long after an observer above the water is unable to see anything but darkness below. While diving in clear water at night I have been

able to distinguish objects above me, using the surface as a contrasting background, when the only source of illumination is starlight. This ability must be considerably more striking in a creature possessing the visual capacities of the seal or sea lion.

An observation made by Bonnot[8] suggests that the California sea lion may indeed take advantage of the relatively light surface in locating prey at night. Bonnot observed a sea lion feeding at night on flying fishes which were milling about under a night light next to his vessel. The sea lion consistently approached the area, swimming on its back, at a depth of approximately 30 ft. On reaching a point directly below the milling fish, the sea lion shot straight up and almost invariably succeeded in capturing a fish. Bonnot recorded the capture of at least six flying fish, with each attack following the pattern described here. Although the presence of the night light here created an atypical nocturnal situation, the unvarying manoeuvre performed by the sea lion in securing its prey suggests this to be a regular hunting pattern.

Captive sea lions and harbour seals at the U.S. Naval Missile Center, Point Mugu, California, also approach targets and baits by swimming in on their backs from below. Even when targets were lowered to a depth of 40 ft., a trained sea lion made its approach only after first descending to a depth greater than that of the target, and then shooting up after swimming in under the target from below. Limited observations at a depth of 240 ft. indicated that the same approach to a target is made at that depth[9].

Such a manoeuvre is advantageous for a predator, not only because its prey is thus viewed against the lighter water above, but also because its attack, being launched from the darker water below, is less readily seen by the prey.

Some predatory fishes use a similar tactic. In the Gulf of California, feeding intensity in the leopard grouper (*Mycteroperca rosacea*) reaches a peak just prior to dawn and shortly after sunset when the groupers concentrate under schools of flatiron herring (*Harengula thrissina*). In spite of the fact that the sky is still quite light at this time, relatively little light penetrates into the water, and from the herring's position near the surface the groupers below are very difficult to see. However, from the grouper's position below, the herring stand out sharply against the light surface. Feeding by the grouper under these conditions is consummated with a sudden rush up into the herring school[10].

It is possible that the prey of these animals are particularly vulnerable to attack from below for reasons other than those given here. For example, an approach from this direction may take advantage of the largest 'blind spot' in the visual field of the prey.

Feeding at night in phocids and otariids may also be aided by bioluminescence. The diet of the northern fur seal *Callorhinus ursinus* during its migrations on the high seas is composed largely of lantern fish and squid[11]. The lantern fish is well known for its luminescent characteristics. During the day the lantern fish and squid live at considerable depths, but rise close to the surface at night where they are preyed on by the seals.

In many areas of the sea at night, a moving object is readily observed due to the luminescence of many minute planktonic organisms, mostly protozoans, which light up when disturbed. These organisms are often so numerous that while making observations underwater I have been able to identify, to species, fishes which swam actively among them. This was not because the fish itself was illuminated, but rather because there were so many minute luminescent organisms about the fish that its form was essentially traced out in tiny flecks of light. Under such conditions, a feeding seal or sea lion might follow the luminescent trail of its fleeing prey in making the capture. Of course, a predator operating under such conditions would experience difficulty catching its prey unawares because its predatory manoeuvres would also be advertised by the luminescent organisms.

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Differential Reaction of *Lolium perenne* Genotypes to Certain Herbicides

THE rapid progress in weed destruction made possible simply by introducing new chemicals has resulted in a concentration on obvious and gross effects. Examples of the selective toxicity of various chemicals between different plant genera and between different species of the same genus[1] have been widely reported. Much less frequently cited are differential responses between varieties of one species. In potatoes, variation in varietal reaction to pre-planting application of trichloroacetic acid (TCA) has been noted[2] and large differences in varietal susceptibility to 2 : 4-dichlorophenoxyacetic acid (2,4D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA)[3] have been found. Hayes[4] has demonstrated the differential response of barley to DDT, susceptible varieties being killed by DDT application. Commercial varieties of sugar-cane differ in physiological tolerance to dalapon[5]. Fiddian[6] gave evidence of differential susceptibility of cereal varieties to some commonly used herbicides, variation occurring in amount of leaf scorch, growth deformity, ripening retardation, ear malformation and spikelet sterility.

In grasses, differential tolerances to various chemicals between species have been recorded. For example, King and Davies[7] have shown that of the common grasses differing in their response to dalapon, *Lolium perenne* and *Festuca* spp. are relatively resistant, while *Nardus stricta* and *Molinia caerulea* are susceptible even to quite low dose rates. The time of season at which the dose is applied and the age of the plants when treated affect species reaction. Within varieties, individual cocksfoot seedlings apparently resistant to dalapon were found by Cooper[8].

Sensitivity of *Lolium perenne* genotypes to various herbicides has been investigated at the Plant Breeding Station, Loughgall, North Ireland, with the view of initiating heritability studies. (The help of Dr. D. W. Robinson, formerly of the Horticultural Centre, Loughgall, is gratefully acknowledged.)

Four genotypes—512, 3002, 3136 and *S*2412—and six herbicides—MCPA, MCPB, mecoprop, dalapon, simazine and diquat—were included in the experiment. In a split-plot randomized-block lay-out of three replications herbicide application constituted the major plots and genotypes the sub-plots.

The genotypes were selected as having an identical date of ear emergence to ensure that, so far as possible, all would be at an equivalent physiological stage of growth

at the time of treatment. The clones were tillered on the same day and plantlets, each made up of ten tillers, were planted in the field in March 1961. The plantlets were placed 1·5 ft. apart in rows 1·5 ft. apart. In each sub-plot a genotype was represented by a single row of seventeen plantlets. The entire trial was surrounded by a border row. Compound fertilizer (N, 15 : P, 10 : K, 10) was applied to all plots at a rate of 3 cwt. per acre. A logarithmic sprayer was used for the application of herbicides. The 4·5-ft. spray boom had one nozzle directly above each genotype row and was carried at 1·5 ft. above ground-level. In each sub-plot the first two plants were shielded from application to act as controls and the sprayer allowed steadily to empty over the next twelve plants. The fifteenth plant was used as a buffer to the last two, which were left as unshielded controls. The peak dosages in lb. per acre of active material were as follows: MCPA, MCPB and mecoprop 8, dalapon 20, simazine 6, diquat 4. Ten days after all plants had been cut back uniformly to remove heads spraying was carried out on July 8, 1961, and 3 weeks later plants were cut with hedge shears 3 in. from ground-level and the mean green weight of each adjacent pair of plants recorded.

The mean yield of the four genotypes, which can be considered to represent the general reaction of *Lolium perenne*, is given in Table 1.

Table 1. MEAN YIELDS OF FOUR *Lolium perenne* GENOTYPES SUBJECTED TO VARIOUS CONCENTRATIONS OF SIX HERBICIDES

Herbicide	Mean green weight of adjacent plants (gm) Percentage of peak dosage applied 73·8	47·5	30·0	18·8	12·5	7·5	Unshielded control	Shielded control
MCPA	113·2	126·7	122·1	142·1	123·3	125·8	123·9	128·3
Mecoprop	128·7	130·0	137·7	144·6	143·3	141·6	141·2	144·2
MCPB	142·1	148·7	152·1	144·2	137·1	137·9	136·6	139·6
Dalapon	12·1	28·3	45·4	72·5	106·6	127·5	134·2	140·4
Simazine	23·3	24·2	33·7	43·8	50·8	61·6	132·8	136·7
Diquat	18·3	17·9	34·1	39·6	46·2	60·4	132·5	136·2

MCPA, MCPB and mecoprop (synthetic growth regulators reported not to harm cereals and other graminaceous crops) had no significant effect on yield. Dalapon, a translocated herbicide toxic to grasses, gave a gradual reduction in yield with increased dosage. Diquat and simazine, characterized by their high toxicity to a large range of plants, reduced yield by more than 50 per cent at even the lowest dosage, further reduction proceeding gradually with increase in dose rate.

No genotypic differences were evident when the yields of the four genotypes in the MCPA, MCPB and mecoprop treatments were examined individually, but very obvious differential effects occurred in the dalapon, simazine and diquat treatments. The magnitude of these effects was so great that no formal statistical analysis was considered to be necessary.

The concentration at which death-point was reached varied with genotype. Simazine (Fig. 1) while causing the death of genotype 3002 at 18·8 per cent peak dose (PD), 3136 at 30 per cent PD and 512 at 73·8 per cent PD affected *S*2412 relatively little, almost half of its normal yield still being found at 73·8 per cent PD. The slope of the curves is indicative of further differential reaction, three of the genotypes rapidly succumbing to even the lowest concentration of simazine while *S*2412 was almost unaffected at these levels of application.

Dalapon killed 3136 at 30 per cent PD, 3002 and 512 at 73·8 per cent PD, while *S*2412, little affected by even 18·8 per cent PD, was still alive with about 25 per cent of normal yield at 73·8 per cent PD. A different reaction was obtained with diquat which, while reducing yield of *S*2412 at 73·8 per cent PD to a level similar to that produced by dalapon at 73·8 per cent PD, did not kill any of the genotypes completely. The relative sensitivity of 3002 to even 7·5 per cent PD of diquat compared with that of the other genotypes revealed further differential reaction.

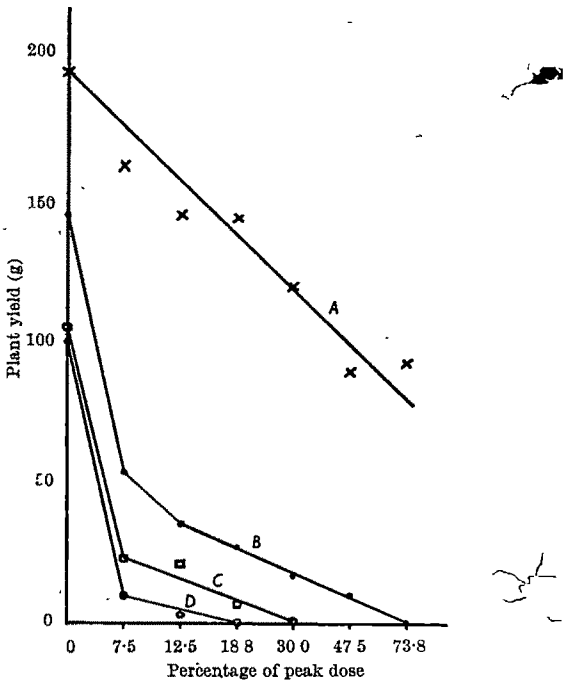


Fig. 1. Effect of various concentrations of simazine on the herbage yield of four *Lolium perenne* genotypes. *A*, *S*2412; *B*, 512; *C*, 3136; *D*, 3002

The agronomic value of grass cultivars based entirely on genotypes resistant to recognized 'grass-killers' could be far reaching. For example, pastures sown with such cultivars could be effectively renovated without ploughing, and contaminant rogues and weeds could be removed by spraying from crops destined to produce certified seed. Should resistant genotypes be found in the species normally sown in lawns the possibility of a simple management for the perfect lawn can be envisaged.

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Stimulation of Flowering in Certain Short-day Plants by Abscisin

ALTHOUGH day-length is known to control flowering in some plant species, and a variety of vegetative responses, including dormancy, runnering and tuberization, in other species the relationship between these different types of photoperiodic effect is obscure. Evidence has been presented that the induction of dormancy in woody plants in response to short days involves the newly discovered growth-regulator, abscisin II ('dormin')[1], and it has been possible to induce the formation of resting buds by application of endogenous inhibitor in birch[2]. Evans has recently reported the inhibition of flowering in the long-day plant, *Lolium temulentum*, by abscisin[3]. Thus it is pertinent to investigate whether abscisin II will stimulate flowering in short-day species. The availability of synthetic abscisin[4] has made it possible to carry out a range of experiments on this problem.

The following experiments have been carried out with the short-day species, *Pharbitis nil*. Scarified seeds were sown in pans of vermiculite and maintained under con-

tinuous illumination from fluorescent lamps at 20° C. When the plants had formed 1–2 leaves and were 8–10 cm high they were removed and their roots washed free of vermiculite. Each seedling was then placed with its roots in a test-tube containing either a solution of synthetic abscisin (a racemic mixture of two enantiomorphs) at a concentration 4 p.p.m. or water alone, and the seedlings were allowed to remain in the tubes under continuous illumination for 7 days. The seedlings were then planted in soil in pots and transferred to a heated greenhouse under 18 h photoperiods. Macroscopic flower buds were visible after 4–5 weeks. The results of three experiments of this type are shown in Table 1.

Table 1. EFFECT OF ABSCISIN ON FLOWERING IN *Pharbitis nil*

	Treated with abscisin		Water controls	
	Vegetative	Flowering	Vegetative	Flowering
Experiment 1	5	7	12	0
Experiment 2	7	18	11	4
Experiment 3	2	10	25	0
Total	14	35	48	4

It is seen that an average of 70 per cent of the seedlings treated with abscisin flowered, whereas only 8 per cent of the untreated control plants did so.

Experiments were also carried out with seedlings of *Chenopodium rubrum*, seed of which was sown on filter paper in Petri dishes. The seedlings were maintained under continuous illumination at 20° C and were supplied with Hoagland's No. 1 nutrient solution (half strength) on alternate days. After 4 weeks, the seedlings were transferred to filter paper in small crystallization dishes, and were supplied with an aqueous solution of abscisin (4 p.p.m.) or with water alone, for 7 days. The seedlings were then continued under the same conditions and supplied with Hoagland's solution on alternate days. Growth was slow under these conditions, but after 6 weeks a high proportion of the seedlings treated with abscisin produced flowers (Table 2). Similar results were obtained in a repeat experiment.

Table 2. EFFECT OF ABSCISIN ON FLOWERING IN *Chenopodium rubrum*

	Treated with abscisin		Water controls	
	Vegetative	Flowering	Vegetative	Flowering
Experiment 1	41	23	49	0
Experiment 2	17	33	26	0

Further experiments have been carried out with larger plants of *Chenopodium rubrum* grown in pots in a greenhouse, and with other short-day species, including soya-bean c.v. 'Biloxi', *Xanthium pennsylvanicum*, and tobacco c.v. 'Maryland Mammoth', in which abscisin was applied at concentrations of 5 p.p.m. using various methods of application, namely: (1) by spraying on the leaves; (2) by feeding through a lip of tissue cut in the stem; (3) by application to the roots of plants grown in sand. No flowering responses were obtained with any of these treatments with these species.

Experiments have also been carried out with plants of blackcurrant, using partially purified endogenous growth-inhibitor extracted from the leaves of other blackcurrant plants grown under short-days. The extraction and purification procedure was similar to that described elsewhere for inhibitors extracted from sycamore and birch leaves[5]. The experimental blackcurrant plants (grown from cuttings) were about 60 cm in height and were grown in pots in a warm greenhouse under 18 h photoperiods. The inhibitor solution (containing the activity from 10 g dry weight of leaves per 1 ml. of water, with 0·01 per cent 'Nonidet *P*42' added as a wetting agent) was sprayed on the apex and leaves in the upper parts of thirty plants. A further thirty plants sprayed with water were used as controls. The treatments were applied for 30 days. The plants were then allowed to remain under long days for a further 60 days, when the axillary buds of each plant were sectioned for examination. It was found that fifteen of the plants treated with inhibitor had initiated clearly recognizable flower primordia, whereas all the control plants remained vegetative.

A further experiment was carried out with blackcurrant, using synthetic abscisin II. Pot grown plants maintained under long days in a heated greenhouse were treated with abscisin at a concentration of 5 p.p.m., applied through a lip in the stem for 15 days and sprayed on the leaves for a further 5 days. Examination of the axillary buds 5 weeks after the cessation of treatment showed that six out of nine plants treated with abscisin had initiated flowers, whereas none of the six control plants had done so.

Thus abscisin II appears to be effective in stimulating flowering in the short-day species *Pharbitis nil*, *Chenopodium rubrum* and blackcurrant, but attempts to induce flowering in other short-day species have so far been unsuccessful.

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ENTOMOLOGY

'Paper Factor' as an Inhibitor of the Embryonic Development of the European Bug, *Pyrrhocoris apterus*

WE have already described a fortuitous combination of events which led to the discovery that American newspapers and other paper products contain a potent analogue of the juvenile hormone of the European bug, *Pyrrhocoris apterus* L. (Heteroptera ; Pyrrhocoridae)[1]. When reared in contact with 'active paper' or when exposed to surfaces impregnated with extracts of the 'paper factor' (PF), fifth-stage *Pyrrhocoris* larvae undergo one or more supernumerary larval moults and finally die without completing metamorphosis or attaining sexual maturity. This same result is observed when 1 μg of partially purified PF is topically applied to young fifth instar larvae[1,2].

The purpose of this communication is to report additional experiments in which PF was tested on *Pyrrhocoris* eggs and adults.

When newly laid eggs were collected from normal, untreated females and placed in contact with active paper, we were astonished to find that few, if any, of them hatched. A typical experiment is summarized in Table 1. It will be observed that, of the 150 eggs exposed to PF, only six completed embryonic development. Experiments of this type have been repeated many times and always with the same result. For example, when 800 freshly deposited eggs were placed on filter paper impregnated with 0·5 mg PF, only four completed embryonic development.

We would emphasize that this inhibition of embryonic development was observed only in experiments performed on *Pyrrhocoris* eggs. For example, PF did not have any

Table 1. EFFECT OF 'PAPER FACTOR' ON THE EMBRYONIC DEVELOPMENT OF THE EGGS OF *Pyrrhocoris apterus*

Exposure to:	No. of eggs	Results: Normal development	Results: Arrested development
Inactive paper (Whatman's No. 1 filter paper)	50	50	0
Active towelling (Scott, Brand 150)	50	6	44
Whatman's filter paper impregnated with 1 mg PF	50	0	50
Microdrop of acetone solution of PF	50	0	50

detectable effect on the eggs of numerous other species of laboratory insects, including two other species of Heteroptera, *Oncopeltus fasciatus* (Lygaeidae) and *Rhodnius prolixus* (Reduviidae). This finding is in accord with conclusions derived from previous experiments performed on larvae—namely, that PF is an effective analogue of the juvenile hormone of *Pyrrhocoris*, but is completely inert for most other species[1,2].

Detailed investigations of *Pyrrhocoris* eggs revealed that, after exposure to PF, embryonic development was initiated and progressed in an apparently normal manner to at least the formation of the germ band. At some later stage, development stopped with the still living embryo short of completion.

The most affected eggs contained a small disk of embryonic tissue afloat on a mass of unincorporated yolk. In somewhat more advanced cases, the embryonic mass showed the rudiments of certain appendages but not of others. Sometimes parts of appendages had formed in the absence of the remainder. Embryos that attained a more advanced status showed rudimentary legs and antennae which were closely appressed to the body surface. The entire, living embryo was, in fact, reminiscent of a miniature lepidopteran pupa. The most advanced embryos were covered with a thin, transparent cuticle overlying epidermal cells in which a red pigment had been synthesized. Hairs and setae were absent and only traces of the compound eyes could be recognized. In some cases, the chorion ruptured at the normal time and the bizarre, non-motile (but still living) creatures were aborted from the eggs.

When PF was topically applied to freshly oviposited eggs, development was blocked at a more uniformly early stage than when eggs were simply placed in random contact with active paper. This implies that the stage at which embryonic development is blocked is related to the uptake of PF.

Freshly laid eggs are maximally sensitive to PF. When eggs were incubated at 25° C for 1 or 2 days and then treated with PF, embryonic development was always less affected. After 4 days of preliminary incubation, the eggs were virtually insensitive to PF, even when high doses were topically applied.

These findings suggested that the eggs might be sensitive to PF while still in the ovary or oviducts. To test this possibility, each of six freshly moulted adult females was treated with a topical application of 200 μg of partially purified PF. The animals were then reared in individual Petri dishes together with adult males. At daily intervals the eggs were collected, counted and incubated at 25° C.

Without exception, the eggs behaved as if they had been directly exposed to PF; that is, development proceeded to a certain stage and then stopped. This implies that, after topical application to adult females, PF is absorbed, translocated to the reproductive tract, and somehow brought into association with the eggs.

We have previously demonstrated the ability of PF to block the metamorphosis or sexual maturation of *Pyrrhocoris* larvae[1,2]. We now see that, for *Pyrrhocoris* eggs either before or immediately after oviposition, PF is an effective and selective ovicide. These findings should encourage a search for juvenile hormone analogues which have similar effects on specific insect pests[3].

The ability of PF to block the metamorphosis of *Pyrrhocoris* is just what would be anticipated on the basis of present knowledge of the mode of action of juvenile hormone[4–6]. As has been pointed out elsewhere[4,5], nearly all the effects of juvenile hormone can be accounted for in terms of its ability to block the derepression, transcription, or utilization of fresh genetic information. Therefore, only in the absence of juvenile hormone can the cells and tissues undertake the new synthetic acts which are prerequisite for metamorphosis but not prerequisite for larval growth. Though the role of hormones in embryonic development is little understood[7–11], this same theory of the action of juvenile hormone can obviously account for the ability of PF to block the metamorphosis of an egg into a *Pyrrhocoris* larva.

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Effect of Removal of the Frontal Ganglion on Corpus Allatum Function in *Locusta migratoria migratorioides* R. & F.

REMOVAL of the frontal ganglion from young adults of *Locusta migratoria* L. prevents normal growth and sexual maturation; the operation is thought to interfere with the activity of the cerebral neurosecretory system[1]. It is possible, however, that the removal of the frontal ganglion affects the activity of the corpora allata which are also essential for maturation in locusts[2,3]. This possibility has been investigated using adults of *Locusta migratoria migratorioides* R. & F.

The frontal ganglion was removed from each of ten newly emerged females, and a further ten newly emerged females were subjected to a sham operation. The insects were reared with males, under conditions described elsewhere[3], for 14 days after which time the survivors were weighed, killed, their ovaries examined, and the volume of their corpora allata measured using Staal's technique (Table 1).

Table 1. FRONTAL GANGLION REMOVAL IN *L. m. migratorioides*.
The mean weights, terminal oocyte lengths, and volumes of the corpora allata of the operated and sham-operated females

	Operated females	Sham-operated females	Comparison of means
Number of locusts	7	8	
Mean weight (g) ± *S.E.*	1·476 ± 0·077	3·020 ± 0·034	$P = < 0·001$
Mean terminal oocyte length (mm) ± *S.E.*	1·21 ± 0·101	6·31 ± 0·263	$P = < 0·001$
Mean corpus allatum volume (arbitrary units) ± *S.E.*	15·8 ± 0·86	34·6 ± 2·15	$P = < 0·001$

The inhibitory effect of the operation on growth and sexual maturation[1] is confirmed, but in addition a new relationship has been established. Removal of the frontal ganglion prevents the increase in corpus allatum volume which normally precedes and accompanies maturation. The effects of the operation are very similar to those observed in adult females of *Schistocerca paranensis* after cautery of the cerebral neurosecretory cells[3]. There may not be a positive relationship between corpus allatum volume and secretory activity in *L. m. migratorioides*, but since the accessory sex glands of the operated females lack secretion it is unlikely that the small corpora allata are active. Additionally Staal has shown that in nymphs of *L. m. migratorioides* corpora allata which are prevented from increasing in volume secrete only at a minimal level.

Clarke and Langley suggest that removal of the frontal ganglion prevents the production and/or release of media neurosecretion from the cerebral neurosecretory system. This withdrawal of neurosecretion may be direct

responsible for the suppression of growth of the corpora allata observed in this work. Indeed in nymphs of *L. m. migratorioides*, small denervated corpora allata grow if placed in contact with the corpora cardiaca[4]. However, median cell neurosecretion has not been identified within the corpora allata of locusts. Furthermore, Girardie has shown that destruction of the cerebral neurosecretory cells causes activation of the corpora allata in nymphs of *L. migratoria*[5].

There is another explanation for the suppression of corpus allatum growth after removal of the frontal ganglion. In *Calliphora erythrocephala* the activity of the corpus allatum can be regulated by varying concentrations of metabolites in the haemolymph[6]. If a similar mechanism exists in locusts, the corpora allata of the operated insects may be expected to remain small since the concentrations of haemolymph metabolites are low following removal of the frontal ganglion[7].

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VIROLOGY

Small Deoxyribonucleic Acid-containing Viruses (Picodnavirus Group)

THE picornavirus group was established in accordance with the decision of the International Subcommittee on Virus Nomenclature for classifying the major groups of viruses on the basis of common biochemical and biophysical properties[1]. Picornaviruses contain ribonucleic acid and protein, are insensitive to ether, and are extremely small in size (15–30 mμ in diameter). The capsids of those members of the group the symmetry of which has been studied appear to display cubic symmetry of the icosahedral pattern[2]. Members of the group derived from humans and lower animals are recognized. Certain plant viruses and the recently isolated RNA-containing bacteriophages have properties which suggest that they might be classified as picornaviruses. The cubic symmetry of the picornaviruses which have been studied is consistent with a capsid constructed from thirty-two morphological sub-units positioned at the vertexes of a rhombic triacontahedron[2] (Fig. 1*a*).

A number of DNA-containing viruses of the same size as the picornaviruses have recently been found, which by analogy might be considered as 'picodnaviruses'. The capsids of one of the small DNA animal viruses appear to be structurally similar to those of the picornaviruses. Vasquez and Brailovsky[3] recently proposed icosahedral symmetry for the Kilham rat virus (RV) based on a hexagonal outline in the electron microscope and a probable capsomere count of thirty-two. The structural symmetry of this virus with a 20 mμ diameter was best represented by the Archimedean dual solid, the pentakis dodecahedron (Fig. 1*b*). Vasquez and Brailovsky believed that the closely related hamster osteolytic *H* viruses (*H*1, *H*3) in all probability presented a similar fine structure.

Members of the hamster osteolytic group of viruses (*H*1, *H*3) are again of very small size (about 20 mμ in diameter). All serotypes produce intranuclear inclusions containing DNA demonstrable by cytochemical staining techniques. Recently Cheong *et al.*[4] have studied the nature of the DNA of the *H*1 prototype virus after purification in a caesium chloride density gradient. They concluded from their biochemical data that the virus consisted of a protein capsid containing a DNA core making up 25 per cent of the complete virion. Green and Karasaki[5] have partially purified *H*1 virus by precipitation at *p*H 4 and have detected evidence of sub-unit structure on the 23 mμ diameter capsid in the electron microscope.

The rat virus *X*14 is serologically related to the *H*3 group of hamster osteolytic viruses. Payne *et al.*[6] banded *X*14 virus at a density of 1·40–1·43 in caesium chloride and examined the virus particles in the electron microscope. The particles were polyhedral in shape and approximately 20 mμ in diameter. The replication of *X*14 was inhibited by 5-fluoro-2′-deoxyuridine[7], thus suggesting that the virus contained DNA, and it has been shown recently by acridine orange staining that the DNA of *X*14 virus occurs in the single-stranded form[8]. This would seem to be the first animal virus to be reported to contain DNA of this type. The fine structure and morphology of the *X*14 virus (Fig. 1*c*) are indistinguishable from RV and from human picornaviruses.

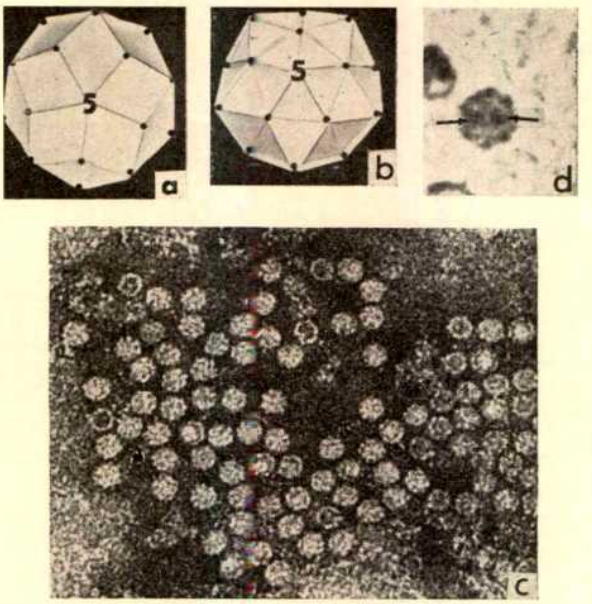


Fig. 1. *a*, Model of a rhombic triacontahedron viewed along a five-fold axis of symmetry. This convex polyhedron belongs to the icosadeltahedral class with triangulation (*T*) number equal to 3. *b*, Model of a pentagonal dodecahedron viewed along a five-fold axis of symmetry. This polyhedron also belongs to class $T = 3$ but demonstrates concavities. *c*, Group of *X*14 virus particles negatively stained with 2 per cent phosphotungstic acid (× *c*. 150,000). *d*, Photographic reversal of a favourably oriented ASV particle from adenovirus *SV*15 negatively stained with 2 per cent ammonium molybdate. Particle is viewed along a two-fold axis of symmetry. Two five-fold axes (black arrows) and six peripheral sub-units are seen. (× *c*. 455,000)

Small 20-mμ particles containing DNA and morphologically similar to *X*14 have recently been observed in preparations of a number of adenoviruses of man and animals[10–13]. Detailed investigation of the particles prepared from simian adenovirus *SV*15 stocks by genetron treatment and density gradient centrifugation revealed that the small particles did indeed possess icosahedral symmetry with as few as twelve morphological sub-units on the capsid[10] (Fig. 1*d*). Under certain conditions, these 20-mμ particles are able to replicate when inoculated into susceptible cell cultures. Both mature adenovirus particles (70 mμ in diameter) and 20-mμ particles are recovered in the material collected. The 20-mμ particles do not occur in the absence of the adenovirions. The particles were banded in a caesium chloride density gradient at 1·43 g/cm³. The band, when fixed and stained with acridine orange, yielded a yellow-green fluorescence consistent with the presence of a double-stranded DNA. The staining reaction was prevented by prior digestion with pepsin and DNase. However, subsequent experi-

Table 1. PHYSICAL AND CHEMICAL PROPERTIES OF SMALL DNA VIRUSES (PICODNAVIRUSES)

Virus	Nucleic acid	Particle diameter (mμ)	Ether resistance	Capsomere diameter (Å)	Buoyant density in caesium chloride	Refs.
RV	DNA	20	+	20	1·38	3
*X*14	Single-stranded DNA	22	+	?	1·40	6,7,8,9
*H*1	DNA	23	+	40	1·39	4,5
Adeno satellite virus from *SV*15	Single-stranded DNA	20	+	35–45	1·43	11,13,19
Adeno satellite virus from human adeno 7	Single-stranded DNA	20	+	?	1·41	10,12,19
φ*X*174	Single-stranded DNA	22	+	70	1·43	16,17,18

ments have shown that the reaction is readily susceptible to DNase digestion even without fixation or pepsin treatment. This is indicative that in addition to the 20-mμ particles a very accessible DNA, possibly even free, but inactive, double-stranded adenovirus DNA, was captured and banded with the small particle fraction. The presence of many degraded adenovirions and free capsomeres in the *SV*15 stocks from which this material was prepared is consistent with this interpretation. When freshly prepared *SV*15 material was monitored in the electron microscope and when only material free from morphological evidence of adenovirus degradation was used, the subsequent bands at 1·43 g/cm³ gave clear 'flame red'-staining and the DNase susceptibility characteristic of single-stranded DNA.

Atchison *et al.*[13] have investigated similar small DNA-containing particles with hexagonal profiles associated with the simian adenovirus *SV*15. These particles were not related antigenically to the adenovirus, but replication of the particles in tissue culture was achieved only when they were inoculated simultaneously with adenovirus. The particles were insensitive to ether.

Similar small virus-like particles containing DNA have been found associated with human adenoviruses[10,12,19]. Again these particles do not appear to be able to replicate unless adenovirus is present. Staining reactions with acridine orange indicate that these particles, like the *X*14 virus, contain single-stranded DNA. The particles, like the *H* viruses, are insensitive to ether but, unlike them, appear incapable of autonomous replication.

Atchison *et al.*[13] suggested that the small particles should be referred to as 'adeno-associated' virus. However, since they behave like the previously described satellite virus of plants[14,15] (in that they are unable to replicate autonomously and their replication is dependent on the presence of a larger virus), the particles should be more fittingly referred to as 'adeno-satellite' virus (ASV). The numerous recent reports on the occurrence of 20-mμ particles containing DNA have prompted us to comment on their structural similarity to the picornaviruses. Certain plant viruses have properties similar to those of the picornaviruses of man and lower animals. Turnip yellow mosaic virus is an example of a small RNA plant virus capable of autonomous replication in a susceptible host. The satellite tobacco necrosis virus (TNSV) is a defective small RNA virus of plants[14,15]. The adeno-satellite viruses are defective DNA viruses of human or monkey origin. Many of their properties are analogous to those exhibited by the satellite tobacco necrosis system.

Selected biophysical and chemical properties of some small DNA virions are shown in Table 1. It should be noted that the buoyant densities of these viruses (approximately 1·40 g/cm³) are greater than those of picornaviruses (1·34) and papovaviruses (1·32).

The staining reactions with acridine orange and relevant enzyme susceptibility patterns of selected DNA virus preparations are shown in Table 2. The yellow-green staining reactions of the purified adenoviruses free from satellite particles are consistent with a double-stranded DNA. On the other hand, the staining reactions of the adeno-satellite viruses and of the *X*14 virus are consistent with a single-stranded molecule. Similar staining reactions were demonstrated by a purified preparation of the well-defined single-stranded bacteriophage φ*X*174. It is probable that the DNAs of RV and of the *H*1 viruses will also be found to exhibit a single-stranded secondary structure.

Table 2. STAINING REACTIONS OF CARNOY-FIXED SMEARS OF SELECTED DNA VIRUS PREPARATIONS

Virus	Staining reactions with 0·01 per cent acridine orange	Enzyme susceptibility RNase	Enzyme susceptibility Pepsin + DNase
φ*X*174	flame red	–	+
*X*14	flame red	–	+
ASV from simian adenovirus *SV*15	flame red	–	+
ASV from adenovirus 7	flame red	–	+
Simian adenovirus (*SV*15)	yellow-green	–	+
Human adenovirus 7	yellow-green	–	+

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Inhibiting Effect of Increasing Body-weight on the Lethal Response of DBA/1 Mice to Vaccinia Virus Infection

IN a previous publication[1] I considered the response of mice of different ages to experimental vaccinia infection; the high degree of resistance of adult mice to this virus, as well as the fact that vaccinia is highly lethal for new-

Table 1. EFFECT OF INCREASING BODY-WEIGHT AND/OR INCREASING AGE ON THE RESISTANCE OF *DBA*/1 MICE TO VACCINIA INFECTION

Infective particles*	Average body-weight of mice‡	Age of the mice in days when inoculated†										
		23	20	19	18	17	16	15	14	12	11	10
5×10^6	3·3–3·9	1/10§			1/4	0/11§	0/8§	0/5		0/5	0/11§	
5×10^5	4·2–4·8		6/8§			2/12§	1/3	0/11§	0/4		0/12§	0/10§
5×10^5	5·2–5·5			3/3	6/9§	7/7§		4/6	0/2			0/6
5×10^5	6·0–6·8				2/3	4/4	3/4				2/2	5/5

* Levaditi neurovaccinia grown in rabbit testes.
† Surviving mice/inoculated
‡ The average body-weight of the mice from each litter was calculated from the total weight of the litter.
§ More than one litter included. The average number of offspring in *DBA*/1 litters is ordinarily five.

born mice. It was also demonstrated that the young mice (non-inbred albino) became resistant to increasingly higher doses of virus as they aged.

The present findings, however, indicate that resistance to vaccinia infection in young mice is determined by their body-weight rather than by their age. The acquisition of resistance to viral infection during the first weeks or days of life has been demonstrated in a variety of systems and it is generally attributed to the effects of increasing age. In some systems, however, it is difficult to establish the precise age at which resistance actually begins. In such systems resistance develops gradually during a comparatively long period of time against increasingly higher doses of virus. This suggests that the very first signs of resistance will scarcely be noticeable as they probably involve the response of the host to minimal doses of virus.

The gradual development of resistance as a dose-dependent phenomenon was clearly demonstrated in experiments in which several strains of Coxsackie virus were titrated in baby mice of different ages[2]. The infective titre of all the strains tested declined gradually in increasingly older hosts until the lowest dilution became non-pathogenic for mice 33–35 days old. In the course of these experiments, it was also noted that differences in body-weight among mice of the same age influenced the host response to viral infection.

The gradual development of resistance was also observed in the experiments with mice infected with vaccinia virus referred to previously[1]. The virus titre declined from 10^5 in newly-born animals to 10^3 in the 1-week-old mice and to $< 10^1$ in the 3-week-old mice. At 4 weeks of age the mice were 100 per cent resistant to the highest virus dose used ($7{\cdot}5 \times 10^6$).

Other examples of dose-dependent host response to viral infection are those in which newly isolated viruses, notably viruses with neoplastic properties, acquired the ability of infecting increasingly older hosts, as well as initially resistant strains or species, when their virulence and/or replicating capacity was increased by repeated animal passage.

From this it appears that differences in the response to certain viral infections among young animals of different age, strain or even species can be overcome by increasingly higher doses of virus and are, therefore, of a quantitative as well as of a qualitative nature.

During recent investigations, a comparison was made between the response to vaccinia infection of baby mice of two strains, non-inbred albino and inbred *DBA*/1, and it was observed that susceptibility to the virus persisted in the latter mice for a considerably longer period of time than in the albino strain.

However, the longer lasting susceptibility of the *DBA*/1 mice to vaccinia infection was apparently due to the fact that these mice are much smaller than the albino mice. Thus, whereas mice from a 23-day-old *DBA*/1 litter weighed on average as little as 3·7 g and were 100 per cent susceptible to the virus, mice from a 12-day-old mouse litter weighed on average as much as 8·5 g and were 100 per cent resistant to the same dose of virus.

It was next determined that the range of body-weight is considerably wide among litters of the same age in both strains but that it stayed within a much lower level until much later in life in the *DBA*/1 than in the albino strain. The average body-weight of 12-day-old mice from albino litters was found to be 4·0–8·5 g, whereas for 23-day-old mice of *DBA*/1 litters it was 3·3–7·5 g. In view of this finding, *DBA*/1 mice were used in the present experiments in order to determine the effect of increasing body-weight in relation to increasing age on the development of resistance to vaccinia infection.

First litters were used of *DBA*/1 mice conceived and born in our laboratory from parents obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. Mice of different body-weights were inoculated at various ages in the fat pad between the scapulae, with 0·05 of a 1 : 10 saline extract of rabbit testes infected with Levaditi neurovaccinia. The dose of virus was about 5×10^5 particles infective for rabbit skin. The average body-weight of the mice was calculated from the total weight of the litter, except when obvious differences in size were observed. Each litter was weighed immediately before inoculation. Autopsies were performed periodically to determine the presence of typical lesions. Deaths occurring either within 48 h or after 15 days of virus inoculation were not recorded as due directly to vaccinia infection.

The results of these experiments are summarized in Table 1. From top to bottom the data indicate the effect of body-weight on the development of resistance to vaccinia infection: from right to left, the effect of increasing age.

It will be noted here that the degree of resistance has been estimated in each group of mice by the percentage of survivals without reference to the incidence of illness among the survivors. Death as well as illness was apparently induced in the present experiments by necrotic lesions in the lungs. The type of lesions induced by vaccinia in young mice varies, depending on the degree of susceptibility of the host and/or the virus dose. The organ distribution and pathology of these lesions have been described elsewhere[1].

The results in Table 1 indicate that increasing body-weight and increasing age compensate for each other in influencing the development of resistance to vaccinia infection in mice, as they exert such an influence in a quantitatively inverse relationship. The greater the body-weight, the younger the animal becomes resistant to the virus. Conversely, the lower the body-weight, the longer it takes for the animal to become resistant. It will be seen that, whereas the mice weighing 6·0–6·8 g were already resistant at the age of 10 days, those weighing 5·2–5·5 g did not become resistant until they were 15–17 days old, and those weighing 4·2–4·8 g until they were 18–20 days old.

These findings indicate that under the present conditions small differences in body-weight seem to have a decisive effect on the response of the mice to vaccinia infection. Thus, whereas most of the 20-day-old mice, which weighed an average of 4·3 g, were resistant to the virus, the mice weighing an average of 3·7 g were still virtually 100 per cent susceptible at the age of 23 days. The long-lasting susceptibility of vaccinia infection of the latter mice is apparently related to their abnormally small size. However, this class of mice, which occurred

in 10–15 per cent of the litters, do not seem to be otherwise abnormal. They are physically well proportioned, as they look rather like miniatures of their normal size counterparts. They are as quick and lively, if not more so, than mice of the same age weighing twice as much. They develop a good set of teeth with which they feed themselves eagerly and bite vigorously when handled for inoculation.

We are trying to determine how long these mice remain susceptible to the virus or fail to reach normal growth. We are also interested in determining how early in life differences in body-weight are apt to influence the response of the mice to vaccinia infection. This information might provide some insight into the difficulties often encountered when attempting to isolate in new-born animals viruses present in the inoculum in unknown amounts. With the virus dose used in the present experiments the 10-day-old mice were the youngest to become resistant to the virus (see Table 1) under the influence of increasing body-weight. With lower doses of virus similar findings have been observed in younger mice. Seven-day-old mice, which on average weighed 4·7 g, survived inoculation with 5×10^3 vaccinia infective particles, whereas 17-day-old mice, which on average weighed 3·3 g, were 100 per cent susceptible to the lethal effects of the same virus dose.

It would seem possible from these findings that differences in the response to experimental viral infection among young animals of the same age and strain as well as of different strains or species might be related from birth to differences in their level of growth. Furthermore, that the age of young hosts is no accurate indication of their probable response to experimental viral infection unless it is correlated with their body-weight.

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[1] Duran-Reynals, M. L., *Biologie Medicale*, 52 (1), 76 (1963).
[2] Howes, D. W., *Austral. J. Exp. Biol.*, 32, 258 (1954).

CYTOLOGY

Chromosome Numbers of *Elaphoglossum* and *Hymenodium*

THE fern genus *Elaphoglossum* is one of the largest in the family Aspidiaceae. Moreover, some taxonomists would like to place it in a separate family, Elaphoglossaceae[1]. Of more than four hundred species of *Elaphoglossum*, most are to be found in tropical America. Some of the species deviate so much from the type of *Elaphoglossum* that they have been separated from the genus, and one of them is the species *Elaphoglossum crinitum*, which has been renamed *Hymenodium crinitum* by Fée and *Dictyoglossum crinitum* by J. Smith. Copeland, however, prefers the name *Elaphoglossum crinitum*[2].

Only very few species of the genus *Elaphoglossum* have been studied cytologically. *Elaphoglossum hirtum* (Sw.) C.Chr., $n = 82$; *E. krajinae* Biswas, $n = 41$; and *E. spathulum* (Bory) Moore, $2n$ = about 160, were studied by Manton and Sledge[3]. Two other species, *E. conforme* Sw., $n = 82$, and *E. latifolium* Bett., $n = 82$, were studied by Bir[4]. All these species seem to have the basic chromosome number of forty-one, although most of them are polyploids.

Material for the present study was collected from El Verde rain-forest in the eastern part of Puerto Rico, in August 1964. The herbarium specimens were identified by Dr. R. Woodbury, of the Agricultural Experiment Station at Rio Piedras. Sporangia were fixed in acetic alcohol (1 : 3) and stained in acetic–iron–haematoxylin solution. The chromosomes were counted at the late meiotic prophase and first metaphase stages.

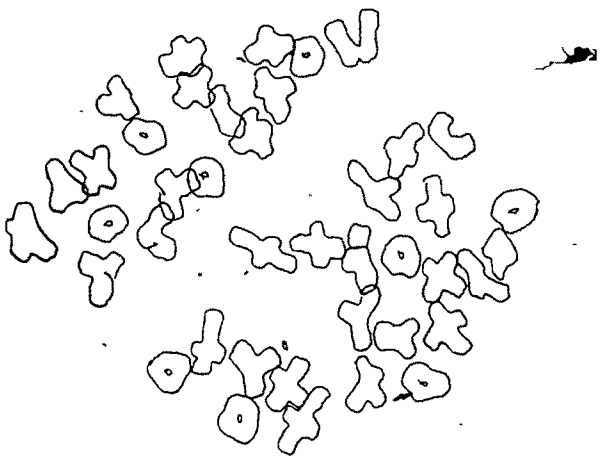


Fig. 1. Diakinesis of *Elaphoglossum flaccidum* (Fée) Moore, $n = 41$. Aceto-iron-haematoxylin squash (× *c.* 2,000)

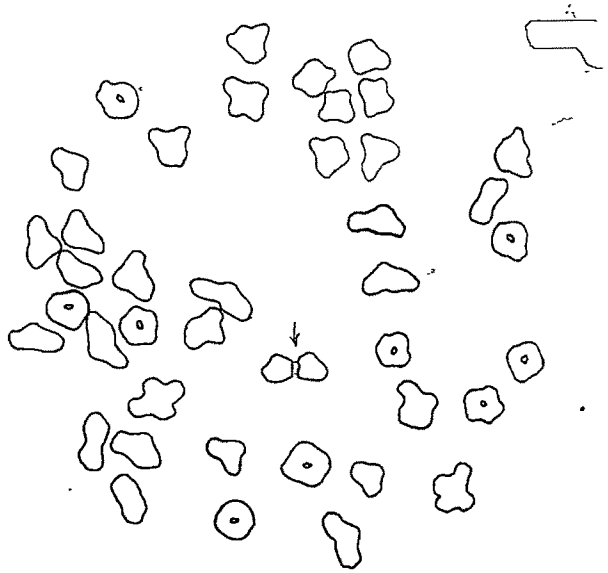


Fig. 2. Prometaphase I of *Hymenodium crinitum* (L.) Fée, $n = 41$ (forty bivalents and two halves of a bivalent). Aceto-iron-haematoxylin squash (× *c.* 2,000)

In both species of *Elaphoglossum* investigated, *E. flaccidum* (Fée) Moore, and *E. firmum* (Mett.) Urban, forty-one bivalent chromosomes were counted. But in the meiosis of *Hymenodium crinitum* (L.) Fée, the chromosome number often seems to be forty-two. Careful investigation of meiosis, however, reveals that the chromosomes of one bivalent very often part company as early as diakinesis. Actually the true number of chromosomes is forty bivalents and two halves of a bivalent, which is the same as in the *Elaphoglossum* species now studied. Another obvious similarity between the meiotic chromosomes of *Elaphoglossum* and *Hymenodium* seems to be the relatively large number of ring bivalents.

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[1] Ching, R. C., *Sunyatsenia*, 5, 201 (1940).
[2] Copeland, E. B., *Genera Filicum* (Waltham, Massachusetts, 1947).
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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, April 18

OPERATIONAL RESEARCH SOCIETY (at the Royal Aeronautical Society, 4 Hamilton Place, London, W.1), at 4.30 p.m., Forum on "O.R. and Accountancy".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. E. C. Claydon. "Electricity for Grain Drying".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.E.–I.E.R.E. COMPUTER GROUPS (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Computer Bulk Storage".

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Annual General Meeting, followed by Dr. B. J. Heywood: "Hydroxybenzonitrile Herbicides" (Chairman's Lecture).

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—"The Days of Whiskey Gap" and "City of Gold" (films).

Tuesday, April 19

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. G. F. Turnbull and Mr. B. E. Jones: "A High Input Impedance Clip-on A.C. Voltmeter": Mr. E. Rawlinson, Mr. P. H. King and Mr. P. K. Richardson: "Automatic Corona Loss Measuring Equipment".

INSTITUTE OF SCIENCE TECHNOLOGY, LONDON BRANCH (in the Surgical Lecture Theatre, New Guy's House, St. Thomas' Street, London Bridge, S.E.1), at 6.30 p.m.—Mr. J. C. Gazet: "Surgery in the Sixties".

Tuesday, April 19—Wednesday, April 20

BRITISH SOCIETY OF SOIL SCIENCE (in the Botany Theatre, University College, Gower Street, London, W.C.1), at 10.30 a.m. daily—Meeting on "Soil Moisture—Physical and Biological Aspects".

Tuesday, April 19—Thursday, April 21

INSTITUTE OF METALS (at Church House, Great Smith Street, London, S.W.1)—Spring Meeting.

SOCIETY OF ENVIRONMENTAL ENGINEERS (at Imperial College, London, S.W.7)—Symposium on "Environmental Engineering and Its Role in Society".

Wednesday, April 20

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. T. Coley, Mr. L. F. Rutishauser and Mr. H. M. Ashton: "A New Laboratory Test for Predicting Low Temperature Operability of Diesel Fuels".

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. B. M. Cobbe: "The West London Area Traffic Control Experiment".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 6 p.m.—Mr. B. D. Armstrong: "Automatic Landing".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, ELECTRO-ACOUSTICS GROUP (at 9 Bedford Square, London, W.C.1), at 6 p.m.—Mr. P. J. Baxandall: "Low Distortion Transistor Audio Amplifiers—Some Important Design Characteristics".

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP—FOOD ENGINEERING PANEL (at R.S.H. Headquarters, 90 Buckingham Palace Road, London, S.W.1), at 6.15 p.m.—Annual General Meeting, followed by Dr. E. H. T. Hoblyn, M.B.E.: Chairman's Address.

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the Society for Analytical Chemistry, at the College of Technology, Park Street, Luton), at 6.45 p.m.—Dr. D. T. Lewis: "Recent Investigations at the Laboratory of the Government Chemist".

Thursday, April 21

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. D. Rees: "The Birational Geometry of Local Rings".

ASLIB (at 3 Belgrave Square, London, S.W.1), at 5.30 p.m.—Dr. J. L. Dolby and Mr. M. B. Line: "University Libraries and the Information Needs of the Research Worker".

INSTITUTE OF PETROLEUM, ECONOMICS AND OPERATIONS GROUP (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. K. Mackrell: "Review of the Year".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Mr. H. E. Pearson: "Satellite Communication" (Silvanus P. Thompson Lecture).

OIL AND COLOUR CHEMISTS' ASSOCIATION, LONDON SECTION (at the Criterion-in-Piccadilly, London, W.1), at 7 p.m.—Annual General Meeting.

TELEVISION SOCIETY (at the Royal Institution, 21 Albemarle Street, London, W.1), at 7 p.m.—Prof. W. D. Wright. "The Implications for Television of Modern Thinking on the Visual Process" (Fleming Memorial Lecture).

Thursday, April 21—Friday, April 22

BIOCHEMICAL SOCIETY (at University College, Gower Street, London, W.C.1)—Annual General Meeting. Symposium on "Instrumentation in Biochemistry". Prof. F. Lynen: "The Role of Biotin-Dependent Carboxylations in Biosynthetic Reactions" (Third Jubilee Lecture). Small exhibition of instruments.

Friday, April 22

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 10 a.m.—Colloquium on "Electrical Services in System Building".

ROYAL ASTRONOMICAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.15 p.m.—Geophysical Discussion on "Physical Origin of Magnetization in Rocks".

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Annual General Meeting, followed by Dr. D. S. Morris: "Coarse Chemistry and Fine Chemicals" (Chairman's Address).

Monday, April 25

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion Meeting on "Breakdown of Large Air-gaps and Effect of Voltage Wave Shape" opened by Dr. F. D. A. Boylett and Dr. D. F. Oakeshott.

PLASTICS INSTITUTE, LONDON SECTION REINFORCED PLASTICS SUB-GROUP (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Mr. P. Lynch: "The Advances of Hot Press Moulding in the U.K."

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Prof. S. Thorarinsson: "An Island is Born".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned.

LECTURER IN THE DEPARTMENT OF PHILOSOPHY—The Registrar, The University, Leicester (April 20).

LECTURER (preferably with a research interest in numerical analysis, astrophysics or fluid dynamics) IN APPLIED MATHEMATICS—The Registrar, King's College (University of London), Strand, London, W.C.2 (April 22).

ORGANIC CHEMIST (with experience in isolation of natural products) for research on coffee berry disease—The Secretary, Wye College (University of London), near Ashford, Kent (April 22).

ASSISTANT EXPERIMENTAL OFFICER (with a degree or equivalent in botany, chemistry, agriculture or related subject) to assist with work on the extraction and purification of plant hormones and the study of their physiological properties—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (April 23).

ASSISTANT LECTURER IN ORGANIC CHEMISTRY—The Registrar, The University, Manchester, 13, quoting Ref. 79/66 (April 23).

CHAIR OF PURE MATHEMATICS—The Registrar, University College of Swansea, Singleton Park, Swansea (April 23).

LECTURER or ASSISTANT LECTURER (climatologist, preferably with an interest in biogeography) IN THE DEPARTMENT OF GEOGRAPHY—The Secretary, The University, Southampton (April 23).

LECTURER (preferably with some research or industrial experience) IN THE DEPARTMENT OF METALLURGY—The Registrar, University College of Swansea, Singleton Park, Swansea (April 23).

ASSISTANT LECTURER IN STATISTICS, preferably in Mathematical Statistics, or Probability Theory—The Registrar, The University, Hull (April 25).

TEACHING FELLOW/DEMONSTRATOR (graduate, preferably with an honours degree in rural science, agriculture, or science with specialization in plant science) IN AGRONOMY at the University of New England, Armidale, New South Wales, Australia—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, April 25)

LECTURER IN THEORETICAL CHEMISTRY—The Secretary of the University Court, The University, Glasgow (April 27).

ASSISTANT LECTURER (with an honours degree in pharmacy and an interest in phytochemistry) IN PHARMACOGNOSY—The Registrar, University of Strathclyde, George Street, Glasgow, C.1 (April 29).

LECTURER (honours graduate in pharmacy or an equivalent qualification, an interest in organic chemistry and experience of the application of modern analytical methods to the solution of pharmaceutical, chemical and biochemical problems) IN PHARMACEUTICAL CHEMISTRY—The Registrar, University of Strathclyde, George Street, Glasgow, C.1 (April 29)

DIRECTOR OF THE UNIVERSITY COMPUTING CENTRE; and a LECTURER IN THE UNIVERSITY COMPUTING CENTRE—The Registrar, The University, Leicester (April 30).

LECTURER and an ASSISTANT LECTURER IN THE DEPARTMENT OF BIOCHEMISTRY, Queen's College—The Secretary, University of St. Andrews, Queen's College, Dundee (April 30).

LECTURER (graduate in metallurgy or physics) IN PHYSICAL METALLURGY—The Secretary, Sir John Cass College, Whitechapel High Street, London, E.1 (April 30).

LECTURER or ASSISTANT LECTURER IN THE DEPARTMENT OF PHYSICS, University of Singapore—The Inter-University Council, 33 Bedford Place, London, W.C.1 (April 30).

LECTURER (Electrical Theory) (with an electrical engineering degree and postgraduate experience in industry or in research) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING, University College, Nairobi, University of East Africa—The Inter-University Council, 33 Bedford Place, London, W.C.1 (April 30).

LECTURER or SENIOR LECTURER (with qualifications in the biological or physical sciences and particular interests in the methods of teaching science) IN SCIENCE EDUCATION in the School of Education, University of Zambia—The Inter-University Council, 33 Bedford Place, London, W.C.1 (April 30).

LECTURER (with a special interest and research experience in plant pathology) IN BIOLOGY IN THE DEPARTMENT OF APPLIED MICROBIOLOGY AND BIOLOGY—The Registrar, University of Strathclyde, George Street, Glasgow, C.1 (April 30).

LECTURERS (preferably with research experience in bacterial genetics or electron microscopy) IN GENETICS—The Secretary of the University Court, The University, Glasgow (April 30).

RESEARCH ASSISTANT (with a qualification, not necessarily a degree, in physics and/or chemistry) IN THE DEPARTMENT OF INORGANIC AND STRUCTURAL CHEMISTRY, for duties which will include the operation of electronic equipment for X-ray crystallography—Prof. H. Irving, School of Chemistry, The University, Leeds, 2 (April 30).

SENIOR LECTURER (Grade I) or LECTURER (Grade II) IN APPLIED MATHEMATICS—The Registrar, University Senate House, Tyndall Avenue, Bristol, 2 (April 30).

SENIOR LECTURER/LECTURER IN MATHEMATICS at Rhodes University, Grahamstown, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, April 30).

SENIOR LECTURER or LECTURER IN SYSTEMS AND CONTROL ENGINEERING; and a LECTURER IN AUTOMATIC CONTROL OR SYSTEM DYNAMICS, including vibration analysis—The Registrar, The University, Leicester (April 30).

CHAIR OF PLANT SCIENCE; CHAIR OF SOIL SCIENCE; and CHAIR OF AGRICULTURAL ECONOMICS IN THE FACULTY OF AGRICULTURE, University of Malaya—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Malaya and London, May 1).

RESEARCH ASSISTANT IN SOIL CHEMISTRY OR PLANT PHYSIOLOGY—The Reader in Soil Science, Soil Science Laboratory, The University, Oxford (May 1).

LECTURERS OR ASSISTANT LECTURERS (with qualifications in either sociology or social anthropology, and for one of the posts an interest in sociological aspects of developing countries or in organizational theory would be an advantage) IN SOCIOLOGY—The Registrar, University of East Anglia, Earlham Hall, Norwich, NOR 88C (May 2).

LECTURER IN BIOCHEMISTRY OR ANIMAL PHYSIOLOGY IN THE DEPARTMENT OF PHYSIOLOGY AND BIOCHEMISTRY, The Royal University of Malta—The Inter-University Council, 33 Bedford Place, London, W.C.1 (May 3).

CHAIR OF GEOLOGY—The Registrar, The University, Hull (May 5).

CHAIR OF MECHANICAL ENGINEERING—The Registrar, The University, Manchester, 13, quoting Ref. 85/66 (May 5).

READER IN PHARMACOLOGY at Guy's Hospital Medical School—The Academic Registrar, University of London, Senate House, London, W.C.1 (May 6).

ASSISTANT LECTURER (preferably with research interests and experience in aspects of the geography of Africa) IN GEOGRAPHY—The Registrar, The University, Sheffield (May 7).

ASSISTANT LECTURER (with a wide interest in cytology so as to be able to lecture to students of both departments) IN CYTOLOGY IN THE DEPARTMENTS OF BOTANY AND ZOOLOGY in the Faculty of Sciences—The Registrar, The University, Manchester, 13, quoting Ref. 84/66 (May 7).

LECTURER or ASSISTANT LECTURER IN MATHEMATICAL STATISTICS, to work with an existing group of five members of staff—The Registrar, The University, Manchester, 13, quoting Ref. 87/66 (May 7).

LECTURER (with a special interest in either electrical machinery or electrical power distribution systems) IN ELECTRONIC AND ELECTRICAL ENGINEERING—The Registrar, The University, Sheffield (May 7).

READER IN BIOLOGY at King's College—The Academic Registrar, University of London, Senate House, London, W.C.1 (May 9).

THE H. W. HOOVER, JR., CHAIR OF MATHEMATICAL STATISTICS AND OPERATIONAL RESEARCH—The Registrar, University College of South Wales and Monmouthshire, Cardiff (May 9).

PROFESSOR IN MICROBIOLOGY; and a SENIOR LECTURER or LECTURER IN MICROBIOLOGY IN THE FACULTY OF AGRICULTURE, University of Natal, Pietermaritzburg—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, May 10).

CHAIR OF CELL BIOLOGY—The Secretary of the University Court, The University, Glasgow (May 13).

ASSISTANT LECTURER or a UNIVERSITY DEMONSTRATOR IN THE DEPARTMENT OF ZOOLOGY—The Secretary, The University, Edinburgh (May 14).

CHAIR OF BOTANY—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (May 14).

LECTURERS/SENIOR LECTURERS/READERS (3) IN THE DEPARTMENT OF MATHEMATICS, University of Tasmania—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, May 20).

LIBRARIAN (preferably science or engineering graduate with library experience) OF THE SCIENCE AND ENGINEERING LIBRARY, Haile Sellassie I University, Ethiopia—The Inter-University Council, 33 Bedford Place, London, W.C.1 (May 22).

SENIOR RESEARCH FELLOW IN THE DEPARTMENT OF THEORETICAL PHYSICS, Research School of Physical Sciences, Institute of Advanced Studies, Australian National University (members of the department are working on theoretical nuclear physics, elementary particles and field theory and many-body problems)—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, May 27).

CHAIR OF BOTANY at the University of Tasmania—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, May 31).

LECTURER (with a postgraduate degree and preferably some experience of teaching biochemistry at university level) IN BIOCHEMISTRY at Lincoln College, University of Canterbury, New Zealand—The Secretary, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or the Registrar of the College (New Zealand and London, June 30).

SENIOR LECTURER or LECTURER (preferably with interests in the genetics of micro-organisms) IN GENETICS—The Secretary, The Queen's University, Belfast, Northern Ireland (June 30).

RESEARCH ASSISTANTS/STUDENTS (graduates or prospective graduates in zoology or physiology) in the following fields: physiology of aquatic adaptations in reptiles; limnology (especially underwater sound); soil zoology; parasites of fish and amphibia; ecology of ticks of British mammals; and insect sound—The Secretary, Sir John Cass College, Jewry Street, London, E.C.3.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Third Special Report from the Estimates Committee, Session 1965–66—Grants to Universities and Colleges. (Departmental Observations on the Fifth Report from the Estimates Committee in Session 1964–65, together with the Comment of the Committee.) Pp. 8. (London: H.M. Stationery Office, 1965.) 9*d*. net. [182

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UPROAR ABOUT TEACHING

IF war is too important to be left to generals, can society continue to entrust education to the teachers? This is one of the many questions thrust on public attention in Britain by the confrontation at Easter between the Secretary of State for Education and Science, Mr. Anthony Crosland, and the National Union of Teachers. For what has been plain for years is now inescapable—the administration of primary and secondary education, and the terms of service of teachers, are too rigid for a society seeking to transform itself.

There were three main issues at the annual conference of the National Union of Teachers at which the disagreement between the profession and the British Government emerged with almost unprecedented abandon. First, there was the complaint that the Government has asked for a reorganization of the pattern of secondary education, and in particular for the replacement of selective by comprehensive schools, without providing the money to ensure that the job is done properly. Mr. Crosland's retort that the teachers themselves have not done what they might have done to awaken public support for public spending on education was fair comment, but no answer. There was also a dispute about the place of ancillary aids in the classroom. In the hope of mollifying the teachers with a vision of what might be, Mr. Crosland only maddened them by saying that the teacher of the future would be a superintendent of a host of teaching aids, some human and some mechanical. Finally, there was the familiar, but legitimate, complaint that teaching is a grossly impoverished profession. So it is, but the teachers themselves are partly to blame.

On reorganization and the cost thereof, the problem is not that the Minister wishes to go too far too fast, but that his thinking may not yet be radical enough. The pattern of secondary education in Britain evolved at the end of the nineteenth century, when few people went to university. It was a considerable pedagogical triumph that secondary schools should have found ways of giving a great many young people a valuable replica of the benefit they would have won from further education. But times have changed. Education after school has become a commonplace. Yet specialist sixth-form teaching in secondary schools remains the pride of British headmasters, the self-justification of a large part of the teaching profession, a determinant of the organization of secondary schools of all kinds and an exacerbation of the shortage of teachers. But—in its present form—is it really necessary? And is the curriculum elsewhere in the secondary schools that best suited to modern needs? At a time when even the French Government has carried through a reorganization of secondary schooling, it would be easier to feel that British disputes about reorganization were worth fighting if there were some evidence that the parties involved knew what secondary education is really for.

When it comes to guessing how innovations of technique could transform the craft of teaching, the angels are mostly on the Minister's side. The possibilities are exciting. There is a bright future for well-designed and well-tested programmed courses of instruction. Computers have a part to play. So also have television and other visual aids. Team teaching needs a thorough trial, and extra-mural activities (such as visits to countries abroad) have a valuable potential. Then there is no school in Britain that could not, for example, profit from the services of laboratory assistants skilled enough to participate in teaching. The promise of these and other innovations is not merely a better use of existing manpower, but a means of catering more sensitively than is at present possible for the increasingly diverse interests and abilities of students at schools. It is exceedingly tiresome that the National Union of Teachers, like many similar organizations in countries other than Britain, should be suspicious of these and other innovations, but it is only fair to add that the British Government has not yet seen fit to back Mr. Crosland's enthusiasm with the substantial sums of money that will have to be spent on development if schools are to make proper use of the new technology of education.

The teachers' distrust of innovation unfortunately seems to spring from arguments closely allied to frustration over terms of service. Their insistence that there is a special relationship between students and those teachers on whom the mantles of Dewey and Piaget have been solemnly laid fits so well with the strategy of labour scarcity pursued by the more primitive Trade Unions that even innocent observers are bound to wonder how much weight to give the argument. In fact, the Union has successfully fought for a nation-wide salary structure and in the process may have done the profession a disservice. One result is that it is difficult to reward really able people. Another is that teaching is less able than it might be to attract those who decide, in middle life, to forsake careers in industry or the armed services. And everybody would be better off if young people entering the profession could look forward to a progressively better salary without having to abandon the classroom for administration or some similar activity.

The teaching profession as a whole would also be much more healthy if it were less obviously cut off from the outside world. Part-time teachers should be welcomed, not only for the relief they bring to the shortage of teachers but also for the experience of the outside world they can import into the classroom. In the same spirit, it has become an urgent need that full-time teachers should be regularly relieved from their responsibilities in class, partly so as to keep up to date but also to refresh themselves intellectually. They must also be freed from the penny-pinching meanness that prevents many of them from being free agents in their classrooms.

These are issues too broad to be debated at annual conferences, and too separate from daily administration to play much part in the making of government decisions. Yet they are important, and they are alive. They deserve more serious attention than they have been given in recent years. There may, after all, be some merit in the suggestion of that otherwise eccentric body, the National Association of Schoolmasters, that the times call for a thorough re-examination of British education. What needs to be decided, of course, is not the payment to be made to schoolteachers, but what function they are to have in modern society and how they are best to be equipped to do their job. No doubt Mr. Crosland was

hoping, at Easter, that an outspoken declaration would jolly the profession along for a few months or so. The growls of discontent he left behind should have convinced him that this is a turning point in the history of British education, and that he should take the lead in finding out what should happen to it next.

TRIBOLOGY FOR ALL

IF you give a dog a good name, you have more hope of sanctifying him. This principle seems to have guided the British working party on lubrication which has now reported to the Department of Education and Science (*Lubrication*, H.M.S.O., 6*s*. 6*d*.). It is no excuse that the editors of the *Oxford English Dictionary* are said to have helped in coining the word 'tribology' for "the science and technology of interacting surfaces in relative motion and the practices related thereto". There is nothing wrong with the word, but only with the amalgam of pomposity and special pleading which had led the working party to undermine a good case by wild exaggeration.

The working party starts off on the wrong foot with the calculation that British industry could save some £515 millions a year by making full use of what is called tribotechnology. Obviously there are savings to be made—in reduced energy consumption, reduced lubrication costs, savings on spare parts and because properly designed and lubricated machinery should last longer. Better tribology could also reduce losses of industrial output caused by unexpected breakdowns. But is it sensible to suppose that industry could realistically hope to win all these benefits by better practice ? Or that it would be wise to try ? There may indeed be many circumstances when bad practice is cheapest and therefore best. As with fuel efficiency or the prevention of corrosion, it is necessary always to balance potential benefits against the costs of attaining them. The working party has naïvely forgotten this rule.

The report does not recover from this bad beginning. It says, for example, that only "a small minority" of the 200,000 technicians in the metal and engineering industries have a "working knowledge" of lubrication, but does not go through what would have been the valuable exercise of saying what a working knowledge should consist of. There is a lot in the working party's argument that there should be more lubrication research in Britain, and better liaison with and a more intimate involvement of the universities. Obviously it would also help a great deal if there could be more short courses for practising engineers and technicians. But it is hard to see what is intended by the recommendation that tribology should sometimes be "offered as an optional subject in the last year of engineering first degree courses". The working group has evidently fallen into the old fallacy of British education—the view that if a topic is thought to be important, it should be possible for a few specialists to study it to the exclusion of most other things. By recommending 'several' institutes of tribology, and "organized professional institution activities in the subject of Tribology", the working party repeats the error of trying to make a portentous speciality out of an activity so important that it should be a part of the equipment of all technologists. It is a relief to see that this report does not finish with the demand that there should be a Minister of Tribology, with a seat in the Cabinet.

CLASSIFICATION IN PSYCHIATRY

Personality and Personal Illness

By G. A. Foulds, in collaboration with T. M. Caine, and with the assistance of Anne Adams and Anna Owen. Pp. xi+344. (London: Tavistock Publications, Ltd., 1965.) 55*s*. net.

CLASSIFICATION is a painful subject in psychiatry. No one can do without it, and no one likes it. At times it has taken the form of multiple divisions and subdivisions, as in medicine generally; Boissier de Sauvages, using a Linnean model, recognized 2,700 types of disease. At other times excessive classification was denounced : Adolf Meyer, the outstanding psychiatrist of the United States, groaned at "veritable debauches of unwarranted systematization" and declared that hankering after premature groupings was "excusable in a naïve statistician but not in a modern clinician". The statisticians, among whom many psychologists may now be included, do not by any means regard themselves as naïve in this matter. They have grappled with the problem, and, as Dr. Foulds clearly shows, have contrived ingenious ways of setting about it. Their work has reached the stage at which there is contention about methods and interpretation. Dr. Foulds is a notable contributor to the subject, who has worked alongside psychiatrists and acquainted himself with their diagnostic procedures, but developed his independent approach.

Initially and throughout he emphasizes the difference between traits of personality and the symptoms and signs of illness. It seems an obvious point, but some psychologists who have worked in this area have overlooked it. He then says, quite rightly, that the system of diagnostic classification used by psychiatrists is logically inadequate, intermingling as it does classes defined by major cause with classes defined by manifest anomalies of behaviour, and with classes defined by course of development or even by adequacy of social compliance. The hotch-potch has its merits and its empirical justification, but Dr. Foulds, like many before him, would greatly prefer a more cohesive system. The product of his efforts in this direction makes up the main body of *Personality and Personal Illness*.

He starts from the premise that every mental illness is a disorder "of the person, not merely of the organism", and that it is characterized by a failure, of varying extent, to maintain mutual personal relationships. He comes to the conclusion that there is a continuum of increasing failure as one passes from normality to personality disorders, neuroses, psychoses, and at last to non-integrated psychoses (by which he means psychoses in which the patient has lost awareness of himself as agent). He has investigated the problem mainly by the use of two questionnaires. One, devised by his collaborator, T. M. Caine, measures a component in personality which he calls 'hysteroid-obsessoid'; the other is an inventory which deliberately does not cover traits but only the symptoms and signs that betoken illness. Using the scores on the questionnaire and this symptom-sign inventory as valid quantitative criteria for mental disorder and personality respectively, he shows that in his subjects the illness measure, that is, the inventory score, changed during treatment, and that personality measures did not change except in some patients who had lengthy 'community therapy' which altered some of their attitudes.

The work is technically well documented and argued—though the reader becomes a little tired of the scholastic flavour of some often repeated phrases. It throws some systematic light into a troubled area, and it examines faithfully the faults inherent in psychological methods of enquiry which do not distinguish between personality which is lasting and mental illness which may be transitory, nor between symptoms and the pathological or psychopathological process manifested through them. Some of Dr. Foulds's statements provoke dissent. He

says, for example, that "the presence of delusions and/or gross perceptual disorder constitutes the necessary and sufficient condition for psychosis"—where, then, does schizophrenia simplex belong? "An involutional melancholia with schizophrenic features is incomprehensible except to those who believe in a schizo-affective syndrome", he writes: and he holds that syndromes must be mutually exclusive, therefore there cannot be a schizo-affective syndrome but "it would, however, be possible for a particular individual to fall into both classes". Where psychiatrists have most trouble in classification, Dr. Foulds's scheme likewise tends to run aground; for example, in distinguishing the varieties of neurotic disorder.

There is, in the main, however, remarkable agreement between the findings arrived at here by the psychologist, determinedly systematic in his procedure, and those of the psychiatrist, using all the information he can and obliged to turn his diagnoses to practical account in each individual patient. Dr. Foulds, in a characteristic aside, says that until the structure of an experimental science of abnormal behaviour is complete, "psychiatrists should bear the confirmation of their observations with benign resignation".

AUBREY LEWIS

MANAGEMENT FOR METALLURGISTS

Management and Cost Control in the Metallurgical Industry

(Lectures delivered at the Institution of Metallurgists Refresher Course, November, 1964.) Pp. x+105. (London: Iliffe Books, Ltd., 1965. Published for The Institution of Metallurgists.) 25*s*. net.

MANAGEMENT and Cost Control in the Metallurgical Industry is comprised of lectures delivered at the Institution of Metallurgists in November 1964. The lectures were: "The Role of the Manager", by E. F. L. Brech; "Management and Cost Accountancy", by J. H. Spooner; "Industrial Relations", by Lord Geddes; and "A Project Officer's Diary", by R. L. Berry.

Mr. Brech attempted to distil his many years of experience as a management consultant into twenty-odd pages, including two appendixes entitled "A Definition of Management" and "The Theory of Management". The latter are a useful record, but until one has had experience it is difficult to turn the precise phrases into the flesh and blood of management in practice. It is bound to be true that the role of the manager varies between organizations, and particularly with the size of the organization and the extent to which technological advance has made an impact on it. It would have been helpful in addressing a group of technologists and scientists to discuss these points and to consider the presently changing role of management.

In the lecture "Management and Cost Accountancy", Mr. Spooner presented the results of twenty years' experience of the United Steel Companies in the development of their standard cost system. This talk gives a useful insight into the objectives of their cost control system and the means by which these are to a greater or lesser extent achieved. An audience of technologists would probably have been interested to hear more about the basic relationships underlying the cost structure of an industrial organization, since without this knowledge a manager is less able to interpret the true meaning of the data presented. Under what circumstances is it reasonable to assume that the total cost/output relationship is linear and the marginal cost per unit of product constant?

Lord Geddes's talk consisted of his presentation of the general case for the Trades Unions in industrial behaviour. This is a most valuable talk for the person with little experience of industrial relations in industry. The speaker managed to get away from the traditional 'two sides' approach to such problems and to explain the basic aims of Union activities. This talk should be read by those on both sides who are inclined towards a stereotyped view of the roles of management and Unions.

In the final talk, Dr. Berry, a director of I.M.I. Ltd., described the changing role of the manager as a particular project progresses from research, through development and pilot plant operation, to full-scale production. The talk suggests how the relationship of the project officer with other functions in the organization must develop, and how his role must change as increasing emphasis is placed on production. The research technologist who may transfer into production management by this route will find the talk most helpful. Dr. Berry not only signposted the route, but also the way in which the individual must change the emphasis that he puts on the qualities and abilities he possesses in order to become a good manager.

In summary, this is a mixed bag containing some useful material and some which would have been more so if it had been aimed more particularly at the needs of a specialist audience. The lack of obvious orientation is reflected in the discussions after the lectures which rapidly got down to the details of the subjects presented rather than retaining the broader overall perspective. Some bibliographies would have been helpful.

J. BANBURY

DETERMINATION OF NITROGEN

The Kjeldahl Method for Organic Nitrogen

By R. B. Bradstreet. Pp. viii+239. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 76*s*.

THERE can be little doubt that the methods most frequently used for the determination of nitrogen in organic substances are based on that originally described by J. Kjeldahl in 1883. Essentially the method involves the conversion of the nitrogen to ammonia by digestion with sulphuric acid, and titration of the ammonia. Speed and simplicity are the principal attractions of the method, but other useful features are its adaptability to multiple determinations and to aqueous solutions of nitrogenous substances, and its sensitivity to low levels of nitrogen. The defects are that many compounds, notably those in which nitrogen is linked to oxygen or to nitrogen, require special treatment which detracts from the essential simplicity, and there are a few types of compound which apparently fail to give correct values with any known modification of the method. Other methods for the determination of nitrogen, although usually applicable without modification to a wide range of substances, lack many or all of the attractive features of Kjeldahl's method. The combustion method of Dumas (1831), although accurate, is slow and complicated even in its micro-modification by Pregl (1912), nor is it of universal application, being unreliable with some heterocyclic compounds and unsuitable for materials containing trace amounts of nitrogen. The ter Meulen (1924) method, based on pyrolysis in a stream of hydrogen, overcomes these two latter objections but remains unattractively complex. The Kjeldahl method seems likely, therefore, to retain its popularity, and it is thus most important that both its potentialities and limitations be widely recognized.

Since the inception of the method, many investigations and modifications have been described, and the literature of the subject is vast and scattered. Thus it is often difficult especially for the inexperienced analyst to find the procedure most appropriate for the material under examination, and it is probable that many procedures in regular use are far from the best that could be devised.

The author of *The Kjeldahl Method for Organic Nitrogen* has set out to ease the task of the analyst by collecting in

monograph form as much as possible of the available information on the Kjeldahl procedure; and in this aim he has been very largely successful. The material is arranged in five main sections. After a short historical introduction, there is a chapter (74 pages) dealing with conditions affecting the sulphuric acid digestion of organic material, which considers, among other topics, acid requirements, effect of salt addition, oxidizing agents, catalysts, and reducing agents. The third chapter (50 pages) is concerned with digestion procedures appropriate to various types of material and also deals with sub-micro methods. In the fourth chapter (21 pages), methods for the determination of ammonia, with and without distillation, are considered. Finally, there is a useful classified bibliography of publications covering the entire period since the inception of the method.

The book is well documented with a numerical list of references at the end of each chapter and there is an adequate subject index, but the absence of an author index is, to say the least, irritating. References up to the year 1963 are listed.

Although there are few if any sins of commission, there are a few of omission. For example, the section dealing with procedures for soils is inadequate in that there is no reference to work later than 1937, and important contributions such as those by Alves and Alves in 1952 and Bremner in 1960 are not mentioned either in the text or in the bibliography. The subject of distillation apparatus would seem to merit more extensive treatment in the text than it has received, although it is well covered in the bibliography, but even here no reference could be found to the Hoskins distillation apparatus which is commercially available in the United Kingdom. Furthermore, there is no mention of adaptation of the Kjeldahl procedure to automatic analysis as described by Whitehead in 1961.

The text is clear and concise in style and the printing and binding are admirable. With only minor faults, this book brings together a mass of information on the Kjeldahl method and is undoubtedly worthy of careful examination by all investigators concerned with nitrogen in organic materials. One is, however, left with the thought that an extension of the book to include all methods for the determination of organic nitrogen would make it even more valuable; perhaps the author might consider this suggestion when a second edition becomes due.

R. I. MORRISON

JETS IN IDEAL FLUIDS

Theory of Jets in Ideal Fluids

By M. I. Gurevich. Translated from the Russian by Robert L. Street and Konstantin Zagustin. Pp. xvi + 585. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 120*s*.

ONLY the word 'jets' in the title of *Theory of Jets in Ideal Fluids* needs amplification, the other words need only emphasis. The author uses the term 'jet-flows' to include any flows with free streamlines, flows caused by hydrofoils and separated flows as well as the kinds of flow more ordinarily thought of as jet-flows, that is, flows from orifices. The book is certainly about theoretical investigations and exclusively about ideal fluids. The author's intention is clearly that of being a compiler of work carried out under the heading which is his title, work which is otherwise scattered in many journals. He disclaims, properly, the aim of being encyclopaedic but he does not always make clear the principles on which he has based his choice of material.

The book appeared in Russian in 1961. More than half of it deals with two-dimensional, steady, incompressible flow. This material is therefore a long account of the many ingonious uses of conformal transformation techniques and is very much for the hydrodynamicist rather than the engineer-scientist working with real fluids. After this long section the final four chapters deal, in sequence, with: unsteady flows; jets in compressible fluids (a topic which perhaps just steps outside the narrowest definition of ideal fluids); axisymmetric flows; and a very short section (26 pages) on heavy fluids and surface effects. From the purely visual point of view it is not an easy book to read. It is produced by photographic reproduction of a typescript original, with the result that apart from the use of upper and lower case letters there is no variety whatsoever of type-face. This monotony of visual impact is increased by the minimal use of headings.

The translators claim, as one of the particular strengths of the book, the comparison of theoretical results with experimental data. This claim is, unfortunately, not justified. There is very little indeed of such comparison and therefore very little assessment of the value of the work in relation to the flow of real fluids. This claim, it must be emphasized, is made by the translators in their preface and not (except perhaps by a most oblique implication) by the author in his preface.

All the flows discussed in the book are ones of which the kinematics are those of shear layers in real fluid flows. Of course there is no consideration of shear forces, the fluid by hypothesis being inviscid, but the flows themselves are all examples of flows with steep velocity gradients. It is just in such flows that viscous effects are of great importance. Where, also, the pressure gradient is adverse, the flow of a real fluid may not be even qualitatively similar to that of an ideal fluid. This situation does make the detailed results of questionable value. If the combined effects of pressure forces and shear forces in real fluids result in flows at least qualitatively the same as the flow patterns of the ideal fluids discussed here, then some features of these ideal flow solutions can be of value. More precisely, it can be the case that the ideal fluid flow results may give a satisfactory outer boundary condition to which the shear layer solution of real fluid flows can be matched. Also, for thin shear layers, the prediction of the pressure field may be satisfactory.

There is an extensive bibliography with many Russian references. The proof reading has been rather rough; a quick glance at the list of authors shows Lord Kelvin referred to as 'Lelvin' and Lighthill has been given one of his degrees (B.A.) as his initials.

R. G. TAYLOR

ANALYSIS OF THE FLYSCH

Sedimentary Features of Flysch and Greywackes

By S. Dzulyński and E. K. Walton. (Developments in Sedimentology, Vol. 7.) Pp. viii + 274. (Amsterdam, London and New York: Elsevier Publishing Company, 1965.) 95*s*.

IT is easy to suppose that the authors of *Sedimentary Features of Flysch and Greywackes* had some difficulty in deciding on its title; and the layman could not appreciate that a previous volume in this series entitled *Turbidites* referred to the same group of rocks. This reflects a fundamental difficulty of definition. The name 'greywacke' is a petrographic term, enveloping a specific group of rock-types, which may loosely be defined as muddy sandstones. 'Flysch' describes an association of rock-types, now recognized as having genetic affinities, and as having been deposited in a particular tectonic environment—that of a deep trough in front of an advancing mountain chain. While many flysch sandstones are greywackes, they are not all; nor are greywackes confined to flysch. While a consensus agrees that turbidity currents are responsible for flysch, it must be conceded that this is not proved, that greywackes can originate by other processes, and that turbidity currents can operate in other environments than that envisaged for flysch. Nevertheless it must be admitted that there exists a genetic associa-

tion of rocks which recurs in a similar tectonic setting throughout geological time and in many parts of the world. A forbidding volume of literature has arisen around these rocks; and the authors have done good service in this timely conspectus.

As was to be expected of two premier experts in the field, this account is definitive and authoritative. It begins with an introduction in which the subject is outlined, and evidences of origin adduced; the authors come down strongly in favour of a deep-water, turbidity current hypothesis. There follow chapters in which the main features of the rocks are described: the descriptions are succinct and are illustrated by a very large number of excellent photographs. Throughout this section genetic implications are discussed, and this is further extended by the chapter on experimental investigations. Finally, the authors give two examples of palaeogeographical reconstructions based on their interpretations and here point the way for further developments in this field. The authors' coverage of the very extensive literature is complete, as is shown by the comprehensive bibliography.

There are perhaps two sources of disappointment. The first is that the treatment of some controversial issues is brief. The authors may well reply that in the limitations of space they could not be discursive: but this has meant that some very fundamental criticisms of the points of view of the authors, for example, those of Cummins (pp. 28–29) and Hubert (p. 253), are incompletely answered. The second omission is that there is scarcely a reference to calcareous flysch, and very little on turbidite conglomerates; yet both of these are widespread and an up-to-date synthesis of the scattered literature would have been welcome. However, perhaps this is to expect too much; we are fortunate in having this clarification of the central aspects of the problem.

J. E. PRENTICE

LIPIDS AND ATHEROSCLEROSIS

Metabolism of Lipids as Related to Atherosclerosis

A Symposium. Compiled by Fred A. Kummerow. Pp. xxxiv+300. (Springfield, Illinois: Charles C. Thomas, Publisher, 1965.) 14.50 dollars.

THE series of papers published in *Metabolism of Lipids as Related to Atherosclerosis* constituted a symposium held on the occasion of the dedication of the new Burnsides Research Laboratories at the University of Illinois, Urbana, in June 1963. Twenty-six scientists and clinicians from the United States and two from Canada contributed, and together they construct a most varied and stimulating picture of modern research into lipid metabolism. The relationship of their researches to atherosclerosis is close in some cases, and not so immediately obvious in others, but there can be no doubting the high scientific standard of every paper. Many disciplines are represented; the challenges of clinical medicine and epidemiology are met by pathologists, histochemists, biochemists, physical chemists, experimental pathologists and others. Modern techniques have opened new avenues of exploration: chromatographic techniques such as thin-layer or gas–liquid chromatography, infra-red and ultra-violet spectroscopy, electron microscopy, radioactive tracer methods and many other recent tools all show their value.

Dr. Irvine Page, in an introductory chapter, warns against enslavement to sophisticated instruments, but the preliminary thought and planning evident in these published papers render the warning unnecessary, and the results show that the instrument is more often the productive slave. Several of the studies are of a speculative nature and necessarily incomplete or inconclusive, but they all point to the possibility of an exceptionally productive new era in lipid research.

Dr. Louis Katz deals with clinical aspects of atherosclerosis, with particular reference to factors which increase the risk, and Dr. G. V. Mann outlines the present position of dietary treatment. The natural history of atherosclerosis, from the fatty streaks of infancy onwards, is discussed by Hartroft of Toronto and by McGill and his colleagues from New Orleans. Kummerow's group throw fresh light on the composition of the extractable and bound (so-called 'ceroid') lipids of the human aorta, and also on lipoprotein denaturation. There are three chapters on the physiological and pharmacological control of cholesterol metabolism: van Itallie and Hashim concentrate on serum cholesterol, Steinberg on cholesterol synthesis and Kritchevsky on the oxidation of cholesterol by rat liver mitochondria. The subjects of other chapters include the relationship between low-density lipoproteins; cholesterol and triglycerides; the synthesis of fatty acids; studies on phospholipids; essential fatty acids; poly-unsaturated fatty acids; coenzyme Q (ubiquinone); and an antithrombin and heparin co-factor. Klein examines the behaviour of sterols at interfaces, in a search for clues to enzymatic reactions and processes involved in the assembly of lipid structures.

Only the lipid chemist will understand the import of every paper, but all who are trying to apply basic knowledge of lipids to biological and clinical situations will appreciate this fine book. It certainly points the way for much future research; since the symposium took place two and a half years ago, much of this 'future research' is presumably well advanced by now. The almost exclusively American references in the various chapters are a tribute to the pace of lipid research in the United States, but workers in other countries perhaps deserve rather more recognition.

The book is compact, very well produced, well indexed and easy to read—altogether an attractive volume.

T. B. BEGG

MAINLY FERREDOXINS

Non-Heme Iron Proteins

Role in Energy Conversion. Edited by Anthony San Pietro. (A Symposium sponsored by The Charles F. Kettering Research Laboratory.) Pp. xiv+475. (Yellow Springs, Ohio: The Antioch Press, 1965.) 12.50 dollars.

THE Charles F. Kettering Research Laboratory is well known to workers engaged in the investigation of photosynthesis. It is becoming known to an even wider circle of research workers through its published symposia, of which *Non-Heme Iron Proteins* is the second. This volume describes the proceedings of a meeting held in March 1965, which included contributions by many of the leading workers in the fields under discussion. It is unfortunate, therefore, that this excellent volume suffers in several respects from a lack of purpose. Ostensibly concerned with non-haem iron proteins, it is in fact mainly concerned with ferredoxins. Ostensibly concerned with the part played by these proteins in energy conversion, this aspect is scarcely touched on, as one of the chairmen was led to remark. Several papers seem out of place in their particular sections, and the order in which they are arranged could be improved on.

These are more than minor irritations, since in general the papers are well written and contain valuable summaries of work in progress. However, they are presented in such a way as to make it extremely difficult to locate specific information (there is, of course, no index). It is impossible to list all the contributions in detail, but I shall try to summarize the contents of the four sections into which the symposium was divided.

The first covers the physical and chemical properties of iron compounds in general, and includes contributions on electron paramagnetic resonance and Mossbauer spectro-

scopy, and on optical rotatory dispersion. This is a most valuable compilation of information on these relatively new and less well-known techniques.

Next follows a section on photosynthesis, concerned mainly with ferredoxin. The papers fall into two groups, dealing with electron transfer and synthetic reactions respectively. Reviews of soluble protein factors in chloroplast electron transport, and of a ferredoxin in photosynthesis, summarize the development of present knowledge, and are followed by several papers on synthetic pathways involving ferredoxin, one on a proposed model of ferredoxin, and one on the kinetics of ferredoxin reduction.

Section 3 deals with nitrogen fixation. Although one can see the connexion with non-haem iron proteins in general, and with ferredoxin in particular, one cannot help feeling that this section was largely off the main line of interest of the symposium. Equally this difficult field still seems to lack sufficient information of a kind to stimulate the attention of the non-specialist reader. The papers report various experiments on aspects of nitrogen fixation, with little interrelationship between them.

The final section deals with a number of dehydrogenases, mostly from cell particles. Again, these are related only by that and the general theme of the section.

There is no record of discussion or comment, and the papers are apparently reproduced essentially as they were presented at the symposium. The remarks of the section chairmen are introductory, and not a summing up of the material presented. One has to rely on reading the individual papers to get an evaluation of the present status of each problem, and one is denied access to any record of the argument and discussion which are often among the most valuable results of such meetings.

The book itself is well produced and seems remarkably free of printing errors. In general there is little that one would wish to comment on so far as the content is concerned. The papers are largely concerned with experimental work, of which this is essentially a progress report, and which should be read in that light. The presentation might have been better, but nevertheless the book provides a much-needed summary of present work on iron proteins, particularly the ferredoxins, and is recommended to all workers interested in the role of iron in biological systems.

M. H. SMITH

VIRUSES, MYCOPLASMAS AND LEUKAEMIA

Methodological Approaches to the Study of Leukemias

Edited by Vittorio Defendi. (A Symposium held at The Wistar Institute of Anatomy and Biology, April 5 and 6, 1965. Symposium Monograph, No. 4.) Pp. ix+225. (Philadelphia: The Wistar Institute Press, 1965.) 7.50 dollars.

SINCE the discovery by Gross in 1951 that leukaemia in a particular strain of mice could be induced by a virus, the search for viruses as the cause of malignant conditions has become a major trend in cancer research. The electron microscope has revealed, in numerous experimental animal tumours, virus-like particles, the oncogenic properties of which have been proved by the injection of cell-free tumour extracts into suitable recipients. With the introduction of new techniques, the number of transplantable animal tumours in which such viruses have been found is increasing rapidly. Furthermore, it has also been found that malignant transformation of cells grown *in vitro* can be initiated by the introduction of virus into the culture. It was a natural development therefore that the search for oncogenic viruses should be extended to human tumours, especially to leukaemia, utilizing the methods used with success in the study of experimental animal leukaemias. *Methodological Approaches to the Study of Leukemias* contains the proceedings of a symposium which aimed at discussing how the various techniques could be used in the search for viruses in human tumours and at spot-lighting the many difficulties, both methodological and interpretative, which beset an investigation of this kind.

Some of the participants dealt with cell kinetics, stem cell differentiation and chromosome anomalies in relation to leukaemia, but most of the contributors focused their interest on subcellular particles shown by electron microscopy to be present in tumour cells cultured *in vitro* or in cells obtained from primary neoplasms. Such particles were identified in cultures of Burkitt's lymphoma or observed in the plasma from leukaemic patients. The principal points of discussion were whether these are the agents which are responsible for the malignant behaviour of cells, whether they are latent viruses which manifest themselves because of the leukaemic condition, or whether they should be looked on only as secondary invaders.

Several contributions were devoted to the mycoplasmas, the smallest micro-organisms so far observed which contain all the enzyme systems and genetic mechanisms compatible with free-living existence on agar. Mycoplasmas are known to be the cause of at least one type of pneumonia in man. This class of organism was first isolated from human tumours in 1937, but gained much importance only recently when mycoplasmas were identified in tissues of leukaemic patients. At the symposium much information was given about the morphology and metabolism of these micro-organisms, which are encountered in the most diverse pathological states. It was admitted that the possible presence of mycoplasmas in tissues under examination makes it even more difficult to investigate the role of viruses in leukaemia. The participants agreed that only future research can determine whether mycoplasma or virus have in fact anything to do with the aetiology of human leukaemia. The proceedings of the symposium are nevertheless a valuable source of information concerning the application of electron microscopy to leukaemia research, and from the volume the reader will also learn about the many potential pitfalls present in such an approach.

P. C. KOLLER

THE ASPERGILLI

The Genus *Aspergillus*

By Prof. Kenneth B. Raper and Dorothy I. Fennell. With a chapter on Pathogenicity by Peter K. C. Austwick. Pp. ix+686. (Baltimore, Md.: The Williams and Wilkins Company, 1965. Distributed in the U.K. by E. and S. Livingstone, Ltd., Edinburgh.) 160*s*. net.

THE aspergilli are a ubiquitous, prominent and notorious component of common mould. They occur everywhere in soil and compost, and cause deterioration of many materials including fabrics, leather, paper and foodstuffs; their fermentation products include sauces and alcoholic drinks, antibiotics and citric acid, and they find a use as a biochemical tool for the detection of minor elements in soil. In addition, some are pathogenic for man, animals (particularly birds) and plants, and others produce toxins which poison livestock and possibly induce cancer in man, while their spores may act as airborne allergens. As aspergilli are of such economic importance and because they are easily recognized in their asexual (imperfect or conidial) state, grow well in culture, and are both macroscopically and microscopically attractive organisms, they have received much attention during the past two hundred years.

The Genus Aspergillus is a third generation monograph. In 1926, Thom and Church published *The Aspergilli*, a volume of 272 pages in which sixty-nine species were accepted; this was replaced in 1945 by Thom and Raper's *A Manual of the Aspergilli* (373 pages, 77 species)

which, in its turn, has been superseded by the present work (686 pages, 132 species). This pattern reflects the steady growth of mycology, and each monograph has in its turn raised the study of the genus *Aspergillus* to a new level.

The most recent version is basically a much enlarged and improved edition of its immediate predecessor. There are well-illustrated descriptions of the species, arranged in eighteen groups each with its own specific keys, while two keys, based primarily on morphology and colour, respectively, are provided for the preliminary sorting into groups. After an excellent and characteristic portrait of the late Charles Thom (1872–1956), to whom the book is dedicated, there are introductory chapters on general aspects of the genus, methods of cultivation, etc. Here, as a new feature, is the most useful chapter by Austwick on "Pathogenicity", but pathogenicity to man and animals only it should be noted, the plant pathogenic members of the genus having to be laboriously sought under the different species. In conclusion there are lists of the twenty-eight generic and more than one thousand specific names which have at one time or another been applied, correctly or incorrectly, to aspergilli, a list of species and varieties at present accepted, a 54-page general bibliography, and an index.

One point that must be regretfully noted is that, as in the *Manual*, insufficient attention is paid to the International Code of Botanical Nomenclature which prescribes the present conventions for ordering the scientific names of fungi. It is surprising that in this comprehensive account of the genus the correct author citation for the validation of the name *Aspergillus* under the Code—Micheli ex Fries, 1821—does not appear. This is presumably because, if it did, it would have embarrassed the authors who use the name of *Aspergillus* for both sexual and asexual states while the name was validated for the asexual state only. This is not an occasion to elaborate the point. The Code is not penal, authors may do as they please, but when they infringe the Code they may find themselves penalized by the names and usages they personally prefer not being given general acceptance.

This technical blemish will not affect the sales or the popularity of an outstanding monograph. Everyone must agree that the purpose of the volume "to facilitate the identification of the Aspergilli by providing descriptions of the recognized species together with appropriate keys" has been most successfully achieved.

G. C. AINSWORTH

BALSAM FIR

Balsam Fir

Abies balsamea (Linnaeus) Miller. By E. V. Bakuzis and H. L. Hansen. (A Monographic Review.) Pp. xix+445. (Minneapolis: The University of Minnesota Press; London: Oxford University Press, 1965.) 76*s*. net.

MUCH has been written about the balsam fir and first records go back 300 years. It is a major coniferous species of eastern North America and it reaches its greatest proportion of total growing stock in south-eastern Canada, especially in Newfoundland and the maritime provinces where it may constitute more than 30 per cent by volume of standing timber. In the United States the tree is most important in Maine and to a lesser extent in New Hampshire, Vermont and New York. Because of its technical properties, balsam fir does not rank as a superior timber, but high-quality pulp and paper products can be manufactured from high-quality pulpwood. No longer is it an important lumber in the United States, but its use for fibre continues to increase. Indeed, some pulp and paper mills formerly geared to spruce as the main raw material for quality products have reorientated their processing technology to use more balsam fir. The position this species holds in the timber industry is indicated by what is described as a conservative estimate that the total merchantable volume of balsam fir in both Canada and the United States is about 36 billion cubic feet. Production figures on a national scale, embracing as they do a variety of sites and intensities of management, are not always very reliable, but even the range of 15–80 cubic ft. per acre represents a vast amount of timber increment. From the blisters on the tree an oleoresin is collected and this Canada balsam has long been known in the scientific world for optical work and for mounting media.

It is usually interesting to learn what caused the initial impetus to compile such a useful work as *Balsam Fir*. In this case it was the uncertainty of the successional role of balsam fir that was the basis for the recommendation to the University of Minnesota School of Forestry, by the Quetico-Superior Wilderness Research Centre Advisory Committee in 1953, that a complete literature survey be made of all research work conducted on this species. The various bodies which sponsored the work and the team responsible for collecting the scattered information and integrating it into one volume must be thanked and congratulated. The result is a coherent picture of balsam fir both in Nature and in forestry practice. This is no mere catalogue, but a most readable account of an interesting and important tree species.

The first three chapters are devoted to the botany, synecology and ecology of balsam fir. Although accepted as a single species, it is possible that more detailed taxonomic work will show that variations exist throughout its wide range of distribution, but the boundaries have not been well established in all the areas where it occurs. Balsam fir is essentially a species of the boreal region, and, even away from it, it retains its boreal character as expressed by its tendency to occupy moist and cool sites. Complex inter-relationships in its ecology have still to be worked out. The chapters on microbiology and entomology indicate that more knowledge is required on the fungi and insects associated with balsam fir. Due to the spruce budworm attacks having reached epidemic status at times, a great deal of attention has been concentrated on this insect. The silviculture of balsam fir is described in two chapters, and many of its characteristics are influenced by its great seeding ability and its shade tolerance. Chapters on growth and yield and on utilization conclude the study. Lists of associated fungi and insects are given and the 1,381 references are appropriately grouped by chapters. Not only has this most useful publication succeeded in its aim but it also directs attention to where further research is needed. Forest biologists will be glad to have such a complete reference work.

C. J. TAYLOR

NATURE'S HANDICAP: THE ANATOMY OF PLANTS BEGUN

The Anatomy of Plants

With an Idea of a Philosophical History of Plants and several other Lectures read before the Royal Society. By Nehemiah Grew. Reprinted from the 1682 edition, with a new Introduction by Conway Zirkle. (The Sources of Science, No. 11.) Pp. xviii+24+304+83 tables. (New York and London: Johnson Reprint Corporation, 1965.) 35 dollars.

SOME of us who were introduced to botanical science by the scholarly exponents of an earlier generation may have nostalgic memories of being shown treasured first editions of Hooke, Grew and Malpighi—the founders of plant anatomy, who did their work in the middle to later decades of the seventeenth century. A few of us, perhaps, still recollect how good, all things considered, some of the illustrations seemed to be. But, typically, we probably took passing note of the musty smell of the

old tomes and then put them aside with the thought that it was nice to know that we had such works in the university library. For me, at least, it has been an agreeable and refreshing experience to look into the excellent reprint of the 1682 edition of Nehemiah Grew's *Anatomy of Plants*, originally read before the Royal Society of 1672 and now reappearing as No. 11 in "The Sources of Science" series.

In a short but excellent introduction, Prof. Conway Zirkle, of the University of Pennsylvania, has reminded us of things that we ought not to have forgotten, and has directed our attention to some new and critical facets of these early studies of plant anatomy. He reminds us, for example, of the odd and remarkable fact that the microscopic study of plants and animals might well have been begun at a much earlier date, for the optical means were already available. A specially valuable part of the introduction is his critical assessment of the contributions made by the three primary exponents. Hooke, with his *Micrographia* published in 1665, was first in the field. He showed what could be achieved by microscopy over a wide range of materials, but his contributions to plant anatomy, although excellently illustrated, were considerably less fundamental than those of Grew and Malpighi. Between these two it was a close thing: their respective studies were submitted for publication almost simultaneously, and Prof. Zirkle has been careful and cautious in handling the relevant questions of priority and alleged plagiarism. The circumstances of the simultaneous publication of two major works make fascinating reading. So also do Grew's accounts of, and reflexions on, the structural features which he observed in his anatomical studies. Among others, for example, he gave us the term "parenchyma"; and some of his morphological observations were very much to the point.

As to the contents of Grew's book, it is with renewed surprise that one realizes how systematically thorough he was in his work and how direct and lucid in his writing. There is still an agreeable freshness, indeed modernity, in his 'discourses'. Thus, in 'the epistle dedicatory' to Charles II, he referred to the precision of the "mathematic lines of a flower"; and he stated explicitly that all we perceive in a plant, the miracle of "Nature's Handicraft" to be seen in the tissues of the meanest walking-stick, the ascent of sap, the distribution of air and of substances in plants and, indeed, many other aspects of vegetation, "are all contrived and brought about in a mechanical way". So we enter into a new world "whereof we see no end". The numerous original illustrations, here reproduced with fidelity, are still impressive because of their observational content, clarity and, not least, their sheer artistic quality. Of course, at 35 dollars, this volume is something of a collector's piece; but many would, undoubtedly, like to possess it. It might be salutary, and it would certainly be no bad thing, if it were made available for at least occasional student reference. C. W. WARDLAW

BIOLOGICAL CONTROL OF ROOT DISEASES

Ecology of Soil-Borne Plant Pathogens

Prelude to Biological Control. Edited by Prof. Kenneth F. Baker and Prof. William C. Snyder. (An International Symposium on Factors determining the Behavior of Plant Pathogens in Soil, held at the University of California, Berkeley, April 7–13, 1963.) Pp. 571. (London: John Murray, 1965.) 105*s*. net.

DISEASED roots of crop plants are less amenable to chemical control than diseased leaves, and they are also more difficult to examine, if only because soil is opaque and complex. Applying fungicides and sterilants on a field scale is usually prohibitively expensive; consequently the possibility of biological control by manipulating the microflora becomes correspondingly attractive, and, although successful in only a few cases with present knowledge, much is promised for the future.

As the editors point out in the foreword to *Ecology of Soil-Borne Plant Pathogens*, exploration of this difficult subject "awaited the overcoming of . . . compartmentalization of the scattered, diffuse, isolated and uncorrelated knowledge" in the disciplines of plant pathology, microbiology, soil science, plant physiology, plant anatomy, biochemistry, bacteriology, nematology, mycology, virology and zoology. For this reason, the Agricultural Board of the National Academy of Sciences–National Research Council, Washington, sponsored an international symposium on factors determining the behaviour of plant pathogens in the soil, with the object of assembling a group of specialists to compile and discuss existing information in preparation for further advances towards biological control. The conference was held at Berkeley, California, in April 1963. Generous financial aid brought together more than forty experts to present formal papers before an invited audience of more than three hundred participants from twenty-four countries.

The papers and recorded discussions are now published in a handsome (and weighty) volume, edited by two of the leading spirits in the group which initiated the conference. The resulting volume provides an invaluable course on the principles of soil microbiology in relation to root diseases. Individual papers are usually introduced by a critical survey of relevant literature, leading on to an exposition of the special topics studied by the author with an account of his own researches and conclusions, and ending often with a more speculative section that could stimulate further developments. There are copious bibliographies and, as inevitable in a symposium of this kind, there is some repetition because certain key papers are cited again and again, as the work of some authors is discussed from different angles.

Readers of the half-million or so words of this book will agree with the opening speaker, Victor R. Boswell, that it represents a landmark in biology. The first main paper, by S. D. Garrett, deals with fundamental concepts and prospects for the future of biological control of root diseases. Thereafter groups of papers deal with topics of increasing complexity. Soil microbes and their behaviour are surveyed by A. Burges, D. K. McE. Kevan, J. H. Warcup, J. M. Hirst, D. Park, A. S. Sussman and J. D. Menzies. Then the soil is considered as an environment for microbes by W. A. Raney and H. D. Chapman. Next, attention is focused on the host plant, particularly its roots and rhizosphere, by Flora M. Scott, H. G. Burström, A. D. Rovira, H. Katznelson, D. J. D. Nicholas, J. L. Harley, P. S. Nutman and A. Kerr. Host and pathogen are then brought together in a series of papers by N. T. Flentje, I. W. Buddenhagen, W. B. Mountain, C. H. Cadman, J. C. Walker, J. E. DeVay and I. A. M. Cruickshank. Mechanisms of microbial antagonism are considered by F. E. Clark, R. A. Fox, R. M. Jackson, M. G. Boosalis and R. Mankau. Theoretical aspects of soil inoculum are developed by R. Baker, A. E. Dimond and J. G. Horsfall. The final section is the most complex and deals with interactions between soil organisms and crop plant in a series of papers by Mary D. Glynne, G. C. Papavizas, Z. A. Patrick, T. A. Toussoun, T. S. Sadasivan, R. A. Ludwig, G. W. F. Sewell, W. A. Kreutzer, S. Wilhelm and A. W. Dimock.

The editors have maintained an exacting standard throughout their formidable task of dealing with papers, discussions, bibliographies, and an index of thirty-six pages. For its wealth of material this splendidly produced volume is a necessity in every centre where plant pathology is taught or practised. As one consequence, the Berkeley symposium has stimulated plans for the First International Congress of Plant Pathology in London in 1968. Another consequence will be eager searching of its records for clues to help fill hungry bellies.

P. H. GREGORY

Science and the Manager

By Prof. R. W. Revans. Pp. xi+160. (London: Macdonald and Co. (Publishers), Ltd., 1965.) 25*s*. net.

IN *Science and the Manager*, Prof. Revans has collected eight essays, written during the past 20 years and addressed to various types of readers. They have been pruned of material which is no longer topical or relevant, but there are neither references nor index and the book is best regarded as essentially new. Prof. Revans himself suggests that the essays illustrate three common themes: the need to apply the methods and attitudes of experimental science to industrial practice; the subsequent emergence of exceedingly difficult problems which require more than known scientific method to solve them; and the comparatively elementary character of the study of management and the development of theory from such study. Even more strikingly, the importance of clarity of thought and expression is emphasized throughout the book and exemplified in Prof. Revans's own prose, pointed and sometimes pregnant with prophetic authority. Beginning with a study of the sampling of attitudes on the shop floor, which strongly emphasizes this point about effective communication, the essays deal successively with decisions, communications and morale; science and the manager; technical knowledge; the pathology of automation; the scope of management control; industrial relations and industrial training; and the nature of operational research.

Prof. Revans's sense of the possibilities of using science in management is matched by his sense of the limitations of the scientific method as well as a deep respect for human personality, and he has a rare capacity for going to the root of things. This study of technical knowledge and the essay on industrial relations and industrial training are perhaps the best in the book and highly pertinent to Britain's present difficulties, outlining what needs to be done immediately. With so much good sense, constructive criticism and insight in this short volume, one wonders what Prof. Revans might have given us had he set out to discuss the technological society on the scale that Jacques Ellul has done. Perhaps he may yet give us a worthy successor to Bavink or Mumford. R. BRIGHTMAN

The Tissues of the Body

By Prof. W. E. Le Gros Clark. Fifth edition. Pp. ix+423. (Oxford: Clarendon Press; London: Oxford University Press, 1965.) 55*s*. net.

THE appearance of another edition of *The Tissues of the Body*, the fifth since its original publication in 1939, is itself some indication that this is no ordinary text-book. Sir Wilfrid Le Gros Clark is, of course, well known for his many and varied contributions to human and comparative anatomy, which gave impetus to the happy revitalization of academic anatomy that is now so much in evidence. Earlier editions of this, probably his best-known book, have exerted an intellectually stimulating effect on many medical students for whom the subject of anatomy might otherwise have seemed as inert as the dissecting-room specimens. In a direct and readable way the book succeeds in conveying the notion of anatomy as an exciting subject, with obvious relevance to bodily functions in health and disease. The author's wide experience of research is evident in the enthusiastic presentation of ideas and theories amenable to experimental investigation, rather than depending on mere preoccupation with morphological minutiae.

The present edition consists, as before, of thirteen self-contained chapters showing anatomy as basically the examination of biological organization at all levels: the cell, the tissue, the organ, the functional system and the integrated organism. The reader is offered not just factual information but reasoned interpretations based on the available evidence, with frequent indications of the actual limits of existing knowledge. There are well-chosen references in the text, which may encourage the student to develop at an early stage the habit of looking up original work. The opportunity has been taken, with little increase in length, to revise many sections in the light of recent findings; arising especially from the application of newer techniques in the field of cell biology. A few electron micrographs, of adequate but not inspiring quality, are now included in the illustrations. One cannot help feeling that perhaps more should have been done to emphasize the phenomenal scope of recent developments at the level of anatomical organization that is represented by molecular and cell biology. Nevertheless, this admirable book remains virtually in a class of its own, and can be recommended unhesitatingly to students of medicine and biology. J. A. ARMSTRONG

Neurospora Bibliography and Index

By Prof. Barbara J. Bachmann and Prof. W. N. Strickland. Pp. vii+225. (New Haven and London: Yale University Press, 1965.) 4.00 dollars; 30*s*.

THE fungus *Neurospora crassa* is of outstanding significance in the development of biochemical genetics and it is attracting increasing attention as a research tool. It is therefore of very great value that a bibliography and index has been prepared as a guide to its literature. The first section of the volume is comprised of the bibliography itself, listing 2,310 papers and taking the record to January 1964. The references are listed alphabetically and each one is assigned a code number which is used in the index to refer to the paper. The second section is an author cross-index. The third section indexes the subject matter of all papers in as much detail as has seemed useful. It is divided into three parts, a general index of subject matter, a mutant index and an index of *Neurospora* species other than *N. crassa*. Tests of the index have shown how effective it is. It appears from the preface that the indexes were the work of Dr. Bachmann, while the compilation of the references was done mainly by Dr. Strickland. Those who use this book (I hope a growing number) will realize what a great debt is owed to the authors. They have provided an easy guide by which all information on a great variety of subjects can be quickly traced in a complex literature.

D. G. CATCHESIDE

Bananas

By N. W. Simmonds. Second edition. (Tropical Agriculture Series.) Pp. xvi+512+106 photographs. (London: Longmans, Green and Co., Ltd., 1966.) 63*s*. net.

WHEN I reviewed the first edition of N. W. Simmonds's book *Bananas* (*Nature*, 186, 48; 1960), one of the "Tropical Agriculture Series", issued under the editorship of Mr. D. Rhind, I stated, with knowledge and conviction, that no book of equal status on this subject had hitherto been published. With the appearance of the second edition, I see no reason to change this view. However, a great deal that is new and interesting has happened in the banana industry since that time, and much of this has been successfully incorporated in the new edition. Accordingly, to those who are closely concerned with banana growing and all that is now involved in the harvesting, packaging, transport and ripening of this sometimes fickle and always delicate crop, the new edition will commend itself as affording ready access to the agricultural and scientific innovations in a highly competitive industry. In fact, it is quite surprising how many new and unexpected developments there have been. The preparation of the present volume has therefore involved extensive revision of the original text, the skilful splicing-in of critical new material at various points, and the incorporation of other quite new information of various kinds in a supplementary chapter—an overall enlargement of some forty-six pages. Like the first edition, this volume will prove instructive to a wide range of readers.

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QUASARS — A MINORITY REPORT

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IT is generally accepted that the large red shifts observed in the spectra of quasi-stellar radio sources (hereafter called quasars) are due to the radial recession of these objects. According to the most widely accepted present view, these red shifts are of cosmological origin, and the resulting distances and luminosities of quasars are so enormous that the primary mechanisms responsible for their energy release are not yet understood within the framework of known physical processes. Proponents of a less popular view argue that quasars could be 'local' phenomena. According to one version of this local picture, the observed Doppler shifts of quasars are the result of recessional velocities imparted to these objects by a gigantic explosion that occurred in the nucleus of our Galaxy in the relatively recent past. This interpretation was first proposed by Terrell[1], and its major advantage lies in the fact that it implies energy requirements for quasars that can be very much less than the energy requirements on the cosmological picture.

It is fair to say that, as yet, no clear-cut case has been made in favour of either interpretation. Recent observational developments, however, justify a reconsideration of the fundamental problem of the distances of quasars. In this article we hope to make some progress towards resolving this question by using accepted procedures of classical astronomy.

Our first task is to attempt to put a reasonable upper bound on the distance of 3*C*273 *B*. Recent radio observations[2] indicate that 3*C*273 *B* undergoes variations in its radio flux having periods of the order of a few months. The simplest conclusion to be drawn from this result is that the diameter of 3*C*273 *B* is considerably less than one light-year. If this were not the case, the differences in light travel times between light emitted from different portions of the object would tend to smooth out radio variations resulting from pulsation of the object as a whole. We are fully aware that it is possible to construct more complicated models of quasars that exhibit variability[3]. However, in attempting to explain a new phenomenon it is desirable first to rule out the possibility of using a conservative, simple model, before considering more bizarre models. Therefore, we assume, provisionally, that the variations in radio flux are caused by oscillations of the radio source as a whole, and we adopt 0·5 light-years as a reasonable upper limit for the diameter of 3*C*273 *B*.

Recent, long-baseline interferometry at Jodrell Bank has established that the radio source 3*C*273 *B* is probably double, and that each component has a diameter of $\sim 0{\cdot}1$ sec of arc[3,4]. Apparently, the components may not have been completely resolved, but for want of a better figure, we will assume that the pulsating radio object subtends 0·1 sec of arc. This implies that the distance to 3*C*273 *B* is about 320 kiloparsec (kpc). At this distance, the proper motion of 3*C*273 would be $\sim 0{\cdot}0015$ sec/yr, which is less than the mean errors of $\pm 0{\cdot}0025$ sec/yr assigned by Jefferys[5]. Thus, our crude estimate of the upper bound for the distance to 3*C*273 using variability arguments is very nearly equal to the lower distance limit set by proper motion studies (if 3*C*273 were ~ 192 kpc from the Sun, the proper motion would be about 0·0025 sec/yr). While this upper bound to the distance of 3*C*273 can be questioned legitimately on several grounds, it will be shown, in due course, that energy considerations do not permit our variability upper limit to be exceeded by more than a factor of 10 if the local hypothesis is to be retained. Consequently, we regard it as encouraging that our tentative upper bound to the distance of 3*C*273 lies between the absolute lower limit set by Jefferys's proper motion studies and the upper limit imposed by energy requirements. A summary of observational data for nineteen quasars with known red shifts is given in Table 1.

Burbidge, Burbidge and Sandage[6] have set forth arguments which suggest that a violent event may have occurred in the nucleus of our Galaxy in the relatively recent past. Therefore, Terrell's hypothesis[1] is a logical one to account for the local distribution of quasars—that is, quasars were ejected from the nucleus of our Galaxy $\gtrsim 4 \times 10^6$ yr ago. In Table 2, we have assembled the distances and visual luminosities of the quasars listed in Table 1 assuming the foregoing time scale of 4×10^6 yr. These luminosities and distances should be regarded as lower limits, since the time scale of 4×10^6 yr places 3*C*273 at the lower distance limit (~ 190 kpc) set by proper motion considerations.

On the cosmological picture, the total energy requirement for each quasar is rather large: at least 10^{60} ergs for 3*C*273 (ref. 7). On the other hand, the local hypothesis, as outlined in Table 2, leads to an absolute visual magnitude for 3*C*273 of $-8^{m}{\cdot}6$, which implies a total energy output in visible light of the order of 10^{54} ergs, if we assume it has radiated at its present rate for 4×10^6 years. At proton–proton burning efficiency, this energy corresponds roughly to the conversion of 100 $M_\odot$ of hydrogen to helium. However, since the visible light represents only a fraction of the total energy associated with quasars, an estimate of a total mass of 1,000 $M_\odot$ /quasar is perhaps more realistic. Using the velocities of Table 1, the average relativistic kinetic energy per quasar of this mass would be $\sim 4{\cdot}3 \times 10^{56}$ ergs. If we postulate that our Galaxy has ejected 100 quasars, then $\sim 4{\cdot}3 \times 10^{58}$ ergs would be contained in the kinetic energy of these objects. This figure may be compared with energies quoted for material in the exploding galaxies *M* 82 ($\sim 10^{55}$ ergs[14]) and *NGC* 1275 ($\sim 10^{61}$ ergs[6]).

If quasars are indeed local, then their distances cannot greatly exceed the distances quoted in Table 2, because the local interpretation requires that the energy output per quasar increases as the cube of the distance from the centre of our Galaxy (energy requirement = luminosity × time, luminosity $\propto r^2$, and time $\propto r$). Their masses must also be increased if a substantial increase in energy is required, and thus the kinetic energy will represent a

Table 1. OBSERVED PROPERTIES OF QUASARS

References are listed below by number. Magnitudes are photoelectric, except for 3C345, 3C380, and *MSH* 14–121, which are estimated *B* magnitudes

Source		Position						Velocity		Photometry					References	
Cat.	No.	h	m	°0	′	*l*	*b*	*z*	*v/c*	*V*	*B–V*	*U–B*	$-Kv$	*V*(0)	*Z*	photometry
3C	9	00	18	+15	23	112	−46	2·012	0·801	18·21	+0·23	−0·74	0·6	18 8	(1)	(2)
3C	47	01	34	+20	42	137	−41	0·425	0·340	18·1	+0·05	−0 65	0·3	18·4	(1)	(2)
3C	48	01	35	+32	55	134	−29	0·367	0·303	16·2	+0·42	−0 58	0·2	16·4	(1)	(2)
3C	147	05	39	+49	51	162	−10	0·545	0 410	17 8	+0·65	−0·37	0 3	18·1	(1)	(2)
3C	245	10	40	+12	20	233	+56	1·029	0·609	17·25	+0·45	−0·83	0·5	17 7	(1)	(2)
3C	254	11	12	+40	58	156	+61	0·734	0·501	17·98	+0·15	−0·49	0 4	18·4	(1)	(2)
3C	273	12	27	+02	22	291	+65	0·158	0 146	12 8	+0·21	−0·85	0·1	12 9	(1)	(2)
3C	279	12	54	−05	41	303	+57	0·540	0·407	17·75	+0·26	−0·52	0·3	18 1	(3)	(2)
3C	286	13	29	+30	40	56	+81	0·848	0·547	17·30	+0·22	−0·84	0·9	17·7	(3)	(2)
3C	287	13	29	+25	24	23	+81	1·055	0·617	17·67	+0 63	−0·65	0 5	18·2	(1)	(2)
MSH	14–121	14	53	−11	02	345	+41	0 938	0 579	17·5			0·4	17 9	(6)	(6)
3C	334	16	18	+17	44	33	+41	0·555	0 415	16·41	+0·12	−0·59	0·3	16 7	(3)	(2)
3C	345	16	42	+40	11	64	+41	0·595	0 436	16·3			0·3	16·6	(3)	(5)
3C	380	18	28	+48	43	77	+23	0·693	0·483	17·0			0 4	17 4	(3)	(5)
3C	446	22	23	−05	24	59	−49	2·07	0·808	18·4	+0 44	−0·90	0·6	19·0	(4)	(2)
	446							1·40	0·704						(4)	
CTA	102	22	30	+11	28	77	−38	1·037	0·612	17·32	+0·42	−0·79	0 5	17 8	(1)	(2)
0106	+01	01	06	+01	19	101	−59	2·095	0·81	18·39	+0·15	−0·70	0·6	19·0	(7)	(2)
1252	+11	12	52	+11	57	306	+75	0·87	0·555	16·64	+0·35	−0·75	0·4	17·0	(3)	(2)

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Table 2. DERIVED PROPERTIES OF QUASARS ON THE ASSUMPTION OF THEIR LOCAL ORIGIN IN A GALACTIC EXPLOSION 4×10^5 YEARS AGO

(1)		(2)	(3)	(4)	(5)	(6)	(7)	(8)
Cat.	No.	γ_c	$\gamma\odot$	Δm_{obs}	Δm_c	(Δm)	$\frac{L}{L_{3C273}}$	$\frac{L}{\bar{L}}$
3C	9	1,044	1,041	5·9	3·7	2·2	0·13	0 65
3C	47	444	438	5·5	1·8	3·7	0·03	0·16
3C	48	396	390	3·5	1·5	2·0	0·16	0·78
3C	147	535	526	5·2	2·2	3·0	0·06	0 31
3C	245	796	793	4·8	3·1	1·7	0·21	1 03
3C	254	652	648	5·5	2·6	2·9	0·07	0·34
3C	273	190	192	0·0	0·0	0·0	1·00	4·95
3C	279	531	534	5 2	2·2	3·0	0·06	0·31
3C	286	713	714	4·8	2·8	2·0	0·16	0·78
3C	287	805	807	5·3	3·1	2·2	0·13	0·65
MSH	14–121	757	764	5·0	3·0	2·0	0·16	0·78
3C	334	539	545	3·8	2·3	1·5	0·25	1·24
3C	345	570	573	3·7	2·4	1·3	0·30	1·49
3C	380	631	633	4·5	2·6	1·9	0·17	0 86
3C	446	1,053	1,056	6·1	2·7	2·4	0·11	0 54
3C	446	918	921	6·1	3·4	2·7	0·08	0·41
CTA	102	800	802	4·9	3·1	1·8	0·19	0·94
0106	+01	1,057	1,056	6·1	3·7	2·4	0·11	0·59
1252	+11	722	724	4·1	2·9	1·2	0·33	1 64

Column number: (1) Identification; (2) present distance from centre of Galaxy; (3) present distance from Sun; (4) observed difference in apparent magnitude, $m_{object} - m_{3C273}$; (5) computed difference in apparent magnitude, $m_{object} - m_{3C273}$, on the assumption that all quasars are of the same absolute magnitude; (6) difference in magnitude differences, $\Delta m_{obs} - \Delta m_c$; (7) ratio of luminosity of quasar to that of 3C273, on the assumption that $\delta(\Delta m)$ is due to a difference in absolute magnitudes between the object and 3C273; (8) ratio of luminosity of quasar to the average luminosity of all quasars, on the same assumption.

large fraction of the total energy of our Galaxy over a cosmological time scale. For example, if we increase the distance of 3C273 to 30 Mpc, the upper bound to the distance recently proposed by Terrell[8], the total energy requirement of 3C273 is more than 3×10^{59} ergs, its mass at least $3 \times 10^7 M_\odot$, and the kinetic energy of all quasars more than 10^{63} ergs. On energy arguments alone, therefore, a reasonable upper limit for the distance of 3C273 is ~ 1 Mpc, meaning that the violent event in the nucleus of our Galaxy must have occurred between a few $\times 10^6$ and a few $\times 10^7$ years ago, converting between 10^{58} and 10^{60} ergs into kinetic energy of quasars.

An additional consequence of the local picture is that no absorption immediately shortward of Ly_α due to intergalactic hydrogen is to be expected, since quasars would be in rapid motion in a hydrogen medium approximately at rest with respect to our Galaxy. Thus, the fact that spectra of some of the highest velocity quasars (3C9, for example) exhibit a lack of total absorption (and, quite possibly, a total lack of absorption[9]) to the blue of Ly_α supports the local interpretation. Whether or not absorption appears to be present in a given spectrum depends on how the continuum is extrapolated from the red to the blue side of Ly_α—a most uncertain procedure, since the intrinsic shape of a quasar's continuum is unknown. (We thank Prof. M. Schmidt for directing our attention to this fact[9].) If some absorption is present, and if the density of the intergalactic medium (assumed to be hydrogen) has the cosmological value of $\sim 10^{-5}$ atoms/cm^3, Rees and Sciama[10] have shown that the temperature of the intergalactic material must be a few $\times 10^5$ °K.

We are justified in ignoring evolutionary effects in evolving cosmological models because of the following happy circumstances: (*a*) if the universe expands adiabatically, then the temperature, T, and number density, N, of the intergalactic hydrogen are related by the expression $T \propto N^{2/3}$; (*b*) the cross-section/hydrogen atom for absorption of Ly_α quanta, σ, is approximately proportional to $T^{-1/2}$; (*c*) in the high-temperature range that we are interested in, N_1, the number density of neutral hydrogen atoms in the ground state, $\propto N/T^{10,11}$. Hence, the Ly_α adsorption/unit length, $N_1\sigma$, goes as $N/T^{3/2} =$ constant, and we arrive at the important conclusion that the Ly_α adsorption should be independent of the evolutionary stage of an adiabatically expanding universe. Thus, Rees and Sciama's temperature bounds for the intergalactic hydrogen in a steady-state universe should apply, approximately, to evolving models as well.

However, since the Ly_α adsorption estimates are uncertain, we are led to one of the following conclusions: (1) quasars are local; (2) if the density of the intergalactic hydrogen at the present epoch is 10^{-5} atoms/cm^3, its temperature must lie between a few $\times 10^5$ °K and 3–4 $\times 10^6$ °K, the upper limit set from X-ray observations[12]; (3) the present density of hydrogen in the intergalactic medium is substantially less than 10^{-5} atoms/cm^3, in which case the temperature bounds are less severe than those already quoted.

Thus far, the local explanation of quasars has been self-consistent, However, Koehler[13] has reported a remarkable observation which, if substantiated, will probably establish the cosmological origin of quasars. In the spectrum of 3C273, he has reported what may be a slight absorption in the 21-cm line of neutral hydrogen by a cloud moving at $\sim 10^3$ km/sec, having co-ordinates which very nearly coincide with the Virgo cluster of galaxies. Since the cosmological red shift of the Virgo cluster is $\sim 10^3$ km/sec, the natural interpretation would be to assume that the cloud is associated with the cluster. Hence, 3C273 would have to be >10 Mpc from our Galaxy, and energy considerations alone would compel us to accept the cosmological interpretation. Consistency with local origin can be obtained only in the unlikely cases that: (1) a hydrogen cloud has been expelled from our Galaxy at 1,000 km/sec in the direction of 3C273, or (2) a cloud of neutral hydrogen

has been somehow focused in the wake of 3C273 as it ploughs through the intergalactic medium.

In summary, the local picture of quasars is consistent with all the observational data excepting for Koehler's result. On the other hand, it is difficult to account for the lack of total absorption to the blue of Ly_α in such quasars as 3C9 on the cosmological picture. Since only a projection observation such as Koehler's can unambiguously confirm the cosmological nature of quasars, it is imperative that such observations be pursued for all quasars for which they are geometrically possible, using any techniques which might extend the accuracy to the limit necessary for this crucial confirmation. Also, in order to eliminate the possibility that the 21-cm absorption reported by Koehler is caused by neutral hydrogen in the wake of 3C273, a search should be made for 21 cm absorption in the spectra of selected quasars that do not appear in projection on a cluster of galaxies. If Koehler's observation is not confirmed, then Terrell's local hypothesis must be taken much more seriously than it appears to be being taken at present.

A possible observational test of the local hypothesis would be to search for faint objects having large ultra-violet excesses surrounding relatively nearby galaxies.

Note added in proof. Using arguments different from ours, Hoyle, Burbidge, and Sargent (*Nature*, **209**, 751; 1966) have concluded that "either the quasi-stellar objects are at distances of ~ 10 Mpc or less, or the physical model associated with these objects must be substantially different from the theories at present in vogue". Our findings tend to substantiate the first alternative.

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DISTRIBUTION OF QUASI-STELLAR RADIO SOURCES IN STEADY STATE COSMOLOGY

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ONE of the most intriguing problems in astronomy to-day is to understand the origin and structure of quasi-stellar radio sources. The starting-point for any discussion of this problem must be a knowledge of the distances of quasi-stellar radio sources. However, it has not yet been possible to measure these distances directly, and three general hypotheses have been proposed concerning the location of quasi-stellar radio sources: (i) they are objects in our own Galaxy; (ii) they are extragalactic but relatively nearby (1–10 megaparsecs (Mpc)); (iii) they are at cosmological distances (> ~ 300 Mpc). We shall call these the galactic, local and cosmological hypotheses, respectively.

When the first quasi-stellar radio sources were discovered it was assumed[1] that they were galactic, both because of their star-like appearance and because of the observed variations[2] in their optical brightness. This situation changed with the discovery[3] that the quasi-stellar radio source 3C 273 has a red shift z of 0·158. Of the fifty or so quasi-stellar radio sources now known, twenty-three have had their red shifts measured[4], and all these red shifts are larger than that of 3C 273—in many cases substantially larger. The possibility that these red shifts are gravitational in origin has almost certainly been ruled out[5], and we are left with the hypothesis that the red shifts are Doppler shifts, signifying that these quasi-stellar radio sources are receding from us with velocities comparable with the velocity of light.

If we are to retain the galactic or local models for these quasi-stellar radio sources, we must suppose that they are debris of a violent explosion which has occurred in our Galaxy or in a nearby galaxy. This possibility has been examined by Schmidt[6], Terrell[7], and Hoyle and Burbidge[8]. It now seems to be implausible in view of Koehler's[9] recent discovery that 3C 273 has a 21-cm absorption line in its spectrum, which is due to absorption by a neutral hydrogen cloud with a red shift $z \sim 3 \times 10^{-3}$, presumably associated with the Virgo cluster of galaxies. It follows that 3C 273 lies behind the Virgo cluster and so has a distance of at least 12 Mpc. Presumably, the other quasi-stellar radio sources, which have much larger red shifts, would be correspondingly more distant. It is therefore natural to adopt the cosmological hypothesis and to attribute the large red shifts of the quasi-stellar radio sources to their partaking of the expansion of the universe. The distance of 3C 273 would then be 470 Mpc (for a Hubble constant of 10^{10} years) while the quasi-stellar radio sources with larger red shifts would be at correspondingly greater distances.

Most workers in the field have, in fact, adopted the cosmological hypothesis from the time when the red shifts of the quasi-stellar radio sources were discovered. It must be recognized that this hypothesis does make it difficult to construct a model of a quasi-stellar radio source which accounts for its size, energy content, optical variations and so on. Nevertheless, along with most other workers, we believe that these difficulties can be overcome. In particular, although it has been stated[10,11] that the radio variations of 3C 273 (refs. 10, 12, 13) are not consistent with the cosmological hypothesis, it is, in fact, possible[14–16] to construct a composite model of this source which is consistent with its being 470 Mpc away. Although such a model still awaits independent observational confirmation, it is inherently a plausible one, and the cosmological hypothesis seems by far the most likely to be correct.

Despite this apparently satisfactory situation, we wish to suggest that many quasi-stellar radio sources may turn out, after all, to be inside our own Galaxy. We have in mind those quasi-stellar radio sources which have not yet had their red shifts measured. We suggest that some of them may have an essentially zero red shift, corresponding to the relatively very small velocities possessed by galactic objects. On this view the population of quasi-stellar radio sources is not homogeneous, but consists of two entirely different and unrelated components. Our reason

for introducing this possibility has to do with the relation between counts of quasi-stellar radio sources and the steady-state model of the universe. This relation is our main concern in this article, but it seems worth pointing out at this stage that the assumption of a composite population for the quasi-stellar radio sources is by no means an arbitrary and implausible complication of an essentially simple situation. We may recall the history of what Sandage[17] has called quasi-stellar galaxies. Their discovery sprang from Sandage's attempt to pick out quasi-stellar radio sources in a rapid manner by using their ultra-violet excess as a means of identification. He soon found, however, that there were many star-like objects of similar colour which were not radio sources, and which he at first called 'interlopers'. These interlopers he took to be stars which have quasi-stellar radio source-like colours. Such stars are indeed known (white dwarfs[18] and *SS* Cygni and other variable stars[19], for example). However, a study of the Haro–Luyten catalogue[20] of blue stars convinced him that most of the stars with apparent magnitude V > 16, which he found to have quasi-stellar radio source-like colours, were actually extragalactic objects—radio-quiet quasi-stellar radio sources which he called quasi-stellar galaxies. This conclusion seemed to be confirmed by Sandage's important discovery that, of the five objects the spectra of which were obtained, two were featureless and three showed large red shifts. However, this conclusion has recently been challenged by Kinman[21], who in a special investigation has found that, of a group of Haro–Luyten objects half of which should be extragalactic according to Sandage's estimates, all are in fact stars (seven white dwarfs, four horizontal branch stars and one hot sub-dwarf). Kinman estimates that only a few per cent of the faint blue stars are, in fact, galactic. A similar conclusion has been reached by Lynds and Villere[26]. If this is correct, it shows that a population of radio-quiet starlike objects with quasi-stellar radio source colours, which was once thought to be homogeneous, is in fact composite, one component being galactic and the other extragalactic. It does not seem unreasonable to make the same assumption about star-like objects with quasi-stellar radio source colours which have significant radio emission. As we have said, our reason for making this assumption has to do with the cosmological interpretation of the radio source counts, and this is the problem to which we now turn.

Radio Source Counts

If all radio sources were extragalactic, uniformly distributed, and at rest in Euclidean space, the number N of sources the flux-density of which at some given frequency exceeds S would be proportional to $S^{-3/2}$. A plot of log N versus log S would then be a straight line with slope $-3/2$. In view of the large red shifts of many radio sources, cosmological corrections are required, and both evolutionary and the steady-state models lead to a progressive flattening of the slope as S decreases. On the other hand, the observed counts[22–25,27] have a steeper slope down to about 0·5 flux units (1 flux unit = 10^{-26} W m^{-2} (c/s)$^{-1}$) (see Fig. 1).

This result has led to much discussion (for surveys, see refs. 28–30). The interpretations are of two types: (i) the steep slope is attributed to an excess of faint sources, which is given a cosmological explanation; (ii) it is assumed that there is a deficit of bright sources, arising from a local irregularity in their distribution.

Ryle and Clarke[31] have argued in favour of (i). Their cosmological explanation is based on supposing that the intrinsic properties of the sources change with the epoch in an evolving universe (distant sources being seen at an earlier epoch than nearby ones). Possible modes of evolution have been constructed by Davidson and Davies[32–33] and by Longair[34]. Now such an explanation is not available to the steady-state model, and so Ryle and Clarke concluded that this model is incompatible with the counts.

This conclusion was challenged by Hanbury-Brown[35], Hoyle and Narlikar[36,37] and Sciama[38–40], all of whom adopted (ii). One of the crucial features of such an interpretation is the required size D of the local irregularity. If all the radio sources form a single population, then their distances (as derived from the red shifts of their optical components) imply[41] that $D \sim 300$ Mpc. This would rule out the Hanbury-Brown model, which was based on the observed irregularity of the galaxies out to ~ 30 Mpc. The Hoyle–Narlikar model invoked irregularities on a scale of ~ 300 Mpc, but on the basis of a very hot intergalactic gas ($T \sim 10^9$ °K) which is now known[42,43] to be inconsistent with the cosmic X-ray background[44] arriving at Earth.

It was pointed out by Sciama[38] that the radio sources need not form a single population. At that time (early in 1963) optical identifications were complete only down to a relatively large flux density (~ 25 flux units) where the slope of the log N–log S curve does not differ sensibly from $-3/2$. He therefore postulated the existence of a second population, intrinsically much fainter than the identified population, and assumed that the irregularity occurred in this population. Only below ~ 17 flux units (where the slope definitely begins to be steeper than $-3/2$) should this second population begin to show up in the identifications. The point of this postulate, of course, is that the required scale of the irregularity would then be much smaller. Now detailed calculations show[40] that if this second population is also extragalactic the counts of Ryle and Neville[23] at low flux densities cannot be accounted for. In fact, Sciama[38] proposed that the second population lies in the Galaxy, and that the scale of irregularity ~ 20 pcs.

The relevance of this discussion to the location of the quasi-stellar radio sources is the following. There are now enough quasi-stellar radio sources identified to make it possible to begin constructing a separate log N–log S curve for radio galaxies and for quasi-stellar radio sources This has been done independently by Véron[45] and Longair[34], and both have found that the slope for radio galaxies $\sim -1{\cdot}5$ and for quasi-stellar radio sources ~ -2 (see Fig. 2). Thus the quasi-stellar radio sources are entirely responsible for the steep slope. In the flux range where this separation has so far been possible most of the radio galaxies do not have very large red shifts, and it

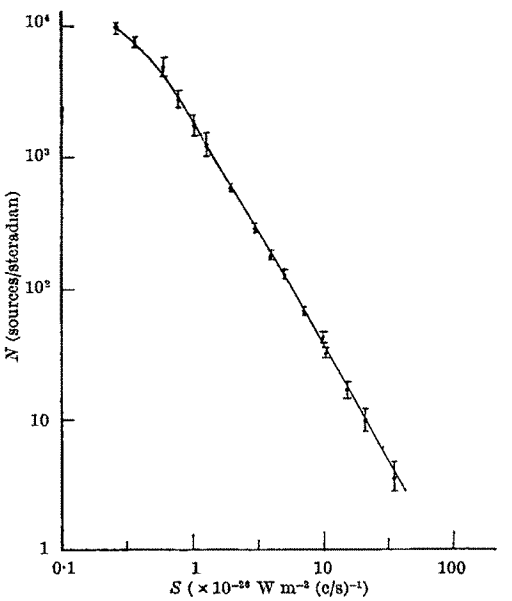


Fig. 1. The number-flux density relationship derived from Cambridge radio source surveys (refs. 22, 23, 27)

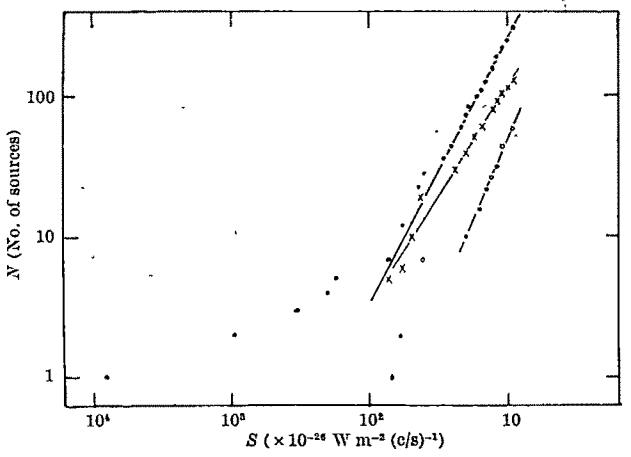


Fig. 2. The number-flux density relationships for quasi-stellar radio sources, ○; radio galaxies, ×, and all the extragalactic sources in the 3C revised catalogue. This diagram is due to Véron (ref. 45) and is based on the identifications listed by Wyndham (ref. 48). The lines have slopes of −2·20, −1·55, and −1·85 respectively

is understandable that they should conform to the three-halves power law. On the other hand, the quasi-stellar radio sources do have large red shifts and according to the evolutionary interpretation of the steep slope it is natural that they should exhibit significant evolutionary effects. Indeed, Longair[34] found that only the intrinsically brightest radio sources (among which the quasi-stellar radio sources are the dominant component) could evolve, if the log N–log S curve is to be explained in this way.

However, we can still maintain the galactic interpretation, and with it the steady-state model of the universe, if we assume that the quasi-stellar radio sources consist of two populations, one extragalactic and one galactic. We thereby make contact with the discussion in the first section of this article. It is worth noting that we could not have maintained the galactic interpretation if the class of objects having a steep slope were obviously extragalactic in optical appearance (for example, if they were radio galaxies). We therefore regard it as encouraging for this point of view that the objects which have a steep slope do have an optical appearance which is consistent with their being galactic. It thus seems worth while to pursue the possibility that many of the quasi-stellar radio sources fainter than ~ 17 flux units are in the Galaxy.

Required Properties of the Galactic Quasi-stellar Radio Sources

It was shown in refs. 38–40 that the radio data serve to fix many of the properties of the required galactic population of radio sources. We now know that these properties must refer to the galactic quasi-stellar radio sources. It seems appropriate to re-discuss this question since some relevant data have recently become available. These new data are as follows: (*a*) the 4C survey for a particular region of the sky[27]; (*b*) an improved luminosity function[46] for the extragalactic sources, made possible by an increase in the number of optical identifications, red shifts and so distances; (*c*) further data on the spectra of sources[47] and of the galactic background[49].

Our procedure is as follows: at high flux-levels ($S > 20$ flux units) nearly all the sources in the 3C catalogue have been identified with radio galaxies or with quasi-stellar radio sources of known red shift. Thus, we are here observing the extragalactic population, undiluted by the galactic sources (which are missing because of the local irregularity in their distribution). We can then calculate the expected number N_e' of extragalactic sources at lower flux-levels by using the red shift correction appropriate to the steady-state model. This red shift correction was computed on the basis of the new luminosity function derived by Longair and Scott[46].

The number N_g of galactic sources was then determined by assuming that at 2 flux units we are looking well beyond the local deficit, and that the total number of missing sources is comparable with the statistical error in the observed counts at this flux-level. It is then sufficiently accurate to take for N_g the difference $N–N_e'$ between the observed counts and the computed extragalactic contribution. We thus find $N_g = 0{\cdot}95\ (\pm\ 0{\cdot}05)\ N_g$, where N_g is the number of extragalactic sources uncorrected for red shift. A similar relation was, in fact, found previously[40], and the new log N–log S relation and the new luminosity functions do not change the analysis appreciably.

The details of the local deficit can now be determined from a comparison between the observed and computed counts, and we find that the galactic population begins to be noticeable at ~ 17 flux units and that beyond ~ 10 flux units there are no more missing sources, the total deficit being ~ 25 sources/steradian (Fig. 3). The model is thus completely determined by the counts down to 10 flux units, together with one count at a lower flux-level, which we have taken as 2 flux units. All the remaining counts between 10 and 0·25 flux units (the lower level of the Ryle–Neville survey) then serve as a test of the model. It will be seen from Fig. 3 that the model survives this test. In particular, the count at 0·25 flux units is correctly reproduced within the uncertainties. Furthermore, calculations with the new luminosity function show that the model is consistent with the identifications down to this flux-level which have been made by Shakeshaft and Longair[50]. This is of interest because Longair had some difficulty in fitting this point with an evolutionary model, despite the large number of adjustable parameters available in that case. Indeed, he had to introduce a very sharp cut-off in the number of sources with red shift $z > \sim 4$. As he suggested, such a cut-off may be associated with the original formation of radio sources, but the position of this cut-off

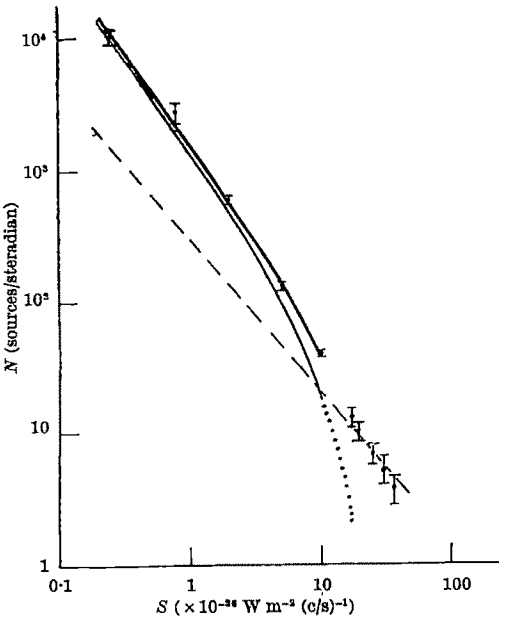


Fig 3 Comparison between predicted and observed radio source counts. – – – – –, Computed counts of extragalactic sources expected on the steady-state model. The curve is normalized to agree with the observed counts above 17 flux units. ●●●●●●, Counts of galactic sources in local irregularity derived from observed counts. ————, Expected counts of galactic sources beyond local irregularity (normalized to agree with observed counts at 2 flux units). ————, Predicted total counts of galactic and extragalactic sources. I, Observed counts of sources

cannot be predicted *a priori*. The present model thus faces a more severe test. In this connexion it is interesting to calculate the expected number counts at a lower flux-level of, say, 0·1 flux units, since the observations should soon penetrate into this region. We find that, according to our composite model, there should be ~ 44,000 sources per steradian brighter than 0·1 flux units (unless the edge of the distribution has been reached, in which case anisotropy should be observed (see below)). By contrast, if we did adopt the model considered earlier, in which the second population is also extragalactic, we should expect only ~ 19,000 sources per steradian brighter than 0·1 flux units. Evolutionary models would also predict far fewer than 44,000 sources.

Since the composite model with galactic sources reproduces the observed counts, it seems worth exploring further the properties of the galactic quasi-stellar radio sources. If P_0 is their median intrinsic radio luminosity and $\rho(P_0)$ the corresponding concentration, then our determination of N_g tells us $\rho(P_0)\ P_0^{3/2}$ up to a factor of order unity depending on the luminosity distribution. We would now like to obtain $\rho(P_0)$ and P_0 separately. This was done in the original discussion of the model in the following way. As was emphasized by Ryle[51] and Ryle and Clarke[21], the parameters of the galactic model are subject to two constraints: (1) the integrated emission of the galactic sources must not exceed the minimum observed galactic background; (2) the galactic sources must not show anisotropy down to about ~ 0·1 flux units, since the radio observations indicate no such anisotropy (statistical arguments are used[52] for flux-levels where individual sources cannot be resolved).

These two constraints act in opposite directions, and Ryle, and Ryle and Clarke, believed that they were inconsistent with one another, so that the galactic model could be ruled out. To see that they act oppositely we proceed as follows. Let r be the effective scale-length of the distribution of galactic sources in the direction of the minimum sky brightness B. Then, if we neglect for the moment the spread in intrinsic radio luminosity, we have:

$$\rho P_0 r \leq B$$

But we also know $\rho P_0^{3/2}$, which is X, say. Therefore:

$$\frac{P_0}{r^2} \geq \frac{X^2}{B^2}$$

Thus we cannot make P_0 as small as we would like to eliminate the anisotropy problem (by arguing that we are observing only the immediate vicinity of the Sun). In fact, at a flux-level less than X^2/B^2 we would be beginning to observe sources beyond the effective distance r, and so this is the critical level at which appreciable anisotropy should set in. The situation is worse if we allow for a spread in intrinsic radio luminosity. If an appreciable fraction of the sources have an intrinsic luminosity ten times the median, which seems plausible, then the critical flux-level will be $10\ X^2/B^2$. We shall adopt this value, while recognizing that it is somewhat arbitrary. Then, in view of (2), we must have:

$$\frac{10\ X^2}{B^2} \leq \sim 0{\cdot}1 \text{ flux units.}$$

Is this inequality satisfied? The value of X has already been determined from the counts. What can we say about the background brightness B? According to Turtle and Baldwin[53] the minimum background temperature at 178 Mc/s (the frequency of the Cambridge radio source counts) is 80° K. Of this, about 23° K has been attributed[54] to the extragalactic background. If we attribute the remaining 57° K to B we find that:

$$\frac{10\ X^2}{B^2} \sim 0{\cdot}1 \text{ flux units.}$$

Thus a solution is just possible if: (*a*) the source counts are anisotropic below ~0·1 flux units; (*b*) most of the galactic background in the direction of the minimum is due to the integrated effect of the sources rather than synchrotron radiation emitted by relativistic electrons moving in an interstellar magnetic field.

Conclusion (*a*) will be tested by future source counts, but (*b*) deserves a little discussion. It was argued by Scott[55] that the background has a different spectrum from the sources, so that (*b*) cannot be correct. However, estimates of the background spectrum have changed since Scott wrote his paper, and while there is not yet complete agreement about the correct spectrum, some workers[49] claim that it is straight from about 18 Mc/s to beyond 404 Mc/s, with a slope ~ 0·65 ± 0·15. On the other hand, most of the sources also have[47] a straight spectrum with a median spectral index ~ 0·7. In any event, exact agreement need not necessarily be expected since intrinsically faint sources contribute more to the integrated emission (through $\Sigma\rho P$) than they do to the sample used for determining spectra (which is selected on the basis of flux density and therefore of $\Sigma\rho P^{3/2}$). Thus, if the faint sources have atypical spectra, this will be reflected in the spectrum of the background.

Another unsettled question is the relative intensities of the halo and disk components of the galactic background. Some observers[56,57] now maintain that the halo is relatively unimportant, while others[58,59] deny this. We have chosen to accept provisionally Baldwin's result[58] that the halo is unimportant and that the disk component has a thickness at the Sun of about 400 pcs on each side of the galactic plane. Because of (*b*) this means that we should take ~ 400 pcs for r. This determines the scale of the distribution of galactic quasi-stellar radio souces and enables us to determine ρ and P_0 separately. We find[38-40]:

$$\rho \sim 10^{-2}\ pc^{-3}$$

$$P_0 \sim 3 \times 10^{10}\ \text{W (c/s)}^{-1}\ \text{ster}^{-1},$$

values closely similar to those adopted[60] in the original radio source hypothesis for the galactic background. Thus, according to this analysis, and allowing for the local deficit, the nearest galactic quasi-stellar radio sources are within 20 pcs of the Sun. Accordingly, their absolute optical magnitude must be very faint, and a possible astrophysical mechanism consistent with this has been proposed[39]. More to the point, they are close enough to have appreciable proper motions. We therefore predict that some of the known quasi-stellar radio sources the red shifts of which have not yet been determined should have detectable proper motions.

An alternative test of the model would be to find some essentially zero red shifts among these quasi-stellar radio sources, but the possibility exists that the spectra of the galactic quasi-stellar radio sources may show no lines. Spectra of this type are indeed found among the quasi-stellar radio sources[5,6] and the interlopers[17]. (In this connexion it is worth noting that some white dwarfs (which have quasi-stellar radio source colours) also have no lines in their spectra (type *DC*)[61].) Unless we are altogether wrong about the value of r (and the distance of the nearest galactic quasi-stellar radio source is proportional to r), it appears that the composite model stands or falls by the presence or absence of detectable proper motions among the known quasi-stellar radio source. Moreover, if the model falls, it looks as though the steady-state theory of the universe falls with it.

We thank Dr. P. A. G. Scheuer for helpful discussions. We also thank Mr. M. S. Longair, whose analysis of the new radio data stimulated us to re-examine the composite model and whose advice greatly simplified our task.

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STRUCTURAL ABNORMALITIES OF THE *Y* CHROMOSOME IN MAN

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DURING the past 5 years we and our colleagues have examined the chromosomes of several thousand individuals. Four have been found with an abnormality which we consider to be a structural abnormality of the *Y* chromosome and in whom there is no evidence of mosaicism. While the findings in two of these have been briefly reported elsewhere, the purpose of this article is to describe the findings in all four individuals and to discuss the implications of these in relation to the male determining role of the *Y* chromosome in man.

Case 1 is a 34-year-old phenotypic female, 155 cm tall, who has never menstruated. Her secondary sexual characteristics have not developed beyond the infantile stage. Abdominal surgery showed her uterus to be underdeveloped and that instead of ovaries there were 'streaks' of gonadal tissue in the infundibulo-pelvic ligaments comparable with those described in females with an *XO* sex chromosome complement. Microscopic investigation of these showed the absence of ovarian elements. The only other noteworthy clinical feature was the presence of a short neck with some slight suggestion of webbing.

Buccal cells were chromatin negative and both cultured lymphocytes and fibroblasts showed the chromosome number to be forty-six. There were only fifteen medium-sized sub-metacentric chromosomes and no normal *Y* chromosome. However, there was a structurally abnormal chromosome which appeared exactly metacentric with the distal parts of both arms showing a characteristically *Y*-like appearance (Fig. 1). Investigations of cells collected from a lymphocytic culture 4 h after the addition of tritium-labelled thymidine showed no evidence of any chromosome synthesizing DNA as late in the *S*-period as is characteristic of a second *X* chromosome. However, it is known that the *Y* chromosome is one of the last of the small chromosomes to complete DNA synthesis, and the labelling pattern of the abnormal chromosome was consistent with its being composed of *Y* chromosome material[1]. Furthermore, in several cells in which the abnormal

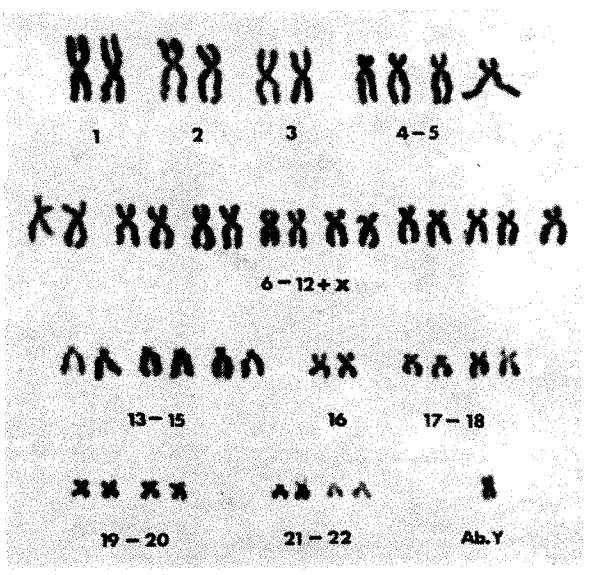


Fig. 1. Karyotype of cultured lymphocyte from Case 1

chromosome was labelled the labelling pattern appeared to be the same on both arms.

Studies of the chromosomes of the two surviving brothers of the patient showed each to have a normal *Y* chromosome.

After considering the clinical findings together with all the cytogenetic evidence, it was concluded that the abnormal chromosome was most reasonably interpreted as an isochromosome for the long arm of the *Y*.

Full details of this case have been published elsewhere[2].

Case 2 is a 62-year-old phenotypic female, 163 cm tall, who has never menstruated. Her secondary sexual characteristics have not developed beyond the infantile stage, and the condition of her gonads and internal genitalia is unknown. Apart from a rather deep voice the patient shows no evidence of masculinization and the only other noteworthy clinical feature is bilateral choroidoretinitis.

Buccal cells were chromatin negative and both cultured lymphocytes and fibroblasts showed the same karyotype as in Case 1. The pattern of DNA synthesis, as shown by the use of tritium-labelled thymidine, again showed no chromosome synthesizing very late in the *S*-period. However, the pattern of replication of the abnormal chromosome was again consistent with its being an abnormal *Y* chromosome, and the labelling pattern appeared to be the same on both arms (Fig. 2).

Studies of the chromosomes of the male relatives of Case 2 have not yet been possible.

After considering all the evidence it was concluded that the abnormal chromosome was most reasonably interpreted as an isochromosome for the long arm of the *Y*.

Case 3 was an 83-year-old man, 152 cm tall. He had a left-sided hare lip which had been repaired, but otherwise he was clinically unremarkable. His external genitalia and testes were normal for his age. He had been married, but his wife died after 9 years of marriage and they had no children. The patient died in his eighty-third year from generalized arteriosclerosis.

Buccal cells were chromatin negative and both cultured lymphocytes and fibroblasts showed a chromosome number of forty-six with no evidence of mosaicism. None of the cells showed a normal *Y* chromosome, but there was an additional chromosome closely resembling the autosomes of pairs 19–20 (Fig. 3). In a few cells it could be differentiated from autosomes 19–20 by the rather *Y*-like appearance of the chromatin material. Autoradiography was not carried out on the cells from this patient and it has not been possible to study the chromosomes of any of his male relatives.

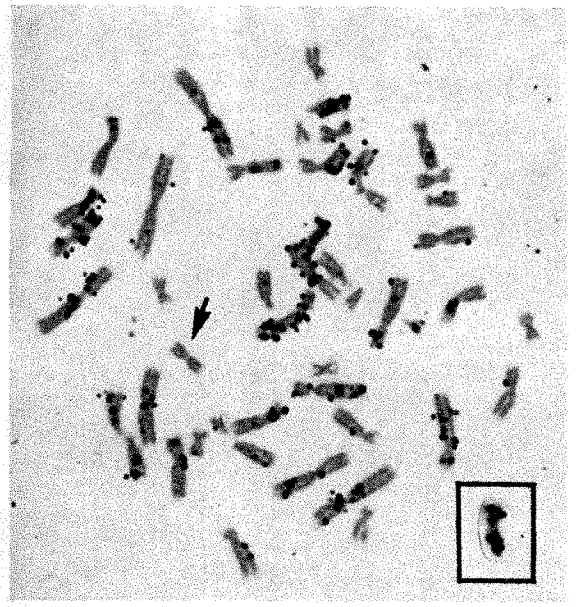


Fig. 2. Metaphase plate of cell from Case 2 after continuous labelling with tritium-labelled thymidine for 4 h before collecting, showing absence of labelling on the abnormal *Y* chromosome. Inset shows the abnormal chromosome from a cell labelled further back in the *S*-period showing symmetrical labelling

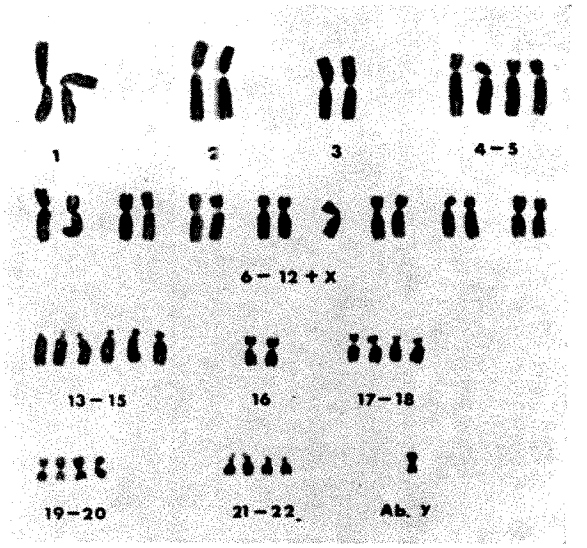


Fig. 3. Karyotype of cultured lymphocyte from Case 3

After considering the clinical and cytological evidence it was concluded that the abnormal chromosome was most reasonably interpreted as a *Y* chromosome in which there had been a pericentric inversion.

Full details of this case have been published elsewhere[3].

Case 4 is a 41-year-old man, 180 cm tall, with atrophic testes and some of the other features of Klinefelter's syndrome.

Buccal cells were chromatin positive and 'drumsticks' were present on his polymorphonuclear leucocytes. Both cultured lymphocytes and fibroblasts showed a chromosome number of 47. There was a normal female complement of chromosomes present but no normal *Y* chromosome. However, there was an additional chromosome resembling the autosomes of pairs 19–20, the chromatin of which often appeared characteristic of that of the *Y* chromosome. Investigations of cells collected 4 h after the addition of tritium-labelled thymidine showed that in 60 per cent of the labelled cells there was a very late synthesizing medium-sized submetacentric chromosome, presumed to be an *X* (Fig. 4). However, the abnormal small metacentric chromosome did not synthesize DNA very late in the *S*-period, but its labelling pattern was consistent with its being an abnormal *Y* chromosome.

The patient is illegitimate and has no known male relatives.

After consideration of the clinical and cytological findings it was concluded that the patient, in addition to having two *X* chromosomes, also has an abnormal *Y* chromosome, and that the additional small metacentric chromosome is a *Y* chromosome in which a pericentric inversion has occurred.

The *Y* chromosome of man is known to be strongly male determining. Thus individuals with an *XO* sex chromosome constitution are phenotypic females. The masculinizing effect of the *Y* chromosome can even overcome the female determining genes on as many as four *X*-chromosomes, as evidenced by the fact that individuals with an *XXXXY* sex chromosome constitution, while having a number of congenital abnormalities, are still undoubted phenotypic males. Furthermore, it is well known that the size of the *Y* chromosome in man can vary considerably in length from one person to another without any apparent phenotypic effect and that this

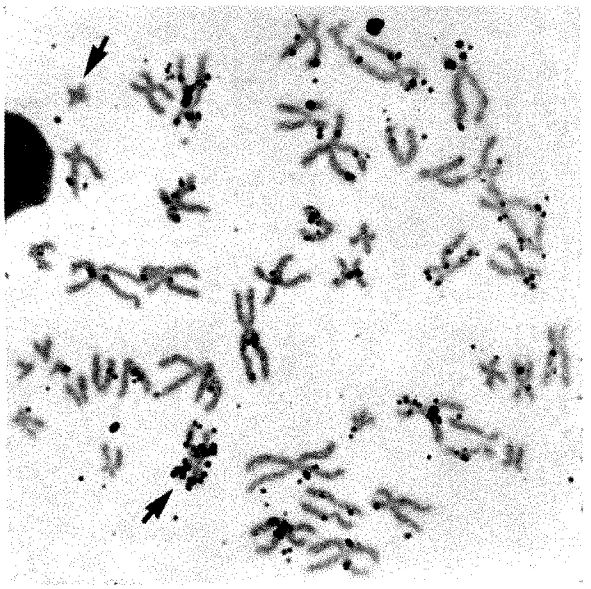


Fig. 4. Metaphase plate of cell from Case 4 after continuous labelling with tritium-labelled thymidine for 4 h before collecting, showing the late labelling *X* chromosome and the absence of labelling on the abnormal chromosome

polymorphism of the *Y* chromosome is an inherited characteristic. The *Y* chromosome usually approximates in size to the autosomes of pairs 21–22, but it may be as small as only half the size of these or it may be as large as the autosomes of group 13–15. These variations in size are due to differences in length of the long arm, the distal part of which often appears rather diffuse and stains faintly, thus suggesting that it is largely heterochromatic. The apparent differences in length of the *Y* chromosome may in some cases be due to genetically controlled variations in coiling of the distal part of the long arm and in others to actual variations in the amount of chromatic material.

With the exception of the variation in length of the *Y* chromosome in normal males, and the presence of a minute chromosome or a small-ring chromosome, which might be formed from the centromeric region of either the *X* or the *Y* chromosome, in one cell line of a number of individuals who have the clinical features of Turner's syndrome, there have been very few non-mosaic individuals described with a structurally abnormal *Y* chromosome. The four individuals described in this report, together with the father of an abnormal son described by Solomon and her colleagues[4], are the only instances known to us. The cytogenetic observations and the phenotypic appearance of these cases have to be considered in the light of the strongly male-determining effect of the normal *Y* chromosome in man and the fact that apparent variations in the length of its long arm are without obvious phenotypic effects.

In order to explain our observations and those of Solomon *et al.*, we would suggest: first, that the male-determining gene or genes in man are situated on the short arm of the *Y* chromosome; secondly, that the genes present on the long arm of the *Y* chromosome are not primarily concerned with the determination of sexual characteristics; and, thirdly, that an individual with a pericentric inversion of the *Y* chromosome is a normal fertile male, but that he may be liable, as a consequence of his abnormal *Y* chromosome, to produce a proportion of chromosomally abnormal sperm which would be expected to result in *XO* daughters and *XXY* sons.

If the abnormal chromosome described in Cases 1 and 2 is an isochromosome for the long arm of the *Y*, these individuals, while they have a double dose of all the genes on the long arm of the *Y*, are lacking the male-determining genes on the short arm. Presumably the latter act by causing the primordial gonads to develop into testes. In the absence of either male- or female-determining factors neither the cortex nor the medulla of the primordial gonad develops and the resulting individual is a phenotypic female who, having no functioning gonadal tissue, fails to develop secondary sex characteristics.

It is of considerable interest to compare females with an isochromosome for the long arm of the *Y* with individuals with an *XO* chromosome constitution, who are also 'genetically neuter'. It would seem reasonable to suggest that any differences between the two groups are due to genes on the long arm of the *Y* chromosome. Individuals with an *XO* sex chromosome constitution are invariably short in stature and often have a number of additional abnormalities such as co-arctation of the aorta, neck-webbing, pigmented naevi and a low hair line. Cases 1 and 2 are both of normal stature and, while Case 1 had a rather short neck with a slight suggestion of neck-webbing, there were no other stigmata associated with an *XO* sex chromosome constitution present in either of them. It would appear, therefore, that the presence of the material of the long arm of the *Y* chromosome prevented the development of dwarfing and many of the other stigmata associated with an *XO* constitution. Furthermore, with the exception of the deep voice of patient 2 there was no evidence of masculinization in either patient.

While Cases 3 and 4 also had a structurally abnormal metacentric *Y* chromosome, this was interpreted as being due to a pericentric inversion. Thus the short-arm material of the *Y* chromosome is still present and the chromosome is functioning as a normal *Y* chromosome. It is unfortunate that in neither case was it possible to examine the chromosomes of a male relative. However, Solomon *et al.* have described a similar abnormal *Y* chromosome in one cell line of a male with Klinefelter's syndrome, the other cell line having no *Y* chromosome. They found the same abnormal *Y* chromosome in his father, a normal fertile male, and while these authors state that "the exact nature of this abnormal *Y* chromosome is not clear" its appearance is very similar to that seen in Cases 3 and 4, and it is reasonable to postulate that it has also arisen by a pericentric inversion.

This interpretation also offers an explanation for the fact that two of the four individuals described with a pericentric inversion of the *Y* chromosome also have an extra *X* chromosome and show the clinical features of Klinefelter's syndrome. The *X* and *Y* chromosome have an end-to-end association in meiosis which appears to be between the short arm of the *X* and the short arm of the *Y* (McIlree—personal communication). A pericentric inversion would presumably interfere with this association and lead to a failure of pairing of the sex chromosomes in the first meiotic division, which would result in a proportion of the sperm having no sex chromosomes and a proportion having both an *X* chromosome and the abnormal *Y*. If these sperm fertilize a normal ova the result will be *XO* females and *XXY* males. This being the case, it is not surprising that two of the males with a pericentric inversion of the *Y* chromosome also have an additional *X* chromosome.

We thank Mrs. J. Bond, Dr. M. J. W. Faed and Dr. D. G. Harnden for the results of chromosome studies on the fibroblasts. We are also grateful to the following colleagues, Dr. T. N. McGregor, Mr. W. Selby Tulloch, Dr. I. S. Stokoe and Dr. J. A. Strong, who referred the patients to us, and to Mrs. Janet Barclay, Miss Muriel Brunton, Miss Gillian Woodcock and Mr. Duncan Davidson for their technical help.

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OBITUARIES

John Anthony Newth

JOHN ANTHONY NEWTH was born on January 17, 1924, and died on November 18, 1965. He was educated at King Edward's School, Birmingham, and Trinity College, Cambridge. After graduation in 1943, he joined the staff of the Department of Physics, University College, Exeter, as an assistant in charge of the training of Signal Corps cadets, later becoming an assistant lecturer. In 1947, he was appointed assistant lecturer in the Department of Physics at Manchester and started research work on the newly discovered V-particles under the direction of J. G. Wilson and P. M. S. Blackett. During the following three years he and his students designed and built a large expansion cloud-chamber and magnet for operation at the Jungfraujoch station in Switzerland.

For the next eight years Newth led an active and successful team, working with this apparatus at the mountain station. During this period were produced a number of important papers on the properties of the strange particles. The large cloud-chamber turned out to be particularly suitable for measuring the lifetimes of particles and the group made a notable contribution in this field. It is common experience that a small group working in the isolated conditions of a mountain laboratory at this altitude is subject to considerable stress of both a physical and an emotional kind. The very high efficiency achieved and the cordial relations that existed between the members of this group throughout the period are a splendid tribute to Newth's leadership, understanding and sense of humour —qualities immediately apparent to anyone spending a few hours in his company. During the final three years of this period he was attached to CERN, where he made many friends and contributed notably to the atmosphere of that laboratory during its crucial formative years.

Newth was appointed senior lecturer at Imperial College in 1958 and reader in 1964. During his first few years at Imperial College, he was engaged in leading a research team using image intensifiers for various nuclear physics applications. His group became one of the world's leading teams in this field, and three major uses for image tubes in high-energy physics were developed. These were the photography of Cerenkov rings produced by single particles, the photography of tracks of particles in scintillating materials, and the use of image tubes to investigate the beam profiles of secondary particle beams at high energy accelerators.

By 1961 it was clear that a new and powerful experimental technique (the spark chamber) was rapidly changing the possibilities for counter experiments, and Newth and his team designed an experiment on the branching ratio of the decay modes $e^+ + e^-$ of the ω^0 meson. This experiment was carried out at the 7-GeV proton accelerator (*Nimrod*) at the Rutherford High Energy Laboratory during the winter of 1964. Anthony Newth's bold leadership and inspiration contributed greatly to the success of this difficult experiment, which exploited spark chamber and counter techniques to the full. At the time of his death he was very actively involved in planning a related experiment to measure the $\varphi^0 \rightarrow e^+ + e^-$ branching ratio.

P. ASTBURY

NEWS AND VIEWS

Nuclear Physics in the University of Lancaster : Prof. A. B. Clegg

DR. A. B. CLEGG, who will shortly take up his position as professor of nuclear physics at the University of Lancaster, was born in 1929 and was educated in Birkenhead (1939–47). He read physics at King's College, Cambridge (1949–55), and after gaining first-class honours in Part II of the Natural Sciences Tripos began research in the Cavendish Laboratory on nuclear structure problems. His interests were in the structure of the light nuclei and he participated in a number of early experiments to test the validity of the isotopic spin quantum number which had recently come into prominence. He left the Cavendish Laboratory in 1955 and went to the new synchrotron laboratory of the California Institute of Technology. There he worked originally on the radio frequency system of the synchrotron and later on elementary particle experiments. He came back to Britain in 1958 as a senior research officer in the Clarendon Laboratory, Oxford, and later in the new Nuclear Physics Laboratory there. His intentions from the beginning were ultimately to work on elementary particle problems on the Rutherford Laboratory's 7-GeV proton synchrotron *Nimrod*, then under construction. He chose as entry to this field, however, a reversion to his earlier study of nuclear structure using the 170-MeV synchrocyclotron at the Atomic Energy Research Establishment, Harwell. With this machine he pioneered much of the study of nuclear structure using intermediate energies and rapidly gained an international position in this new field. Despite his very considerable success with the intermediate energy work, he stuck to his original intention and transferred his efforts, in February 1964, to high-energy work when *Nimrod* became operational. His work on *Nimrod* has used spark chambers and has been concerned with the interactions and decay of pions and kaons. In going up to Lancaster, his research activity will transfer to the 4-GeV electron synchrotron *Nina* at the Daresbury Laboratory of the Science Research Council and his interest in elementary particle physics will continue.

Genetics in University College, Swansea : Prof. J. A. Beardmore

PROF. JOHN A. BEARDMORE has been appointed to the newly established chair of genetics in University College, Swansea. Prof. Beardmore was a student of the University of Sheffield, where he took his B.Sc. with special honours in botany and where he received his Ph.D. degree in genetics under Prof. J. M. Thoday in 1953. Beardmore's scientific work has been mainly in the field of population genetics, and he has published a number of experimental studies on *Drosophila*. Some of his later work was done at the laboratory of Prof. Dobzhansky, whose teachings in population genetics and evolution have greatly influenced a whole generation of geneticists. He spent two years at Columbia University, with an interruption of five months for an assignment as visiting professor at Cornell University. He was then appointed lecturer at the Universities of Sheffield and Leicester, and afterwards moved briefly to the University of Rochester, New York, as a visiting research associate. In 1961, he was appointed to the chair of genetics at the University of Groningen, where he succeeded Prof. Sirks. The task he took on himself was far from easy. He had to introduce new fields of research

in an old Institute not too well equipped for the purpose, and he was confronted with a foreign tongue. Without a trace of hesitation, he solved all problems speedily and elegantly, while at the same time he was, from the beginning, actively concerned with various aspects of genetics in The Netherlands, including the editing of the journal *Genetica*. He and his family were on the way to becoming true Dutchmen, except perhaps for the inborn necessity of a sabbatical retreat. The irresistible call to Swansea clearly came at the right time, although it is not a propitious one to genetics in The Netherlands, and the move is a disappointment to Dutch colleagues.

More German Research Funds

GOVERNMENT expenditure on scientific research is to be increased in 1966 by about 30 per cent compared with 1965. The budget of the Ministry for Scientific Research, now published, shows that the expenditure planned for 1966 is the equivalent of £116 million. This sum is, of course, separate from that spent on scientific research and development by other agencies of the West German Government. In 1965 it is estimated that a total of about 2 per cent of the West German gross national product was spent on scientific research and development, which implies an expenditure equivalent to £800 million a year.

On the funds available at the Ministry for Scientific Research during 1966, £32 million will be spent on the expansion of universities and other establishments of higher education, £44 million for the support of nuclear energy for peaceful purposes, and a total of more than £22 million on space research, including the development and construction of a German Earth satellite.

Grants for Industrial Scientists

THE Science Research Council in the United Kingdom has announced two schemes clearly intended to make it easier for industrial scientists to obtain further training and postgraduate qualifications. Thus the Council has announced a new scheme of industrial studentships for which young scientists working in industry are eligible. These awards will be available either for 6- or 12-month courses for advanced training, or for longer periods of up to 3 years for training in research normally leading to a higher degree. The scheme will start in October 1966 and it is thought that at least a hundred awards will be made. To obtain an award an industrial scientist must have been employed professionally for at least a year, and must also be academically suitable in the eyes of the Science Research Council and the intending university or college of advanced technology. The most welcome innovation in the new scheme may be that the Science Research Council has decided that industrial employers (if they wish) can supplement the amount of an industrial studentship so as to increase the student's income to that which he would have received at his job.

The second scheme devised by the Science Research Council is intended to encourage young graduates in science and technology to gain industrial experience before embarking on postgraduate training. The intention is that suitable young graduates should be given a guarantee of support from the Science Research Council for a course of postgraduate study to begin at any time from 1 to 5 years after graduation. The Council hopes this arrangement will enable young graduates to start an industrial career and to undertake further academic training at what they and their employers consider the most suitable time. Here, again, the Science Research Council hopes that employers will give their moral and financial blessing to further education.

Obviously the success of both ventures will be determined partly by the willingness of industrial companies to respond and partly by the willingness of young graduates, together with universities and colleges of advanced technology, to acknowledge that a ready exchange of people between academic life and industry can be advantageous. Thus even if the schemes should not—as the Science Research Council hopes—enhance the academic background of industrial scientists, they will provide a searching test of the honesty of recent protestations of goodwill towards industry by academic institutions.

Institute of Geological Sciences

A NEW organization called the Institute of Geological Sciences has been formed in Britain by the National Environment Research Council. The Institute is really an amalgam of the Geological Survey in Great Britain, the Museum of Practical Geology and the Overseas Geological Surveys. These organizations have worked closely together ever since the formation of the National Environment Council which followed the recommendation of the Brundrett Committee in uniting the home and overseas geological surveys in June 1965. The National Environment Research Council hopes that the title of the geological institute will emphasize the breadth and the range of scientific disciplines used in modern geological survey and research. It is intended to extend the field of activity of the existing organizations under the guidance of the Geology and Geophysics Committee of the Council, and steps are also being taken to see how best the headquarters of the new organization (but not the Museum) can be moved out of London.

Strontium-90 Levels in Australia, 1961–63

THE Atomic Weapons Tests Safety Committee in Australia has published the results of a survey of the amount of strontium-90 in the Australian environment during 1961–63 (*Austral. J. Sci.*, 28, No. 2; 1965). Strontium-90 fall-out over Australia during the period under review arose from nuclear weapons tests carried out in the Northern Hemisphere before 1959, and from the U.S.S.R. and United States test series of 1961–62, the total and fission yields of which were greater than those from all previous tests. Until the middle of 1962, there was no significant fall-out over Australia from tests later than 1958. During the remainder of the period under review, increasing levels of fall-out were recorded; they have been attributed mainly to the United States programme in the North Pacific Ocean during 1962, but towards the end of 1963 strontium-90 from tests by the U.S.S.R. may have been making a significant contribution. It is expected that all fall-out over Australia from nuclear weapons tests held during 1961–62 will eventually be comparable with that from all previous testing. The mean annual deposition of strontium-90 in precipitation at the chosen monitoring sites in Australia remained in the region of 1 mc./km² from 1958 to 1962 but rose to about 1·5 mc./km² in 1963. Regular differences in the annual strontium-90 fall-out between the sites are now well established: during the period of constant fall-out (1958–62), Townsville was lowest at 0·4 mc./km² per annum and Sydney highest at 1·3 mc./km² per annum, with the other stations in between in the ascending order, Darwin, Perth, Hobart, Adelaide, Melbourne and Brisbane. These levels are comparable with those for the period before 1960, as assessed by the National Radiation Advisory Committee. Levels of strontium-90 in Australian human bone are lower than those in the populations of the United Kingdom and the United States. However, it is of interest that the ratio of strontium-90 to calcium in bone tissue or in total diet, relative to the level of strontium-90 in precipitation, is higher in Australia than in the United Kingdom or the United States. Although fall-out levels continue to be much lower in Australia than in the United Kingdom and the United States, examination of substantial bodies of data indicates that the transfer of strontium-90 from fall-out through the food-chain to diet may be approximately twice as efficient in the Australian environment as in the United

Kingdom and the United States. This anomaly may occur in all foodstuffs, but it has been noted mainly in milk, where it has been attributed to marked differences in dairying practices.

No Mutations in Kerala

A SEARCH for possible genetic consequences of the comparatively intense background radioactivity in Kerala, South India, has yielded a negative result. The study and its results are described in a report now published in Britain by the Medical Research Council (*A Search for Genetic Effects of High Natural Radio-activity in South India*. H.M.S.O. 11*s*.). Within the limits of its statistical significance, the study bears on the suggestion, first given prominence in the late 'fifties, that the genetic consequences of small but chronic doses of radiation should be most apparent in populations of living things in exceptionally radioactive environments. A report of the committee on radiation appointed by the World Health Organization, and which reported in 1959, was one of several to urge that such exceptional populations should be studied with great care. Kerala commends itself as a site for such an investigation because of its monazite sand, rich in thorium.

The study has been carried out by a team under Prof. H. Gruneberg, honorary director of the Experimental Genetics Research Unit of the Medical Research Council at University College, London. The report explains that the study was concerned with wild populations of the black rat *Rattus rattus* L., because this animal is the only mammal other than man to occur in large numbers in the region. Wild rats were trapped at eight villages on the narrow coastal strip of monazite sand, and at eight nearby villages away from the strip. A total of 438 rats were trapped at the monazite villages, and 458 at those used as controls. The numbers of males and females were insignificantly different. Measurements were made of anatomical quantities such as the sizes of the crowns of molar teeth, which are substantially independent of age, and of other skeletal dimensions for which it was necessary to make a correction for age. Skeletons were also scrutinized for certain more or less well-defined variations of shape, while attempts were made to estimate fertility and pre-natal mortality from the incidence of pregnancy in female rats, and from characteristics of the unborn litters.

The intensity of the gamma-radiation on the coastal strip, equivalent to 1·6 r. a year, was seven and a half times that in the control areas inland. Measurements revealed that both populations of rats are heterogeneous, but that there are no systematic differences between the two groups that would suggest either systematic genetic differences or a larger genetic variance in the more heavily irradiated group. The authors of the report point to four hypotheses consistent with their results—the existence of a 'threshold' of radiation doses below which genetic damage does not occur; the possibility that genetic mutations due to radiation might be almost exactly balanced by natural selection; the possibility that an increase of genetic variance due to radiation might be almost exactly counterbalanced by a reduction in environmental variance; and the possibility that accidents of sampling may obscure a real effect. An extremely tentative calculation suggests that a difference of 0·3 per cent in the means of measurement from the two groups might be concealed by sampling errors. The authors echo a statement in an introductory note by the Medical Research Council to the effect that there is nothing in this apparently negative study conclusively to suggest that low doses of radiation have no genetic effects. Because of the way in which genetic effects may be masked by effects due to the environments or natural selection, however, the authors offer little hope that further studies of this kind will yield decisive results.

Aflatoxin in Malaysia

RESEARCH in progress at several institutions in Kuala Lumpur has shown that aflatoxin is present in various groundnut products on sale in Malaysia. In view of the social implications and the need for an interdisciplinary approach to them, the Malaysian Scientific Association has set up a committee with the following terms of reference: "To encourage investigation of aflatoxins in Malaysia and to collect and disseminate information on this problem. To formulate recommendations for the reduction of hazards arising from aflatoxins in this country".

Problems of Food Aid

A STUDY of some of the problems encountered in the organization of food aid to developing countries has been made by D. A. FitzGerald of the Brookings Institution, Washington, D.C. The study is published in a report, *Operational and Administrative Problems of Food Aid*, the fourth in a series of World Food Program Studies by the Food and Agriculture Organization of the United Nations (Pp. viii+63. Rome: Food and Agriculture Organization of the United Nations; London: H.M.S.O., 1965. 5*s*.; 1 dollar). The study covered the background to food aid from its beginning with the passing of an amendment to the United States Mutual Security Act in 1953, to the establishment of the World Food Programme in 1961 by the United Nations General Assembly. The purpose of the study was to analyse the experience gained in the organization of food aid and to draw conclusions for the guidance of member governments in deciding whether the World Food Program should be continued beyond its original three-year period. Assistance to developing countries has been supplied either as bulk-supply, project-oriented or emergency food aid, the first of these categories being by far the major part of all food aid in the first 6 years. The problems encountered in organizing each of these three types of aid are considered under separate headings.

Electronics Star Catalogue

THE Smithsonian Astrophysical Observatory has now published in the United States a comprehensive star catalogue giving the positions and proper motions of more than a quarter of a million stars. The *Smithsonian Astrophysical Observatory Star Catalog* is available either in printed form, occupying four volumes, or as two reels of magnetic tape compatible with IBM 729 units. The books, available at the United States Government Printing Office, Washington, D.C., cost 20 dollars; the tapes cost 50 dollars, and are to be had from the Smithsonian Astrophysical Observatory.

The availability of the catalogue on magnetic tape is in accord with the extent to which the Institution has relied on computers in the compilation of the new catalogue. Work began more than 8 years ago and a computer programme was used to correlate information about the positions and proper motions of stars given in various catalogues and to refer these to the Epoch and Equinox 1950·0. In effect, the new catalogue is a combination of several earlier catalogues, including these catalogues: the *FK* 4, *FK* 3, *GC*, *AGK* 2, *AGK* 1, Greenwich *AC*, Yale, Cape, Cape Zone, *Me* 3, and *Me* 4. Transferring this mass of information into print was accomplished by means of a Stromberg Carlson computer which was programmed so as to present an image of one page of print at a time on the face of a cathode ray tube. This was then photographed to produce lithographic plates for printing. At the Smithsonian Astrophysical Observatory the information is now being transferred to sky maps which are expected to be complete by this summer. The density of stars is such as to provide an average of six stars per square degree. The average standard deviation of the position of the stars at the time of their first observation

is one-fifth of a second of arc. The average standard deviation of positions for the present epoch obtained by using the proper motions given in the catalogue amounts to half a second of arc.

Mössbauer Spectroscopy

THE U.S. National Bureau of Standards is now issuing a reference standard intended as a standard absorber in Mössbauer spectroscopy. The standard consists of a single crystal of sodium nitroprusside. This material has been selected on account of its chemical stability, very low temperature coefficient, the narrowness of the X-ray lines, and because of its doublet line structure. The standard is intended to be used in comparison with measurements with other materials, and may be purchased for 150 dollars.

University and College News:

Battersea College of Technology

DR. K. HOSELITZ has been appointed to the first visiting professorship of the College, while remaining deputy director of Mullard Research Laboratories. Dr. Hoselitz will be associated with the work of the Solid State Physics Group which recently received a grant of £25,000 from the Science Research Council for work on the structure and chemical properties of crystalline polymers. This appointment is one of the very few so far in Britain to have been designed to bring industrial scientists more fully into academic work. Dr. M. B. Waldron, group leader in physical metallurgy at the Atomic Energy Research Establishment, Harwell, has been appointed to the chair in and headship of the Department of Metallurgy and Materials Technology as from September 1.

Cambridge

PROF. W. R. HAWTHORNE, Hopkinson and I.C.I. professor of applied thermodynamics at the University of Cambridge, has been appointed chairman of the Advisory Council on Scientific Research and Technical Development of the British Army, as from April 1. He succeeds Sir Charles Dodds, president of the Royal College of Physicians, who has been chairman for the past five years.

Lancaster

THE University has announced the appointment of Dr. K. D. Tocher as its first industry-based part-time professor. Dr. Tocher, head of the Department of Operational Research, The United Steel Companies, Ltd., Sheffield, will work in the Department of Mathematics. The University regards this appointment as the beginning of the establishment of a liaison between the University and industrial scientists.

London

THE following appointments have been made: Dr. C. C. Booth, senior lecturer in medicine at the Postgraduate Medical School of London, to the chair of medicine, tenable at that School; Prof. D. T. Donovan, professor of geology in the University of Hull, to the Yates-Goldsmid chair of geology, tenable at University College.

Appointments

MR. R. G. VOYSEY has been appointed director of the United Kingdom Scientific Mission in Washington, D.C., and counsellor (scientific) at the British Embassy in Washington. He replaces Dr. J. A. Saxton, who is returning to the United Kingdom after holding this post since January 1964.

DR. J. A. SIMMONS has been appointed head of the Metal Physics Section at the National Bureau of Standards.

DR. D. W. MATHIESON, reader in pharmaceutical chemistry in the University of London, has been appointed professor of pharmaceutical chemistry as from September 1.

Announcements

A CONFERENCE on "Calculation of the Properties of Vacancies and Interstitials" will be held in Washington during May 1–5. Further information can be obtained from A. D. Franklin, National Bureau of Standards, Washington, D.C.

THE eighteenth international symposium on "Crop Protection" will be held in Ghent on May 3. Further information can be obtained from Prof. J. Van den Brande, Rijksfaculteit der Landbouwwetenschappen, Coupure Links 235, Ghent.

A MEETING of the Society for Analytical Chemistry, devoted to short papers on "Research Topics in Analytical Chemistry", will be held in the University of Bristol on May 4. Further information can be obtained from the Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

AN international seminar on "Research and Education in Chemical Engineering and Physical Chemistry" will be held in the Technical University of Karlsruhe during May 2–July 15. Further information can be obtained from the United Nations Educational, Scientific and Cultural Organization, Place de Fontenoy, Paris 7.

A SYMPOSIUM on "Graphite", organized by the Electrothermics and Metallurgy Division and the Industrial Electrolytic Division of the Electrochemical Society, Inc., will be held in Cleveland as part of the Spring meeting of the Society during May 1–5. Further information can be obtained from S. W. Bradstreet, Air Force Materials Laboratory, Wright Patterson Air Force Base, Ohio.

THE annual general meeting of the Polarographic Society will be held at the Imperial College of Science and Technology, London, on April 27. The programme will include a lecture on "Polarographic Characteristics of Organic Compounds in Organic Solvents", by Dr. M. E. Peover. Further information can be obtained from Mrs. B. Lamb, 42 Blenheim Gardens, Wembley, Middlesex.

DR. W. J. ECKERT, director of the Watson Laboratory of the International Business Machines Corporation at Columbia University and professor of celestial mechanics in the University, has been awarded the James Craig Watson Medal of the National Academy of Sciences for his contribution to the scientific application of electronic computers and to the theory of the motion of the Moon.

ERRATUM. In the article entitled "Finding Metarchons for Pest Control" by R. H. Wright, which appeared on p. 103 of the July 3, 1965, issue of *Nature*, lines 16 and 17 of the fourth paragraph should read, "likewise completely ineffective in preventing the fruit-flies from congregating".

CORRIGENDUM. In the article entitled "Typography of Bacteria", which appeared on p. 332 of the October 23, 1965, issue of *Nature*, in section 13 (*c*), the word '*kauffmanii*' should read '*kauffmannii*', and in section 13 (*d*), the example should read '*Salmonella kauffmanni* [serotype] London'.

CORRIGENDUM. In the article entitled "Is the Early Evolution of Life Related to the Development of the Earth's Core?" by Prof. C. Sagan, which appeared on p. 448 of the May 1, 1965, issue of *Nature*, in lines 13 and 14 of paragraph 4, '125 km' should be '260 km' and '30 km' should be '200 km'. In line 8 of paragraph 7, '2×18^8' should be '2×10^8'.

THE CARNEGIE INSTITUTION OF WASHINGTON

YEAR Book 64 of the Carnegie Institution of Washington, covering the activities of the Institution in the year ended June 30, 1965, includes, besides the President's review, reports from the several Departments of Terrestrial Magnetism, Plant Biology and Embryology, the Mount Wilson and Palomar Observatories, the Geophysical and Cytogenetics Laboratories, the Committee on Image Tubes for Telescopes and the Genetics Research Unit*. There is also a bibliography of publications during the year, the report of the Executive Committee and a list of officers and staff.

The president comments that the work of the Institution again illustrates the important part played to-day in many scientific fields by programmes of relatively low cost built around the work of the gifted individual investigator, and refers first to the Institution's activities in the planetary sciences, astrophysics and astronomy (see also *Nature*, 209, 743; 1966). Spectacular red-shifts were observed for some of the quasi-stellar radio sources first catalogued by radio astronomers, and much progress was made towards the optical identification of radio sources. An analysis of the new data suggests that we may be on the verge of conclusive evidence about the validity of important hypotheses concerning the dynamic structure of the Universe. A theoretical investigation of background cosmic light from galaxies completed during the year indicated that the combined light of the zodiac and the galaxy would be more than 100 times stronger than the cosmic signal.

The staff of the Observatories were actively concerned with investigations for the site of a possible new major observatory in the Southern Hemisphere. Two prospective sites in Chili offer superlatively good observing conditions, and the Institution is prepared to co-operate in establishing such an observatory. A 200-in. mirror-size instrument would be the most effective, subject to appropriate sponsorship. The Committee on Image Tubes for Telescopes has now met the rather stringent requirements by a 'cascaded' image tube developed for the Committee by the Electron Tube Division of the Radio Corporation of America. Twenty of the tubes were delivered during the year, and the Committee has now designed five different optical systems for efficiently presenting the optical image from a telescope to the tube and for projecting its output on to a photographic plate for recording; these tubes have been made available to five observatories. The Mount Wilson and Palomar Observatories continued their investigations of the spectra of Mars, Venus, Jupiter and Galilean satellites, the asteroid Vesta and the Moon.

The Geophysical Laboratory continued its investigations into the magnetic properties of the Earth meteorite minerals and the organic components of ancient rocks. Much attention was given to present and potential petrographic applications of discriminant function analysis, with particular emphasis on the interesting properties of linear and higher-order discriminants in the closed array. The influence of water on the melting behaviour of simple silicate systems was studied as a contribution to the understanding of volcanic eruptions. A detailed investigation was begun, at the recently developed Strathcona Mine at Sudbury, Ontario, into the formation of copper–iron–nickel sulphide ore. Investigations of the melting relations of the members of volcanic basalt–trachyte associations have confirmed a direct relation between liquid iron enrichment and liquidus temperatures for the rocks of the oceanic alkali provinces of Hawaii and Tristan da Cunha.

The Department of Terrestrial Magnetism continued its Andes seismic programme, and efforts were made to extend the scope of indigenous analysis procedures for the local groups by establishing a 'Carnegie Analysis Centre' in Antofagasta. The nuclear physics programme continued to be directed towards the interactions of polarized nuclear particles. It was observed that the size–frequency-of-occurrence pattern of the foreshocks of the large Chilean earthquake of 1960 differed remarkably from the pattern of aftershocks for the same region. Apparently each seismically active region has a characteristic 'normal' ratio of small shocks to those of moderate intensity. Investigations of heat flow and thermal properties at the bottom of Lake Superior and of Seneca Lake have suggested a possible new method of measuring the outward flow of heat from the inside of the Earth's crust that avoids the costly drilling of large numbers of very deep holes. The Biophysics Group of the Department has further examined the processes of replication and preservation of the genetic information which is transmitted from cell to cell in the coded form of base sequences in the deoxyribonucleic acid of the cell. Animal deoxyribonucleic acid was found to possess numerous copies of, or very nearly identical, gene sequences when fragmented in suitable suspension and eluted from single-stranded deoxyribonucleic acid immobilized in agar. The degree of homology indicated by the varying firmness of binding is tied to the degree of sameness or identity of the gene fragments, both for the same species or for related animals. Selective repression of lysogenic cells among susceptible bacteria can be effectively obtained by: (1) introduction of defects into deoxyribonucleic acid by radiation or radiomimetic drugs, such as mitomycin; (2) inhibition of repair mechanisms in the deoxyribonucleic acid by agents like hydroxyurea or phenethyl alcohol inhibiting the deoxyribonucleic acid synthesis; (3) inhibition of protein synthesis by drugs such as chloramphenicol, puromycin, or amino-acid analogues; (4) addition of chelators, such as ethylenediamine sodium tetraacetate to amplify the increasing effectiveness of the initial radiation or radiomimetic agents. It remains to be seen if this combination chemotherapy can be effective in inhibiting solid tumours of animals and man.

In the Department of Plant Biology the chemical composition and the function in photosynthesis of the pigment complexes in plants have been a major concern, and efforts were intensified to separate the two pigment systems without altering their characteristic absorption spectra or photochemical activity. Attempts were made to determine what step in the photosynthetic process is responsible for oxygen utilization, and changes in rates of oxygen uptake lasting some minutes were induced in *Chlorella* by illumination for only 1 sec, but not in cells treated with glucose or with antimycin. The location of the copper-containing protein plastocyanin in the electron transport chain of photosynthesis has been more specifically established, while in experimental taxonomy differences continued to be found in the photosynthetic ability of plants native to various climates. Races of *Mimulus lewisii* from high altitudes require considerably higher light intensities to saturate photosynthesis than races of *Mimulus cardinalis* from lower altitudes. Further investigation of the mechanics of light absorption and utilization in *Mimulus* showed that the capacity to absorb light varies between races, and the study of evolutionary relations in *Mimulus* has been extended to the genetics

* Carnegie Institution of Washington. *Year Book 64*. Pp. xii + 64 + 596 + 21 plates. (Washington, D.C.: Carnegie Institution of Washington, 1964–65) 1.50 dollars.

of other related species in the Erythranthe section of *Mimulus*.

The Department of Embryology continued to explore the relation of the egg deoxyribonucleic acids to the somatic deoxyribonucleic acids of the eggs of *Xenopus laevis* and *Rana pipiens* and also the mechanism and site of action of actinomycin *A* in inhibiting the developing heart and somites in chick embryo. A mitochondrial factor was isolated which affords protection against anmycin and also prevents the action of antimycin in monolayer cultures of embryonic skeletal muscle. Evidence was obtained that myoblasts can be infected by Rous sarcoma virus. Experiments are in progress to determine the amount and rate of viral synthesis in muscle at each major point in its development and to compare the infectivity of muscle clones with clones of cartilage cells and with clones of fibroblastic cells. Studies of the fate of small fragments of radioactively labelled chick embryos transplanted orthotopically in unlabelled hosts showed that cells of the epiblast may be found in autoradiographic preparations in sites approaching the primitive streak and leaving it and in the streak itself.

In the Genetics Research Unit, progress was made towards the completely accurate physical mapping of phage genes. Work on the structure and function of phage deoxyribonucleic acids showed that the deoxyribonucleic acid fragments in the defective bacteriophage *T*4 particles terminate at random points in the genome of the phage and that the deoxyribonucleic acid molecule and the genome are collinear. Experiments with phage lambda afforded a first physical description of the sequence in the activity of different parts of the phage genome, and other work in the Unit was concerned with the components of actin of the regulators *Spru* and *Ac* present in maize. The Cytogenetics Laboratory reports investigations of the fine structure of chromosomes, the mitotic cycle and deoxyribonucleic acid replication of *Haplopappus gracilis*, the deoxyribonucleic acid of mitotic chromosomes of *Drosophila* and the base composition of heterochromatic deoxyribonucleic acid in *Drosophila melanogaster*.

THE ROYAL SOCIETY OF NEW ZEALAND

THE Royal Society of New Zealand Act, 1965, which came into force on January 1, 1966, consolidates and amends the previous Act of 1933. The Society was established as the New Zealand Institute in 1867 and had three primary functions. First, it provided scientists in New Zealand with an independent forum for scientific discussion, and an open channel for publishing their research findings and, secondly, it offered an independent and authoritative voice to the community and to the Government on scientific matters. Its third function was to act as a body through which New Zealand scientists were linked to the world organization of science, and it is the New Zealand section of the International Council for Scientific Unions.

From its inception, the Society has been federal in structure, linking the activities of regional scientific institutes or societies in the main New Zealand Centres. The new Act and constitution place the policy and control of the Society in the hands of the Fellows while retaining the links established with the member bodies. The Fellowship of the Society, established in 1919, is a self-perpetuating body, at present limited to one hundred scientists, and their part in administering the Society has hitherto been restricted to the appointment of two out of about thirty councillors.

Under the new Act, control of the Society is vested in a council of fourteen, of whom four officers and eight councillors are elected by the Fellows from among their number. The two other councillors represent the member bodies and are appointed by a Member Bodies Committee acting as an electoral college.

The objects of the Society are defined as to promote science, to encourage and stimulate high standards of scientific endeavour and achievement and to give recognition to notable achievements in the advancement of science in New Zealand; to inform the Minister of fields in which, in the opinion of the Council, the scientific effort of New Zealand should be increased, and to suggest how this may be done; to initiate and maintain contacts between New Zealand scientists and scientists in other parts of the world, especially through the unions of the International Council of Scientific Unions and other scientific bodies overseas; to administer funds for scientific research or scientific projects; to initiate meetings or participate in meetings of scientists in New Zealand; to initiate and maintain contacts between scientists in New Zealand, and provide liaison between co-relating efforts of its member bodies and other scientific bodies in New Zealand; and to publish and disseminate scientific knowledge and make available sources of scientific information. The present Council remains in office until the appointment of officers and councillors under the new Act at the annual general meetings of Fellows in May 1966, and one of the last acts of the Council was to adopt new rules which have now been published in the *New Zealand Gazette* of December 22, 1965. Besides the branches of the Royal Society itself, the member bodies listed in the first Schedule to the Act include the Auckland Institute, the Geological Society of New Zealand, the New Zealand Institute of Chemistry, the New Zealand Economic Society and the New Zealand Society of Soil Science.

BRITISH REPRESENTATIONAL SERVICES OVERSEAS

THE debate on a Ministry of External Affairs in the House of Lords on December 21 was opened by Lord Gladwyn, who asked whether the Government had now concluded that the time had arrived for establishing a single Ministry of External Affairs. After referring to the recommendation to this effect in the Plowden Report on Representational Services Overseas, Lord Gladwyn urged that it was now even more important for overseas matters to be handled by a single Ministry of External Affairs.

Lord Caccia supported him, and urged that all that had happened in the past 2 years strengthened the argument. He thought that the present pattern of events was unlikely to change.

Lord Inchyra, while agreeing with Lord Gladwyn, pointed out that the Foreign and Diplomatic Services had already been amalgamated and that the process seemed to be proceeding more smoothly than was anticipated; the time had come, he said, for change. Lord Sherfield also did not see why a single office should not be so organized as to avoid any additional burden on the Secretary of State. He was also supported by Lord Hankey, who thought that if the change was made it might be necessary to have an extra minister in the Foreign Office, though not necessarily a Cabinet Minister.

Lord Walston said that he had an open mind about the actual process of co-ordination of the two Offices, but he

emphasized the importance of paying due consideration to the views of other countries in the Commonwealth. He thought, on the grounds of efficiency, however, that there was much to be said for one office.

With the creation of an integrated diplomatic service, the main recommendations of the Plowden Committee had already been put into effect. Since January 1, 1965, eighty-eight members of the former Foreign Service had been posted to posts in the Commonwealth Relations Office and forty-two members of the Commonwealth Relations Office had been posted to Foreign Office posts. The communications system and the cyphers had been unified, as had the information services of the two offices, and significant progress had been made in the making and implementation of policy, but he could not give Lord Gladwyn any assurance that the two offices would be merged within the next year. The Government was in fact working towards an organization in which, whether or not there were two Departments of State, and two Secretaries of State, the co-operation between the two was such as to eliminate very largely the inefficiencies and illogicalities.

REFORM OF SCOTTISH UNIVERSITIES

THE Universities (Scotland) Bill received the Royal Assent on March 9. The Bill has as its purpose to implement some specific proposals of the Robbins Report for the development of higher education in Scotland. As Lord Hughes explained in moving the second reading in the House of Lords, its aim is to modernize the constitutions of four old Scottish universities and to pave the way for establishing a new university at Dundee. After looking at the constitutions of the existing universities, the Robbins Committee recommended that the four older Scottish universities should be free from the cumbersome outmoded procedures, under the Universities (Scotland) Acts, 1858 and 1889, by which they still had to conduct academic and other business. This procedure involved consultations between the four universities, laying ordinances before Parliament, and approval of them by the Privy Council. This process could take anything from 6 months to a year, and extended into such business as the founding of new chairs and making regulations for new degrees.

There is no controversy about the basic aim of the Bill to modernize the constitutions of these four old universities, although there had been a sharp difference of view on the means by which this should be achieved. The present university authorities asked only for certain minimum reforms to the existing acts, but the Association of University Teachers urged that no really satisfactory reforms could be achieved unless the universities revised their constitutions by drawing up charters.

Lord Hughes said that the Bill was essentially an enabling one, adjusting the balance of power within the Universities to give all the members of a university a due share in its government, and leaving it to the university to decide the rate and nature of constitutional reform. Part I empowered each of the four universities to contract out of the present legislation by petitioning for a Royal Charter whenever it judged the time to be right, provided that the views of all members of the university were canvassed before any such move was made.

In Part II, the essential minimum reforms to the present constitutions were made. By Clauses 2 and 7 the composition of the main organs of university government, the court and the senate, were revised, and non-professorial staff—readers and lecturers—were given a larger share in the management of the university. Clauses 4 and 6 swept away the outmoded and time-wasting procedures at present obtaining and replaced them by new procedures which distinguish between matters of public importance and academic and other business lying properly within the discretion of the university.

Part III of the Bill took the legislative steps necessary to separate Queen's College, Dundee, from St. Andrew's University when the new University of Dundee came to be founded by Royal Charter. This part of the Bill is wholly non-controversial but somewhat complex and technical in character.

LATITUDE-PERIOD DEPENDENCY OF GEOMAGNETIC MICROPULSATIONS

By T. J. HERRON and DR. J. R. HEIRTZLER
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IN the analysis of geomagnetic micropulsations in the period range of ten to several hundred seconds, various authors have commented on the dependence of period on latitude. The nature of such a dependence, if one exists, has not been fully resolved. The subject is of importance to theories of origin which attribute micropulsations to hydromagnetic oscillations in the magnetosphere. The study of period difference at latitudinally separated stations has involved the direct comparison from analogue records of simultaneous micropulsations, the comparison of power spectra of simultaneous micropulsation wavetrains, and the comparison, over an interval of several months and at various latitudes, of the predominant periods of micropulsations that are not necessarily simultaneous. The conclusions reached have been varied. Voelker[1] recorded micropulsations simultaneously with three similar instruments (48·9°–54·6° geomagnetic latitude) and reported a systematic increase of period with latitude for the H-component of Pc-type oscillations and for the H-component of a damped pulsation named 'pulsation single effect'. Obayashi and Jacobs[2] compiled a statistical scatter diagram of period versus latitude from data of eleven observatories (28°–67° geomag. lat.) which, despite considerable scatter, showed an increase of period with latitude. Duncan[3] compared the predominant periods at three Australian stations (29°–52° geomag. lat.) and found an increase of period with latitude. Other investigators, however, have not found such an effect. Ellis[4], studying the data from the three Australian stations used by Duncan[3], found no latitude-period dependence. Komack *et al.*[5] computed power spectra of magnetotelluric micropulsations at stations separated in latitude (22°–40° geomag. lat.) and reported little or no difference in the period.

To study the latitude-period dependency, the Geomagnetism Group of Lamont Geological Observatory recorded micropulsation activity simultaneously at pairs of stations ranging from 36° to 61° geomagnetic latitude. Because the results of Obayashi and Jacobs[2] indicated a much more rapid period increase near the auroral zones

Table 1. STATIONS OCCUPIED

Station	Geographic Lat. N.	Geographic Long. W.	Geomagnetic latitude	Recording interval, U.T., 1963
Miami, Florida	25° 45′	80° 30′	36°	2300 Aug. 7–2400 Aug. 8
Folkston, Ga.	30° 49′	82° 02′	42°	1400 Aug. 5–2100 Aug. 6
Smithfield, N.C.	35° 14′	78° 08′	46°	0100 Aug. 3–1500 Aug. 4
Lebanon, N.J.	39° 38′	74° 32′	50°	Simultaneous with other stations
Plattsburgh, N.Y.	44° 43′	73° 15′	55°	0200 July 28–0900 July 29
St. Leonard, Que.	46° 02′	72° 22′	57°	1600 July 20–1800 July 21
St. Ambrose, Que.	48° 30′	71° 19′	60°	2300 July 29–1200 July 31
St. Felecien, Que.	48° 34′	72° 28′	60°	1900 July 24–0900 July 25
Chibougamau, Que.	49° 46′	74° 26′	61°	1700 July 22–1600 July 23

(nearly a doubling of period from 50° to 60°), the Lamont stations were established over a latitude range sufficiently wide to test the dependency found in these earlier studies. A rubidium vapour magnetometer was operated at a base station in the Lebanon State Forest on the New Jersey coastal plain. A second rubidium magnetometer was operated at other sites (Table 1) approximately on a north–south line through Lebanon. Recording was continued for at least 24 h at each station pair during a 2·5 week interval of generally high micropulsation activity. Only those station pairs that showed micropulsation activity throughout a major part of a diurnal interval were used in this analysis. Each instrument recorded on a strip chart recorder at a paper speed of 1 in./min and a sensitivity of 1·3 γ/in. The rubidium magnetometers measured the components of magnetic variation in the direction of the total field (mainly the vertical component at the higher latitudes where the magnetic lines of force approach the vertical).

Investigation of Individual Micropulsations. The records for each station pair were examined throughout the recording interval and the highest amplitude oscillations occurring at both stations were selected for study. The period of an amplitude group, usually 5–10 cycles in duration, was determined by numbering the peaks and troughs of the oscillation and plotting the occurrence times of the peaks and troughs versus the cycle number. The slope of the best-fit line through the plotted points was the average period. The ratio of the period at the high-latitude station to the period at the low-latitude station was plotted against time of day. Fig. 1 gives the results for three pairs of stations in which the field stations are at lower latitudes than Lebanon. Fig. 2 presents the

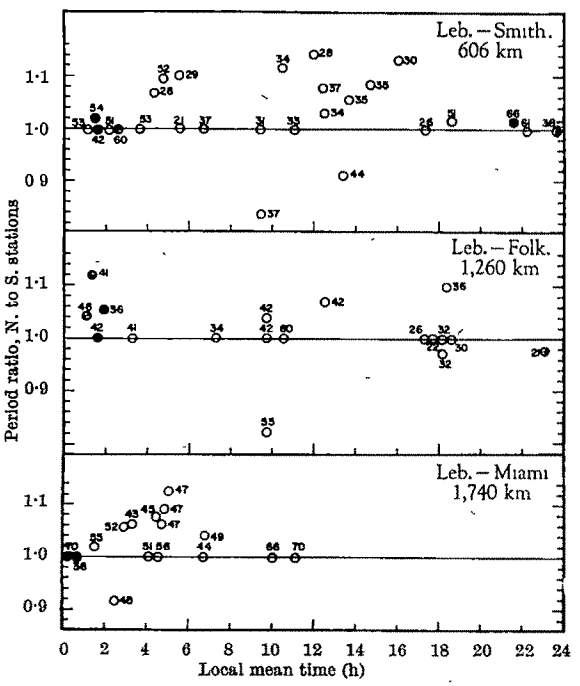


Fig. 1

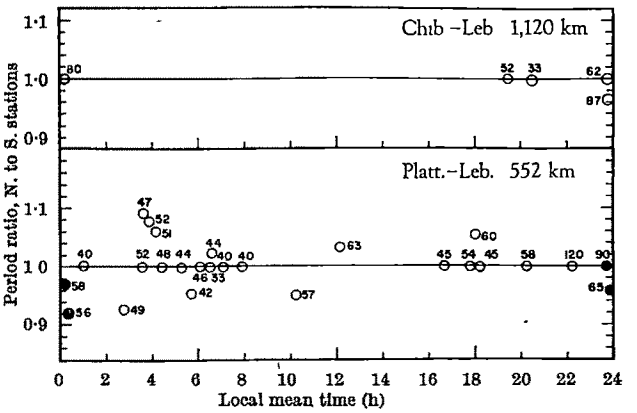


Fig. 2

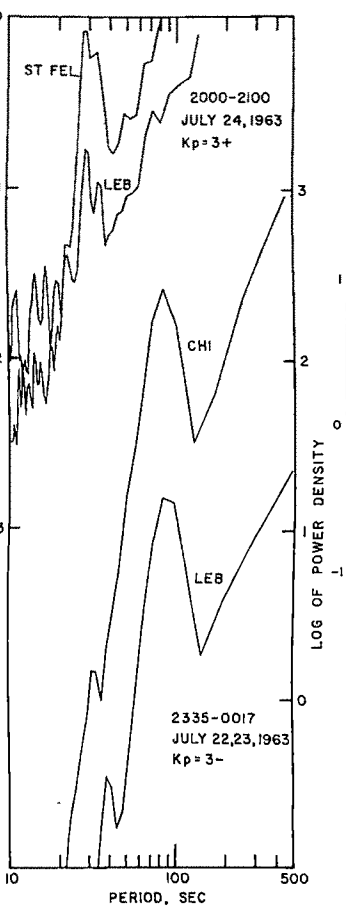


Fig. 3

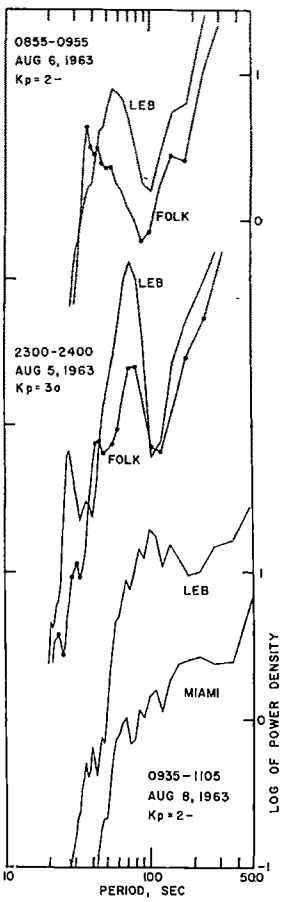


Fig. 4

period ratios for two of the four stations at higher latitudes than Lebanon. The open circles indicate *Pc*-type oscillations. The solid points indicate *Pt*-type oscillations. The number next to each point is the period at the lower-latitude station.

For most of the station pairs in Figs. 1 and 2, more points fall above the 1·0 axis than below; this could indicate a tendency for the period to be longer at the higher-latitude station. However, the average period resolution for the method used is no better than about 5 per cent allowing a somewhat greater error in the ratio determination. The scatter of ratio points is mostly within a 10 per cent variation and is no greater for the more widely separated stations, indicating that the scatter is probably caused by error in measuring the periods. Thus, within the accuracy of the method, there is no dependence of period on latitude for the simultaneous micropulsation groups.

Power Spectral Analysis. Power density spectra were computed of approximately 1-h intervals of strong micropulsation wave-trains occurring at both the base and field stations. Digital sampling intervals of 1–3 sec were used. The data were pre-whitened before the spectral analyses were performed by use of a filter that attenuated at 6 dB/octave toward the long periods. The maximum lag used was 10 per cent. Smoothing of the raw spectrum was done with a hamming window. The spectra were restored after analysis to compensate for the pre-whitening. Power density was computed in units of $\gamma^2/c/s$.

Fig. 3 presents two pairs of spectra for field stations at higher latitudes than Lebanon. The Lebanon–St. Felecien pair in the upper part of the figure shows strong spectral peaks at the 30-sec period. The Lebanon–Chibougamau pair in the lower part of the figure shows very strong spectral peaks at about the 86-sec period. For each pair, the spectral peaks at Lebanon (the lower latitude station) are at periods 2–3 sec longer.

Fig. 4 presents three pairs of spectra for two field stations at lower latitudes than Lebanon. The Lebanon–Miami pair at the bottom of the figure shows a group of spectral peaks between the 60- and 100-sec period. There is no measurable difference in period of the peaks between stations. The Lebanon–Folkston pair in the middle of the figure shows large spectral peaks at the 74-sec period at Lebanon and at the 76-sec period at Folkston. The Lebanon–Folkston pair at the top of the figure shows a concentration of energy at the 55-sec period at Lebanon and the 37-sec period at Folkston. Closer examination of this upper pair reveals that the broad spectral maxima between 30 and 70 sec are a superposition of spectral components of 37, 45 and 55 sec (and perhaps other) periods. The components are evident in both spectra, if only as minor humps. The 37-sec component has the greatest amplitude on the Folkston spectrum and the 55-sec component has the greatest amplitude on the Lebanon spectrum. The first impression of a period dependence on latitude, in this case, would appear to be rather a case of amplitude dependence on latitude of a number of distinct spectral components. The Lebanon–Miami pair clearly shows that a group of distinct spectral peaks can occur at both of two widely separated stations. Previous investigations summarized by Troitskaya[6] have shown that amplitudes of micropulsations usually increase with latitude. In agreement with this, the five pairs of spectra show that, except for several narrow-band peaks, the higher-latitude station had higher amplitudes over a broad period range.

In summary, ten pairs of power spectra at north–south stations were computed, of which the five pairs shown are typical. Cases where the higher latitude station had slightly longer periods were observed as were cases where the lower-latitude station had slightly longer periods. Both the investigation of power spectra and the investigation of individual micropulsation groups indicate no sizable or consistent difference in period between latitudinally separated stations. These results apply to geomagnetic latitudes between 36° and 61°, to the component of variation in the direction of the total field, and to the time of year indicated in Table 1. The results do not preclude a period-latitude dependence for any type of rarely occurring micropulsation or at some other season of the year or in a different component of the field. The conflicting results of this and previous investigations indicate the necessity of carrying out a more extensive study involving three orthogonal components of the field recorded over a wide latitude range at several seasons of the year.

We thank Mr. M. Davidson, Mr. J. Chute, and Mr. J. Foster for help in collecting and processing the data. The work was supported by a contract from the U.S. Office of Naval Research and by a grant from the National Science Foundation.

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ICE SHELF THEORY OF PLEISTOCENE GLACIATIONS

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A. T. WILSON[1] developed a theory for the origin of the ice ages. In his theory, Antarctica plays an important part. Although he is able to give an excellent explanation of highly important features of the ice ages, his theory is open to some criticism.

Wilson's theory. Briefly, the main points of Wilson's theory are:

(1) Antarctica is covered by an ice sheet several kilometres thick. The pressure on the rocks below the ice sheet is very high. Computations show that where there is little precipitation the ice at the base of the cover will reach its pressure melting-point; however, where there is considerable precipitation on the surface of the ice sheet, the pressure melting-point will not be reached at the bottom. Thus a decrease in surface precipitation may cause instability of the ice sheet.

(2) If instability occurs the ice sheet will flow radially outwards into the surrounding sea. A large ice shelf will be formed, covering both Antarctica and the ocean around it. As a consequence of the increased albedo of the earth, cooling will occur and also extensive glaciation will be induced in the northern hemisphere.

(3) When the expansion of the southern ice shelf stops, the ice cover of the ocean will slowly start to melt. After some time, only the ice cover of Antarctica will be left. Clearly it will be much thinner than before the outflow. Because of the slow decrease of the albedo, the Earth will slowly return to its interglacial temperature. In the subsequent warmer period the Antarctic ice cover will grow again by precipitation, until a sudden decrease of precipitation induces a new instability. In this way the cause of the ice ages would resemble a kind of relaxation mechanism, like the 'flip-flop' in electronics. The 'controlling pulse' would be the relatively sudden decrease of precipitation.

(4) The fact that no ice ages occurred more than 1 million years ago is explained as follows: Wilson's mechanism needs a large area of rocks and mountains close to the south pole. Because of continental drift (Wegener's hypothesis) Antarctica would have arrived close to the pole several million years ago. At that time Wilson's mechanism could start. First, small ice shelves would be produced, but as Antarctica gained a more symmetric position with respect to the pole, larger ice shelves would be produced which would result in ice ages.

(5) The coincidence of the ice ages in the northern hemisphere with the insolation-minima computed by Milankovitch[2] can be explained as follows: the decrease of

precipitation would be caused by a decrease of 'insolation'. Thus a decrease of insolation would be the real 'controlling pulse' of the 'flip-flop' mechanism.

My criticism is directed against this last-mentioned point. Milankovitch computed his insolation curves for the northern hemisphere. Because the astronomical causes of variations in insolation have different consequences for both hemispheres, the insolation curves for the southern hemisphere will in general be entirely different from the northern. This can be seen in Fig. 1.

In order to demonstrate why the insolation changes are different in the two hemispheres, I shall now review Milankovitch's theory.

Milankovitch's theory. The changes of the insolation are caused by two different mechanisms:

(1) The obliquity ε of the ecliptic oscillates between 21° 58·6′ and 24° 36′, with a period of 40,400 years. This is a consequence of secular perturbations caused by the major planets. If ε is maximal the high latitudes on both hemispheres will receive considerably more solar radiation during a year than when ε is minimal. Thus the situation with minimal ε is favourable for the occurrence of glaciations on both hemispheres. Changes in ε give rise to equal insolation changes for both hemispheres.

(2) Owing to the precession, the perihelion length Π of the Earth's orbit increases by 360° in about 21,000 years. If $\Pi = 0°$, then the lengths of summer and winter are equal for both hemispheres (see Fig. 2). In this case there will be no difference in insolation. However, 5,250 years later, when $\Pi = 90°$ (and if the eccentricity e of the orbit does not equal zero), the northern summer will last longer than the winter. In this event, the northern summer will be long and cool, and the winter will be short and mild. The southern summer will be shorter than the winter. The summer will be warm and the winter cold. The difference in duration between summer and winter is:

$$\Delta T = \frac{4e}{\pi} \sin \Pi \,.\, 365{\cdot}24 \text{ days} \qquad (1)$$

Milankovitch[2] claims that in times of cool summers the yearly amount of precipitation is large. During a hot summer and the subsequent cold winter the amount of precipitation is small. Since glaciers grow by precipitation, the situation with $\Pi = 90°$ will be favourable for the occurrence of extensive glaciation in the northern hemisphere, and unfavourable for it in the southern hemisphere.

If $\Pi = 180°$, there will again be no difference in insolation between the hemispheres. If $\Pi = 270°$, the situation

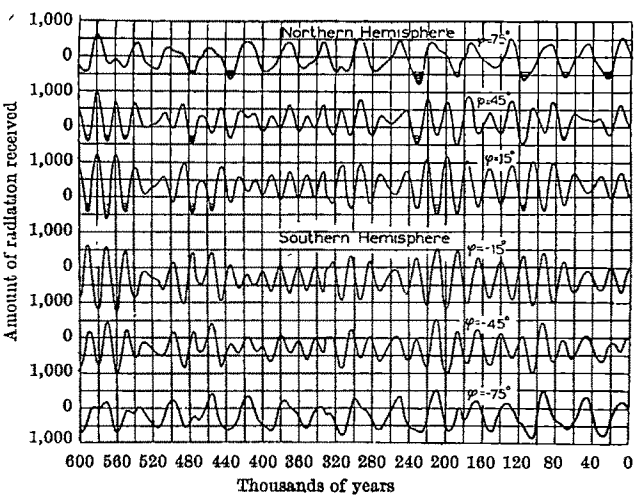


Fig. 1. Milankovitch's insolation curves for the past 600,000 years. The figure represents the curves for geographical latitudes $\varphi = \pm 15°$, $\varphi = \pm 45°$ and $\varphi = \pm 75°$. Note that the curves for $+15°$ and $-15°$ show opposite variations; the amounts of radiation are given in 'canonic' units[2]. The curves for $\varphi = +75°$ and $\varphi = -75°$ show maxima and minima at almost the same times. The curves for $\varphi = +45°$ and $\varphi = -45°$ are irregular and show no correlation. The same holds for the curves for latitudes between $\pm 45°$ and $\pm 75°$

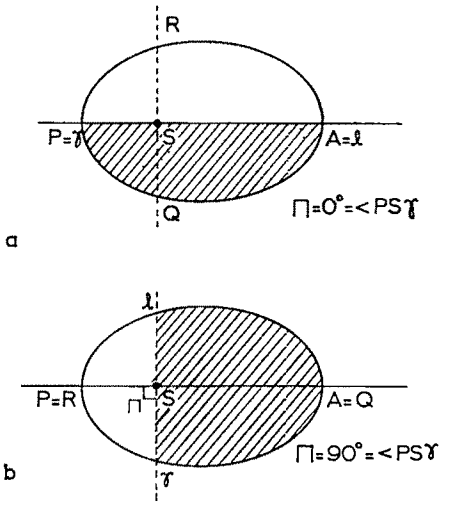


Fig. 2. *a*, If the perihelion length $\Pi = <PS\gamma = 0°$, the Sun is in the equatorial plane when the Earth is in the point P of its orbit. (γ is the equinox, S is the Sun.) The Sun reaches its maximal angular height above the equatorial plane when the Earth is in the point Q (mid-summer) and is again in the equatorial plane when the Earth is in the point A (aphelion). Mid-winter in the northern hemisphere occurs if the Earth is at the point R. Thus if $\Pi = 0°$, the length of the summer (area PQA) is just equal to the length of the winter (area ARP). The same occurs if $\Pi = 180°$. In that case $A = \gamma$ and $P = \mathcal{A}$, while R and Q are interchanged. *b*, If $\Pi = 90°$, mid-summer for the northern hemisphere occurs when the Earth is in the aphelion A and mid-winter when it is in the perihelion P. Thus the northern summer (area $\gamma A \mathcal{A}$) will be long and cool, the winter short and mild. For the southern hemisphere the winter is long and cold, the summer short and hot. The situation for $\Pi = 270°$ is just opposite to that for $\Pi = 90°$. As a consequence of the precession and the proper motion of the perihelion the angle Π increases by 360° in a time-interval of about 21,000 years

will be favourable for the occurrence of glaciations on the southern hemisphere and not the northern. According to the value of e (between 0·0000 and 0·0677) the contrasts between the northern and southern mean temperatures will be small or large (see equation 1). If $e = 0$, there will be no contrast at all. Thus the ε-variations act symmetrically on both hemispheres and the Π-variations act unsymmetrically.

The occurrence of ice ages. Since the periods of the ε-variations and the Π-variations are different, the insolation curves for the northern hemisphere will have an irregular character: sometimes the ε-variations will act in the same direction as the Π-variations, in which case the insolation will reach an absolute minimum or an absolute maximum. If the variations act in opposite directions, no extreme insolation situation will occur. The ice ages for the southern hemisphere are expected to occur if a minimum of ε coincides with times at which $\Pi = 270°$ and if simultaneously e is large.

On the northern hemisphere an ice age is expected if a minimum of ε coincides with a moment at which $\Pi = 90°$, and if simultaneously e is large.

Milankovitch's curves and Emiliani's minimum temperatures. By computing the diagrams for summer insolation, Milankovitch obtained curves as shown in Fig. 1. The minima of the curves for medium latitudes indicate the presumed times of the ice ages. From Fig. 1 it can be seen that for latitudes smaller than 70° the curves have an irregular character. The minima of the southern insolation curves do not coincide with those of the northern curves. Only for geographical latitudes larger than 70° are the curves for the northern and southern hemispheres more or less the same. This is due to the fact that in Arctic regions the insolation variations caused by changes of the obliquity ε of the ecliptic are much larger than the insolation variations caused by changes in Π. Thus for latitudes higher than 70°, one expects insolation minima at the same times for both hemispheres. Milankovitch[2] postulated that the insolation variations at latitudes of about 65° are the most important indications for the occurrence of the glaciations in the hemispheres, since the absolute insola-

tion variations are maximal at these latitudes. The 65°-curves are different for both hemispheres.

Emiliani[3] determined very carefully the changes in the temperature of the North Atlantic Ocean for the past 300,000 years. He obtained the temperatures by measuring the contents of oxygen-16 and oxygen-18 in ocean bottom sediments. His curve shows minima which coincide more or less with the insolation minima for 65° northern latitude. The minima in Emiliani's curve show a periodicity of about 40,000 years. They also coincide more or less with the minima of ε.

The contradiction in Wilson's theory is that the northern ice ages do not coincide with the southern insolation minima : if the northern ice ages were caused by an instability of the Antarctic ice cover it would be expected that they would coincide with the southern insolation minima and not with the northern insolation minima as is actually observed.

However, this criticism is not so serious as it seems, since it can be removed by considering the Antarctic instability mechanism in more detail or by changing somewhat Milankovitch's theory.

(*A*) Let us first consider the Antarctic instability mechanism in more detail. One can read Milankovitch's northern summer insolation curves as southern winter insolation curves since a northern summer coincides with a southern winter and inversely.

Milankovitch claims that in times of long and cool northern summers ($\Pi = 90°$) the yearly amount of precipitation in the northern hemisphere is enhanced. At the same time, the southern winters are long and cold, the summers short and hot, causing the yearly amount of precipitation on the southern hemisphere to be reduced. For this reason the minima of yearly precipitation for the southern hemisphere coincide with Milankovitch's northern insolation minima. Hence antarctic instability will just occur at times at which Milankovitch expected the northern ice ages to occur. It would therefore be quite logical that also according to Wilson the ice ages coincided with Milankovitch's northern summer insolation minima, which in fact indicate southern precipitation minima. This coincidence is only a question of mere chance due to the fact that northern summer insolation curves can be read as southern winter insolation curves. This removes my criticism. The issues of Milankovitch's and Wilson's theories, however, remain entirely different, and previously it would seem that these theories contradicted each other.

(*B*) The second possibility would be to use the fact that, according to Emiliani[3-5], the northern ice ages coincide more or less with the ε-minima. Milankovitch postulated that the insolation variations at 65° latitude are the most important. One could, however, abandon this postulate and assume that the variations in arctic regions are more important. Because the insolation curves for latitudes greater than 70° are almost the same for both hemispheres, the northern and southern insolation minima would coincide in that case. If now the instability of the antarctic ice cover would occur at times of minimal southern insolation, it would also occur at times of minimal northern insolation and again there would be a coincidence between the northern insolation minima and the ice ages.

Conclusions

Neither of the proposed adaptations of Milankovitch's theory to that of Wilson contradicts Emiliani's temperature determinations. Only a careful search for synchronism between variations in northern and southern temperatures will provide information that will make it possible to decide which of the two theories is correct. If the northern glaciations were to follow the 65° northern insolation curve and if the southern one were to follow the southern insolation curve, then obviously Milankovitch's theory would be right.

If, on the other hand, synchronism were found, then Wilson's theory would be right. Synchronism with a periodicity of 40,000 years would mean that variations in insolation in arctic regions were more important than those at 65° latitude. Only perfect synchronism between the 65° northern summer insolation curve and the ice ages in both hemispheres would be a decisive indication that Wilson's theory is right.

So far, however, it is only possible to say that there seems to be a correlation between the occurrence of the northern ice ages and the secular summer insolation variations in the 65° latitude region of the northern hemisphere.

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THE GRANITIC ROCKS OF HAIG FRAS

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IN an account recently published, Smith, Stride and Whittard[1] describe an area of granitic rocks situated about 60 miles west-north-west of the Scilly Isles. The specimens from this area, which appears to be surrounded by Devonian or Carboniferous rocks, include: (*a*) Seven granites and thirteen gneisses collected by scientists from the National Institute of Oceanography embarked in R.R.S. *Discovery II* in 1962. (*b*) Two granites and one gneiss obtained by University of Bristol scientists in R.V. *Sarsia* in 1962 and 1963. All *Discovery's* specimens were obtained by dredge; one of *Sarsia's* specimens was obtained by dredge and two by corer. An account of the petrology of all the rocks is given in an appendix by Dr. P. A. Sabine[1].

I have been fortunate to have the opportunity to examine in some detail the rocks collected by *Discovery II* and to have been given enough material from some of them for chemical analysis. Unlike *Sarsia's* specimens, which were taken from three sites, respectively in the north-west, east and south of the area, those recovered by *Discovery II* came from a single station in the north central part. (Ref. 1, Fig. 4.)

The Granites. In view of their position and age (277 ± 10 m.y.), Smith, Stride and Whittard conclude that the Haig Fras granitic rocks comprise a westerly addition to the granites of South-west England. It is therefore appropriate that some comparisons should be made.

From both the mineralogical and the chemical standpoints, certain similarities are apparent; for example, both suites of rock contain tourmaline as a normal constituent and both have a characteristically low potassium : rubidium ratio[2]. However, the greater part of the granite of the mainland is coarse-grained and porphyritic, with megacrysts upwards of about 3 cm in length, whereas the Haig Fras specimens are fine-grained, the largest crystals being no longer than about 3 mm. *Sarsia's*, as well as *Discovery's*, samples are reported to be fine-grained and it may be that no large mass of coarse rock is exposed

in the area. Further, the coarse rock of the mainland is distinctly potassic, only rarely becoming sodi-potassic[3], while the Haig Fras rocks are generally sodic (Table 2, columns 1–3, 6). It would appear, therefore, that the Haig Fras rocks do not resemble the bulk of the south-west granites either texturally or compositionally and that similarities must be sought among less common varieties.

Comparing them first with the fine-grained granites that occur in all the main outcrops, it is found that there is a reasonably close textural correspondence, apart from the foliation of the Haig Fras rocks[1], which is unusual. In composition, however, the fine-grained varieties of the mainland are manifestly more mafic and more potassic than the submarine specimens (Table 2, columns 1–4).

Two other groups of rocks might be regarded as possible counterparts; these are the fine-grained early and late sodic facies of the main granites.

Worth[5] suggested that the felsites of southern Dartmoor constitute an early chilled facies of the main granites there, a view later endorsed by Brammall and Harwood[6] in the light of their chemical analyses which gave $K_2O : Na_2O$ ratios respectively of 0·47, 0·53 and 1·1 for the Legis Tor, Ludbrook and Broadall Lake felsites. These values are very like those of the Haig Fras rocks (Table 2, columns 1–3, 6). In all other respects, however, the felsites are entirely different.

Among the youngest rocks of the granitic suite of South-west England is a group of leucogranites which occurs in the form of sheets and veins, seldom more than 4 or 5 ft. thick and intrusive into both granite and country-rock. Such rocks have been seen in all the major intrusions including that of the Scilly Isles, which is the nearest to Haig Fras. From the analyses in Tables 1 and 2 (columns 5 and 7) it can be seen that although there are some notable differences in mineralogical composition, there is very good chemical agreement between these rocks and the Haig Fras granites (Table 2, columns 1–3). It is also evident that the Haig Fras 'aplogranite' (Table 2, column 6) is chemically close both to the mainland leucogranites and to the Haig Fras granites. The most striking mineralogical difference lies in the proportions of the two feldspars. While not wholly illusory, this difference is in fact less important than it first appears because of the perthitic nature of the potash feldspar, a phenomenon which is not taken into account in the mode and which also explains some of the seemingly anomalous alkali ratios. The anorthite content of the Haig Fras plagioclases (An_{10}) is identical with that of the Bodmin Moor leucogranite (Table 1, column 7) in which the potash feldspar is largely microcline, as it is in the Haig Fras rocks, although not commonly in the other south-west granites. Finally there is a tendency for the leucogranites to develop a planar structure somewhat similar to the foliation in the Haig Fras rocks, although not so marked.

Fine-grained varieties occur among granites of similar age in Brittany but these are generally much more mafic, have a rather different feldspar content and contain little or none of the characteristic tourmaline.

The Gneisses. Injection and permeation gneisses and schists are not associated with any of the granites on the mainland, such metamorphism as there is being mainly

Table 1. MODAL ANALYSES (VOL. %)

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Quartz	33·4	31·7	33·8	33 4	37·6	41·9	43·6	52 0
Potash feldspar	30 5	21·9	41·0	28·1	42 5	38·7	18·9	21·3
Plagioclase	27·8	39·6	19·2	24·0	10 8	16 5	27 3*	14·8
Muscovite	4·7	0·7	2·7	2 4	3 7	0 3	6 0	1·3
Biotite	0·5	—	2 4	5 6	2·0	—	—	8 6
Secondary mica	1·0	1·0	1·5	6 1	—	—	1 1	1·9
Tourmaline	2·2	5·0	—	Tr.	2·0	2·6	3 0	—
Apatite	Tr.	Tr.	—	Others	Others	—	Tr	—
Ore	Tr.	0·1	Tr	0 4	1·0	—	—	—
	100·1	100 0	100 1	100 0	100 0	100 0	99·9	99·9 †
Chayes[4] I.C. No.	93	67	70	—	—	45	90	—

For key to columns (1) to (7) see Table 2; for column (8) see Table 3. Columns (1)–(3) and (6)–(8), analyst C. S. Exley. Column 4, analyst M. Stone. Column (5), analyst H. F. Harwood.

* Includes 1·7 per cent of clay/mica aggregates.

† Includes trace of epidote.

Table 2. CHEMICAL ANALYSES OF GRANITES
(Oxides as wt. %; elements as p.p.m.)

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
SiO_2	73·32	71·61	73·44	72·1	74·68	74 77	73·45
TiO_2	0 06	0·05	0·05	0 28	0·16	0 07	0·09
Al_2O_3	15·42	14 92	15 19	14 29	13·25	14 91	15·20
Fe_2O_3	0·04	0·83	0·55	1·11	0·72	0·57	0 30
FeO	0·80	0·47	0·31	1 16	0·71	0 26	0·99
MnO	Tr	Tr.	Tr	0·04	0·02	Tr.	0 01
MgO	1 54	0·64	1·38	0 51	0·25	1·33	1·85
CaO	0·99	0·80	0 62	0 50	0·48	0 59	0 57
Na_2O	4 75	4·95	4·20	2·45	2·66	4·75	3·47
K_2O	3·15	5 07	2·75	5 98	5·42	2·61	3 63
P_2O_5	0·17	0·19	0 14	0·25	0·19	0·15	0·14
H_2O+	0·31	0 58	0·40	0 53	0·93	0 37	1·11
H_2O-	0·12	0 02	0 10	—	0 60	0 09	0·03
CO_2	0 06	0 14	0 08	—	—	0·13	0·09
Ga	22	22	19	—	—	22	20
Cr	5	11	*N.D.*	—	—	9	6
Li	54	33	34	—	Tr. %	33	65
Cu	9	9	7	—	—	11	5
V	25	46	26	—	—	30	*N.D.*
Zr	*N.D.*	*N D.*	101	—	—	*N.D.*	Tr.
Sc	12	21	11	—	—	11	1
Sr	88	101	122	—	—	85	138
Ba	184	189	248	—	Tr. %	121	285
Rb	347	386	334	—	—	180	366
K : Rb	75	108	68	—	—	120	82
$K_2O : Na_2O$	0 66	1·02	0·65	2·44	2·04	0·55	1 04

N.D. indicates 'not detected'; Tr. indicates 'Trace'.

(1) Granite; Haig Fras. Geol. Surv. No. *F*6698. Analysts A. J. C. Steventon and G. M. Power.

(2) Granite; Haig Fras. Geol. Surv No. *F*6700. Analysts A. J. C. Steventon and G. M. Power.

(3) Granite; Haig Fras. Geol. Surv. No. *F*6704. Analysts A. J. C Steventon and G. M. Power.

(4) Fine porphyritic granite, Godolphin Hill. Exley and Stone, 1965, Table 5, col. (3). Analyst M. Stone.

(5) Fine leucogranite; Haytor Rocks, Dartmoor. Brammall and Harwood, 1932, analysis 3. Analyst H. F. Harwood.

(6) Aplogranite; Haig Fras. Geol. Surv. No. *F*6713. Analysts A. J. C. Steventon and G. M. Power.

(7) Fine leucogranite; Bodmin Moor. Analysts A. J. C. Steventon and Mrs. M. A. Humphreys.

thermal, with some metasomatism. This is true both of the envelopes of the granites and inclusions within them. In particular, metasediments of the Devonian and Carboniferous when metamorphosed bear no resemblance to the gneisses and schists of Haig Fras. A similar situation was observed on White Island in the Scillies by Osman[7], who described 'pneumatolysed' inclusions of schist and concluded that, far from being part of the Devonian or Carboniferous roof of the granite, they were part of the much older floor. Comparing the White Island inclusions with the schists of the Lizard, he claimed that the resemblance in both structure and mineralogy was 'striking'.

The fragments of Haig Fras metamorphic rock are too small to be very helpful, but a comparison between the fragments themselves, Dr. Sabine's description of them[1], and Osman's description of the White Island inclusions[7], indicates that the former have been much more extensively granitized than the latter. This is confirmed by the chemical analysis in Table 3; unfortunately there is no analysis of the White Island rock, but a cursory examination of an analysis of an Old Lizard Head Series pelitic schist (Table 3, column 2) shows how extensive an alteration would be required to produce a Haig Fras-type injection gneiss. On the other hand, the analysis of a Porthallow granite-gneiss (Table 3, column 3) is not dissimilar, especially in its alkali content. The possibility of sodium metasomatism is suggested by the low $K_2O : Na_2O$ ratio in both these cases and is supported by the nature of the plagioclase, which is albite or oligoclase in the Porthallow rock and oligoclase (An_{11}) in the Haig Fras gneiss. A difference in age between this gneiss (Table 3, column 1) and the associated granites (Table 2, columns 1–3) is hinted at by its relatively high zirconium and barium content.

The Position of Haig Fras. The igneous and metamorphic area lies more than 40 miles to the north of the projected south-westerly trend of the known granites of the south-west. This may be due to either of two causes. On the one hand the Haig Fras granites may belong to a mass which is quite separate from the elongated 'batholith' underlying Devon, Cornwall and the Scillies, while on the other there may be a dextral fault, or series of

Table 3 CHEMICAL ANALYSES OF GNEISSES
(Oxides as wt. %; elements as p p m)

	(1)*	(2)	(3)
SiO_2	71·39	55·92	74·35
TiO_2	0·18	0 88	0·18
Al_2O_3	15·51	22·14	14·70
Fe_2O_3	0 70	3·75	0·79
FeO	1·91	3·72	0·62
MnO	Tr.	0 21	0·07
MgO	2·16	1·77	0·81
CaO	0·88	0·30	0·70
Na_2O	4·80	2·25	5·29
K_2O	1·65	4·26	1·67
P_2O_5	0·07	0 07	0 12
H_2O+	0 39	3·94	0 72
H_2O-	0 04	0 43	0·11
CO_2	0 08	0 21	*N.D.*
Ga	20	—	—
Cr	11	—	—
Li	39	Tr. %	Tr %
Cu	7	—	—
V	31	—	—
Zr	181	—	—
Sc	20	—	—
Sr	108	—	—
Ba	428	*N.D.*	180
Rb	295	—	—
K : Rb	37	—	—
$K_2O : Na_2O$	0 32	1·89	0·32

(1) Injection gneiss; Haig Fras. Geol. Surv. No. *F*6709. Analysts A. J. C. Steventon and G. M. Power.
(2) Mica-schist; Porthallow, Lizard. J. S. Flett (ref. 8, p. 43), anal. I. Analyst E. G Radley.
(3) Granite gneiss; Porthallow, Lizard. J. S. Flett (ref. 8, p 60), anal. I. Analyst W. Pollard.
* The modal analysis of this rock is given in Table 1, col. 8.

faults, under the sea between Haig Fras and the Scillies. Such a structural feature would be in keeping with the pattern of wrench-faults postulated by Dearman[9] for the mainland.

As Smith, Stride, Whittard and Sabine[1] have said, the Haig Fras granitic rocks represent a suite of granites and related gneisses resulting from granitization of schists. However, the former show strong mineralogical and chemical affinities with one of the youngest suites of granitic rocks in Devon and Cornwall, and probably comprise small, late intrusions which have penetrated through an earlier granite phase of which there is so far no evidence. If this is the case, only the uppermost part of the granite body has been revealed by erosion. By analogy with what is seen on the mainland, the gneisses seem much more likely to represent part of the 'basement' than metamorphosed Devonian or Carboniferous metasediments; this may mean that the high ground of Haig Fras is flanked by faults which have brought the metasediments into juxtaposition with the older metamorphics or, more probably, that it is flanked by an unconformity between these two groups of rocks.

I thank Mr. A. H. Stride for directing my attention to the Haig Fras rocks, for discussing them with me and for his helpful comments on these notes; also the Director of the Geological Survey for permission to examine and analyse the specimens; Mr. A. J. C. Steventon for the major element analyses; and Mr. G. M. Power and Mrs. M. A. Humphreys for the trace element analyses.

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CHEMICAL COUPLING OF ENZYMES TO CROSS-LINKED DEXTRAN ('SEPHADEX')

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ENZYMES and other organic catalysts may be chemically attached to insoluble polymers with retention of all or part of their catalytic activities[1–6]. Such modified catalysts possess certain properties which make them attractive as tools for synthesis and degradation of biopolymers. They are often more stable than original enzymes. A great advantage of their use in polymerization and depolymerization reactions is the fact that the products can be freed rapidly and completely from the enzyme simply by filtration.

The coupling reaction to be used for binding the catalyst must proceed under mild conditions. Extremes of *p*H must be avoided and the coupling must be performed within a narrow range of temperature in aqueous solution.

The insoluble polymer must fulfil certain requirements. It must be stable, should not significantly inhibit the enzyme and should not cause disturbing unspecific adsorption. Since the enzymatic reactions take place in aqueous solutions, hydrophylic polymers are preferred as enzyme carriers[4,6]. A cross-linked dextran, 'Sephadex', would act as an almost ideal matrix except for possible screening effects caused by steric hindrance. To ascertain whether this is the case we have synthesized a number of products which we expected to show hydrolytic activities. These substances include polymer-fixed histidine peptides, chymotrypsin, trypsin and β-amylase.

We have described elsewhere the synthesis of isothiocyanate derivatives of dextran and 'Sephadex' (from *AB* Pharmacia, Uppsala, Sweden) and their conjugation with peptides and proteins[7]. With different selectively protected amino-acids it was shown that, with the coupling and washing procedures used, the main product is a substituted thiourea. Therefore, the coupling of a protein occurs mostly at its α-amino groups and at the ε-amino groups of lysine. The content of bound protein was determined after acid hydrolysis according to the method of Spackman, Moore and Stein. The high content of carbohydrate does not disturb the analysis.

Preparation of p-nitro-phenoxy-hydroxypropyl ethers of 'Sephadex G200'. A mixture of 10 g 'Sephadex *G*200' and 2 g *p*-nitro-phenylglycidyl ether[8] was suspended in 10 ml. toluene, and 40 ml. 1 M sodium hydroxide was added. The suspension was heated at 70° C for 18 h. When less substituted products were desired the reaction was run for only 6 h. The product was acidified with acetic acid, washed with water, shrunk by dropwise addition of ethanol to the stirred water suspension, filtered, washed with acetone and then dried. The content of nitro-groups was determined by titration with titanium chloride[9]. The values agreed with the results of nitrogen determinations performed according to Dumas. 'Sephadex *G*100' and '*G*25' were converted to the nitro-derivatives in the same fashion, except that only 20 ml. and 10 ml. 1 M sodium hydroxide were used, respectively.

Reduction. Reduction of the *p*-nitro-phenoxy-hydroxypropyl-ethers of 'Sephadex' was effected on a preparative scale using sodium dithionite. 10 g polymer was swelled in 50 ml. water and 20 ml. 5 M sodium hydroxide, and 1 g sodium dithionite was added. The reaction was carried out at 65° C for 1 h with stirring. After cooling, the reaction product was collected on a filter and washed with water, 0·1 M hydrochloric acid, and again with water. It was then suspended in water, allowed to sediment, and

the finer beads were decanted. The heavier particles, about 80 per cent of the total, were treated with ethanol and acetone and were then dried. The consumption of titanium chloride on titration of the amino polymer was about 10 per cent of that observed in titrating the original nitro-polymer.

Conversion to isothiocyanate derivatives. The amino groups were converted to isothiocyano groups by treatment of the amino-polymer swelled in 3·5 M potassium hydrogen phosphate buffer (*p*H 6·8) with a 10 per cent solution of thiophosgene in carbon tetrachloride. The suspension was agitated thoroughly at room temperature with a vibrator during the reaction. 1 g of the swelled amino-'Sephadex' was vibrated for 20 min with 1·5 ml. thiophosgene solution. A second 1·5 ml. portion of thiophosgene was then added and the agitation was continued for 20 min. The gel was washed on a filter with water, acetone, 0·5 M sodium hydrogen carbonate (5 min) and again with water. The product can be used directly for coupling reactions or it can be shrunk for storage by adding acetone to the water suspension and then dried at 100° C under vacuum. The isothiocyanate content was determined by the iodometric titration of the hydrogen sulphide evolved on acid hydrolysis of the dried product.

Fixation of peptides and proteins. The isothiocyano-'Sephadex' was tested by coupling with some model substances. The polymer was swelled in 0·3 M sodium hydrogen carbonate and a five-fold excess of glycyl-leucine was added. The reaction was allowed to proceed for 24 h at room temperature. After washing and drying the content of leucine was determined. In some other experiments, insulin in 1·5-fold excess was coupled to 'Sephadex *G*100' and '*G*200' derivatives. The results are listed in Table 1.

Table 1. DEGREE OF SUBSTITUTION OF 'SEPHADEX' DERIVATIVES IN THE SYNTHETIC SERIES NITRO- (AMINO-), ISOTHIOCYANO-PHENOXY-HYDROXYPROPYL-'SEPHADEX', AND YIELD ON COUPLING WITH MODEL SUBSTANCES

'Sephadex'-derivatives	Grade of 'Sephadex' *G*25	*G*100	*G*200
p-nitro-phenoxy-hydroxypropyl-'Sephadex' Nitro-group determination (μmole/g)	360	240	230 (190)
p-isothiocyano-phenoxy-hydroxypropyl-'Sephadex' Isothiocyanate determination (μmole/g)	290	180	190 (150)
Amount (μmole/g) of coupled Gly-Leu (5-fold excess of peptide in 0·3 M $NaHCO_3$)	232	155	173 (112)
Amount (μmole/g) of coupled insulin (1·5-fold excess of insulin in 0·3 M $NaHCO_3$)	—	29·5 170 mg/g	30 173 mg/g

The reaction time in the synthesis of the nitropolymer was 18 h or 6 h (values in parenthesis).

Chymotrypsin was coupled to *p*-isothiocyano-phenoxy-hydroxypropyl-'Sephadex *G*200' by the following procedure (preparation *CHY* : 1). 100 mg isothiocyano-'Sephadex' was swollen in 3 ml. 0·05 M borate buffer of *p*H 8·6, to which 50 mg crystallized, salt-free chymotrypsin was added over 30 min. The reaction mixture was stirred with a slow stream of nitrogen for 15 h at 5° C. The gel was poured into a column, where it was washed successively with the borate buffer (24 h), 10^{-3} M hydrochloric acid (24 h), 0·5 M sodium chloride (6 h) and 10^{-3} M hydrochloric acid (24 h). The washing solutions were delivered with a peristaltic pump at 10–15 ml./h. 50 per cent of the gel was then suspended in water and shrunk by slow addition of acetone. After filtration, the shrunken gel was kept in acetone for 30 min and then was dried at 40° C in vacuum (Product *CHY* : 1*a*). The content of coupled enzyme was determined (Table 2). The remainder of the swelled gel was transferred to a smaller column and was treated with 0·2 M phosphate buffer of *p*H 7·6 containing 0·02 per cent sodium azide. This material was used for several experiments at +5° C (hydrolysis of *p*-nitro-phenylacetate, methionine methyl ester and *N*-acetyl-tyrosine ethyl ester). After 4 weeks the gel was washed with water, shrunk and dried (Product *CHY* : 1*b*). A suspension of *CHY* : 1*a* in 0·2 M phosphate buffer of *p*H 7·6 was stirred for 6 h and the gel was centrifuged down. The supernatant showed no activity toward *N*-acetyl-tyrosine ethyl ester. The

Table 2. CHEMICAL FIXATION OF ENZYMES TO *p*-ISOTHIOCYANO-PHENOXY-HYDROXYPROPYL-'SEPHADEX'

Enzyme-'Sephadex'	Preparation No.	Content of isothiocyanate μmole/g	Polymer/enzyme ratio by the coupling	Content of fixed enzyme μmole/g	mg/g
Chymotrypsin-'Sephadex *G*200'	*CHY* : 1	190	100 mg/50 mg	8·9	220
Chymotrypsin-'Sephadex *G*200'	*CHY* . 2	190	200 mg/50 mg	3 7	93
Chymotrypsin-'Sephadex *G*100'	*CHY* : 3	180	200 mg/50 mg	3·7	93
Chymotrypsin-'Sephadex *G*25'	*CHY* : 4	290	200 mg/40 mg	3·6	90
Trypsin-'Sephadex *G*200'	*TRY* . 1	150	200 mg/50 mg	2 6	62
β-Amylase-'Sephadex *G*200'	—	150	200 mg/70 mg	0·15	23

trypsin-'Sephadex', β-amylase-'Sephadex', and the other chymotrypsin-'Sephadex' samples listed in Table 2 were prepared in the same way.

Determination of activities. Measurements of catalytic activities of the insoluble chymotrypsin toward *p*-nitro-phenylacetate were made and compared with some other histidine-containing derivatives of 'Sephadex' and with imidazole and free chymotrypsin. The rate of hydrolysis of a $5{\cdot}5 \times 10^{-5}$ M *p*-nitro-phenylacetate solution in 0·2 M phosphate buffer of *p*H 7·6, containing 5 per cent (by volume) dioxane at 22° C in the absence or presence of catalyst (10^{-4}–10^{-6} M in histidine), was measured and the rate constants for these reactions, k_0 and k_c, respectively, were calculated.

The β-amylase-'Sephadex' was assayed with 1 per cent soluble starch (Merck) in 0·02 M sodium acetate at *p*H 4·8, using 3,5-dinitro-salicylic acid as a colour reagent[10]. The β-amylase-'Sephadex *G*25' was inactive.

The esterase activity of chymotrypsin-'Sephadex' was determined under the following conditions with tyrosine ethyl ester as a substrate: 2×10^{-3} M tyrosine ethyl ester (0·02 M Ca^{++}) in 0·05 M *tris* buffer of *p*H 7·0 at 22° C. The activity of trypsin-'Sephadex' was determined toward *N*-benzoylarginine ethyl ester: 10^{-3} M ester (0·02 M Ca^{++}) in 0·05 M *tris* buffer of *p*H 8·0 at 22° C (ref. 11). The activities of the enzyme-derivatives were compared with the activities of the corresponding free enzymes. Proteolytic activity was determined by the casein digestion method[11].

Catalytic properties. The catalytic constants $(k_c - k_0)/c$, *c* being the concentration of histidine[12], were calculated for various products and are compiled in Table 3. The *p*-amino-phenoxy-hydroxypropyl-'Sephadex *G*200' shows no catalytic effect toward *p*-nitro-phenylacetate.

The catalytic activities of histidine-containing polymers are of the same order as reported for the corresponding polypeptide polymers prepared by Katchalski *et al.*[13]. Thus the nature of the polymer does not appear to influence very much the catalytic action displayed by the fixed groups. It is also interesting to note the similar catalytic constants of chymotrypsin 'Sephadex *G*25' and '*G*200'. This finding indicates that the chymotrypsin buried in the interior of the matrix ('Sephadex *G*200') exhibits the same or closely the same activity as the 'surface-fixed' enzyme ('Sephadex *G*25'). The activity ratios listed in Table 4 were determined within 5 days after the coupling product had been shrunk and dried once. The activities of the preparations *CHY* : 2 and *TRY* : 1 dropped by about 20 per cent after storage under 10^{-3} M hydrochloric acid for 1 month at +5° C.

Table 3. CATALYSIS OF THE HYDROLYSIS OF *p*-NITROPHENYLACETATE AT 22° C IN 0·2 M PHOSPHATE BUFFER OF *p*H 7·6 (5 PER CENT BY VOL. OF DIOXANE) OF SOME DERIVATIVES OF 'SEPHADEX' COMPARED WITH IMIDAZOLE AND CHYMOTRYPSIN

Catalyst	Preparation No.	Histidine-content of the polymer μmole/g	$\frac{k_c - k_0}{c}$ l./mole/min
Imidazole	—	—	28
Chymotrypsin	—	—	10^4
Gly-His-'Sephadex *G*25'	—	184	4·7
His-His-'Sephadex *G*25'	—	350	4·8
Chymotrypsin-'Sephadex *G*200'	*CHY* : 1*a*	17·8	2,000
Chymotrypsin-'Sephadex *G*200' (after 4 weeks)	*CHY* : 1*b*	17·8	1,600
Chymotrypsin-'Sephadex *G*25'	*CHY* : 4	7·2	2,100

Table 4. COMPARISON OF RATES OF HYDROLYSIS OF SOME SUBSTRATES BY FREE ENZYMES AND CORRESPONDING 'SEPHADEX' DERIVATIVES

Enzyme-'Sephadex' (Preparation No.)	Substrate	Activity ratio bound to free enzyme (%)
Chymotrypsin-'Sephadex G200' (*CHY* . 1*a*)	Tyrosine ethyl ester	14–25
Chymotrypsin-'Sephadex G200' (*CHY* · 1*a*)	Casein	3–5
Chymotrypsin-'Sephadex G200' (*CHY* : 2)	Tyrosine ethyl ester	19–34
Chymotrypsin-'Sephadex G25' (*CHY* : 4)	Tyrosine ethyl ester	20–33
Chymotrypsin-'Sephadex G25' (*CHY* : 4)	Casein	2·5–4
Trypsin-'Sephadex G200' (*TRY* : 1)	*N*-benzoyl-arginine ethyl ester	17–24
Trypsin-'Sephadex G200' (*TRY* . 1)	Casein	2 5–4
β-Amylase-'Sephadex G200'	Soluble starch	0

The activity was not affected by five cycles of swelling with water and shrinking with acetone performed at room temperature over a period of 2 days.

The activity of the dried polymer *CHY* : 1*a* was unaltered after storage for 5 weeks at −10° C, but a sample *CHY* : 1*a* lost 80 per cent of its activity after storage at room temperature for the same time.

The coupling of chymotrypsin and trypsin to 'Sephadex' by way of isothiocyanate derivatives can be performed with retention of enzymatic activity. The method should be applicable to other enzymes. The synthesis of the corresponding soluble dextran derivatives is under investigation.

The results in Table 4 confirm the expected effect of steric hindrance. Soluble starch apparently is excluded from the enzymatically active regions of β-amylase. The low molecular weight substrates are hydrolysed more efficiently than the substrate polymers. This discriminating effect on the activity towards substrates of different molecular size should make applications possible utilizing this behaviour to advantage. Occasions might arise, for example, where preferential digestion or other selective action is desirable.

Another fascinating potential of solid phase fixed enzymes might be mentioned, although it must have occurred to students in the field and has probably been stated elsewhere. If the space distribution of the enzyme could be controlled, it should be possible to build up protein sequences in a properly ordered manner so as to make possible syntheses similar to those occurring in Nature. For many reaction pathways this may perhaps be effected simply by mixing separately prepared fixed enzymes in granular form or by using stratified beds. Reaction routes including very short-lived intermediates may require more sophisticated methods for production of satisfactory functioning multiple enzyme systems.

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ALTERED PROPERTIES OF DEOXYRIBONUCLEIC ACID NUCLEOTIDYLTRANSFERASE AFTER INFECTION OF MAMMALIAN CELLS WITH HERPES SIMPLEX VIRUS

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THE activity of DNA nucleotidyltransferase (*EC* 2.7.7.7) increases markedly in mammalian cells after infection with vaccinia virus[1,2], with pseudorabies virus[3] and with herpes simplex virus[4]. In the last-mentioned case, the increase has been related in time to an increase in the activity of deoxyribonuclease (*EC* 3.1.4.5), and to the appearance in the infected cells of complement-fixing antigens, herpes DNA, morphological particles and infective virus[5]. Measurements of nucleotidyltransferase activity in soluble preparations derived from sub-cellular fractions of the herpes-infected cells have shown that the activity increases by 600 per cent in the nuclei, by 200 per cent in the mitochondria-microsome fraction and by only 50 per cent in the cell sap fraction[4].

We now have evidence which indicates that the DNA nucleotidyltransferase activity in herpes-infected cells is attributable at least in part to an enzyme different from that in the uninfected control cells.

*BHK*21 (*C*13) cells[6] were grown as previously described[5], and the monolayers were infected *in situ* with herpes simplex virus strain α at a multiplicity of exposure of 10. Four hours later, the cell sheets were washed with phosphate-buffered saline, removed from the glass with versene (0·5 mM in phosphate-buffered saline), washed twice with phosphate-buffered saline by centrifugation at 0° C, and stored at −70° C. The incubation period of 4 h (after addition of the virus) was chosen because previous results had indicated that by this time the DNA nucleotidyltransferase activity has increased substantially while the post-infection increase of deoxyribonuclease activity is as yet minimal[4]. Because the high levels of deoxyribonuclease activity which develop in the infected cells by 8 h after infection could interfere seriously with the nucleotidyltransferase assay by damaging the DNA-primer and the DNA-product, the gathering of the cells 4 h after addition of virus represents a safeguard which allows valid comparison of control and infected nucleotidyltransferase preparations.

The frozen control and infected cell batches were thawed at 0°–2° C and were quickly homogenized in 10 volumes of 0·02 M *tris*-hydrochloric acid *p*H 7·5, 0·001 M versene, 0·005 M 2-mercaptoethanol. Magnesium sulphate and potassium chloride were immediately added to final concentrations of 0·004 M and 0·15 M, respectively, and

the preparations were centrifuged for 1 h at 105,000*g*. Each supernatant fraction (*S*) representing the initially soluble material was collected, and the sediments were homogenized vigorously for 30 sec in 0·02 M *tris*-hydrochloric acid buffer *p*H 7·5, 0·001 M versene, 0·005 M 2-mercaptoethanol, sonicated for 30–60 sec (ref. 7), and centrifuged for 1 h at 105,000*g*. The supernatant fraction (*P*), representing the material solubilized in the course of the re-extraction procedure, was collected and made 0·004 M in magnesium sulphate and 0·15 M in potassium chloride. An important feature of the re-extraction is that potassium chloride and magnesium sulphate are excluded during homogenizing, ultrasonication and centrifugation (see ref. 8).

DNA nucleotidyltransferase was assayed by a standard procedure[9,10] involving measurement of incorporation of [^{32}P] thymidine 5′-monophosphate from [α-^{32}P] thymidine 5′-triphosphate in the presence of the 5′-triphosphates of deoxyadenosine, deoxycytidine and deoxyguanosine, and denatured DNA-primer.

The heat-inactivation data for DNA nucleotidyltransferase plotted in Fig. 1 were obtained as follows: The enzyme preparations at *p*H 7·5 were heated at 45° C or at 50° C for various times in the presence of DNA, and cooled to 0° C. The four triphosphates and other standard assay components were then added and the mixtures were incubated at 37° C for 1 h for assay of nucleotidyltransferase.

The data suggest that: (i) the infected preparations are much more heat-resistant than are the corresponding control preparations; (ii) the *P* preparations are more heat-resistant than the *S* preparations; (iii) the infected preparations, but not the controls, are activated after 30 min pre-incubation at 45° C; (iv) the activity of the control *S* fraction actually increases during the first 5 min at 45° C (the *P* fractions behave somewhat similarly at 10 min).

The effect of pre-incubation was investigated at 50° C. The results (Fig. 2) show: (i) more than 90 per cent of the control cell nucleotidyltransferase was inactivated by 15–20 min pre-incubation; (ii) the infected preparations were again much more heat-resistant than the controls, 20–25 per cent of the initial activity surviving heating for 80 min; (iii) the infected preparations, but not the controls, clearly exhibited increased activity after 20–30 min. Omission of DNA during pre-incubation resulted in quick and drastic loss of activity from all samples (more than 50 per cent after 2 min at 45° C, and more than 90 per cent after 9 min at 45° C).

Two features stand out from the heat-inactivation data. First, at 45° C there is apparently a brief heat-activation phase after about 5–10 min; we attribute this tentatively

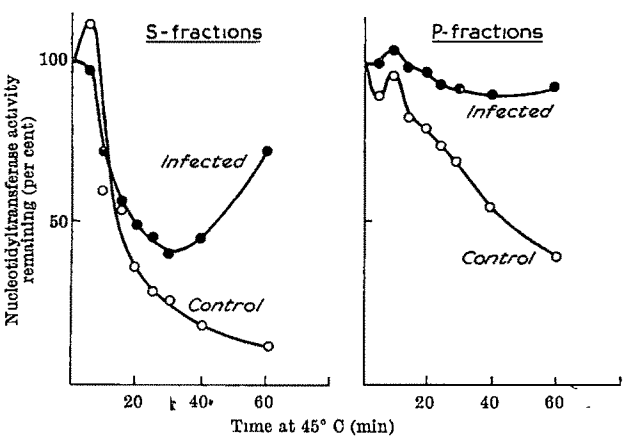


Fig. 1. Heat-inactivation of DNA nucleotidyltransferase. The enzyme preparations were heated at 45° C in the presence of 80 μg of denatured DNA, and cooled rapidly to 0° C. The triphosphates were then added together with 52 μg of denatured DNA, and incubation then followed at 37° C for 60 min for measurement of enzyme activity by the standard procedure (refs. 9, 10)

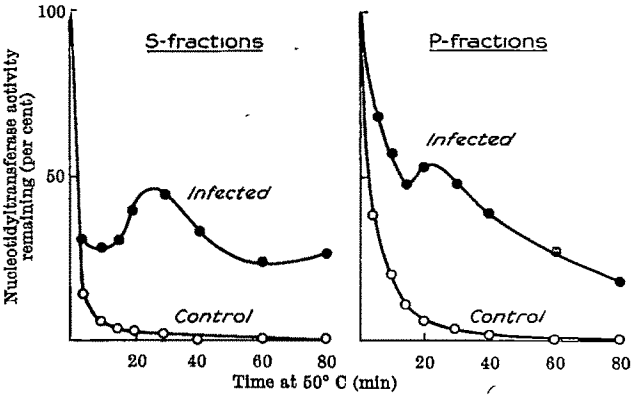


Fig. 2. Heat-inactivation of DNA nucleotidyltransferase at 50° C. The experiment was conducted according to the plan described in Fig. 1 except that the prior heating was at 50° C

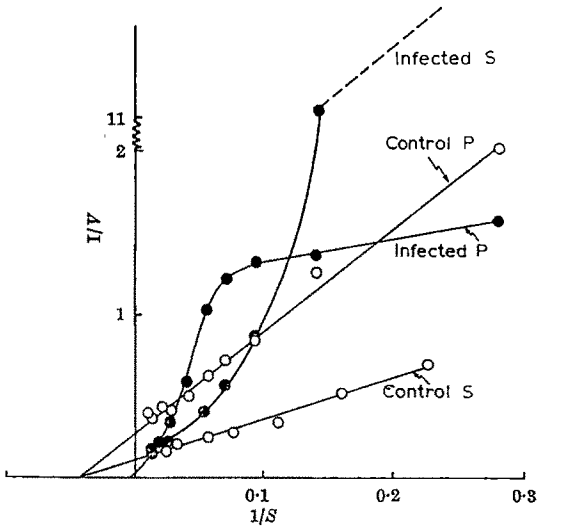


Fig. 3. Nucleotidyltransferase activity at various concentrations of DNA-primer

to activation of the heat-labile, host cell nucleotidyltransferase that is present in control and infected cells. Secondly, further activation occurs at 45° C in the infected samples only, beginning at about 40 min. At 50° C, the early brief activation observed at 45° C is no longer detectable and activation is confined to the infected samples only, now beginning at 15–20 min. This later activation effect induced by both the 45° C and the 50° C pre-incubations we attribute either to a herpes virus-coded nucleotidyltransferase or to the host enzyme modified by virus action.

When *HEp* 2 cells (human epithelioid carcinoma) were used as host cells, enzyme prepared from control and infected cells yielded results similar in all main aspects to those illustrated in Fig. 2 for *BHK* 21 host cells (hamster fibroblasts). Since the genetic origins of the *HEp* 2 and *BHK* 21 cells are quite different, while the same stock virus was used, this evidence strongly supports our hypothesis that the heat-stable nucleotidyltransferase in infected cells is virus-coded.

The rates of DNA nucleotidyltransferase activity at various concentrations of DNA-primer were measured for the control and infected samples, and the results are expressed as double reciprocal plots[11] ($1/v$ versus $1/S$) in Fig. 3. The plots are linear for the controls, but bimodal for the infected preparations.

Other evidence of different behaviour between DNA nucleotidyltransferase preparations from control (*BHK* 21) and infected cells stems from preliminary investigations of the magnesium ion requirements of the reaction. These showed that the infected *S* and *P* preparations have essentially one Mg^{2+} optimum (20–24 mM) while the

control *P* preparations have two (8–10 mM and 20–24 mM). The control *S* preparations show only the lower Mg^{2+} optimum. Pre-incubation of the infected preparations (or of the control *P* fraction) at 50° C results in a shift of the Mg^{2+} optimum from 20–24 mM to 8–10 mM.

In concurrent experiments using nucleotidyltransferase preparations from control and infected *HEp* 2 cells, we have tested the priming capacities of native herpes virus DNA, and of native and heat-denatured Landschutz ascites-tumour cell DNA. We have consistently observed that native DNA, though a very poor primer for the control enzyme preparations, is a highly effective one for the enzyme prepared from infected cells. Moreover, preliminary results indicate that native herpes DNA is a better primer than native mammalian DNA for the infected cell enzyme. In contrast, heat-denatured DNA serves as a good primer for both enzyme preparations. Similar results with respect to priming by native and denatured DNA from the ascites-tumour cells have been obtained for enzyme prepared from control and infected *BHK* 21 cells.

Consideration of the data presented in Figs. 1–3, of the Mg^{2+} requirement data, of the investigations of DNA priming, and of the similar behaviour of nucleotidyltransferase prepared from infected hamster cells and infected human cells, prompts us to suggest the following hypothesis. Nucleotidyltransferase as obtained from infected cells at the time when they are most actively synthesizing herpes DNA (see ref. 5) exists principally in a native conformation characterized by possession of two polymerizing sites which operate interdependently in the native enzyme. One catalyses nucleophilic attack by the 3′-hydroxyl group of the substrate triphosphate on the α-phosphate group of a 5′-triphosphate terminus on one of the growing daughter DNA strands (see refs. 12 and 13), the other concurrently catalyses nucleophilic attack by the 3′-hydroxyterminal group of the other growing daughter strand at the α-phosphate of another incoming substrate triphosphate. The concerted and interdependent action of the two catalytic sites on the enzyme thus allows unidirectional and semi-conservative replication along the parent DNA double-helix producing two daughter double-helices each identical with the parent. This implies that the two catalytic sites are not identical. An extension of the hypothesis is that the native enzyme can undergo a transition such that the two active sites are no longer fully interdependent (for example, a dimer to monomer transition). The transition may occur naturally, *in vivo*, or it may be induced *in vitro*, for example, by pre-incubation at elevated temperatures. We postulate that the uninfected cell nucleotidyltransferase is of a similar nature but comparatively much less stable, thus in our experiments is already mainly in monomer form.

In conditions in which the two sites are no longer interdependent, that is, after the postulated dimer to monomer transition, polymerization along denatured single-stranded template (as in the standard nucleotidyltransferase assay) should proceed at an increased rate. This increase is in fact observed (Figs. 1 and 2) with heat-denatured DNA as primer, and it suggests that the postulated dimer to monomer transition proceeds much more readily with the host enzyme (after 5 min at 45° C) than with the herpes-induced enzyme (after 20–30 min at 50° C).

Interdependence operates only when both sites are actively engaged in replication of one primer molecule of double-stranded DNA, or of two primer molecules of single-stranded DNA held in close apposition by the binding sites on the enzyme.

The infected cell enzyme was always isolated at a time when it could be expected to be fully engaged in production of herpes virus DNA (4 h after infection). Thus we would expect it to possess the ability to use native and denatured DNA with equal efficiency, because it is expected to have been obtained almost entirely in the active or dimeric form. On the other hand, only a relatively small proportion of the control cells would have been actively engaged in DNA synthesis at the time of preparation of the enzyme, since these cells were not synchronized with respect to the growth cycle and therefore most of the control nucleotidyltransferase would be expected to be found degraded to the monomeric conformation. This accords with our observation that the control preparation has only a very limited ability to accept native DNA as primer.

Similar reasoning may be applied in interpretation of our results obtained with Mg^{2+} concentration requirements. Thus, the higher Mg^{2+} optimum is ascribed to the requirement of the intact, dimeric nucleotidyltransferase; the higher optimum is displayed by the herpes virus-induced enzyme and also by some of the control *P* fraction. The latter would be expected to contain enzyme from the cell nuclei and the nuclei would reasonably be expected to contain all of that amount of enzyme of the control cells that was collected while in the phase of DNA synthesis. These ideas are supported by the observations: (i) that pre-incubation of the infected preparation at 50° C or of the control *P* fraction at 45° C results in a fall of the Mg^{2+} optimum from 20–24 mM to the lower level of 8–10 mM, and (ii) that the control *S* fraction exhibits only the lower optimum.

The data shown in Fig. 3 are difficult to interpret with real confidence, but it may be noted that the lower limbs of the infected plots show a curvature that is concave upwards while the control preparations are virtually linear. If the points on the curved plots of the infected samples are replotted as $1/v$ versus $1/S^2$ they plot for both the *P* and *S* fractions as a straight line; thus these data fit the hypothesis that the action of infected-cell nucleotidyltransferase is bimolecular with respect to single strands of DNA. The upper limb of the infected-*P* plot in Fig. 3 is clearly linear on the $1/v$ versus $1/S$ plot, and therefore, at these low DNA concentrations, the linearity might be regarded as due to nucleotidyltransferase molecules with only one of the two binding sites occupied by a strand of DNA.

In conclusion, we believe that the results presented here are informative in two ways: First, they suggest that the increase of DNA nucleotidyltransferase activity observed after infection with herpes simplex virus can be attributed either to elaboration of a virus-coded enzyme or, less likely, to induction of modifications in the host enzyme by the infective process. We favour the former view not only for genetical reasons but also because modification probably could not account for the increased level of activity which rises to 5–7 times above the control level at 5–6 h after infection[4]. Secondly, the behaviour of the two nucleotidyltransferase systems under the experimental conditions used here has suggested a hypothesis for the mechanism and control of DNA synthesis *in vivo*.

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LIPIDS OF PHOTOSYNTHETIC SYSTEMS

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THE chloroplast has one of the highest contents of lipid (~ 50 per cent dry weight) of all cell organelles, but unlike the pigments, quinones and tocopherols, the function of acyl lipids has not yet been defined even though their structures are now well understood (see Benson[1] and Carter[2]). These lipids could function as either (or both) chemical or structural components of the photochemical system.

The ideal approach to this problem would be to isolate whatever unit of the chloroplast is capable of carrying out all the known photochemical reactions as well as the associated lipid syntheses, and to fully define all its protein, enzyme, and lipid-soluble components. Since this is difficult in any single laboratory we have adopted the indirect approach of investigating the fatty acid and acyl lipid composition of a variety of photosynthetic systems, that is, photosynthetic bacteria, yellow, blue-green and green fresh-water algae, marine algae, mosses, and leaves from widely different species of higher plants. In addition, we have studied the effect of light on etiolated leaves and algae so far as their fatty acid composition, rate of biosynthesis of unsaturated fatty acids and turnover of the acyl lipids are concerned. These data, together with those already published, enable some conclusions to be reached as to the function of these lipids in the photosynthetic particle.

Acyl lipids as chemical co-factors in photosynthesis. Chemical involvement of acyl lipids in the electron transport chain would require some easily oxidizable component, and for this role the unsaturated fatty acids are the most likely candidates. We might then expect photosynthetic systems of the same type to contain unsaturated fatty acids of similar structure and that there should be some form of light-stimulated production of these components paralleling that of some of the photosynthetic pigments.

Table 1*A*. UNSATURATED FATTY ACIDS OF PHOTOSYNTHETIC SYSTEMS

	Photosynthetic bacteria	Marine algae			Freshwater algae													
					Yellow	Blue-green		Green										
Fatty acid	*A,B,C,D,E,* refs. 17, 31	*Rhodophyta* ref. 18	*Phaeophyta* ref. 18	*Chlorophyta* ref. 18	*F* ref. 21	*G* refs 13, 20	*H* refs 13, 22	*I* ref. 23	*J* ref. 3 Normal	*J* ref. 3 Mutant	*K* Light grown refs. 3,24,25	*K* Etiolated refs. 3, 25	*L* ref. 19	*M* ref. 3	*N* (Y2 mutant) ref. 3 Light	*N* (Y2 mutant) ref. 3 Dark	*O* ref. 8 Heterotrophic growth	*O* ref. 8 Photosynthetic growth
16:1 3	0	+[19]	+[19]	+[19]	?	0	0	?	+	+	+	0	?	?	?	?	−	+
16:1	+9	+	+	+	+	+9	+	+	+	+	+7/9/11	+7/9	+	+	+	+	+	+
16:2		+	+	+			+				+	0	+				+	+
16:3				+							+		+					
16:4				+							+	0						
18:1	+11	+	+	+	+	+9	+	+	+	+	9/11	+	+	+	+	+	+9	+9
18:2		+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
18:3 αγ		+	+	+	0		+α	+α	+(α)	+(α)	+α	+	+	+(α	+γ)	+(α+γ)	+α	+α
18:4		+	+	+							?							
20:2											+	+						
20:3		+	+	+	+			+			+	+		} ?	} ?	} ?		
20:4		+	+	+	?			+			+	+						
20:5	+	+	+								+	+						
22:4			+															
22:5				+							+							
22:6				+														

A, Rhodopseudomonas capsulata
B, Rhodopseudomonas spheroides
C, Rhodopseudomonas gelatinosa
D, Rhodopseudomonas palustris
E, Rhodospirillum rubrum
F, Monodus subterranus
G, Anacystis nidulans
H, Anabaena variabilis
I, Ochromonas danica
J, Scenedesmus D$_2$
K, Euglena gracilis z
L, Chlorella pyroidenosa
M, Chlamydomonas reinhardi
N, Chlamydomonas reinhardi (Y2 mutant)
O, Chlorella vulgaris

Table 1*B*. UNSATURATED FATTY ACIDS OF PHOTOSYNTHETIC SYSTEMS

Fatty acid	Plant leaves										Chloroplasts (pea leaf)	Protoplasts (castor leaf)
	Spinach refs. 10, 11	Castor ref. 6 Normal	Castor ref. 6 Etiolated	Lichens ref. 26	*Ginkgo biloba* ref 26	Nettle ref. 6	Moss refs. 9, 26	Holly ref. 9	Pine* ref. 6	Ferns ref. 26	ref. 6	ref. 27
16:1 Δ3	+	+	0	?	?	+	+	+	+	?	+	0
16:1	+	+	+	+	+7/9	t	+	+	+	+	+	+
16:2	+				+7:10		+			+	+	+
					+9:12							
16.3	+				+7:10:13							
					+9:12:15					+		
16.4												
18:1	+	+	+	+	9/11	+	+	+	+	+	+	+
18:2	+	+	+	+	+9:12	+	+	+	+	+	+	+
18:3 αγ	+α	+α	+α	+α	+α	+	+	+	+	+	+	+
18:4							+					
20:2		} Traces		?	+5:11		+			+		
					+11:14							
20:3					+14:17		+			+		
					+5:11:14							
20:4					+5:11:14:17		+		?	+		
20:5							+			+		
22:4							±			+		
22:5												
22:6												

* Blackpine, *Pinus thunbergii*

A summary of the unsaturated fatty acids occurring in photosynthetic systems is given in Table 1*A* and *B*. Of these, only monoenoic fatty acids occur in the photosynthetic bacteria and in one of the fresh-water blue-green algae (*Anacystis nidulans*), whereas all the other systems contain a variety of *cis*-polyunsaturated fatty acids of chain-lengths ranging from C_{16} to C_{22} having usually (but not invariably) a methylene-interrupted sequence of double bonds.

Two classes of photosynthetic organisms occur, one group comprising the photosynthetic bacteria, and the other the algae and plant leaves. Both are believed to have similar fundamental photochemical pathways but only the latter is able to evolve oxygen under the influence of light (the Hill reaction).

However, the blue-green alga *Anacystis nidulans* can carry out the Hill reaction but its only unsaturated acids are monoenoic. Thus there is no correlation between the distribution of any type of polyunsaturated fatty acid and the occurrence of the Hill reaction. This suggested correlation was put forward by Erwin and Bloch[3] on the basis of a study of the distribution of fatty acids in plants and algae, and on the effect of light on the biosynthesis of α-linolenic acid.

While it is true that exposure to light of an etiolated leaf (Table 2) or transference of green algae to an entirely photosynthetic mode of existence (Table 3) does cause a stimulation of α-linolenic acid synthesis, this acid is already present in dark-grown leaves and heterotrophically grown *Chlorella*. Also, α-linolenic acid is found in all plant tissues and is not specifically associated with chloroplast-containing structures[4]. The action of light on etiolated leaves causes a rapid synthesis of chlorophyll, β-carotene, lutein and zeoxanthin[5] and the production of a specific fatty acid, *trans*-Δ-3-hexadecenoic acid[6]. This fatty acid was first identified by Debuch[7] in lipid extracts

Table 2. FATTY ACID COMPOSITION OF THE LIPIDS OF LIGHT-GROWN (*L*) AND DARK-GROWN (*D*) CASTOR LEAVES

		Percentage of total fatty acids									
		12:0	14:0	16:0	18:0	16:1 Δ3	16:1 Δ9	16:2	18:1	18:2	18:3
Monogalactosyl diglyceride	*L*			6·0	t				0·7	0·8	91·0
	D	*t*	*t*	6·6	3·6		3·2	*t*	6·7	14·2	66·8
Digalactosyl diglyceride	*L*			11·1						3·7	85·3
	D		*t*	14·0	7·3		2·0		8·3	20·6	51·0
Sulpholipid	*L*	*t*	*t*	53·9	4·8		*t*		3·6	4·9	32·6
	D	4·0	3·7	28·9	12·3		4·5	1·1	14·5	12·2	17·2
Phosphatidyl glycerol	*L*	0·9	1·3	47·3	4·4	18·5	*t*		12·0	3·5	19·8
	D	1·7	2·1	37·8	9·0		5·3	*t*	10·8	19·6	13·5
Phosphatidyl ethanolamine	*L*			36·4	1·6				1·4	21·1	39·5
	D		*t*	21·4	5·2		3·6		7·7	40·7	18·1
Phosphatidyl choline	*L*			37·5	2·8		*t*		2·8	23·8	32·6
	D		*t*	14·8	9·0		1·9		10·9	37·3	23·9
Phosphatidyl inositol	*L*			53·8	4·8		4·1		7·8	6·4	34·2
	D	3·0	3·9	32·0	10·6		5·1		14·2	23·0	10·6
Cardiolipin	*L*										
	D	3·8	5·8	24·0	10·9			*t*	15·2	28·6	8·7

Table 3. FATTY ACID COMPOSITION OF THE LIPIDS OF *Chlorella vulgaris* GROWN IN THE LIGHT IN ORGANIC MEDIUM (*O*) AND INORGANIC MEDIUM (*P*) (ref. 8)

Lipid		Percentage of total fatty acids									
		14:0	16:0	18:0	16:1 3	16:1 9, 7	16:2	16:3	18:1	18:2	18:3
Monogalactosyl diglyceride	*P*		4·5	1·9	0	1·5	18·9	10·0	2·8	16·8	44·6
	O	1·4	3·7	0·7	0	14·3	21·4	1·8	18·4	29·9	8·0
Digalactosyl diglyceride	*P*		7·6	3·4	0	2·7	5·6	1·5	2·6	34·9	36·6
	O	3·5	15·0	3·6	0	4·3	5·9	1·9	14·3	39·7	7·1
Sulpholipid	*P*		32·4	3·4	0	4·8	2·7	2·5	10·0	25·2	15·4
	O	2·4	36·2	4·3	0	5·9	2·9	2·6	20·3	17·5	2·5
Phosphatidyl glycerol	*P*		31·3	5·8	15·7	5·2	*t*	—	10·0	25·3	4·5
	O	2·3	46·2	2·0	*t*	6·7	*t*	*t*	28·3	11·0	0
Phosphatidyl ethanolamine	*P*		28·9	2·8	0	5·5	1·5	1·5	8·7	33·3	14·7
	O	0·7	19·0	3·4	0	3·1	2·8	2·6	13·2	52·0	2·7
Phosphatidyl choline	*P*		15·8	4·5	0	2·8	2·8	1·5	8·6	44·3	17·6
	O	1·3	10·3	4·9	0	3·6	4·3	2·9	20·4	44·9	4·6
Phosphatidyl inositol	*P*		30·2	6·4	0	11·2	4·0	*t*	23·1	10·0	3·5
	O	4·6	44·2	4·9	0	4·2	1·9	*t*	18·1	9·2	0
Cardiolipin	*P*		24·2	13·7	0	8·5	*t*	*t*	27·3	27·3	5·9
	O	3·6	32·4	3·6	0	13·3	1·1	0·8	20·9	18·3	1·4
Neutral lipid	*P*	8·7	34·4	12·3	0	3·9	*t*	*t*	21·3	10·6	4·7

from both leaves and green algae. We[6,8,9] and others[10,11,16] have found it to occur only in the phosphatidyl glycerol of green leaves and algae (Tables 2 and 3), and to be completely absent from etiolated systems. There was thus more reason to associate this fatty acid, rather than α-linolenic acid, with some active role in photosynthesis, particularly as the fatty acids of phosphatidyl glycerol turn over more rapidly than those of any other lipid in photosynthetic systems[6,12]. However, *trans*-Δ-3-hexadecenoic acid does not occur in some blue-green algae[13], so that unless there is some discrete difference between the mechanisms of photosynthesis in green algae and leaves and in blue-green algae, even this acid cannot be involved.

As an alternative explanation for the wide distribution of polyunsaturated fatty acids, it should be pointed out that leaves, and to a lesser extent algae, must be able to function even at low ambient temperatures, since they cannot regulate their own temperature. A high content of unsaturated fatty acids would ensure that their lipoprotein structures were fully mobile over a wide temperature range.

Lipids as structural components. Lipids can be considered as having two possible functions as components of the protein–enzyme–pigment–lipid complex of the chloroplast: (*a*) As specific structural components maintaining the lipid soluble pigments in correct spatial alignment with one another and their associated enzymes. The existence of specific interaction forces would require a similar lipid or group of lipids to occur in all the photosynthetic systems of a given type. (*b*) As relatively non-specific micellar elements which exclude water and in which the pigment–protein–enzyme complexes could be partially or completely embedded. Such water-free areas could allow the operation of electron transport chains that would be inhibited by free water. The lipid would thus act as an organized medium of low dielectric constant.

The formation of complex micelles that provide water-free areas could be done by a variety of ampiphatic lipids. Thus the different types of photosynthetic tissue could contain a wide variety of lipids performing similar functions.

Distribution of acyl lipids in photosynthetic tissues. The distribution of compound lipids in the different photosynthetic systems is shown in Table 4. There is a clear differentiation between the two groups defined by the Hill reaction. Those capable of evolving oxygen always contain mono- and di-galactosyl diglycerides, sulpholipid (6-sulpho-α-D-quinovosopyranosyl-(1–11)-diglyceride[14]), and also phosphatidyl glycerol, whereas the systems incapable of the Hill reaction have only phosphatidyl glycerol in common. Phosphatidylcholine, -ethanolamine, -inositol and cardiolipin occur in green algae and leaf extracts but are absent from both blue-green algae and washed isolated leaf chloroplasts. Investigation of the fatty acid composition of the individual lipids from these sources has shown that polyunsaturated acids and particularly α-linolenic acid are concentrated in the galactosyl diglycerides. In the case of *Anacystis nidulans*, however, which contains only saturated and monoenoic acids, there is no such particular concentration of unsaturated fatty acids[13].

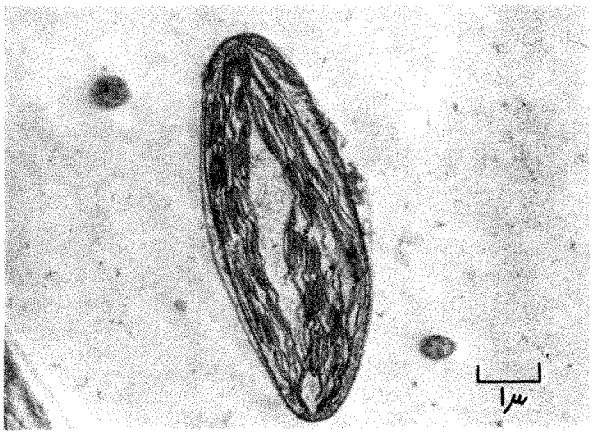


Fig. 1. Electron micrograph of chloroplast in *Ricinus communis* leaves

Leaves grown in the absence of light do not possess the normal layered structure of the chloroplasts seen in Fig. 1 but a lattice-like organization[15] having what appears to be a hexagonal structural unit (Fig. 2). This etiolated system possesses the four acyl lipids present in chloroplasts although their fatty acid composition is somewhat different, as already detailed (Table 3). During exposure to light trans-Δ-3-hexadecenoic acid, chlorophyll, pigment and α-linolenic acid particularly are synthesized. The electron micrograph shown in Fig. 3 demonstrates that after exposure to light the prolamellar structure breaks down to give a number of membranes which rearrange to give the normal lamellar structure of the mature chloroplast. The fact that very similar lipids (though in altered proportions) form part of such different structures suggests that they do not determine the detailed microstructure.

The photosynthetic bacteria operate the non-oxygen-evolving parts of the photosynthetic process with quite

Table 4. LIPID COMPONENTS OF PHOTOSYNTHETIC SYSTEMS

Lipid	Photosynthetic bacteria ref. 17					Salt water alga ref. 6	Fresh-water algae					*J* ref. 21	Wide variety of plant leaves refs. 6, 11, 28	Leaf chloroplasts ref. 29
	Rhodopseudomonas						Yellow	Blue-green		Green				
	A	*B*	*C*	*D*	*E*		*F* ref. 21	*G* ref. 13	*H* ref. 13	*K* ref. 11	*O* ref. 8			
Monogalactosyl diglyceride						+	+	+	+	+	+	+	+	+
Digalactosyl diglyceride						+	+	+	+	+	+	+	+	+
Sulpholipid	+					+	+	+	+	+	+	+	+	+
Phosphatidyl glycerol	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phosphatidyl ethanolamine	+	+	+	+	+	+	+	0	0	+	+	+	+	
Phosphatidyl choline	+	+	+			+	+	0	0	+	+	+	+	
Phosphatidyl inositol					+		+	0	0	+	+	+	+	
Cardiolipin		+		+	+	+					+	+	+	
Triglyceride							+				+	+	+	
Unknown lipid								+	+					
o-Ornithyl phosphatidyl glycerol	+	+	+	+	+									

Symbols as in Table 1*A*

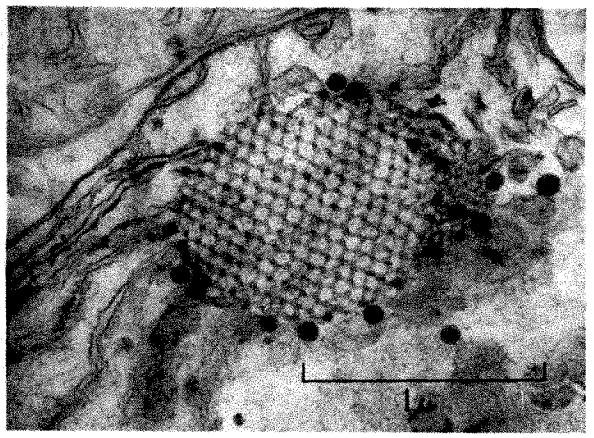


Fig. 2. Electron micrograph of the photochloroplast in etiolated pea testa

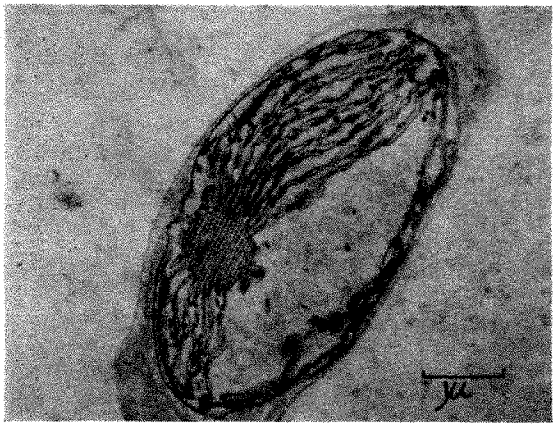


Fig. 3. Electron micrograph of the photochloroplast in etiolated pea testa, after exposure to light showing breakdown of the hexagonal structure to give a series of ribbon-like filaments

different lipids (Table 4), suggesting that part of lipid function is to provide water-free structures into which the photochemically active components can fit. If this is the case, an exact stoichiometric relationship between the amounts of the different lipids, the pigments and protein (originally suggested by Lichtenthaler and Park[32]) is unlikely.

The data collated here do not support the concept that unsaturated fatty acids are chemically involved in any part of the photosynthetic processes. Four acyl lipids, namely, mono- and di-galactosyl diglycerides, sulpholipid and phosphatidyl glycerol are specifically associated with photosynthetic organelles capable of the Hill reaction and are the sole lipids of blue-green algae, but no general pattern of fatty acid composition in this group is discernible. Apart from the oxygen-evolving system, photosynthesis seems able to operate with a wide range of ampipathic lipids the general pattern of which is diglyceride linked to a polar group, suggesting that the real requirement is for non-aqueous complexes capable both of solubilizing the pigments, quinones and tocopherols and of non-specific interaction with water-soluble macromolecules such as proteins and polysaccharides.

We thank Mr. M. Stubbs for permission to reproduce his electron micrographs.

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DYNAMIC ASPECTS OF GROWTH AND METABOLISM IN *Candida utilis*

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THE microbe, as an independent unit of life, possesses many unique advantages that make it a useful agent for investigating growth and metabolism in a cell, and for more than a century microbes have played an important part in the investigation of metabolism. Investigation of the metabolism of the single cell has been impracticable, however, and the microbial culture, comprising the cell population and its substrate, has been used instead. The traditional batch culture has served this purpose and provided the material for investigation.

In the closed system of the batch technique, cells grow in a limited amount of medium; as these cells multiply and form a population, which flourishes initially but subsequently diminishes, they produce a sequence of change in the culture that is often referred to as the 'growth cycle'. In this sequence two relatively constant phases of growth have been recognized that produce the 'proliferating cells' and the 'resting cells' of the biochemist, and much of the contemporary knowledge of microbial growth and metabolism has been obtained by their use. The batch technique, despite many limitations, has been a keystone in the development of microbiology and related disciplines.

New methods have recently been developed for cultivating micro-organisms in open systems of continuous culture[1]. In these methods, cells grow in a continuous

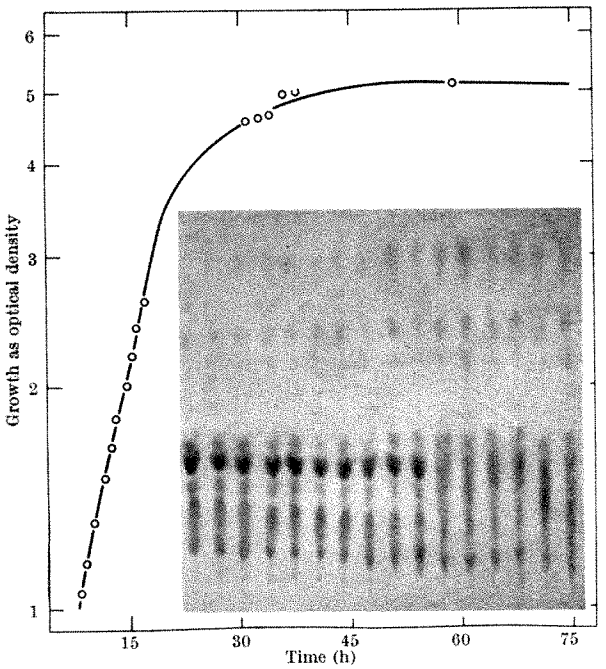


Fig. 1. Batch growth of *C. utilis* on glucose (N-limited) medium. Inset shows paper chromatogram of amino-acid pools corresponding to the points on the growth curve

flow of medium in arrangements that use a constant volume of culture in a steady-state condition of growth. One technique, now generally referred to as chemostat culture, enables the microbial growth to be examined in any one of an infinite number of steady states that are definable, constant, and reproducible. The system confers advantages that are in sharp contrast to the transient changes of the batch growth and the often erratic reproducibility of that method.

Another important advance in methods for cultivating micro-organisms is the recent introduction of synchronization techniques to phase all, or most, of the cells in a culture, so that they divide together ('in step')[2]. In this way, the growth of a cell can be magnified by the size of the population in the culture, and the cell cycle thus becomes available for analysis by conventional methods.

The ultimate expression of cell metabolism is growth and multiplication, and in consequence these new techniques for growing microbial cells offer fresh approaches for studying the growth metabolism of the cells, especially for investigating the integrated metabolic systems of the cell *in vivo*. Because of this, the new techniques could be of fundamental importance for the future development of microbiology, biochemistry and cell physiology.

The important part that enzymes play in cell metabolism has long been recognized, but it is only recently that some indications of the nature of their formation and function in cell metabolism and cell regulation have been obtained[3]. Changes in growth and metabolism in a cell require changes in enzyme constitution, and these, reflected in the protein synthesis of the cell, must ultimately be linked to the supply of the precursors, that is, of amino-acids arising by *de novo* synthesis, turnover, or by incorporation from the medium. Variations in the synthesis of other macromolecules in the cell will likewise require a changing supply of precursors, so that changes in metabolite pools, besides those of the amino-acid pool, might be expected under different circumstances.

Various workers have reported changes in microbial amino-acid pools under different conditions of medium, and recently Holden[4], reviewing the accumulated information on the subject, concluded that the apparent lack of constancy in the composition of the pools was likely to be a consequence of the dependence of the pool on the external environment and of the ease with which this could be changed. In much of the work covered by his review, batch cultures were grown on media that contained amino-acids, and interpretations of the results were, in consequence, often complicated by inadequately defined growth and problems associated with transport and permeation phenomena. These difficulties would largely be eliminated if cells were grown in chemostat and closely controlled batch cultures using simple chemically defined media devoid of pre-formed metabolites, that is, amino-acids.

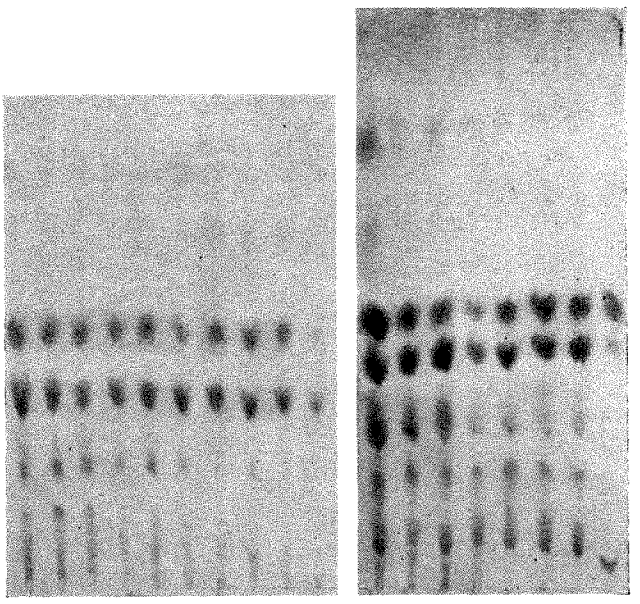
The possibility of using such techniques to examine the behaviour of various metabolic pools, as a method for examining *in vivo* metabolism in a microbe, was investigated using the yeast *Candida utilis*[5].

Cells of *Candida utilis* were grown in nitrogen-limited batch culture under defined conditions of aeration and temperature, on a simple medium that contained glucose and mineral salts. The cells obtained from samples of the culture collected during the exponential, retardation and stationary phases of growth were extracted, and the amino-acid pools obtained were chromatographed on paper. Details of the methods have been published elsewhere[6]. It can be seen (Fig. 1) that changes occurred in the spectrum of the amino-acids constituting the pool for different phases of growth, but the pool appeared to be of constant composition during the exponential phase.

When cells were grown in chemostat culture on the same medium under identical experimental conditions, but at different growth rates, the chromatograms of the extracted pools showed changes in the amino-acid spectrum with growth rate. In Fig. 2*a* the changes for nitrogen-limited growth are illustrated, and in Fig. 2*b* are shown the changes on the same medium, but under carbon-limited conditions.

Further characteristic patterns of change in the pool, with changes in growth rate, were obtained when glucose was replaced in the medium by other different carbon sources such as glycerol, ethanol, acetate and lactate, in chemostat cultures under similar nitrogen-limited conditions of growth.

Profiles, obtained by gas chromatography[6], of the amino-acid pools extracted from cells of the same doubling time (7·0 h) grown under identical conditions of culture



(*a*) (*b*)

Fig. 2. Paper chromatograms showing changes in the intracellular amino-acid pool of *C. utilis* grown on (*a*) glucose (N-limited) medium and (*b*) glucose (C-limited) medium for residence times between 2·5 and 12·5 h

in the chemostat on different media are illustrated in Fig. 3. The widely different spectra would suggest that metabolism is determined by the medium conditions and not by linkage to the growth rate.

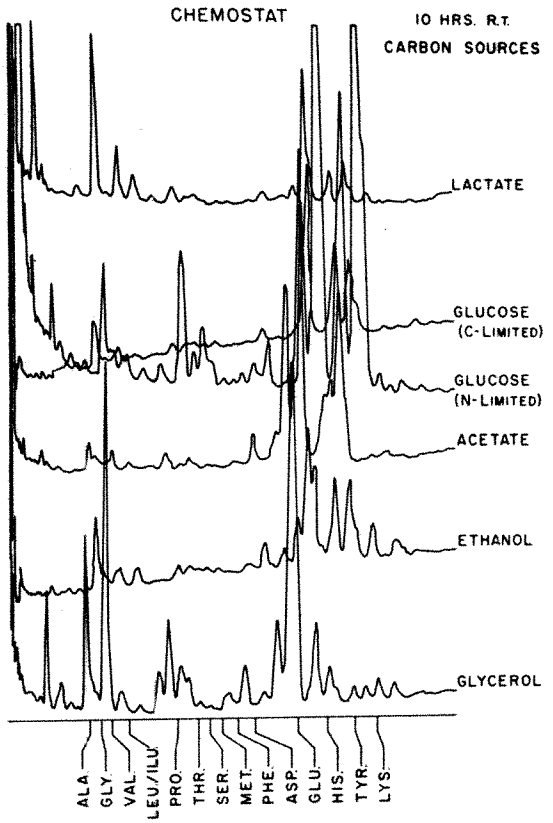


Fig. 3. Profiles of amino-acid pools, obtained by gas chromatography, for chemostat cultures of the same growth rate (residence time 10 h*) on different (N-limited) media

* Doubling time of 7 h.

A method of continuous phased culture has been devised, and is described in another paper[7], which enables the population of a chemostat culture to be maintained in a very high state of synchrony over periods of many months. Amino-acid pools extracted from sequential samples of cells grown over a cycle in this apparatus, when chromatographed, gave a pattern of change over the cycle. Variations in this pattern occurred with changes in the cycle time; that is, with the growth rate. Fig. 4 shows spectra obtained for doubling, or cycle, times between 2 h and 8 h 20 min on glucose nitrogen-limited medium, under conditions of culture identical with those used for the batch and chemostat experiments.

The pools extracted at intervals over a cycle of phased growth, when combined, produce an average pool, the spectrum of which corresponds with that obtained from cells grown in a chemostat at the same doubling time. This establishes a common basis for the two techniques[5].

The foregoing results show changes in the amino-acid pools of cells grown on the same medium, in the same culture vessel (a cyclone column fermenter)[8], and under identical cultural conditions, but in different growth systems; that is, as batch, chemostat, and phased cultures.

These results show that the amino-acid pool, in addition to being characteristic of the medium, of the environmental conditions, and of the growth rate, as already reported in a previous publication[6], also underwent a pattern of change during the cell cycle that was characteristic for the conditions operating during that cycle.

The intracellular amino-acid pool, first demonstrated in microbes by Gale[9] and extracted by his simple technique of boiling a suspension of washed cells in distilled water, was found to be adequate as a register of the overall metabolism of a culture and for detecting small changes in metabolism. However, since McQuillen *et al.*[10] had reported a rapid synthesis of cell protein in microbial cells, accelerated procedures of extraction and isolation of the pool appeared to be advisable, if the pool was to be representative of that in the cell. A rapid procedure based on the method of Gale, but modified to use membrane filtration techniques, was therefore devised

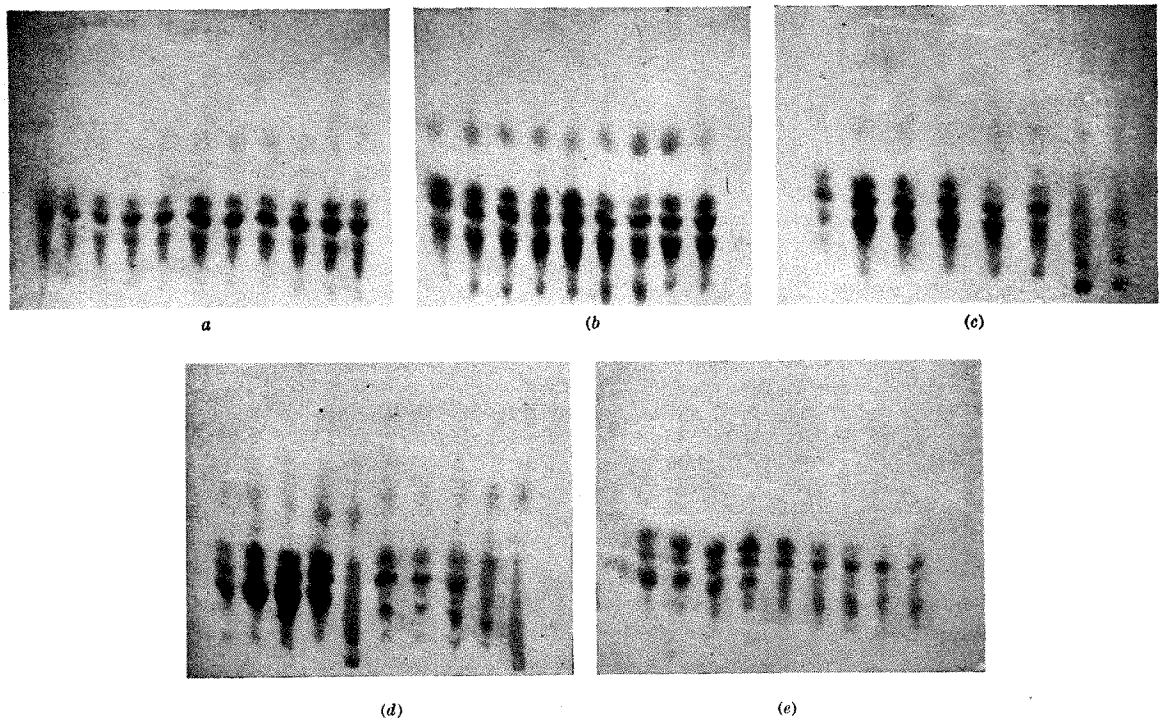


Fig. 4. Chromatograms of amino-acid pools extracted from cells of *C. utilis* growing on glucose (N-limited) medium. Sequences obtained by interval samples from phased growth for cycle times of (*a*) 2 h, (*b*) 2 h 40 min, (*c*) 3 h 15 min, (*d*) 6 h 30 min, and (*e*) 8 h 20 min

and used to extract the amino-acid pools in the foregoing experiments.

Membrane filters are resistant to a number of solvents, and the extraction method described earlier was adapted to give a serial fractionation of cells, so that empirical fractions as pools of amino-acids, nucleotides (as materials absorbing at 260 mμ) and lipids were obtained; however, freeze–thaw techniques were used instead of boiling water for the initial extraction[5].

When cells, grown by the different cultivation procedures of batch, chemostat, and phasing, were extracted in this serial manner, and systematically examined in a fashion analogous to those already employed and described for the amino-acid pools, it was found that the other constituents, nucleotides, lipids and other compounds, also changed and varied in amount, and in ways analogous to those of the amino-acid pool[5].

Fig. 5 illustrates the changes taking place in the pool of nucleotide materials during a batch culture in glucose (N-limited) media, and during a synchronous cycle of 4 h 15 min in phased culture on glycerol (N-limited) medium.

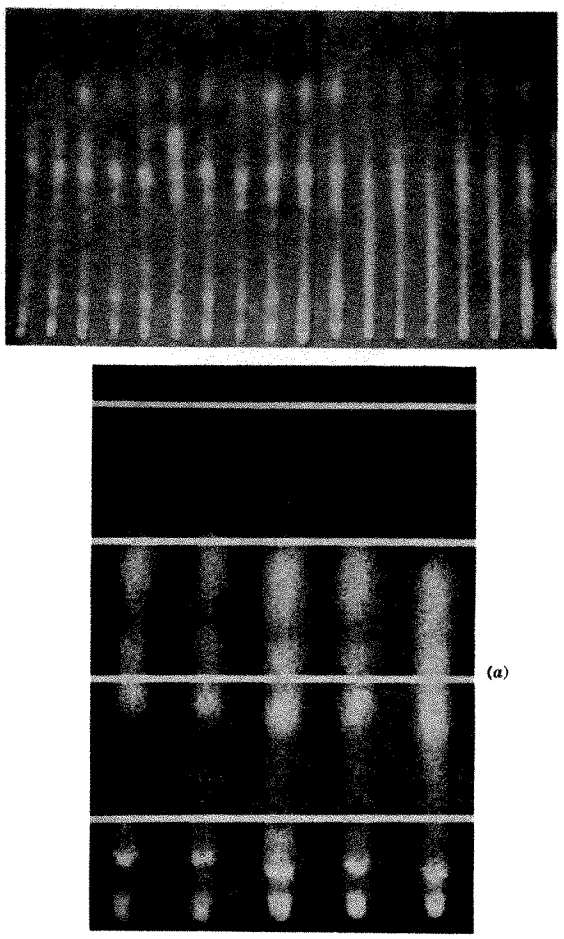
In Fig. 6 changes taking place in various other pools of the same growth are illustrated by a series of chromatogram sequences, and further aspects of this growth are summarized in Table 1.

Changes in the lipid components will be published in detail elsewhere.

It is evident that continuous variations were taking place in the cell metabolites throughout the whole of the cell cycle. These changes were repeated cycle after cycle, and the pattern was constant under unchanged conditions of cultivation, that is, of medium conditions, of growth rate and of environment. Controlled changes in the experimental parameters produced alterations in the cycle patterns under the different chosen conditions, and were analogous to the steady states obtainable in a chemostat. Continuous phased cultures operated indefinitely, like those of a chemostat, and a unit was working for over 18 months.

In the experiments reported here, the use of simple chemically defined media and closely controlled conditions of growth ensured that the metabolites extracted from the cells were products of the cell metabolism and directly reflected the active metabolism of the cells. The amino-acid and other metabolic pools, as registers of the cell metabolism, showed that the metabolism of the cell was in continual flux throughout the whole of its growth, whatever the rate of that growth. The results established that whereas the batch growth outlined the metabolic range of the organism and chemostat cultures enabled selected steady states to be closely examined, continuous phased cultures enabled this to be done on the basis of the cell cycle. The pattern of metabolism over the cell cycle, as portrayed by the pool changes, was characteristic of the environment of the cells and was constant, as a repeating pattern over the cycle, when the environment was constant.

The advantage of the continous methods for examining cell metabolism, by stabilizing growth conditions, was demonstrated by these experimental results: the techniques provide convenient methods for the systematic study of integrated metabolic systems *in vivo*, in cultures—



(b)

(a)

Fig. 5. Paper chromatograms showing materials absorbing at 260 mμ extracted from successive samples of cells of *C. utilis* (*a*) growing batch-wise on glucose (N-limited) medium, and (*b*) during phased growth on glycerol (N-limited) medium for a cycle time of 4 h 15 min

by the chemostat, or in the cell—by the phasing method. These techniques offer new dimensions for experimental explorations of the cell and open up new horizons for microbiology, biochemistry and cell physiology.

It will be apparent that the randomized conditions of the cells in conventional culture does not reflect a constant performance from the cells but only an average pattern of behaviour; that is, stabilized in chemostat and changing in batch growths. This difference will have important repercussions for many experiments using tracers where uniform or steady-state conditions in a cell have been assumed, and, as Mitchison[11] has already pointed out, much published work will need to be re-examined in the light of the dynamic changes now demonstrated as taking place in the cell over the cell cycle.

As discussed more fully elsewhere[5], the amino-acid pools do not reflect the pattern of amino-acids directly used in the current synthesis of protein taking place in the cell, but rather reflect the overall balance between the

Table 1. PHASED GROWTH OF *C. utilis* DURING A CYCLE TIME OF 4 H 15 MIN ON GLUCOSE (N-LIMITED) MEDIUM—SUMMARY OF RESULTS

Period in cycle (min)	0	30	60	90	120	150	180	210	240	254
Absorbance (*O.D.* 1/25)	0·26	0·28	0·29	0·29	0·30	0·31	0·35	0·40	0·42	0·44
Dry wt. cells (mg/ml.)	3·0	2·82	3·49	3·92	4·37	4·72	4·57	5·09	5·28	—
Total amino-acid*†	72·5	27·5	13·0	12·0	22·0	32·0	42·0	55·0	64·0	—
Total amino-acid†‡	61·0	22·0	10·0	10·0	16·0	22·0	28·0	33·0	34·0	—
Ultra-violet light (260 mμ)*‡	1·3	0·14	0·11	0·15	0·16	0·19	0·22	0·24	0·30	—
Amino-acid pool§	Min				Max				Min	
Nucleotide pool‖	Min								Max	
Lipid pool	Min								Max	

* Boiled water extraction method. † After Yemm and Cocking (ref. 19): total amino-acids reported as percentage transmittance at 570 mμ of ninhydrin positive materials. ‡ Freeze–thaw extraction method. Absorbance at 260 mμ measured in 1 cm cell.
§ See Fig. 6*a*. ‖ See Fig. 6*b*.

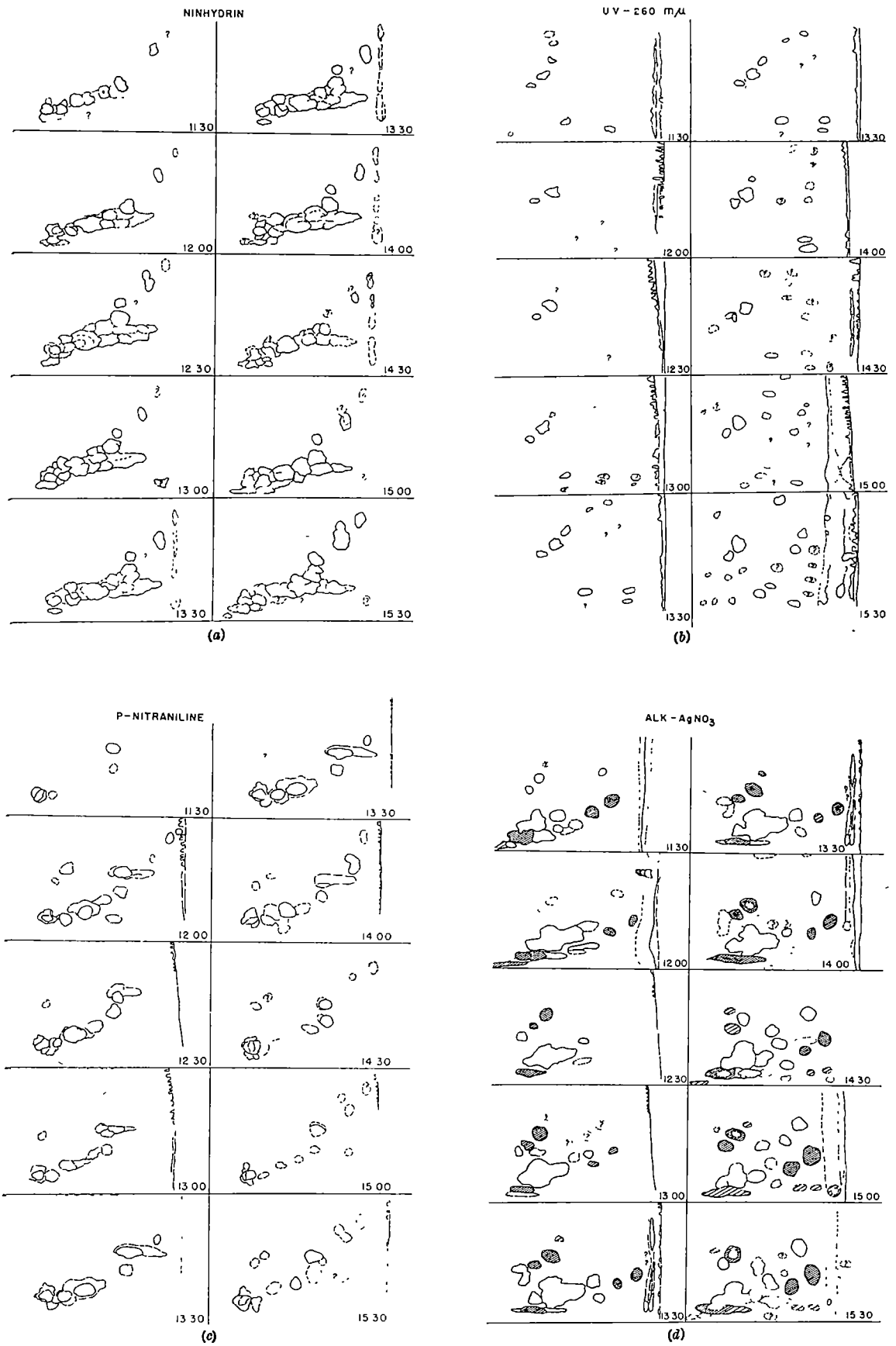


Fig. 6. Phased growth of *O. utilis* on glucose (N-limited) medium. Four sequences of two-dimensional chromatograms prepared from extracts obtained at intervals of 30 min during a cycle time of 4 h 15 min. The papers were run in the same solvent mixture (first dimension butanol/ethanol/water: 60/15/25, and second dimension isopropanol/hydrochloric acid/water. 65/17/18) by the ascending technique The four sets were developed for: (*a*) ninhydrin positive substances; (*b*) materials absorbing at 260 mμ; (*c*) materials reacting with *p*-nitraniline; (*d*) materials reducing alkaline silver nitrate (shaded) and clear areas (unshaded)

supply and demand of these components in the cell metabolism, and more probably serve as an indication of a pattern of feed-back in the cell. Such a role would be in harmony with the suggestions of Stent and Brenner[12], and of Kurland and Maaløe[13], that the transfer-RNA molecules, or the activating enzymes themselves not saturated with their amino-acids, could serve as repressors of RNA synthesis. The changing spectra of other cell metabolites, operating in like fashion, could also have important significance in the overall regulation and control of metabolism in the cell.

The metabolic pathways leading to the precursors used for macromolecular synthesis in the cell are closely integrated at the lowest levels and largely comprise what Davis[14] has called the amphibolic area of cell metabolism. Our knowledge of the interplay and overall patterns of feed-back mechanisms is still rudimentary, but evidence is available of the possibility for integrated controls of different pathways, for example of nucleotides by amino-acids and vice versa[15–18]. The description by Magasanik[18] of the elegant control of the histidine and guanylic acid biosynthetic pathways by purine and amino-acid components, respectively, is an outstanding example of how feed-back mechanisms might be integrated.

The possibilities offered by an integrated network based on twenty channels of amino-acid feed-back and five or six channels of nucleotide feed-back lead to a system that would suffice to co-ordinate a wide range of metabolic activity, and serve to link the amphibolic metabolism with the biosynthesis of the vital macromolecules, proteins and nucleic acids. In such a system, feed-back mechanisms could operate back from a master template, to order and modulate the supplies of precursors required for the ultimate duplication of the template. The arrangement, linked to cell intermediates and connected to other areas of cell metabolism, could satisfactorily account for the phenotypic variations of the genotype observed in cellular metabolism and growth under different conditions.

In recent articles in *Nature*, Dean and Hinshelwood[20–22] have discussed the possible integrations and implications of cell reactions, cell organization and cell regulation. The work described in this article, in demonstrating a characteristic and organized behaviour of intracellular pools, lends support to their general thesis and emphasizes also the lack of information available from contemporary investigations of cell metabolism in relation to the neglected but important question of the overall supply of building units for the architecture of the cell.

The investigation of metabolic pools is an empirical exercise; their nature, origin, behaviour and complexity are unknown. It would appear that a closer investigation of metabolic pools, and of other aspects of metabolism *in vivo*, might now be attempted with advantage, possibly by using some of the methods described here, in a systematic manner.

I thank Drs. C. G. Youngs and B. M. Craig, of this laboratory, for the determinations of amino-acids and fatty acids by gas chromatography, and E. G. Darichuk for technical assistance.

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POSSIBLE IMPLICATION OF MYRISTICIN AS A PSYCHOTROPIC SUBSTANCE

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WITHIN the past decade there has been a rapid expansion of knowledge of psychotropic medicine. The development of the ataraxics, and more recently of the 'psychic energizers', has depended on chemical syntheses in areas almost totally unknown in Nature.

A third field of investigation concerns those psychotropic materials known as the hallucinogenic drugs. Substances that could 'to an extent duplicate the syndrome of insanity' or that might 'permit one's capacities to transcend the limits of the sensorium' have long been in existence, but serious study of them, under the respective classifications of psychotomimetics and psychedelics, has only very recently been started. Most of these latter chemicals, in contrast to the tranquillizers and antidepressants above, come at least originally from natural sources. The pursuit and exploitation of the folk-lore that describes the many intoxicants of Nature has resulted in a disproportionately small number of new structural families. In many instances it has become apparent that the active material in an intoxicating plant is the same as, or is closely related to, a previously known system. Thus a small handful of chemical individuals, disguised by minor chemical distortions, account for the pharmacological effectiveness of a large variety of plants.

Investigations of the Amazon intoxicant ayahuasca (cappi, yaje) have led to a study of the genus *Banisteriopsis*. The analysis of these vines has revealed a series of alkaloids related to harmine which, with its hydrogenation products harmaline and tetrahydroharmine, is claimed to be the central stimulant present in *Prestonia amazonicum*. Harmine has also been isolated from the seeds and roots of the biologically active *Peganum harmala*, of both a different family and a different continent (*Zygophyllaceae*, rather than *Malpighiaceae;* Asia, rather than South America). Two additional intoxicating plants, of yet different genera (*Tetrapterys methystica* and *Mascagnia psilophylla*), probably embody this same alkaloid group.

The extracts of *P. amazonicum*, mentioned above, have also been found to contain *N*,*N*-dimethyl-5-hydroxytryptamine (bufotenine). This presumed psychotomimetic also occurs in *Lespedeza bicolor* and is one of the toxic principals in the snuffs made from the seeds of *Piptadenia peregrina*, *P. colubrina* and *P. macrocarpa*. This latter genus serves as yet another connecting link, for from its various species has been isolated *N*,*N*-dimethyltryptamine. The implication of this indole as the responsible agent in a narcotic drink concocted from

the closely related plant, *Mimosa hostilis*, must be questioned, as the purified alkaloid is effective only on parenteral administration.

Mescaline is usually associated exclusively with the peyotl button from *Anhalonium lewinii* from which it was originally isolated, but more recent investigations into other cacti, *Gymnocalycium gibbosum* and *Opuntia cylindrica*, have revealed that here, too, the same phenethylamine is a major component.

The search for the sacred Mexican intoxicating mushrooms known as teonanacatl successfully led to the investigation of several members of the genus, *Psilocybe*. One of the most potent examples, *P. mexicana*, has afforded a new hallucinogenic chemical, psilocybin. This compound, the phosphate of a positional isomer (*N*,*N*-dimethyl-4-hydroxytryptamine) of bufotenine (v. s.), has now been shown to be responsible for the narcotic effects of a large number of active mushrooms of the same genus. It has been shown to be present in *Stropharia cubensis* and is suspected to occur in the genus *Panaeolus*.

d-Lysergic acid diethylamide, LSD, is perhaps the most widely studied of all the hallucinogens and was first prepared by synthetic modification of alkaloids derived from the ergot fungi. The lysergic acid structure was long thought to be unique to this plant phylum, but recent analysis of the native (Mexican) drugs ololiuqui and tlitliltzen has led to the investigation of various *Convolvulaceae* (specifically the morning glory plants *Rivia corymbosa* and *Ipomoea violacea*), wherein the intoxicating chemicals were also found to be amides of lysergic acid.

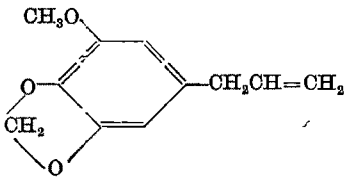
Thus, research into the active components of a score of psychotropic plant sources has revealed perhaps a half-dozen chemicals and their closely related analogues. It is possible that a similar set of circumstances, outlined later, may implicate the simple chemical myristicin (3-methoxy-4,5-methylenedioxyallylbenzene) in a comparable role.

An intoxicating snuff, yakee or paricá, is used by Indians in Colombia, and the origins of this material have been established to be a species of the genus *Virola*, *vis.*, *V. calophylla*, *V. calophylloidea*, and possibly *V. elongata*. These trees are members of the family Myristicaceae, and the essential oil myristicin has been mentioned as a possible suspect in the explanation of the biological activity. Secondly, increasing attention is being drawn, in the medical literature, to the use of nutmeg as a mentally disorganizing material. The seed of *Myristica fragrans*, on ingestion, produces cycles of delirium and giddiness. The components generally believed culpable lie in the non-terpinacious volatile oil fraction, of which the primary component is myristicin. Very recently a new, synthetic, psychotomimetic has been described, 3-methoxy-4,5-methylenedioxyamphetamine (MMDA). This base possesses the precise carbon and oxygen skeleton of myristicin, from which it can be theoretically derived, by the addition of a molecule of ammonia.

Thus, the natural essential oil myristicin may serve a psychotogen role in being a possible component of the virola tree, as a probable active agent in the nutmeg, and as a potential *in vivo* origin of MMDA. The following discussion is intended to present a complete definition of myristicin, both chemically, botanically, and pharmacologically. Further, the known biological effectiveness of it, its cogeners, its botanically related analogues, and the oils in which these have been found, is outlined and is discussed in the light of possible involvement in human intoxication.

Chemical Definition

In early descriptions of natural products, the name 'myristicin' referred to the solids that crystallized from old samples of oil of nutmeg on prolonged standing. These are now known to be myristic acid, $CH_3(CH_2)_{12}COOH$ (ref. 1). Semmler, in his initial investigations of the structures of components of oil of nutmeg[2], used the term to refer to a substance he had isolated after base isomerization and which is now known to be isomyristicin. The name was then applied to the material as found in nutmeg, and is at present used in this sense to represent 3-methoxy-4,5-methylenedioxyallylbenzene, (I).



I

Myristicin has usually been isolated from natural oils by fractional distillation, as it is crystallized only with difficulty even at very low temperatures. The first insight into its structure was achieved by Semmler, who demonstrated that the base-isomerized material from oil of nutmeg could be oxidized to a nine-carbon aromatic acid, the methoxy methylenedioxy ether of gallic acid. Misleading elemental analyses suggested the presence of a four-carbon olefinic chain. A decade later, Thoms[3], working with material isolated from oil of mace, correctly established the side-chain as being an allyl group, in analogy to the then established ether from parsley, apiole. His confirmation of Semmler's further work established that the latter had been working with the conjugated isomer, isomyristicin.

Two routes have been employed in the synthesis of myristicin. The first, reported in 1939 by Trikojus and White[4], used the monoallyl ether of 3-methoxy catechol. Claisen rearrangement yielded 5-allyl-3-methoxy catechol which on reaction with methylene iodide afforded myristicin. A second synthesis of myristicin used eugenol as a starting material[5]. Reaction with hexamine yielded the corresponding salicylaldehyde which was oxidized to the catechol described above. The over-all yield of myristicin was 8 per cent.

Myristicin reacts with bromine to form a tetrabromo derivative (m.p. 130° C) which is frequently used for characterization; the oxidation with permanganate may yield either myristicin aldehyde (m.p. 130°) or myristinic acid (m.p. 208°–210° C). Interaction with methyl magnesium iodide leads to the opening of the methylenedioxy ring with formation of 3-methoxy-4-ethoxy-5-hydroxy allylbenzene[6]. This substance, myristinol*, retains the allyl group intact as is demonstrated by its normal conversion to the conjugated isomyristinol.

Treatment of myristicin with either potassium hydroxide in alcohol or metallic sodium converts the allylic side-chain into the conjugated propenyl group. This latter compound, isomyristicin (m.p. 44° C), is convertible into both a dibromo (m.p. 109° C) and a tetrabromo product (m.p. 156° C) (ref. 3). The oxidation of isomyristicin with ozone yields myristicinaldehyde; oxidation with permanganate leads to the corresponding acid as well. Reaction with nitrous acid leads to a pseudo-nitrosite, which is further convertible through a β-nitro styrene to the corresponding phenyl acetone[7].

Myristicin is a clear, mobile, colourless oil that does not normally freeze even at very low temperatures, although a recent report[8] describes its purification by repeated crystallization at −30° C. It is best isolated from oil of parsley by vacuum distillation (b.p. 173°/40 mm, 126°/4 mm) and shows an $n_D^{20°}$ 1·5407 and a $d^{20°}$ 1·442. It has a faintly aromatic odour often reminiscent of the botanical origin. This is due, without doubt, to the inefficiencies of

* Myristinol (occasionally mylistinol) is not to be confused with myristicol, the name given to a fraction, b.p. 212°–218°, from oil of nutmeg. This alcohol (Wright, *J. Chem. Soc.*, 26, 549, 1873) has been shown to be a mixture of 1-terpinen-4-ol, borneol, and α-terpineol.

vacuum distillation. Although generally believed to be stable on storage, gradual changes in a synthetic sample have been determined by a bio-assay technique[9]. The physical properties of myristicin as isolated from oil of nutmeg must allow for the presence of elemicin as a concomitant contaminant[10].

Botanical Definition

The most important plant family containing myristicin is the Umbelliferae (syn. Ammiaceae or Apiaceae), which embraces many of the rooty vegetables and common herbs. Of the some 250 genera known in this family, about 10 per cent have appeared in the literature with some form of systematic analysis of components. Myristicin has been shown to be present in the species of each of six of these listed directly below; closely related polyalkoxy aromatics have been observed in at least three additional genera. Several of the parsley plants (*Petroselinum hortense*, *P. sativum*) yield essential oils rich in myristicin, the latter possessing the distinct advantage of yielding a distillation cut, unlike that from oil of nutmeg, that is free of elemicin. The oil of lovage that is derived from the Scotch lovage (or sea parsley) *Levisticum scoticum* contains myristicin as a major component. The rhizomes have been reported to yield well over 1 per cent of their total weight as myristicin[11]. The more common lovage, *L. officinale*, contains eugenol, but no aromatic nucleus more highly substituted. Myristicin has not been reported as a component of most of the more common fennel plants, that is. *Foeniculum vulgare* (in its several varieties, known as bitter, sweet, and Roman fennel), *Crithmum maritimum* (sea fennel), and *Phellandrium aquaticum* (water fennel). The genus *Ridolfia* has been found to be an excellent source, however, in the plant known as harvest fennel (*R. segetum*). After the removal of acidic and carbonyl-containing components, the essential oil consists of 33 per cent myristicin. The commercial dill plant *Anethum graveolens* yields essential oils derived either from the above-ground portions (known as dill-weed or dill-herb oil) or from the seeds. The dill-weed oil contains sizable amounts of myristicin as well as several related aromatics listed below. The edible variety of parsnip (*Pastinaca sativa*) has recently been shown[12] to possess properties of an insecticide and of an insecticide synergist. Analysis of a non-polar solvent extraction revealed that myristicin was the active component, although it was present in the whole parsnip to the extent of only 0·02 per cent. Many species of the genus *Oenanthe* (relatives of the dropwart) have undergone analysis as to their composition, namely, *Oe. crocata*, *Oe. stolonifera*, *Oe. californica* or *sarmentosa*, *Oe. aquatica* and *Oe. phellandrium*. The first of these species has been the most thoroughly studied because of its extreme toxicity to both animals and man (see discussion in the portion on toxicology and pharmacology), although *Oe. crocata* has never been reported to contain myristicin, but *Oe. stolonifera* has[13]. The compound is located in the fruit, and represents perhaps 1 per cent of the total weight.

In the family Labiatae (syn. Lamiaceae or Menthaceae), the genus *Orthodon* alone has been observed to contain myristicin. *O. grosseseratum* Kudo contains about 0·2 per cent volatile oils, which contain a small amount of myristicin. *O. asaroniferum* Fujita, so named because of an appreciable amount of asarone in its 0·3 per cent volatile oil fraction, has been equated to *O. asaniferum* by a virtually identical oil composition, which includes a small amount of myristicin[14].

Analysis of the 'Nepal camphor wood' of India, *Cinnamomum glanduliferum* (fam. Lauraceae), has revealed three components, safrole, elemicin and myristicin[15]. Identification of the latter was made by comparison of the tetrabromide with a reference specimen.

The only plant classified under the family Myristicaceae that has been documented as containing myristicin is the nutmeg tree. This source (*Myristica fragrans* as well as many additional species) yields through its seed (the nutmeg) or the sheath of the seed (mace) a volatile oil fraction that contains about 4 per cent myristicin. If the source of the drug yakee (paricá) can be assigned to trees of the genus *Virola* (as mentioned in the introduction) and the presence of myristicin demonstrated, then the aforementioned species will constitute a second occurrence in the family Myristicaceae.

Within these botanical families there are frequent occurrences of many compounds that may be considered as close analogues of myristicin. The term analogue here defines aromatics containing at least three oxygen functions and a three-carbon olefinic chain. The propenyl analogue of myristicin itself, isomyristicin, is known to accompany myristicin in *A. graveolens*. It is most unlikely that it could be generated from myristicin in the course of isolation of the essential oil fraction, as other oils of the Umbelliferae, isolated in similar ways and containing allyl compounds equally easily isomerized (see following), are not found to contain the propenyl isomer.

Elemicin, 3,4,5-trimethoxyallylbenzene, is the analogue of myristicin, in which the methylenedioxy group has been replaced with two methoxy groups. It has been mentioned earlier as a congeneric contaminant of myristicin in the oil of nutmeg. Further, it is one of the principal components of yet another of the orthodon oils, *O. elemiciniferum*. It has yet to be demonstrated in any member of the family Umbelliferae. The positional conjugated isomer of elemicin, asarone, is 2,4,5-trimethoxypropenylbenzene. It occurs in the orthodon group, as mentioned here, as the definitive component of *O. asaroniferum*. In the family Umbelliferae, it is found in the oil of carrot (*ex Daucus carota*). The strict isomer of myristicin and elemicin, namely, 2,4,5-trimethoxyallylbenzene, is unknown in Nature.

Among the more highly substituted analogues of myristicin, both 2,3,4,5-tetramethoxyallylbenzene and apiole* (2,5-dimethoxy-3,4-methylenedioxyallylbenzene) are observed as components of the oil of parsley, *P. sativum*[3]. The positional isomer of apiole, 2,3-dimethoxy-4,5-methylenedioxyallylbenzene (dillapiole), accompanies myristicin in *Anethum graveolens*. It also occurs in *A. sowa* (dill-seed oil) as well in the sea-fennel *Crithmum maritimum*. In the family Labiatae, *Orthodon formosanum* contains only 0·1 per cent oil, but this is 65 per cent dillapiole.

Pharmacological Definitions

The pharmacology of these materials may be divided into three groupings: that of botanicals known to contain myristicin, that of isolated myristicin concentrates, and that of chemicals closely allied (in both molecular structure and in natural origin) to myristicin.

Plants of the genus *Oenanthe* have long been known to be highly toxic both to animals and to man. Oenanthotoxin has been isolated as the toxic principal of *Oe. crocata* and has been established as a multiply unsaturated diol[16]. The toxic symptoms are characterized as initial nervous and intestinal disturbance followed by emesis, mydriasis, cyanosis and convulsions. In humans, a lethal dose is effective within 2 h of ingestion. The purified material, oenanthotoxin, shows a pharmacological spectrum parallel to that of a tincture of the natural tuber but cannot be the sole toxic principle, as the extract devoid of any oenanthotoxin still produces toxic symptoms in man[17]. A non-toxic ketone, crocatone(II), has been isolated which bears a close resemblance to myristicin. Another species of water parsley, *Oe. sarmentosa*, is reported to be non-toxic[18], but no attempt has been made to establish the presence of myristicin. Thus, one must conclude that any responses to myristicin as in these plants would be obscured by the presence of oenanthotoxin.

* The term apiole, representing the compound 2.5-dimethoxy-3,4-methylenedioxyallylbenzene, is not to be confused with the same word (often without the final 'e') used to describe the crude green oil of parsley.

The parsnip, *P. sativa*, has only recently been shown to contain myristicin[12]. It has long been known that, in sensitized individuals, contact with the juices leads to actinic dermatitis. Various furocomarins have been isolated and two of them display biological activity; these are pastinacin, which shows spasmolytic and vasodilating properties, and xanthotoxin, which has been used in the treatment of vitiligo (non-congenital leukodermia)[19]. It appears that, here again, any responses of the myristicin present would be masked by active, but unrelated, congenors.

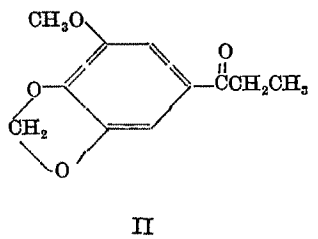
The pharmacology of *A. graveolens* (dill) is virtually unknown. The oil has been recommended as a remedy for hiccoughs and emesis, and as an aqueous solution, as a carminative. Toxicity due to excess is unreported.

The most frequently investigated form of parsley is, rather than the distilled oil, the oleoresin which is obtained by extraction. This fraction is called 'apiol', 'liquid apiol', or green oil of parsley. This natural extract received a clouded reputation in the 1930's when a popular claim of effectiveness as an abortifacient coincided with a commercial product which contained some 30 per cent *o*-cresyl phosphate as an impurity. The polyneurites, paralyses and deaths associated with its misuse during this period have been blamed on this contaminant. The uncontaminated extract produces a fall in blood pressure, tremors, and weakness of extremities similar to nutmeg poisoning. This is discussed later.

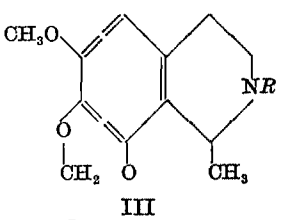
Although various fennels have long been established in the medical world, the only one that contains myristicin, *R. segetum*, has a totally unknown pharmacology. Similarly, studies on the genus *Orthodon* have been conducted solely as a guide to taxonomic arrangement. *L. scoticum*, too, is unexplored although the related lovage, *L. officianele*, apparently dates back for centuries in medical history.

The seed of the myristica has long been enveloped in a folklore of emmanogogic and ecbolic potency, and consequently there is a large body of literature available concerning its misuse. As the animal and human pharmacology have recently been reviewed[21], only a generalized syndrome of human intoxication will be described. The ingestion of about 5 g of the whole seed (approximately one large nutmeg) leads after a period of a few hours to a more or less severe physical collapse, characterized by a weak pulse, hypothermia, clamminess of the extremities, giddiness, vertigo, nausea and a feeling of congestion and pressure either in the chest or abdomen. For some 12 h there is an alternation of delirium and stupor, and this is usually resolved by a heavy sleep. For a period of several days there may be headaches and perhaps spells of dizziness. Cases resulting from the ingestion of powdered nutmeg seem to parallel this general syndrome.

It is of interest to note that two of the components of *A. lewinii*, the source of mescaline, possess the same aromatic substitution pattern as that of myristicin. The isoquinolines lophophorine and anhalonine (III; R = CH_3, R = H, respectively) are natural components of the native cactus. Their pharmacologies are virtually unknown.



II



III

The only myristicin concentrates that have received any pharmacological evaluation have been obtained from nutmeg. Animal responses have been summarized[21] and reflect, apart from liver disruption, a modest hypotensive response. The first of the two reports on effectiveness in humans describes a total dose of 400 mg 2 or 3 h following ingestion there were suggestions of elation or of anxiety in several of the experimental subjects[22]. The second report involves a preliminary employment in diagnosed mental illness[23] but does not elaborate on the psychotropic effectiveness.

Apiole is the most carefully investigated of any of the related polyalkoxylated aromatic propenes. The syndrome of human poisoning includes, besides a transient intoxication, headaches, indigestion, loss of appetite and fever. The isomeric isoapiole, as a pure chemical, produces similar effects in human subjects. There is heart excitement and an expanded pulse becoming dicrotic on higher dosages (0·6–0·8 g). Again, the intoxication is short-lived although other of the toxic phenomena may persist for several days[24].

Of the several tri- or higher alkoxylated alkyl- or propenyl benzenes mentioned, only isomyristicin, tetramethoxyallylbenzene, and recently *cis*- and *trans*-asarone have received any pharmacological investigation. None of these has been reported to have been explored in human subjects, although recent studies in primates have shown *trans*-asarone to possess tranquillizing effects similar to chlorpromazine. Dosages of 5 mg/kg produced a catatonic indifference lasting several days[25].

Theoretical Modes of Action

The possibility must be considered that myristicin *per se* is not the form in which the substance is found in Nature. Thus, the physical isolation of the oil from nutmeg, parsley, etc., may employ techniques sufficiently strenuous to liberate myristicin from a precursor 'protomyristicin'. This natural form may then be either the actual toxicant in the instance of human ingestion, or may generate the active product *in situ*, perhaps at a site or in a concentration not otherwise attainable. These speculations are in accord with the inability to imitate nutmeg intoxication with synthetic myristicin. On the other hand, the isolated apiole from parsley has an effectiveness not enjoyed by the whole plant. This explanation can only be supported by an increasingly careful analysis of the nature source of these materials.

An appealing explanation of the action of myristicin involves the potential addition of a molecule of ammonia to the allyl chain. Such a reaction would readily explain the position of the synthetic drug MMDA, IV (ref. 26). Amination of the analogous ether elemicin has been shown to yield 3,4,5-trimethoxy amphetamine(TMA) *in vitro* by way of a bromo intermediate[27]. Further, a similar addition with an available substituted amine would lead easily to a corresponding N-substituted amphetamine.

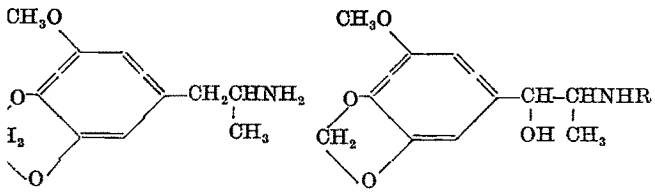
The facile isomerization of an aromatic allyl group to the conjugated propenyl counterpart is assumed to occur in the intact organism[28]. Thus the production of an aromatic acid as a metabolic product of allyl compounds presumes such a preliminary isomerization (that is, safrol → piperonylic acid). The propenyl analogue, isomyristicin, would again be capable of accepting either ammonia or amines as is true with the allyl isomer here, leading to the *in situ* generation of substituted amphetamines. Many other types of reactions at such a double bond are possible, such as oxidation or the addition of non-nitrogenous nucleophiles (alcohols→ethers, mercaptans→thioethers).

Hydrogenation of the double bond in either the allyl or propenyl isomer is improbable. Such a mechanism is not known in human detoxication, and in those instances wherein an olefinic material may be compared directly with the saturated counterpart, the latter is less potent and more slowly acting (for example, anaesthetic ether, barbiturates).

Oxidation could occur either on the ring of myristicin, or on the side-chain with or without chain cleavage. Ring oxidation, a well-recognized biological mechanism, would lead to a demethylated analogue either of apiole or

of dill-apiole. In the light of the known human effectiveness of apiole, this route has appeal, although its mode of action in turn must still be explained in terms of the present discussion of myristicin. Chain oxidation would lead to either 3-methoxy-4,5-methylenedioxyphenyl acetic acid or the corresponding benzoic acid. Both types of products have frequently been observed in the metabolism of three-carbon chains, but in no instance has either type represented anything other than a biological end-product. Hydroxylation of the chain would lead, in the instance of the propenyl isomer, to the enol form of the corresponding propiophenone or the phenylacetone. The former has been mentioned here (crocatone) and is a non-toxic component found in *Oenanthe*. Recently, however, the acetophenone analogue of crocatone was synthesized and found to be of comparatively high toxicity to mice[29]. Using the transaminase enzyme system, the phenyl acetone isomer could easily be converted again to the MMDA. This process has been mentioned in connexion with elemicin[21].

Recently, propenyl aromatics have been found to react readily, after epoxidation, with aliphatic amines, to form various isomers of ephedrine[30]. A parallel reaction with isomyristicin would lead to a mixture of isomers of the corresponding ethanolamines (V):



IV V

Such compounds are not known with the methylenedioxy substitution although analogous trimethoxy compounds have been synthesized[31].

A metabolic pathway which must be considered as a potential explanation of the function of myristicin in the body involves hydrolytic de-alkylation of the ring. Mention has been made here of the easy disruption of the methylenedioxy heterocycle, and a similar reaction *in vivo* employing existing hydrolytic enzymes may explain the increased potency of a methylenedioxy group over the corresponding dimethoxy analogue.

Summary

The purpose of this review has been to delineate the limits of the present knowledge concerning the aromatic ether, myristicin. An appealing suggestion exists that, as a potential factor in two natural intoxicants and as a chemical precursor to a synthetic one, it may be implicated as the responsible agent. Preliminary human pharmacology suggests that it may indeed possess psychotropic properties, but this is yet to be expanded on, and is so far quantitatively inadequate in the explanation of the effectiveness of nutmeg or of paricá.

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SYNTHESIS OF VIRUS AND MACROMOLECULES BY RUBELLA-INFECTED CELLS

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RUBELLA virus grows *in vitro* in a wide variety of mammalian cell types. A recent report[1] describes the growth of this virus in clones of a baby hamster kidney cell line (*BHK* 21) with the production of virus titres which permit an investigation of one-step growth and of virus host-cell interaction.

Rubella virus was concentrated from *BHK* 21 or RK_{13} cell supernatants[1,2] by dialysis against 'Carbowax 20-*M*' until an approximately ten-fold concentration was obtained. The virus was then dialysed overnight against phosphate buffered saline and centrifuged for 4 h at 4° C and 25,000 r.p.m. in an '*SW*39' Spinco rotor, on a cushion of 0·7 M sucrose in phosphate buffered saline. In this way, most of the serum was removed. The sucrose as well as 1 ml. of the adjacent supernatant solution were taken out by a syringe and dialysed against phosphate buffered saline. By these concentration procedures, virus titres were increased fifty or one hundredfold, up to between 10^7 and 10^9 TCD_{50}/ml.

The cultures were infected with this virus-concentrated pool at a multiplicity of 5 or 10 TCD_{50} per cell. Fig. 1 shows the growth curves of cell-associated and extracellular rubella virus in two cell lines after infection with 5–10 TCD_{50} per cell. The virus multiplication is presented as an increase of titre above the virus present intracellularly by 10 h after infection. Although the biological characteristics of the two cell lines[3,4] differ considerably, no basic difference was observed in the time-lapse for the appearance of the new virus. It has been shown by Parkman *et al.*[5] that a true diploid cell culture, such as that of an African green monkey kidney inoculated with 10^4–10^5 InD_{50}, responds in the same manner, the end of the eclipse period occurring at about 12 h after infection. This growth curve also seems to be valid for a continuous line of Rhesus monkey kidney cells (LLC-MK_2)[6]. However, with the same input multiplicity of infection, the maximum intracellular titres obtained in the four systems vary considerably, being 10^4 TCD_{50} in green monkey kidney, 10^5 TCD_{50}

in *LLC-MK*$_2$ cells, 10^6 *TCD*$_{50}$ in *RK*$_{13}$, and $10^{6.8}$ *TCD*$_{50}$ in *BHK* 21.

On the basis of the growth curves it was determined that rubella virus synthesis is not inhibited by actinomycin *D* and that infection has little effect on cellular synthesis of macromolecules. When 2·5 γ/ml. of actinomycin *D* (Merck, Sharp and Dohme) were added 3 h after infection of *RK*$_{13}$ cells, no reduction of titre was observed when cells were collected at 18 h, and only a slight reduction at 24 h, when cytotoxicity was beginning to be visible. In green monkey kidney cells the addition of actinomycin at 12 h, at the time of appearance of the first newly formed virus, did not lead to a reduction of titre in material gathered at 48 h, the peak of virus synthesis (Table 1). The concentration of actinomycin used in these experiments, namely 2·5 γ/ml., is sufficient to depress host-cell RNA synthesis, as shown by the lack of incorporation of ^{3}H-uridine (plus a thousand-fold excess of thymidine) into the ribosomal RNA of green monkey kidney cells. In view of the fact that rubella virus has some properties which place it in the myxovirus group, it is interesting that like Newcastle disease virus it is not inhibited by actinomycin.

Table 1. LACK OF EFFECT ON VIRUS SYNTHESIS OF ADDITION OF ACTINOMYCIN *D* TO RUBELLA-INFECTED TISSUE CULTURES

Cell line	Time of drug addition (h after infection)	Stage of growth-cycle	Collection time (h)	Titre (% of control)
RK$_{13}$	3	Eclipse	18	100
RK$_{13}$	3	Eclipse	24	60*
GMK	12	Early exponential	48	100
GMK	24	Middle exponential	48	100

* Visible signs of cytotoxicity.

It is well known that rubella virus can multiply for weeks in a variety of tissue cultures without marked disturbance of cell growth. This has been confirmed on the macromolecular level by experiments in *BHK* 21/*WI* 2 cells[1]. Cells grown on coverslips were infected by rubella virus at an input multiplicity of 5–10 *TCD*$_{50}$ per cell. Infected cells and controls were pulse-labelled for 20 min at various times after infection with 0·5 μc/ml. of ^{3}H-uridine (plus a thousandfold excess of thymidine) or with 5 μc./ml. of ^{14}C-labelled algal protein hydrolysate. Duplicate experiments were processed following the technique of Bablanian *et al.*[9] and the radioactivity

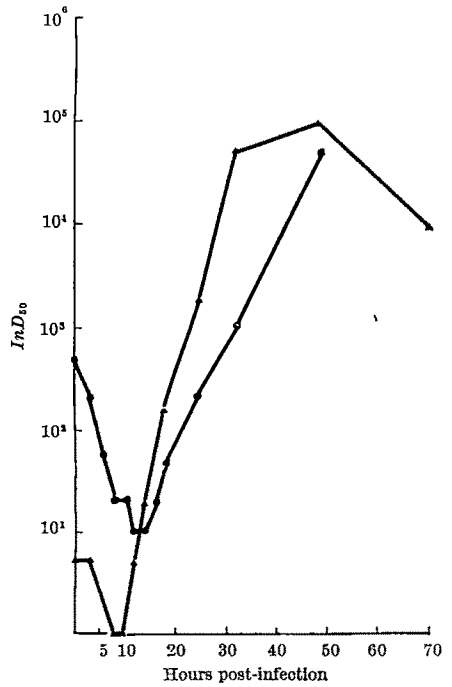


Fig. 1 One-step growth curves of rubella virus in *RK*$_{13}$ cell line grown in monolayer. Titres reported as increase of *TCD*$_{50}$ above the virus intracellularly present during the eclipse period. ▲, Intracellular levels; ●, extracellular levels



Fig. 2. One-step growth curves of rubella virus in *BHK*21/13 cell line grown in monolayer. Titres reported as increase of *TCD*$_{50}$ above the virus intracellularly present during the eclipse period. ●, Intracellular levels; ○, extracellular levels

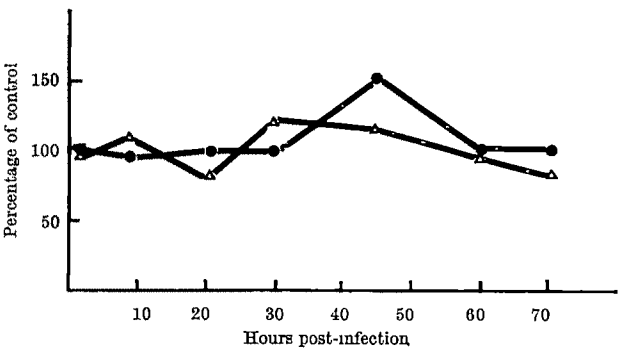


Fig. 3. Lack of inhibition of cellular synthesis of RNA and protein after rubella virus infection. △, ^{3}H-uridine uptake, ●, ^{14}C-protein hydrolysis uptake. For details, see text

measured[8]. No overall inhibition of protein or RNA synthesis was observed during the first 70 h after infection (Fig. 3), at which time the cells started to come away from the glass and the experiment was terminated. It should be noted that the detached cells were still alive as observed by Trypan blue exclusion; the same cell line grown in suspension has, in common with many other systems, the ability to sustain growth of rubella virus without cell destruction for at least several weeks[1,2,5].

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EFFICACY OF 'VIRACTIN' IN PREVENTING RESPIRATORY DISEASE

A DISTILLATE of the fermentation products of *Streptomyces griseus* has been named 'Viractin' by Leach *et al.*[1], who suggested that it prevents influenza and upper respiratory tract disease. The claim was based largely on clinical trials, the validity of which has been questioned[2].

This efficacy of 'Viractin' in cultures and laboratory animals has been investigated at the Common Cold Research Unit in Salisbury, England. Since a strong protective effect had apparently been detected in patients we attempted to show an antiviral effect, using laboratory methods, against some of the viruses known to cause the clinical picture of influenza and minor upper respiratory diseases.

In preliminary experiments the minimum toxic concentration of 'Viractin' was first determined using unstained roller tube cultures of various types of cell (Table 1).

Table 1. TOXICITY OF 'VIRACTIN'. MINIMUM CONCENTRATION OF 'VIRACTIN' CAUSING CYTOPATHIC EFFECT AFTER 4 DAYS' INCUBATION WITH INDICATED CELLS

	Human diploid	Human embryo kidney	HeLa	Calf kidney	Chick embryo fibroblasts	Monkey kidney
Concentration in medium	300	300	100	300	300	100

As a result of these tests it was decided to use a concentration of 30 μg 'Viractin'/ml. of medium in tests for antiviral activity. Leach *et al.* postulated that 'Viractin' acted on cell receptors, making it impossible for viruses to attach to cells. Therefore several types of cell in tissue cultures were treated with medium containing 'Viractin'. Control tubes were left for the same length of time in medium without 'Viractin'. Then serial dilutions of virus suspensions were made and added to the appropriate tubes. Following inoculation, the highest dilutions of the virus suspensions which infected the cultures with and without 'Viractin' were found. Table 2 shows that there was no evidence of an antiviral effect in such tests using viruses drawn from all the biological groups which are known to be concerned in the causation of influenza and upper respiratory disease.

Table 2. EFFECT OF 'VIRACTIN' ON VIRUS GROWTH IN TISSUE CULTURE

Virus used	Result of titration (TCD_{50}/ml.) in cultures containing 30 μg 'Viractin'	No 'Viractin'
Rhinovirus *M* (HGP)	$10^{5.5}$	10^{5}
Rhinovirus *H*	$10^{4.5}$	$10^{5.5}$
Influenza *A*	10^{6}	10^{6}
Influenza *B*	10^{4}	10^{5}
Parainfluenza 1	$10^{5.5}$	10^{5}
Adenovirus 5	10^{4}	10^{4}
Adenovirus 7	10^{3}	10^{2}

Leach *et al.* showed no antiviral activity on adeno-measles and polio-viruses in aqueous suspension. However, viruses which infect the respiratory tract are believed to be transmitted as droplets so we tried to detect inactivation of virus in droplets suspended in an atmosphere containing 'Viractin'. Influenza *A* virus was sprayed into a cupboard and later extracted as previously described[3,4], the coarse, more rapidly falling particles, which correspond to the size range trapped in the upper respiratory tract, were collected in a pre-impinger, and the finer, more slowly falling particles in an impinger. The air in the cupboard had a very high concentration of 'Viractin', obtained by putting saturated swabs in the cupboard. Such an atmosphere could not have been tolerated for long by people because of the very unpleasant smell of 'Viractin'. Virus was also sprayed into a cupboard containing a normal atmosphere and collected in the same way. Virus was titrated for infectivity in tissue cultures. The results showed that there was no reduction in the titre of influenza *A* virus extracted from the cupboard containing 'Viractin' compared with the titre of virus recovered from the cupboard without the compound. This experiment was repeated with a representative of the picornavirus group, coxsackie virus *A*21. The titre of the virus exposed to 'Viractin' was ten-fold less than the control. This apparent reduction in virus titre could not be confirmed in a further experiment, in which the concentration of 'Viractin' used was the same as that used in the clinical trials, that is, 80,000 O.D.U./1,000 ft.^{3}. It was concluded that no viricidal effect could be demonstrated (Table 3).

Table 3. EFFECT OF 'VIRACTIN' ON COXSACKIE VIRUS *A*21 IN DROPLET FORM

Material titrated	Virus titre (TCD_{50}/ml.) in sampling fluids collected from virus in atmosphere with 'Viractin'	without 'Viractin'
Tissue culture fluid remaining in spraying apparatus	$10^{5.75}$	10^{6}
Fluids recovered from		
(*a*) Pre-impinger sampler	10^{3}	$10^{1.5}$
(*b*) Impinger sampler	$10^{1.75}$	$10^{1.25}$

Atmosphere treated with 'Viractin' 80,000 O D U./1,000 ft. (ref. 3) overnight before virus sprayed in. Samples collected 10 min later. Bacterial spores were added to the spray fluid as a tracer and showed that identical volumes of fluid were sprayed and recovered in each test.

It was thought possible that 'Viractin' might have an effect on virus growth in ciliated epithelium although it had none on dedifferentiated cells. Organ cultures of ferret trachea were therefore prepared[5] in disposable plastic Petri dishes and maintained in medium 199 with or without 30 μg/ml. of 'Viractin' at 33° C. After several hours influenza *A* and *B* viruses were added. Medium from the dishes was collected and renewed daily and each sample was titrated for infectivity in tissue culture (Table 4). A similar amount of virus was produced by treated and by untreated cultures; in addition, 'Viractin' had no effect on the ciliary activity of uninfected cultures or on the time at which ciliary activity was stopped by the virus.

Table 4. EFFECT OF 'VIRACTIN' IN ORGAN CULTURE. TITRATION OF MEDIA COLLECTED DAILY FROM ORGAN CULTURES INOCULATED ON DAYS WITH INFLUENZA *A* AND *B* VIRUSES

Day	Influenza *A* MEL 'Viractin'	Influenza *A* MEL Control	Influenza *B* 'Viractin'	Influenza *B* Control
1	$10^{1.5}$	$10^{1.5}$	0	0
2	$10^{2.5}$	$10^{2.5}$	0	0
3	$10^{2.5}$	$10^{2.5}$	$10^{2.5}$	10^{2}
4	$10^{1.5}$	$10^{2.5}$	$10^{2.5}$	10^{1}
5	$10^{2.5}$	$10^{2.5}$	$10^{2.5}$	$10^{2.5}$

Although no protective effect of 'Viractin' in cultures had been detected it was possible that inhaled 'Viractin' might alter the host response to a virus. Therefore groups of 12–14 g *Ajax* strain white mice were put into boxes and left in a room with an atmosphere containing 'Viractin' in a concentration of 80,000 O.D.U./1,000 ft.^{3}. Two days later they were inoculated intranasally under light ether anaesthesia with serial dilutions of influenza *A* MEL virus. Almost all the mice died. After 15 days the reciprocal survival times and the means of the reciprocal survival times were calculated (Table 5). There was no evidence that the mice in 'Viractin' atmosphere survived longer than those in an atmosphere without 'Viractin'.

In view of the uniformly negative results of these laboratory experiments it was decided that there was

Table 5. SURVIVAL OF MICE INFECTED WITH INFLUENZA *A* VIRUS IN ROOM CONTAINING 'VIRACTIN'

Virus dilution	Mean survival time in days 'Viractin'	Mean survival time in days Control	Mean of reciprocal survival time 'Viractin'	Mean of reciprocal survival time Control
10^{-1}	1·7	2·5	0·72	0·44
10^{-2}	3·2	3·3	0·32	0·31
10^{-3}	4·2	3·5	0·25	0·29
10^{-4}	6·4	4·9	0·19	0·22
10^{-5}	8·5	(9·5)*	0·12	0·08

Mice exposed to atmosphere containing 80,000 units 'Viractin'/1,000 cu. ft. for 2 days before being inoculated with 0·05 ml. influenza virus.
* Two mice in this group survived.

insufficient evidence to warrant a clinical investigation of the efficacy of 'Viractin' in acute respiratory disease in man.

The 'Viractin' used was kindly provided by Dr. L. W. Byers, Department of Psychiatry and Neurology, Tulane University School of Medicine, New Orleans, Louisiana.

We thank Miss P. Ball for technical assistance.

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THE data presented by Drs. Walker and Tyrrell on their tissue culture and animal investigations of 'Viractin' confirm that this substance has no significant *in vitro* activity by the test systems used. Our similar results were reported in the first publication on this subject[1]. We have another publication in the press giving further data on 'Viractin' in tissue cultures infected with adenovirus[2].

I realize that the preponderance of opinion is such that animal studies must precede clinical trials. This may be thought of more as an ethical rather than a scientific matter. It clearly involves a feeling that efficacy must be proved in some animal species or tissue culture before it can be investigated in man. It is my contention that this is not necessarily true, and this has been demonstrated with 'Viractin' to a limited extent in that the *in vitro* tests have been negative, but the clinical investigations have demonstrated a sufficient degree of efficacy to warrant further investigations. We are continuing our efforts to extend the preliminary investigations reported earlier.

Litchfield[3] points out some of the limitations in our thinking about the correlations of findings between laboratory animals and man. He also feels that the net result of depending on tests in laboratory animals to find compounds for the treatment of diseases in man is that relatively few effective and safe drugs will emerge. Could it be that our screening programmes are not altogether effective?

Looking back over the past fifty years or so it is difficult not to be impressed with the distinct possibility that a number of drugs which are useful and are being used daily would probably have never reached a clinical trial by to-day's standard. Digitalis, without prior knowledge of its pharmacological properties, would probably have been discarded because it had a variety of toxic effects on the heart and a slight overdosage of it would produce fatal cardiac arrest. I would predict that to-day it would never have progressed to the stage of clinical trials. Another very useful drug, quinine, might not have been tested clinically because of its extreme toxicity to dogs. The foregoing are but two examples of valuable drugs that came to use first by observations in man.

I wish to mention one antibiotic which is being used clinically that did not show any significant activity in animals. This is *cyclo*serine; it did not have *in vitro* activity against most bacteria, but was active against *Mycobacterium tuberculosis*. When *cyclo*serine was tested in guinea-pigs infected with experimental tuberculosis, it was inactive. In man it is effective against a wide variety of bacterial infections and is also one of the effective agents against tuberculosis. The reason for the lack of activity in animals is that D-alanine is a specific antagonist for *cyclo*serine, and man does not have this metabolite[4].

It would seem to me that the foregoing illustrations point to the fact that we may be depending too much on our screening methods to select drugs for clinical trials. This may be the case with 'Viractin' since the limited clinical tests have shown that it holds promise as an effective prophylactic agent for upper respiration infections.

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HERPES VIRUS SPECIFIED RIBONUCLEIC ACIDS

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IN general, when a virus particle successfully infects a host cell, the viral genome first initiates synthesis of new nucleic acids, some of which then programme or induce qualitative or quantitative changes in the synthesis of proteins. The metabolism of the cell thus undergoes a sequence of changes which lead to the production of progeny virus particles. As a result, the host cell is usually destroyed.

While most present-day knowledge of this situation is derived from extensive investigations of infection of bacterial cells by *T*-even bacteriophage[1–3], recent work indicates that mammalian cells respond to virus infection in an analogous fashion. Thus, in the case of HeLa cells infected with vaccinia virus, the normal patterns of synthesis of RNA specified by the cell genome is gradually destroyed, while new messenger-RNA functional in the synthesis of virus-specified protein is concomitantly transcribed from the viral genome in the cytoplasm[4,5].

The present article describes the changes in RNA synthesis induced by herpes simplex virus, which grows in the nucleus of the infected mammalian cell.

Earlier investigations with *BHK* 21 cells infected with herpes simplex virus established[6] the time course of certain virus-induced events, in particular the appearance of herpes DNA, virus-specific protein and mature virus particles. It was also observed that the activities of DNA nucleotidyltransferase (*EC* 2.7.7.7) and deoxyribonuclease (*EC* 3.1.4.5) increase markedly after infection[7]. We have recently obtained evidence which suggests that these increased activities are due to virus-specified enzymes[8].

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Other laboratories have provided evidence that herpes virus codes for or induces a new thymidine kinase (*EC* 2.7.1.21) (ref. 9).

Present-day theory predicts that a DNA virus, like herpes virus, initiates events leading to the manufacture of virus-specified proteins and ultimately to progeny virus, by way of transcription of specific RNA. Preliminary work[10] in *BHK* 21 cells demonstrated that synthesis of rapidly labelled, rapidly sedimenting RNA (45*S*, 35*S*) declined soon after infection with herpes simplex virus, reaching a low value 4–5 h after infection. However, a peak (20*S*) of rapidly labelled RNA, not present in uninfected cells, was observed 90 min after infection. We now have evidence concerning the kinetics of production and the genetic origin of the RNA in the 20*S* peak.

BHK 21 (*C*13) cells[11] grown in monolayer culture[6] were dispersed and infected in suspension with herpes simplex virus (strain α) at a multiplicity of exposure of 16 plaque-forming units (P.F.U.) per cell. After adsorption for 20 min at 37° C standard batches (3×10^7 cells) were plated in flat bottles and gassed with 5 per cent carbon dioxide. At suitable time intervals after infection, batches were exposed to a 30-min pulse of tritiated uridine (200 μc./bottle). The cells were then scraped from the glass, washed in cold buffer and frozen to −70° C in 0·01 M sodium acetate buffer *p*H 5·2 containing 0·11 per cent w/v sodium dodecyl sulphate and 0·2 per cent w/v bentonite. For control cell batches, [^{14}C]-uridine was used instead of tritiated uridine.

RNA was prepared by the method of Eason, Cline and Smellie[12]. Infectivity was measured on parallel cell cultures plated on glass.

Fig. 1 shows that RNA synthesis following infection declines to 60 per cent of the control rate by 1·5 h and

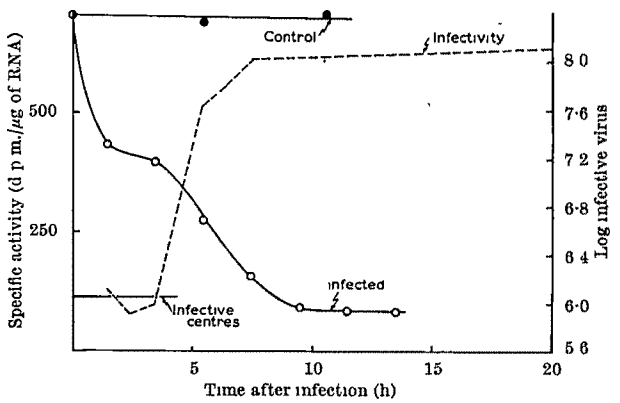


Fig. 1. RNA synthesis and infectious virus synthesis in *BHK* 21 cells infected with herpes virus. RNA synthesis in uninfected cells is also shown. Cells were exposed to tritiated uridine for 30 min and then collected for isolation of RNA. Initial infection was assessed by plating singly dispersed cells for infective centres in the presence of antiserum after mixing with 4×10^6 control cells per plate. Efficiency of plating of washed infective centres was 30 per cent. About 10^4 infected cells were also plated on glass without control cells but with antiserum present, but none formed a colony. Synthesis of infectious virus was assessed by plating batches of $3{\cdot}75 \times 10^6$ infected cells on Petri dishes and incubating in 5 ml. of growth medium. At various times all but 1 ml. of medium was removed from two plates, the cells scraped off the glass, the two 1-ml. portions pooled, the cells disrupted by sonication, and infectivity then titrated (ref 13). ●, Specific activity of RNA from control cells, ○, specific activity of RNA from infected cells; ---, infectivity; ——, infective centres

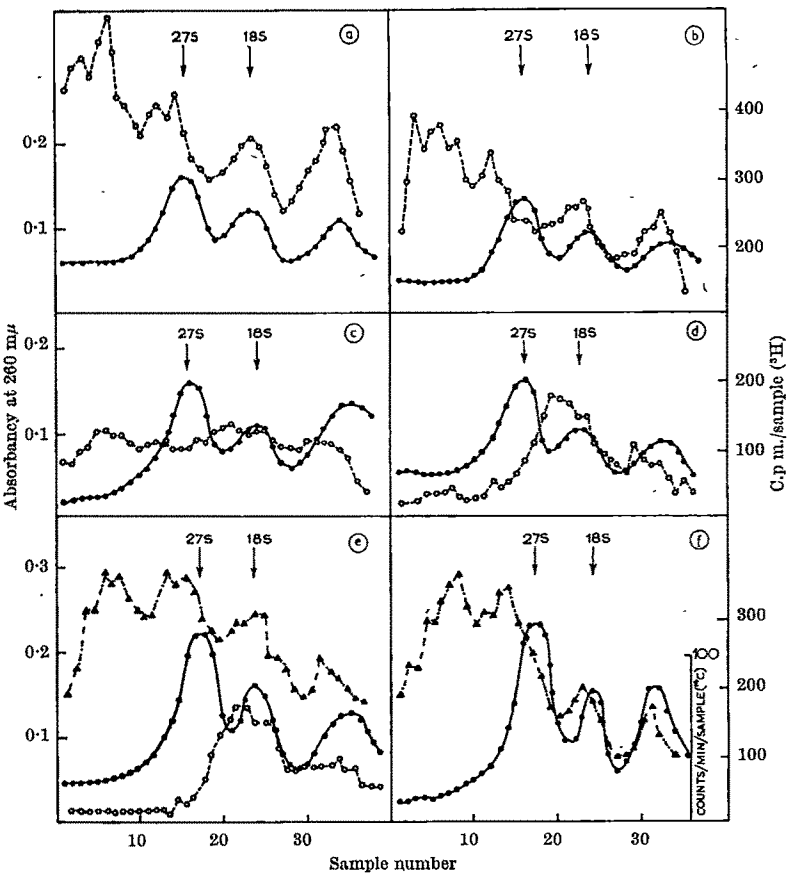


Fig. 2. Synthesis of rapidly labelled RNA in control (*BHK* 21) cells and in cells infected for various times with herpes virus. Infected cells were pulsed with tritiated uridine from: (*a*) 0·75 to 1·25 h; (*b*) 1·25 to 1·75 h; (*c*) 3·25 to 3·75 h, (*d*) 5·25 to 5·75 h; (*e*) 7·25 to 7·75 h after infection. Uninfected cells were pulsed with [^{14}C] uridine from (*e*) 7·25 to 7·75 h and (*f*) 0 to 0·50 h. Part (*e*) of the figure thus represents the sedimentation analysis of [^{14}C]RNA from control cells mixed with an equal amount of [^{3}H]RNA from infected cells. From each pulse sample approx. 300 μg of RNA was layered on to a linear gradient of sucrose (5–25 per cent w/v, pretreated with bentonite) and centrifuged at 21,000 r p m. for 11·5 h. 0 11-ml. fractions collected sequentially from each gradient were measured for absorbancy at 260 mμ, and for radioactivity (ref. 15). Approximate *S* values (see text) were calculated (ref. 16) with reference to ribosomal RNA. ●, Absorbancy at 260 mμ; ○, c p.m. (^{3}H) per fraction (RNA from infected cells); △, c.p m. (^{14}C) per fraction (RNA from control cells)

retains this level until about 3·5 h. A further steady fall occurs up to 9 h when 12 per cent of the control rate is reached, which is maintained for at least a further 5 h. Infective particles first appear between 4 and 5 h and reach the maximum titre at 7·5 h.

RNA samples were analysed by sedimentation through sucrose density gradients[14] (i) to identify the fractions of rapidly labelled cell RNA the syntheses of which declined after herpes infection, and (ii) to characterize any new RNA arising from virus-directed transcription. Results are shown in Fig. 2. The absorbancy patterns indicate the positions of the ribosomal RNA species (27*S* and 18*S*) and of 4*S* RNA. Incorporation of [^{14}C]-uridine into the rapidly labelled RNA of uninfected cells gives rise to a pattern which characteristically involves two main peaks of high specific activity at 45*S* and 35*S*, a peak in the 4*S*–6*S* region, and further peaks of incorporation coincident with the ribosomal RNA components (Fig. 2*e*, *f*). Fig. 2*a*, which presents the analysis of RNA from cells infected with herpes virus and then exposed to tritiated uridine from 0·75 to 1·25 h, is qualitatively the same. A qualitative change in the 20*S* region first appears in the 1·25–1·75 h sample (Fig. 2*b*). By 3·25–3·75 h infected cells show a marked decrease of incorporation into 45*S* and 35*S* RNA (Fig. 2*c*), while a broad peak at 20*S* is now evident; no comparable peak is present in uninfected cells. Two hours later (5·25–5·75 h), RNA synthesis is confined to this broad peak at 20*S*, and to some extent to 4*S* material, while 45*S* and 35*S* RNA synthesis has

fallen to a very low level (Fig. 2*d*). In the 7·25–7·75 h sample (Fig. 2*e*), 45*S* and 35*S* RNA can no longer be detected, and the rates of synthesis of 20*S* RNA and 4*S* have fallen relative to the 5·25–5·75 h sample. This is also found as late as 13·25–13·75 h but with an even lower rate of synthesis (see Fig. 1).

The initial decline in incorporation shown in Fig. 1 is probably due to the fall in synthesis of 45*S* and 35*S* RNA apparent from Fig. 2, whereas the later decline may represent a decrease in the rate of synthesis of the 20*S* RNA species or, more likely, it may reflect the metabolic death of a proportion of the infected cells.

DNA–RNA hybridization experiments. The specific genetic origin of these rapidly-labelled species of RNA formed before and after infection was investigated using DNA–RNA hybridization techniques[17]. DNAs of *BHK* 21 cells and of herpes virus (grown in *HEp* 2 cells) were tested for hybrid formation with RNA from uninfected or infected *BHK* 21 cells.

Uninfected cells. 45*S* RNA from *BHK* 21 cells formed a hybrid with *BHK* 21 cells DNA (Fig. 3*a*) but not with DNA from Landschutz ascites-tumour cells (Fig. 3*b*). This indicates that the rapidly labelled, rapidly sedimenting RNA was transcribed from the *BHK* 21 cell genome; moreover, analysis of its base ratios[20] gave a guanine + cytosine content of 68 per cent, unlike the base composition of *BHK* 21 cell DNA (43 per cent guanine + cytosine), but similar to 27*S* ribosomal RNA from these cells (67 per cent guanine + cytosine); this composition has not altered significantly 3 h after infection.

Infected cells. Analogous DNA–RNA hybridization experiments were carried out using RNA from infected cells (Table 1). Most of the 20*S* RNA which associated with the DNA of herpes virus withstood the action of ribonuclease (*EC* 2.7.7.16). Some of the 20*S* RNA also formed ribonuclease-resistant associations with *BHK* 21 DNA, though not with Landschutz DNA. Hybrid formation with *BHK* 21 DNA was to be expected in this particular experiment as the sedimentation analysis at this sample time showed that some 45*S* and 35*S* material was still being synthesized. (This suggests that not all cells were infected at the beginning of this experiment.) Corroborative evidence of hybridization between 20*S* RNA from infected cells and DNA of herpes virus has also been obtained using the procedure of Becker and Joklik[5]. We conclude that *BHK* 21 cells, 7·25–7·75 h after infection with herpes virus, synthesize virus-specified RNA which sediments as a broad band at 20*S*.

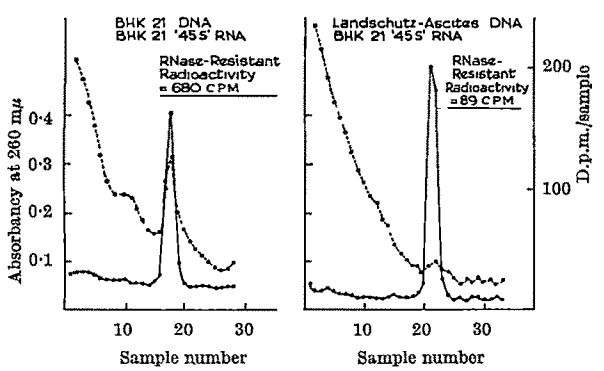


Fig. 3. Hybrid formation between DNA and rapidly labelled RNA from uninfected *BHK* 21 cells. DNA was isolated (ref. 18), and checked for purity (ref. 19) in the Spinco model *E* ultracentrifuge (buoyant density, 1·695 g/cm³). It was then denatured at 100° C. [¹⁴C] uridine-labelled RNA was isolated (ref. 12), fractionated on sucrose density gradients and the 45*S* peak collected. 5×10^4 d p.m. from this peak were mixed with 50 μg of denatured *BHK* 21 cell DNA (or Landschutz ascites-tumour cell DNA), incubated at 65° C for 5 h and then slowly cooled to 25° C during the next 10–12 h. Half of each sample was treated with ribonuclease (free from deoxyribonuclease), then precipitated with acid collected on 'Millipore' filter membranes, washed and counted. The remaining half of each incubation mixture was adjusted with caesium chloride to a density of 1·71 g/cm³ and centrifuged for 60 h to equilibrium. 0·1-ml. fractions were collected sequentially, measured for absorbancy at 260 mμ, and acid-precipitable radioactivity was then counted. ●—●, Absorbancy at 260 mμ; ● - - - ●, d.p.m. per sample

Table 1. HYBRID FORMATION BETWEEN 20*S* RNA FROM CELLS INFECTED WITH HERPES VIRUS AND DNA FROM VARIOUS SOURCES

Source of DNA	Fractions pooled after caesium chloride gradient centrifugation	Total d.p.m. (tritium) in pooled fractions: Before treatment with ribonuclease	After treatment with ribonuclease
BHK 21 cells	Bottom five fractions	390	0
	A_{260} peak fractions	262	40
Landschutz ascites-tumour cells	Bottom five fractions	675	6
	A_{260} peak fractions	75	0
Herpes simplex virus	Bottom five fractions	375	0
	A_{260} peak fractions	225	107

DNA was extracted from purified herpes virus (ref. 21); its buoyant density (ref. 19) was 1·752–1·727 g/cm³. 50 μg portions respectively of denatured *BHK* 21 DNA or Landschutz DNA or herpes DNA were incubated with 20*S* RNA (10^4 d.p.m.) from *BHK* 21 cells exposed to tritiated uridine 7·25–7·75 h after infection. Incubation mixtures were held at 75° C for 1 h, then 65° C for 2 h and then cooled slowly to 22° C. Caesium chloride density gradient centrifugation was carried out on each mixture (see Fig. 3). Fractions from the bottom of each gradient (containing unhybridized RNA) were pooled, and likewise the fractions containing the DNA peak. After dilution to 0·2 M caesium chloride acid-insoluble radioactivity was measured on one-quarter of each pool. After ribonuclease (free from deoxyribonuclease) treatment of the remainder, ribonuclease-resistant material was precipitated with acid, washed and measured for radioactivity.

Table 2. SYNTHESIS OF RIBOSOMAL RNA IN UNINFECTED AND HERPES SIMPLEX VIRUS-INFECTED *BHK* 21 CELLS

Time after infection (h)	Specific activity of ribosomal RNA*: Control ³H	Control ¹⁴C	Infected ³H	Infected ¹⁴C	Infected Control ³H	Infected Control ¹⁴C
0·5–2·0	2·2	1·2	1·6†	1·3	0·73	1·08
2·0–3·5	1·7	1·3	0·92	1·35	0·54	1·03
3·5–6·0	1·8	1·4	0·7	1·45	0·39	1·03

Cells were exposed to [¹⁴C] uridine for 2 h, removed from the glass (ref. 6), infected in suspension with herpes virus, and after 25 min adsorption, plated on glass and labelled with [³H] uridine from 0·5 to 2·0 h, 2·0 to 3·5 h and 3·5 to 6·0 h; control cell samples were treated identically, but were not infected. ¹⁴C and ³H disintegrations were separated as described by Hendler (ref. 15).

* C.p.m. × 10^{-3} per unit of absorbancy at 260 mμ.

† Values for ribosomal RNA synthesis after infection are probably overestimates, because we have evidence that not all cells were infected initially in this experiment.

At present, we cannot rule out that some host-directed synthesis of 20*S* RNA occurs in cells 7·25–7·75 h after infection. Nevertheless, assuming that all RNA elaborated at this time is virus-specified, we can express its amount as a percentage of that formed at the start of the experiment, when all RNA synthesis is cell-specified. The calculation, which relates counts incorporated to the absorbancies of the peaks of ribosomal RNA, yields the following estimate. Between 7·25 and 7·75 h after infection, virus-specified 20*S* and 4*S* RNAs amount to 14 per cent and 4 per cent, respectively, of the total host-specified RNA synthesis under parallel conditions. As this time is probably later than the time of maximum synthesis of virus-specified RNA, these percentages should be considered minimum estimates; for example, it can be calculated from the data in Fig. 2 that virus-directed RNA synthesis between 5·25 and 5·75 h is 23 per cent (20*S*) and 5 per cent (4*S*) of control.

Ribosomal RNA. The stability of ribosomal RNA and the effect of infection on its synthesis have also been investigated. Experimental details are given in Table 2, which shows that ribosomal RNA synthesized before infection was stable up to 6 h after infection (^{14}C specific activities); and that ribosomal RNA continued to be synthesized in the cells following infection. The rate of synthesis rapidly falls as infection proceeds, and reaches 39 per cent of the control-level in the period 3·5–6·0 h after infection (tritium specific activities). This decline paralleled the time course of disappearance of 45*S* and 35*S* rapidly-labelled RNA (Figs. 1 and 2). The rate of incorporation of tritiated uridine into ribosomal RNA of control cells during the period immediately following plating of the dispersed cells (0·5–2·0 h, Table 2) is higher than that found afterwards. This may be a consequence of increased metabolic activity in the cells following dispersal and plating.

S-RNA. The origin of 4*S* RNA labelled after infection of *BHK* 21 cells with herpes virus (see Fig. 2) has also been investigated[21]. The experiments show that 4*S* RNA species with several transfer-RNA properties are specified by the viral genome. At the same time it was demon-

strated that some synthesis of host-specific 4*S* RNA also continued after infection. Assuming that between 7·25 and 7·75 h after infection all 4*S* RNA synthesized is virus-specified, this represents 38·5 per cent of 4*S* RNA synthesized in control cells; between 5·25 and 5·75 h after infection, the figure is 48 per cent.

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EXPERIMENTAL AMYLOIDOSIS AND TOLERANCE

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RECENT work on experimental amyloidosis (induced by means of repeated antigenic stimulation) has produced general agreement that this lesion is produced during the course of a protracted immune response[1]. However, this statement should be made with caution, since the immune response which is apparently related to experimental amyloidosis does not seem to proceed in the orthodox manner[2]. Investigations of the serological changes that occur during the development of experimental amyloidosis have not clarified the mechanism of formation of amyloid[3], and early experiments that cautiously suggested amyloid substance to be antigen–antibody complexes[4] lost ground as a result of recent chemical[5], histochemical[6] and ultra-structural[7] studies of the lesions. Rather, the indisputable presence of γ-globulin in amyloid seems to correspond to a secondary deposit and not to a determinant factor in its production[8]. Finally, irradiation, treatment with steroid or adrenocorticotrophic hormone (ACTH) and antimetabolite administration increase the frequency of amyloidosis in animals subjected to prolonged antigenic stimulation, particularly when applied after this stimulation has begun[1]. However, if this latter condition is observed, these procedures no longer act as immune depressors, and irradiation may even enhance the immune response[9].

Acquired tolerance constitutes a highly specific depression of the immune response, since it excludes only the response against the antigen used, fully respecting the remaining immunological potentialities of the animal[10]. In order to investigate further the relationship between experimental amyloidosis and the immune response, it seemed of interest to test the possibility of inducing this lesion in animals previously rendered tolerant by exposure to the antigen during the neonatal period.

Non-inbred *NMRI* mice of either sex were used throughout the experiment. New-born mice were kept with their respective mothers until weaning at 3–4 weeks of age. The animals were then sexed and placed in groups of five per cage. All animals with signs of infection were excluded. The following antigens were used: complete Freund's adjuvant (Hyland Laboratories), 'reinforced' Freund's adjuvant[11] (3 g per cent *Mycobacterium butyricum* (Difco Laboratories) in complete Freund's adjuvant) and casein–adjuvant mixture (four parts of 5 per cent casein (Hoffman La Roche *A.G.*) in 0·25 per cent sodium bicarbonate solution to one part of complete Freund's adjuvant). All antigens were kept and injected under sterile conditions. For the induction of tolerance, 0·03 c.c. of either of the three substances was injected subcutaneously under the skin of the back at the day of birth, and at 12 and 24 days of age. Repetitions of antigen administration were carried out, since it is known that the maintenance of a tolerant state requires uninterrupted exposure to the antigen[10]. In order to detect eventual effects of this neonatal treatment, a small number of animals (five) corresponding to each group were killed at the end of this stage of the experiment, and studied histologically as detailed later. No lesions were observed and no further mention is granted them. Induction of amyloidosis was begun at the age of five weeks according to one of the following methods: (*a*) weekly subcutaneous injections (0·3 c.c.) of complete Freund's adjuvant[12]; (*b*) weekly subcutaneous injections (0·3 c.c.) of the casein-adjuvant mixture[13]. A total of eight injections was given in both cases. Table 1 shows the different groups instituted according to the various combinations of new-born (tolerance-inducing) and adult (amyloid-inducing) treatment, as well as the respective control groups. Animals were killed by decapitation one week after the last amyloid-inducing injection. (This corresponds to 14 weeks of age in the groups not receiving the latter treatment.) Blocks for the histological examination were taken from spleen, liver, kidney, lymph nodes, lungs and heart. Organs were fixed in 4 per cent formalin, embedded in paraffin and stained with haematoxylin and eosin. Congo red slides were made with frozen sections and observed under polarized light. Numerical evaluation of the results was assessed on splenic involvement only, since it was found that splenic amyloidosis was the most consistent lesion, never failing in those few cases where amyloidosis was found in other organs. No attempt was made to evaluate the degree of the lesions. The percentage of animals revealing splenic amyloidosis is shown in Table 1. Results were statistically analysed by means of a conditional test of binomial distribution.

As the result for group 1 shows, no spontaneous amyloidosis was found in this experiment. Like some other authors[14], but contrary to the findings of Tal and Laufer[12], we were not able to produce amyloidosis in normal animals

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Table 1. DEVELOPMENT OF SPLENIC AMYLOIDOSIS IN THE DIFFERENT GROUPS

Group No.	No. of animals	Neonatal treatment (tolerance-inducing)	Adult treatment (amyloid-inducing)	No. amyloid	Percentage amyloid
1	16	None	None	0	0
2	22	None	Complete adjuvant	0	0
3	21	None	Casein–adjuvant	14	66
4	15	Complete adjuvant	None	0	0
5	34	Complete adjuvant	Complete adjuvant	0	0
6	20	Complete adjuvant	Casein–adjuvant	19	95
7	20	Reinforced adjuvant	None	0	0
8	12	Reinforced adjuvant	Complete adjuvant	1	8·3
9	9	Reinforced adjuvant	Casein–adjuvant	7	77
10	8	Casein-adjuvant	None	0	0
11	36	Casein-adjuvant	Casein–adjuvant	12	33

by means of repeated injections of complete Freund's adjuvant (Group 2). These authors used a different strain of mice, so the discrepancy could be explained on this ground, particularly after the demonstration that strain susceptibility is a pre-eminent factor in the development of experimental amyloidosis[15]. On the other hand, and in accordance with the results of Christensen[13], normal animals treated with the casein–adjuvant mixture showed amyloidosis in a significant number (Group 3). Neonatal treatment with complete Freund's adjuvant did not modify the outcome of the adult treatment with the same substance (Group 5), nor did it alter the evolution of those animals receiving no further treatment (Group 4). The same is also true for Groups 7 and 8, where neonatal treatment was achieved with reinforced adjuvant. It should be mentioned here that pure complete Freund's adjuvant is in itself antigenic on account of its bacterial content[16] and that it has been possible to induce tolerance to this antigen by injecting complete Freund's adjuvant in neonates[17]. Group 6 revealed a somewhat unexpected result, because neonatal treatment with complete Freund's adjuvant significantly increased the frequency by which amyloidosis was produced in the adult with the injection of the casein–adjuvant mixture. We do not have a plausible explanation for this 'enhancing' effect, but believe it merits further study. On the other hand, neonatal treatment with 'reinforced' adjuvant did not significantly modify the outcome of adult treatment with the casein–adjuvant mixture. This suggests that the aforementioned 'enhancing'-phenomenon does not necessarily depend on the bacterial component of the adjuvant or is limited to an optimal dose of it. Finally, when compared with the results of Groups 3, 6 or 9, Group 11 shows that the neonatal treatment with the casein–adjuvant mixture significantly diminishes the frequency of amyloidosis produced by injections of the same mixture at adult age. The same neonatal procedure, not followed by any adult treatment, failed to produce lesions at all (Group 10).

Usually, such findings would suggest that the phenomena had an immune character[17]. However, in this case one is reluctant to fully accept this in view of the facts mentioned in the introduction of this article. Nevertheless, the results point to the fact that production of experimental amyloidosis requires the existence and function of a specific immune response, since its selective elimination impairs the development of the lesions (Group 11).

According to Teilum[1], experimental amyloidosis represents the deposit of a glycoprotein produced *in situ* by mesenchymal cells at the final or 'exhaustion' stage of an abnormally prolonged immune response. This process follows a stereotyped sequence of events which can be brought about by means of a protracted antigenic stimulation, and can be accelerated through radiation, steroid treatment or antimetabolite administration, particularly when they are applied after antigenic stimulation has begun. Our experiment may bear the interpretation that the frequency of experimental amyloidosis was decreased by a state of acquired tolerance against the casein–adjuvant mixture, although this tolerance state was not tested by other means.

We thank W. Gulbinat (Institut für Medizinische Biometrie, Tübingen) for the statistical analysis of the results, and Dr. K. Willms for her valuable technical assistance.

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ANATOMIC ADAPTATION OF THE ALIMENTARY TRACT OF THE RAT TO THE HYPERPHAGIA OF CHRONIC ALLOXAN-DIABETES

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CHESLER and Tislowitz[1] briefly mentioned that at autopsy chronic alloxan-diabetic rats had longer intestines and larger stomachs than normal rats. In this communication we would like to document more fully some of the striking changes that take place in the alimentary tract of chronic alloxan-diabetic rats and discuss possible factors that could cause these changes.

A group of young white rats (40–90 g) were injected intravenously with alloxan (60–70 mg/kg body-weight). A number of these rats developed severe chronic diabetes as judged by the criteria of glucosuria, high fasting blood sugar, cataract development and general depression of growth. Those that did not become diabetic were used as controls. Both groups were allowed *ad lib.* access to water and stock diet (Diet 86, 'Oxoid'). After 6–12 months the animals were anaesthetized, their abdominal cavities opened and the stomachs, small intestines and caeca were removed separately. They were then washed free of debris, blotted, and dried in an oven at 100° C for approximately 24 h.

Fig. 1 shows a typical alloxan diabetic rat (*B*) compared with a rat of similar age (*C*) and a rat of similar weight (*A*). The small stunted body of the diabetic rat with its pronounced distended abdomen contrasts clearly with the normal development of either the age or weight control. The abdomens were distended because the

Table 1. DRY WEIGHTS OF STOMACH, SMALL INTESTINE, AND CAECUM FROM DIABETIC AND TALC-FED RATS

	Body-weight (g)	Dry-weights (g) Stomach	Small intestine	Caecum
Chronic diabetic (8)	249 ± 20	0·38 ± 0·01	2·55 ± 0·21	0·27 ± 0·02†
Controls (11)	340 ± 24	0·33 ± 0·01	1·31 ± 0·05	0·22 ± 0·02
Talc diet* (6)	342 ± 8	0·34 ± 0·01	1·43 ± 0·03	0·23 ± 0·02
Controls* (6)	293 ± 7	0·32 ± 0·01	1·30 ± 0·04	0·21 ± 0·02

Results expressed as arithmetic mean ± standard error. Figures in parenthesis represent number of rats.
* Dry weights obtained after extraction with ether.
† Seven animals.

alimentary tracts usually contained large amounts of food and faeces while the intestine itself was grossly enlarged both in diameter and length (control intestines 80–100 cm, diabetic intestines 100–150 cm). The histological sections in the lower part of Fig. 1 illustrate the change in diameter of the diabetic intestine (*b*) compared with that of the age control (*c*) or the weight control (*a*) that has been maximally distended by injection of fixative into its lumen. Further evidence of the large changes in the alimentary tract is seen in Table 1, which shows the dry-weights of the stomachs, small intestines and caeca of the diabetic rats and their controls. Table 1 also includes data from talc-fed rats and their controls, which will be discussed later. The results for the diabetic group show that the largest increase in weight (95 per cent) occurs in the small intestine ($P < 0.001$). There is a small (15 per cent) but significant increase in stomach weight ($P < 0.05$) but the increase in the weight of the caeca (23 per cent) is not significant ($P > 0.05$).

Although it is possible that the endocrine imbalance of chronic diabetes may affect the growth of the small intestine *per se*, this is likely to be of minor importance compared with the effect of the changed dietary intake. Measurements of the food intake of the diabetic rats indicated that they consumed 2–3 times more food per day per rat than did their controls. Because this increase

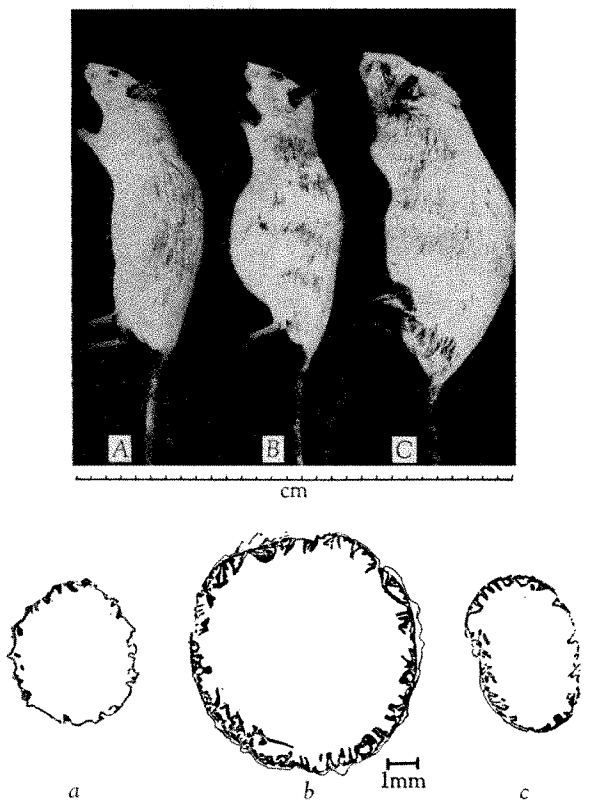


Fig. 1. Photograph shows (*B*) a typical chronic alloxan-diabetic rat compared with (*A*) a rat of the same weight and (*C*) one of the same age. Illustrations below show sections of the intestines from these rats. *a*, Weight control; *b*, chronic diabetic; *c*, age control. For section *a* the intestine was maximally distended by the injection of fixative into the lumen

in the bulk could induce the changes observed in the small bowel, a group of young white rats (50–60 g) were fed for 6–7 months on a specially prepared bulked-out diet made by adding an equal quantity of talc (B.D.H. acid washed) to the stock diet. At the end of the feeding period both the controls (fed the stock diet) and the rats on the bulked-out diet were anaesthetized and treated as before to obtain the dry weights of the various parts of the alimentary tract. The weights are shown in Table 1. The largest significant increase ($P < 0.02$) was seen in the small intestine but even this was small (10 per cent). It therefore appears that in our experiments bulk is not an important factor in causing gross anatomical changes in the small bowel of the rat. This is similar to the findings of Addis[2] and Wierda[3] but differs from those of Friedman[4], who found that bulked-out diets fed to mice caused a large increase in the weight of the small intestine.

Many other experimental conditions have been shown to cause anatomical or weight changes in the small intestine. The small bowel hypertrophies when food is fed intermittently[5], when appetite is stimulated by insulin[6], by lactation[7] or by keeping rats in the cold[8], when rats or mice are made hyperphagic by lesions in the hypothalamus[9,10] or when rats are made hyperthyroid[11]. Surgical removal of small intestine leads to dilatation and hypertrophy of the remaining bowel in the rat[12] and in the dog[13]. That all these conditions cause hyperphagia and thus induce a work hypertrophy of the intestine does not satisfactorily explain how the increased food intake stimulates intestinal growth. As bulk has little effect on growth our investigations suggest that the stimulus is the presence of increased amounts of nutrients. Campbell and Fell[7] speculated that the "enlargement of the small intestine might be a direct response to the amount and rate of passage of one or more specific dietary components".

In all the cases of work hypertrophy more nutrient than normal is present in the lumen of the gut. To cause growth this material must stimulate mitosis in the crypts of Lieberkuhn. This might be brought about in a number of ways. The nutrient might: (1) enter the crypt cells and directly increase their available energy supply; (2) enter the cells and relieve the effects of intracellular mechanisms that suppress mitosis; (3) increase the removal of extracellular tissue-specific mitotic inhibitors (as postulated by Bullough[14]) from around the crypts by the increased blood and lymph flows generated during absorption.

In the case of (1) and (2) the degree of stimulation of mitosis will depend on the amount of nutrient available to the crypt cells. Increases in food intake would increase the amount of nutrient to these cells, stimulate division and thus the formation of new absorptive cells. These, when functioning on the villi, would compete for the nutrient in the lumen. Thus the amount available for the crypt cells would be reduced and the stimulus to division would fall. The size of the intestine would level out at the point where the 'nutrient/intestinal absorptive surface ratio' was similar to that found in normal conditions. Such a system would provide a means by which the intestine could grow to a size commensurate with the food load presented to it. In the case of (3) a limiting factor controlling intestinal size would be the maximal increase in blood and lymph flows that could be generated during the absorption of food. If the condition of the animal makes it eat continually (chronic diabetes) or if food stored in the stomach is continuously released to the intestines, the increased blood flow would be sustained for long periods and support intestinal growth.

Although the processes we have described for the stimulation of intestinal growth are likely to be oversimplifications as factors other than nutrient intake and blood supply (for example, hormones[15] and intestinal flora[16]) affect the intestine, it is interesting to note that massive resection brings about an increase in the mitotic

index[17]. Such a removal of tissue would cause an increased 'nutrient/absorptive surface' ratio and hence stimulate mitosis. Fasting, on the other hand, causes a decrease in mitosis[18]. This could be explained by the reduction in the ratio and hence lowered nutrient supply to the crypt cells.

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EFFECTS OF IRRADIATION ON SYNTHESIS OF DEOXYRIBONUCLEIC ACID AND MITOSIS IN SYNCHRONOUS CULTURES OF HELA CELLS

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RECENT investigations have indicated that the ability of cells to survive ionizing radiation varies during the cell cycle; for example, Terasima and Tolmach found in HeLa cells that the periods of maximum radioresistance were at the beginning of G_1- and at the end of the S-periods[1], and Sinclair and Morton found in Chinese hamster cells that maximum radioresistance occurred at the end of the S-period[2]. It has not been possible so far to correlate these variations with any biochemical processes. The availability of synchronous populations of HeLa cells as described in our previous article[3] has made it possible to examine the effect of radiation on DNA synthesis at different stages of the cell cycle.

Preliminary observations, with asynchronous cultures, on the colony-forming ability of single cells after γ-irradiation showed the usual exponential relationship between dose and survival with a shoulder at low doses. The biochemical investigations described here were made following doses in the range 500–1,000 rads, with a dose rate of 100 rads/min, at which survival was from 10 per cent to 1 per cent. The changes in rate of DNA synthesis observed after irradiating an asynchronous population of cells with 600 rads are seen in Fig. 1. The cells were irradiated in suspension in spinner flasks and the rate of DNA synthesis was measured by assaying the amount of radioactivity incorporated into the cold acid-insoluble cellular material separated on 'Millipore' filters[3] after incubating the cells for 10 or 15 min with tritiated thymidine. As the rate of thymidine incorporation falls immediately after irradiation, it follows that the rate of DNA synthesis in the cells which are in the DNA synthetic phase must be immediately affected by the irradiation.

That this was a direct effect on DNA synthesis and not an artefact produced by an alteration to the composition of the precursor pool was confirmed by examination of the cold acid-soluble radioactivity where it was found that the nature and size of this pool were unaffected by irradiation of the cells.

This finding was confirmed by irradiating a synchronous population of cells in suspension during the period of DNA synthesis (S), and measuring the rate of thymidine uptake at various times during the cell cycle. An apparently immediate large effect on the rate of DNA synthesis was observed (Fig. 2). On the other hand, when irradiation was carried out at mitosis, that is, immediately after collecting the cells, no marked effect on the pattern of the subsequent DNA synthesis was observed, except for a small dose-dependent delay in the position of the S-period (Fig. 3). Cell division at the end of the same cell cycle was, however, found to be markedly delayed and depressed in a dose-dependent fashion (Fig. 4).

Observations were then made over two complete cell cycles, irradiation being given at mitosis, during the period G_1 (7 h after mitosis) and during the S period (11 h after mitosis). Measurements of the rates of DNA synthesis at various times are shown in Fig. 5. It will be seen that only when the cells are irradiated during the S-period is there

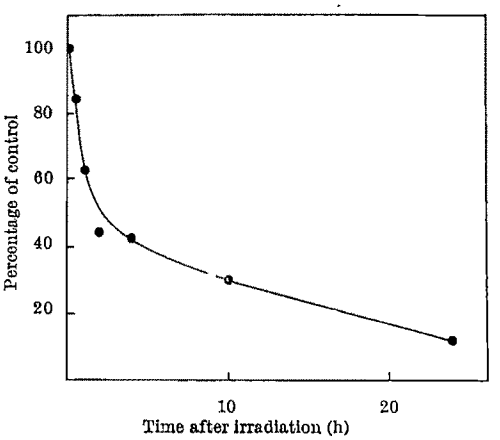


Fig. 1. Incorporation of tritiated thymidine into asynchronous cells in 15 min at various times after irradiation with 600 rads expressed as a percentage of control

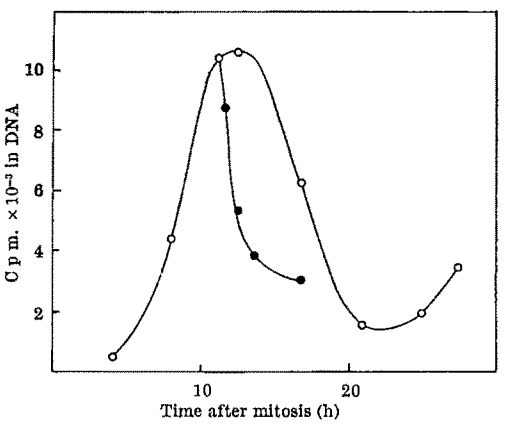


Fig 2 The rate of incorporation of tritiated thymidine in synchronous populations at various times after mitosis —○—, Control experiment; —●—, culture irradiated with 1,000 rads 11 h after mitosis

a marked effect on DNA synthesis during the same cell cycle. In some experiments, such as the example shown, it was noted that there is a small recovery in the rate of DNA synthesis towards the end of the *S*-period. During the second cycle the rate of DNA synthesis is depressed irrespective of the time of irradiation, despite the fact that cells irradiated before the *S*-period have apparently replicated their DNA completely in the first cycle. In the case of the cells irradiated during the *S*-period of the first cycle autoradiographic studies were made from which it was found that during the remainder of the first cycle the percentage of labelled cells was the same as for a control culture (90 per cent at the peak of *S*), but the extent of tritiated thymidine incorporation was reduced. During the second cycle both the percentage of cells labelled and the amount of labelling were reduced compared with a control culture.

The analysis of the situation in the second cycle is complicated by the presence of cells which have not undergone mitosis, but these results clearly indicate that there are two separable effects of radiation: (1) If the cell is irradiated in *S*, DNA replication is largely inhibited; this will prevent cell division and ultimately lead to cell death. (2) If the cell is at mitosis or in G_1 when irradiated, DNA replication in the same cell cycle apparently proceeds normally, but cell division is nevertheless delayed and depressed and only a limited amount of DNA synthesis occurs in the second cycle.

It follows that the DNA replication mechanism is more radiosensitive during the *S*-period than at other times.

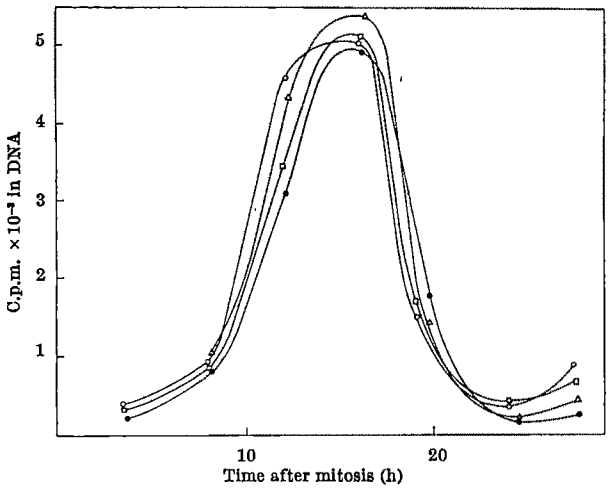


Fig. 3. The rate of incorporation of tritiated thymidine into synchronous populations at various times after mitosis. In all cases the cells were irradiated with various doses immediately after mitosis. —○—, Control, —□—, 300 rads; —△—, 600 rads; —●—, 900 rads

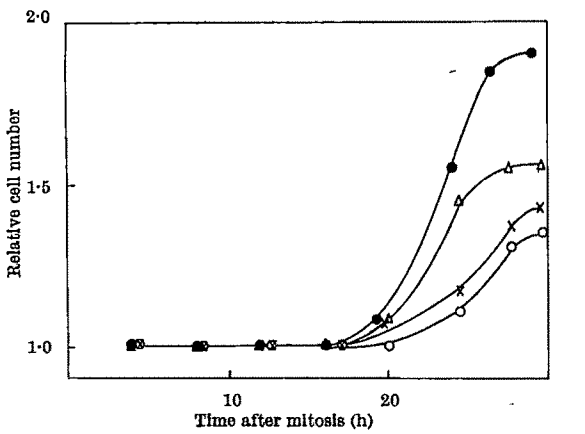


Fig. 4. Cell number in synchronous populations at various times after irradiation at mitosis. —●—, Control; —△—, 300 rads; —×—, 600 rads; —○— 900 rads

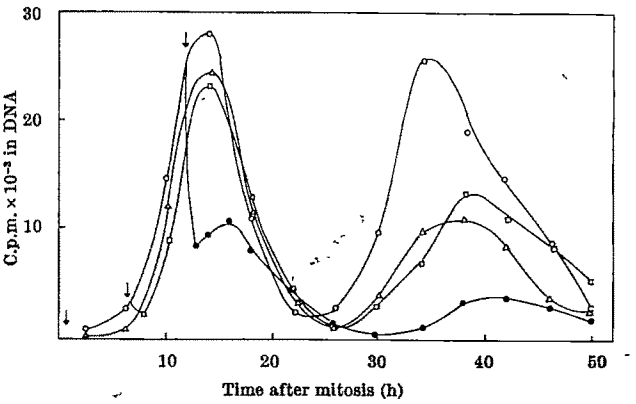


Fig. 5. The rate of incorporation of thymidine in synchronous populations at various times after mitosis. Irradiation with 1,000 rads were carried out at the following times (arrowed): —△—, mitosis; —□—, 7 h after mitosis; —●—, 11 h after mitosis. Control culture, —○—

One possible explanation of this is that the replicating site itself, that is, a region of DNA together with the replicating enzyme, is particularly sensitive at this time. The target size which would be required, however, to account for the sensitivity is large. It can be calculated that a dose of 1,000 rads, which is effective in stopping the synthetic process, provides one 'hit' per 5×10^5 nucleotide pairs (that is, molecular weight $\approx 10^9$). This is much too large to be considered as the system which is at any one moment taking part in DNA replication.

It must therefore be supposed that damage anywhere within a considerable stretch of the DNA twin helix will rapidly block the replicating process. That this is not unreasonable is shown by the following considerations. An estimate of the rate at which a replicating system might move along a DNA molecule can be obtained from the known rate of DNA synthesis in *E. coli*. In this case, a figure of 10^5 nucleotide pairs per minute has been calculated[4]. Thus, on the assumption that synthesis cannot proceed beyond a damaged region, it would be expected that synthesis would cease in a time of the order of 5 min following irradiation with 1,000 rads. A time lag of this order would not be detected in these experiments and therefore the results of these calculations are not incompatible with the 'immediate' effect which is observed.

We have still to account for the lower sensitivity of the replication system when irradiated prior to the *S*-period. This might be due to greater compactness of the DNA at this time. During the synthetic process the DNA fibres must be well dispersed and increased sensitivity might be due to secondary ionizations received from surrounding water molecules. Comparison of these results of irradiation with those obtained with more densely ionizing radiation, where the direct effect will be greater, might clarify this point.

Another possible explanation, which is perhaps more likely, however, is that a repair mechanism exists, such as has been proposed by Setlow and Carrier[5]. This would have to operate rapidly enough to eliminate the damage caused by irradiation in the presynthetic stages in the time available before DNA synthesis begins. So far as the present experiments go, it would appear that damage done 1–2 h before the synthetic period is reached can be repaired. On the other hand, such a process would be too slow to effect repair after irradiation in the *S*-period in the time available before the replicating sites reach the radiation lesions.

It has already been noted that there were some signs of recovery near the end of the synthetic period, after irradiation during the earlier part of this period. This could also be explained by partial repair during the interval. It could, however, be due to imperfect synchrony of the cells, and a more precise analysis would be required to establish this.

It can be concluded that in these experiments it is possible to distinguish two kinds of damage (*a*) to the mitotic apparatus or its precursor, and (*b*) to the DNA in relation to its replication. During mitosis (*a*) is clearly more sensitive to the irradiation than (*b*). During replication of DNA, while the DNA mechanism is clearly more sensitive than it was during the mitotic stage, the sensitivity of the mitotic process at this stage cannot be clearly distinguished.

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HAEMOLYMPH PIGMENTS OF APHIDS

By DR. J. H. BOWIE, DR. D. W. CAMERON, DR. J. A. FINDLAY and DR. J. A. K. QUARTEY
University Chemical Laboratory, Cambridge

SOME years ago, an extensive investigation[1,2] by Todd *et al.* directed attention to a range of remarkable colouring matters found in the haemolymph of various species of aphids. These substances were observed to fall into a number of groups, one of which has been investigated chemically in considerable detail and the structures of its components elucidated[3–5]. This group, termed aphins, is of comparatively wide occurrence, having been found in eighteen of the forty-two species of the Aphididae originally examined. Less attention has been paid until recently to the several non-aphin haemolymph pigments, which frequently occur in equally large proportions. This was due, in some cases, to their instability and in others to their relative inaccessibility. Despite these difficulties, enough of these substances has now been obtained for systematic chemical study, which is in progress. Though not yet complete, it has advanced sufficiently far to show interesting structural and biogenetic similarities affecting all groups of aphid pigments and to permit a limited survey of their occurrence to be made. We are concerned here with establishing what these similarities are. It is not proposed to discuss the chemistry of new compounds in detail except in so far as is necessary to characterize them adequately.

The chemistry of the aphins[3–5] was considered chiefly with reference to the aphins-*fb*, which were obtained from the bean aphid, *Aphis fabae* Scop., and from a number of other species. (Addition of the suffix *-fb* was used to distinguish them from the isomeric aphins *-sl*, obtained from the willow aphid *Tuberolachnus salignus* Gmelin.) These substances occur in the living insect as a glucoside, protoaphin-*fb* (I), $C_{36}H_{38}O_{16}$. After the death of the insect, this compound suffers enzymatic hydrolysis of the glucosidic linkage, together with extensive rearrangement, and is converted successively into xantho-, chryso-, and finally the stable, highly condensed erythroaphin-*fb* (II; R = H).

Careful examination of *A. fabae*, so as to avoid enzymatic decomposition processes, shows that, in addition to protoaphin-*fb*, two other glycosidic components are present in substantial amounts. One of them is a dark blue-green substance, the presence of which was detected in earlier work[2] but which was not then investigated. For this we now propose the name 'aphinin'. The other is a colourless component which fluoresces strongly in ultra-violet light. Protoaphin and aphinin, between them, account for virtually the entire soluble pigment content of the insects. (The small amounts of yellow fat-soluble constituents[2], which are invariably found on extraction of aphids, correspond spectroscopically and in partition behaviour to carotene hydrocarbons. Usually they are present in such small amounts, typically 0·005 per cent of the live insect weight, that we have not examined them in detail.)

Other highly pigmented aphid species, for example, *A. sambuci* L. (elders), *A. rumicis* L. (dock), *A. farinosa* Gm. (*Salix*) and *A. corniella* HRL (dogwood), contain the same three glycosidic constituents as *A. fabae*, but not in the same proportions. In general, black-coloured aphids, for example, *A. fabae* and *A. rumicis*, seem to contain more protoaphin relative to aphinin than dark green species, for example, *A. sambuci*. Variation in pigment content can indeed occur between different individuals of the same species. *A. sambuci*, for example, shows considerable variability in colour, ranging from dark blue-green to reddish-brown, the proportion of each being dependent on external factors, for example, time of season or temperature[6]. Examination of specimens of each of these extreme colour forms shows them to differ only in the relative amounts of the three glycosides already mentioned. A more striking example of colour variation is found in *A. farinosa* which ranges from deep blue-green to dark yellow. Specimens of the former type contain all three glycosides in significant quantities; the latter differ in that the proportion of protoaphin is much reduced, while the amount of aphinin present is so small as to be scarcely detectable.

Relatively non-pigmented, pale green insects, for example, *Brevicoryne brassicae* L. (cabbage), *Hyalopterus pruni* Geoffr. (plum), *Macrosiphoniella artemisiae* (*Artemisia vulgaris*) and *Megoura viciae* Buckton (bean), chemically fall into the same category as those already discussed. Not surprisingly, they contain little protoaphin or aphinin. Indeed, the presence of the former cannot be detected; the latter, for which more sensitive methods of detection are available, is present to the extent of only *c.* 0·02 per cent of the live insect weight. The main component of such species is the colourless, fluorescent glycoside, chromatographically similar to that in *A. fabae* and present in substantial quantity (up to 1 per cent of the live insect weight).

A sufficient quantity of aphinin for chemical study is most conveniently obtained from *A. sambuci*, whence it can be isolated to the extent of 0·2 per cent of the live insect weight. In the solid phase aphinin-*sm* is dark blue-green, almost black, and, in solution, absorbs strongly at 638 mμ. It is exceedingly unstable towards heat, light and most oxidizing conditions. It readily undergoes reversible reduction to a yellow hydroquinone. It can be separated from protoaphin by virtue of its greater acidity. On mild treatment with acid it is hydrolysed to the corresponding aglucone and glucose. Unlike protoaphin it is not hydrolysed enzymatically by extracts of crushed insects. Its probable molecular formula is $C_{36}H_{36}O_{16}$ and its structure almost certainly is based on the extended quinone (III; R = glucose). A compound based on the system (III; R=H) has been synthesized in connexion with other work[7]. It is spectroscopically almost identical

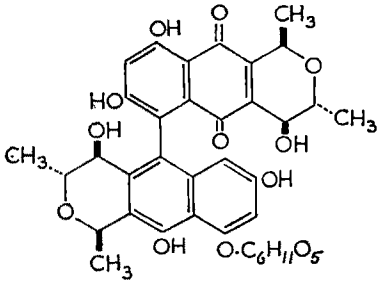
with aphinin, and the chemistry of the two compounds, inasmuch as it affects their chromophores, is completely parallel. For example, very mild, controlled oxidation of either leads to the chromophore (IV). The product obtained from aphinin-*sm* in this way has the formula $C_{36}H_{36}O_{16}$ and absorbs at 590 and 628 mμ. The corresponding aglucone, $C_{30}H_{26}O_{11}$, being less unstable and more symmetrical than its precursors, is more suitable for further chemical study which is in progress. The nature of the non-aromatic residues which substitute the aphinin nucleus (III) has not been established. Spectroscopic considerations, however, strongly suggest that they are closely related to the aliphatic side-chains in protoaphin (I).

The colourless, fluorescent glycosidic component, which accompanies protoaphin and aphinin in the insect species so far considered, has as yet been examined only cursorily. It has not been obtained pure, nor has its homogeneity been established. It is most conveniently isolated from relatively non-pigmented species, for example, *H. pruni*. It is very unstable towards oxidation and less acidic than either of its congeners. Its light absorption is that of a hydroxylated naphthalene, being somewhat similar to the spectrum of derivatives of 1,3,8-trihydroxynaphthalene, obtained by degradation[3] of protoaphin (I).

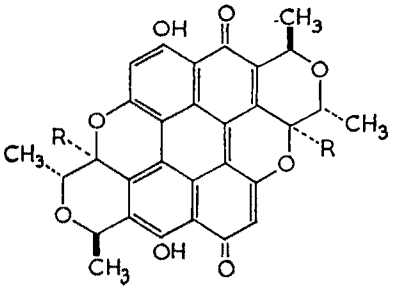
In addition to these substances, two other series of aphid pigments have been found. These were confined to specific genera, although the possibility that they may occur elsewhere cannot be excluded in view of the geographical limitations that attended collection of insects. The pigments derived from two species of *Hormaphis* (previously known as *Hamamelistes*) have already been described[8]. A red glycosidic heteroaphin, present in the living insects, is converted enzymatically after death into a red fluorescent aglycone, rhodoaphin. Recent work suggests that the latter has the molecular formula $C_{30}H_{22}O_{10}$ and confirms that it is closely related to dihydroxyerythroaphin-*fb* (II; R = OH).

A second series of pigments is evidently restricted to the genus *Dactynotus*. They have been found in *D. jaceae* L. (knapweed), *D. cirsii* L. (thistle)[1,2], *D.* (*Uromelan*) *taraxaci* Kaltenbach, *D. tenaceti* L.[9] and in the North American species *D. rudbeckiae* Fitch and *D. ambrosiae* Thomas[10]. *D. jaceae* has been examined in greatest detail. It contains aphinin and a colourless fluorescent glycoside, but no protoaphin. Instead, a new glucoside and its enzymatically derived aglucones constitute a complex series of pigments for which we propose the name 'dactynaphins'. These amount to 0·75 per cent of the live insect weight. Using similar conventions of nomenclature as for the aphins, we refer to the glucosidic precursor as protodactynaphin-*jc*-1, $C_{36}H_{38}O_{17}$. This compound on enzymatic hydrolysis yields a mixture of two aglucones, a reddish orange rhododactynaphin-*jc*-1 and a yellow xanthodactynaphin-*jc*-1, each having the molecular formula $C_{30}H_{28}O_{12}$ and convertible one into the other. Smaller amounts of an analogous pair of aglucones are also isolable from *D. jaceae*. These are termed rhodo- and xantho-dactynaphins-*jc*-2, $C_{30}H_{28}O_{11}$. The properties of the former are similar to those reported for a substance, rhododactynaphin A, which was obtained by Weiss and Altland[10] from the two North American species mentioned. Through the courtesy of Dr. U. Weiss we have been enabled to compare these two compounds. They are indeed identical, as are rhododactynaphin-*jc*-1 and Weiss and Altland's rhododactynaphin *B*. Rhododactynaphins are readily and reversibly reduced to pale yellow hydroquinones; xanthodactynaphins, on the other hand, are stable to mild reduction. The chemistry of these substances is exceedingly complex and is not to be discussed at length here. Evidence is available, however, to support the structure (V; R = OH) for rhododactynaphin-*jc*-1 and (V; R = H) for -*jc*-2. Alkaline degradation of the former leads to the naphthaquinone (VI; R = H), the structure of which has been unambiguously established. For present purposes it is sufficient to note that the major chromophore in rhododactynaphin-*jc*-1 is (VI), linkage of which to a further C_{15} unit is effected through ethereal oxygen.

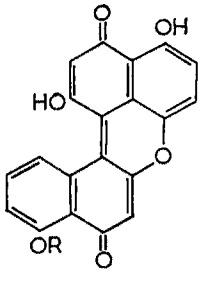
Despite the considerable structural variety in these haemolymph pigments, certain unifying features are, nevertheless, already apparent. All occur in the insects as glycosides, hydrolysis of which invariably leads to C_{30} aglycones. All are quinones or close relatives, as evidenced by their redox properties and general chemistry. All can



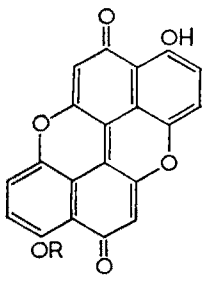
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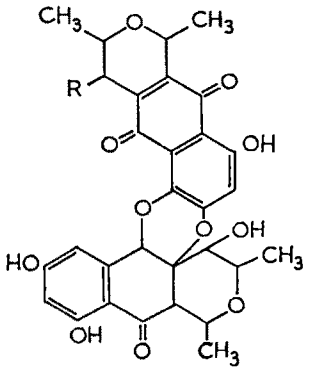
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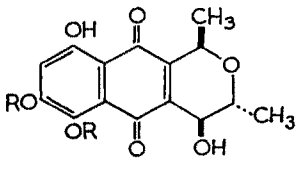
III



IV



V



VI

be visualized as derived formally by oxidative coupling of two C_{15} naphthalenic units, in particular derivatives of 1,3,8-trihydroxynaphthalene. Indeed, the structural variety that occurs is evidently the result of the various possible ways that this coupling process can be effected. In the aphins, aphinin and rhodoaphin, coupling involves carbon–carbon bond formation, in the dactynaphins, carbon–oxygen. In indirect support of these conclusions, similar reactions leading to syntheses of proto- (I) and erythroaphins (II) and several related systems have readily been effected *in vitro* under exceedingly mild conditions[7]. Further, the presence in aphids of colourless glycosides possessing hydroxynaphthalene spectra is of considerable interest. They may be regarded either as pigment precursors or as close relatives formed by similar processes. Besides testing these hypotheses, the completion of structural work in this general area may also provide a basis for speculation on a more fundamental question, namely, the function played by such substances in the insects themselves.

We are grateful to Lord Todd for his interest and encouragement in this work. We thank Mr. H. L. G. Stroyan for identification of aphid specimens and helpful advice, and also Fisons Pest Control, Ltd., Mr. R. N. B. Prior, and Dr. H. Descimon for specimens of *M. viciae*, *A. farinosa*, and *M. artemisiae*, respectively.

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'SKIN DIGESTION' AND EPIDERMAL ABSORPTION IN IRREGULAR AND REGULAR URCHINS AND THEIR PROBABLE RELATION TO THE OUTFLOW OF SPHERULE-COELOMOCYTES

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(1) *Echinocardium cardatum* (Pennant)

When analysing the sand content of the gut of *Echinocardium cordatum*, Lafon[1] was surprised to find how little organic matter was present. Certainly what was present was inadequate for the metabolism and growth of the animal. I endeavoured to determine how *Echinocardium* gathers and absorbs its food by carrying out some investigations on specimens at the Marine Laboratory of Luc-sur-Mer. The specimens had been collected from the same beach as Lafon had collected his; it was possible to keep them for some months in good condition in a sand aquarium supplied with aerated sea-water. In their active state, the animals would push their funnel through the surface: it was obvious that Yonge[2] was right in considering it as a trap for organic particles which, exciting the movements of the dorsal podia, are sucked down into that tunnel of dorsal spines vaulting over the odd ambulacral furrow. There the small prey is mixed with superficial sand (often replaced in my experiments by crushed red brick) and an abundant mucus produced mainly by the clavulae of the internal fasciole. Whenever I tried to trace the trapped particles later on, I was not able to find them, so that the possibility of a superficial 'predigestion' had to be considered. I then carried out some experiments on individuals incompletely buried in the clean sand of a glass vessel continually percolated with sea-water. A great variety of food particles were introduced into the dorsal furrow and I endeavoured to follow their transportation and transformations, for several hours, under a binocular lens. Among the material given were thin pieces of gelatine, egg-white, agar, pieces of worm or mollusc, muscles of crustaceans, crumbs of bread or cheese, flakes of *Ulva lactuca*, and surgical cat-gut soaked in antibiotics. All these were successfully 'dissolved' by the animals provided they were active and the food particles very small or very thin. The particles were impelled along the dorsal furrow by the beat of its small bottom-spines, but being stopped for a time behind the barrier of the internal fasciole where digestion appeared to be especially efficient. If the pieces were too large to be fully digested there, they were sorted out at the frontal notch and rejected laterally into the limy mucus cap which covers the flat dorsal spines. The mucus runs around the frontal humps of the test, comes up the anterior petals and flows backwards over the posterior ambulacra, becomes mixed with the mucus production of the anal and sub-anal fascioles and ends its course in that sticky trail with Nichols[3] calls a 'sanitary drain'. These 'flow-lines' were detected by means of various techniques using crushed brick, carmine, methylene blue, sudan-coloured oil emulsions, etc. They did not fit in with Nichols's description of a diverging flow from the apex. In the 'limy cap', digestion, as well as absorption, proceeded especially in the region of the periproct and the sub-anal tuft and podia. But the dissolved nutrients sometimes slid down the lateral spines to the cocoon of clear mucus which is centred around the mouth, enriched with the secretion of the forty papillate podia of the floscelle.

(2) *Coloured reactions on living heart-urchins. Behaviour of the coelomocytes.*

(*a*) *Proteins.* Egg-albumen, slowly combined with acid fuchsin, turns on heating into a white coagulum which gives a red colour as soon as it is digested. Such coagulum flakes were laid into the dorsal furrow or on the clavulae of an *Echinocardium*; they became light orange while a red tinge appeared in the podia, spines and clavula tips. That general tinge had to be distinguished from the appearance or accentuation of many tiny red spots due to a rush of red spherule-coelomocytes through the epithelia on alimentary excitation. They 'crept' out by means of their mobile lobed pseudopods to reach the external mucus where they eventually disintegrated into red small debris which blackened on treatment with hydrogen peroxide. Kindred[4] had observed this phenomenon in the 'branchial' tufts of *Arbacia*, but I witnessed it everywhere with a feeding urchin—on spines, pedicellariae, podia or clavulae. Other pale coelomocytes with bulky lemon-yellow spherules (responsible for the general colour of *Echinocardia*) behaved in just the same way. The 'sally' of both these cells could be easily observed in the transparent tip of a clavula. A third type of spherule-

coelomocytes showed rounded granules of a greeny-brown tint: but these soon disintegrated under the epithelia into bunches of spherules. Some of the latter were ochre-coloured and might well be pycnotic nuclei. These fragile coelomocytes and their rounded debris were the first to redden when fuchsin-albumen was digested nearby. If the experiment was continued for a sufficient length of time, the connective fibres also reddened and finally the epithelial cells took on a yellowish tinge. It was difficult, however, to be certain that the red coelomocytes grew redder than before and the lemon-coloured ones sometimes showed two or three coloured spherules.

Analogous results were obtained with gelatine flakes soaked with 5 per cent ninhydrin. While they were slowly turning purplish-brown, the neighbouring spines, podia or clavulae showed purple spots and a violet hue; here also the microscope revealed that the greeny-brown spherules were the first to stain dark purple followed by the connective fibres—the other spherule-coelomocytes and even epithelial cells turned violet later on.

To meet the possible objection that bacteria present in the mucus might be the real cause of that proteic liquefaction, pieces of sterile cat-gut or pieces of well-washed annelids were dipped into a solution of penicillin + streptomycin + 'Moldex' + methylene blue + ninhydrin. Half of each piece was left in putrid sea-water to act as a control (where it remained unaltered for weeks), while the other half was given as food. After losing its methylene blue through diffusion, this material gradually turned violet-purple before being dissolved in the limy mucus.

To detect the proteolytic enzymes involved was not easy, yet I was able to detect leucine-aminopeptidase in living material by a histochemical technique. Folk and Burstone's substrate[5] (leucyl-beta-naphthylamide) was incorporated into gelatine flakes deposited in the dorsal furrow. After an hour, the animal was placed in sea-water with a little fast blue *B*. The expected diazonium reaction occurred dyeing the mucus of the furrow, on the neighbouring spines, podia and clavulae an intense red colour. Under the microscope it could be seen that on living transparent clavulae the red colour was first associated with the greenish granules of the disintegrating coelomocytes and possibly the red ones (these turned violet with copper sulphate); later a red dye diffused into the connective tissue and the external mucus.

(*b*) *Glucides.* Agar pieces and small blades of algae, the hardy cuticle of which had been stripped off, were attacked. Filter-paper and starch were more slowly attacked than algal galactosides and polyuronides. When agar gel was added with Schiff reagent it was quickly dissolved, and a cloud of red fuchsin was released.

(*c*) *Lipids.* A greasy mixture of cream cheese, peanut oil and black sudan when dabbed in the dorsal furrow proceeded along the aforementioned flow-lines, turning into a homogeneous emulsion which eventually covered the spines with a thin continuous film. Under the microscope, dark droplets could be seen in the epithelial cells while many disintegrated spherules became purple or reddish. Later on, this red metachromasy appeared even more conspicuously in the muscular and connective fibres of the oesophagus.

(*d*) *Translocations within the body.* Animals which had been submitted to coloured nutrients for some hours were dissected in order to determine the manner in which any absorption had occurred. The oesophagus, the vertical mesentery and the oval gland, the rectum and the clogged coelomocytes spotting the ampullae were densely stained. while the ampullae and the intestine were but lightly tinted. Thus, the intensity of the coloration seemed to be proportional to the density of spherule-coelomocytes. That would support Liebman's view[6] concerning the role of the spherule-coelomocytes (called by him 'trephocytes') in the elaboration and translocation of nutrients. The greeny-brown ones may play such a part, but the red and lemon-yellow cells which do not disintegrate before they are taken up by the external mucus would seem to act as a digestive vanguard.

(3) *Psammechinus miliaris*

Although regular urchins live in the open sea, their podia and epiderm also produce a thin mucous coating which the ciliae push along the spine-grooves. In *Psammechinus*, it has strong digestive powers for artificial food with coloured reagents as well as for living prey. Pieces of egg-white gelatine, crustacean or molluscan tissues, agar, or bread, deposited on the epiderm were soon enclosed by the convergence of the neighbouring spines and soaked with mucus. They were dissolved within 24–48 h. If the prey was too cumbersome or solid, such as a coverslip with coagulated fuchsinated egg-white or the frond of an *Ulva*, the podia would take hold of it, draw it against the spine-tops and the organic matter would be attacked both by the spines and the podial disks, leaving their hollow prints in it. Those prints were sharply brought out in orange-red if fuchsin-albumen was used; and when a frond of *Ulva* had been kept tight for several days, it was possible to observe, using a low-power microscope, several holes and rounded prints where the 'attacked' cells showed a pinkish hue. In aquaria, the urchins are said to be 'disguised' with shells, stones, algae, polyzoa, etc. After some time the shells become cleared of the polyzoa, barnacles or organic detriti which they previously 'wore'. Even a mussel shell itself is attacked, and if its margin comes in contact with the test it becomes as jagged as an old knife. I was able to follow the process of digestion of a small *Natica catena*: within 2 days only a very clean empty shell was left. Still quicker was the skin-digestion of a commensal, *Flabelligera affinis*, provided the worm was removed from its mucous protective bag, which forced it to become attached to the test. When the skin is half destroyed a red stomach can be observed to be still reacting; but soon it loses its colour and becomes dissolved (2·5 h). Here, too, an alimentary excitation of the tegument provokes an outflow of coelomocytes. The red spots on the spines and pedicellariae grow in number and density. Under the microscope, the gradual emergence of red spherule-coelomocytes as well as of colourless spherule-coelomocytes (which replace the lemon-yellow cells of the heart urchin) can easily be observed in podia, pedicellariae, branchial tufts and small crushed spines. Here too the greenish-brown spherulocytes disintegrate in the connective tissue and their diffused pigment can be fixed in the skeletal rods of the spines as 'spinochromes', which are chemically similar to the echinochrome of the red cells. Debris of the latter can only be found on the epithelial surface. But despite this strange 'aboral feeding', regular urchins are essentially 'oral' grazers. Sticking to the bottom with many podia and short spines, they surround a real 'digestive pouch' between their oral surfaces and the floor. In this, the so-called branchial tufts emit large quantities of spherulocytes. So do the lips and the buccal pouches with their mucus-producing villi. The organic matter on the floor can thus be digested externally before being sucked in, the lantern intervening only for cracking hard material. I have stuck the teeth of a sea-urchin together with plastic dental cement ('Ivoclar Justi', kindly supplied by Dr. Bourdin), and although its lips and persistomial podia were injured in the operation, the specimen was able to survive for 16 days. But 24 h sufficed to dissolve out an empty circle under its whole oral face in the coagulated albumen coating a Petri dish, and podial prints were visible in the thinned albumen all around.

A search for leucine-aminopeptidase by means of Burstone's substrate and fast blue *B* gave results similar to those obtained with *Echinocardium* (reddening of the mucus, of the disintegrating cells and possibly the red coelomocytes).

For lipids, I used the same sudan-blacked greasy mixture. The thin black emulsion obtained was pushed up the spines and the same metachromatic colorations were observed inside.

Must one conclude from all this that the gut of the sea-urchin is a useless 'luxury'? Actually, the thick folded epithelium of the pharynx and oesophagus is rich in mucus cells as well as in pervading coleomocytes, so that Stott[7] was able to claim that these wandering cells come out into the lumen to pick the food and then return to the coelom with their 'shopping'! However, although I observed hundreds of red and colourless spherulocytes 'creeping out', I never saw one on its way back. They disintegrate while probably releasing digestive enzymes. The pharynx and oesophagus keep up the epidermal digestive work and probably more efficiently. But in the 'stomach' very few coelomocytes are to be seen either in sections or on the living tissue among the long loose cells of the epithelium. Some of the latter become rather club-shaped and become filled with granules and large vacuoles. Yet they have a ciliate brush like their leaner sisters and do not show the reactions of muco-polysaccharides. Therefore, their content seems to be absorption products rather than secretions. Farman-farmaian's experiments[8] on *Str. purpura-tus* buccally fed with carbon-14 labelled red algae show that the stomach walls are an important seat of reserves, while Lasker and Giese[9] have shown on that same species that digestion was completed in the first two loops—it is possible that this may be a belated digestion due to the action of mucus and coelomocytes proceeding from the oesophagus. The siphon which directly connects the oesophagus to the upper intestine allows some already digested nutrients and also oxygen to reach the festooned loops surrounding the genital glands—the only really internal organs of an urchin.

(4) *Asterias rubens*

Cuénot[10], in his early work, noticed that small annelids could be captured by the rosettes and their pedicellariae and were soon killed. But he went on to comment that the dead prey was then released as it was of no use as food. Actually, if one manages to keep in close contact with the tegument pieces of worms, molluscs, bits of ninhydrinated gelatine, threads of cat-gut, etc., an epidermic digestion will be observed within a few hours. Whenever ninhydrin is used, a violet tinge appears in the tegument which is more conspicuous in the thick epithelium covering the rosettes. If the organic pieces are set into an ambulacral groove where they are soon shut in by the lowering of adambulacral plates over the contracted podia, digestion will be still quicker and if ninhydrin is used at a sufficient concentration several podia turn violet. With Burstone's substrate and fast blue *B*, an intense reddening is obtained in the mucus, the podial mucus-glands and the mucus-cells of the epithelium of the pedicellariae. The 'secretory packets' of Chaet and Philpot's electron microphotographs[11] might be a source of digestive enzymes. They originate from vacuolary cells situated under the epithelia of the disks, which it is possible may act as some kind of spherule coelomocytes. The host of strong active podia surrounding the everted stomach of a feeding starfish may help cardiac digestion chemically as well as mechanically. I gave specimens of *Asterias* living mussels with bolted shells and others enclosed in nylon bags. All were completely digested without being pulled open, for either the dorsal ligaments or the conchyolin margins on the ventral side of the valves had been dissolved. All this is in agreement with J. C. Ferguson's[12] results with *Asterias forbesi* fed with radioactive clams. The study of autoradiographic sections demonstrated that "large quantities of nutrients are absorbed directly from the external medium by epidermal cells, especially those of the tube feet and aboral body-wall": my findings are in agreement with these conclusions.

(5) *Ophiothrix fragilis*

In aquaria, the long spined arms of these ophiurids are either stretched to catch passing particles or tightly fixed to mussels, patellae, lime-covered stones or shells which they eventually clear up. When kept in Petri dishes smeared with egg-white, agar or decked with filter-paper, they leave the bottom clean within a week. To eliminate any gastric emanation through the mouth, I enclosed the disk of a specimen within the cavity of a plastic stopper pressed against a coverslip: five marginal notches allow the arms free movement on the nutritive smear of a Petri dish under sea-water. If that smear consisted of ninhydrinated albumen or gelatine soaked with Burstone's substrate and fast blue *B*, not only did the arms, within a few days, dig their print in it, but staining appeared on their ventral side, the cores of the barbed spines, and more especially in the podia. In them these golden disintegrating granular cells, which grew darker on alimentary excitation and showed a green metachromasy with toluidine blue, were the first to take the dye. Further research is needed to determine whether Buchanan's '*A*' and '*B*' bodies[13] play a part in the production of digestive enzymes. In one experiment, the distal articles of an arm were cut off, and the animal withdrew the proximal part of it within the stopper. The latter, like the disk, was not stained. This indicates brachial digestion and absorption. Large diatoms deposited on the oral surface of an arm, next to a podion and observed for several hours under a lens eventually faded away.

(6) *Conclusions*

I have not yet tested holothurians or crinoids. But there is no reason for these 'ciliary mucus feeders', which have about the same type of coelomocytes as urchins, to behave differently. The large surface area for catching in the case of a crinoid, with its forked arms bristling with pinnules and podia, is out of proportion with the small U-shaped gut of the animal and should have a greater nutritive efficiency. When it is considered that most of the tissues of echinoderms are exoskeletal and that the animals have only a very simple digestive tube without differentiated glands, the necessity of an epithelial nutrition and respiration can be understood. For the fragile unprotected skin, devoid of any cuticle or shell, digestive 'aggressiveness' provides the animal with a means of defending itself against prospective parasites. Actually, the superficial mucus of a living urchin is poor in bacteria and ciliates. Only a few specific commensals (*Urceolariae* for ciliates, a few copepods and amphipods) are to be found. Real digestive organs, respiratory chambers or gills require a circulatory system to drain them; but in this respect M. Burton[14] has shown that the haemal system is not a circulatory network. If a diffuse type of digestion and absorption were to be found with all echinoderms, it can be claimed that evolution shows a tendency to an 'oralization' of the digestive process. It is evident in regular urchins, the Aristotle's lantern of which may be an adaptation to coralligenous environments, and in the large predatory starfish that succeed in engulfing large prey (*Luidia* and *Marthasterias*) and store their gains in their long folded caeca.

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MEMORY AND SPONTANEOUS ALTERNATION IN THE RAT

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IT is well known that rats have a tendency to alternate their choices in a T-maze; that is, if given two choices in succession, they tend to choose first one arm of the maze, then the other, rather than the same arm on both choices. They do not, of course, always choose according to this tendency since, for example, a rat rewarded in one arm only will usually choose that arm. We may thus regard choice in a T-maze as being determined in two possible ways, depending on whether or not the rat is choosing according to the alternation tendency.

There are several experiments showing that when rats alternate they usually vary the places they go to, rather than the responses they make[1,2]. Therefore, in order to alternate, the rat must, in some sense, remember which arm of the maze was last entered, and this has led Dember and Fowler[3] to suggest that spontaneous alternation might be used to study memory. This suggestion has not yet been explicitly followed up, though some of the studies on spontaneous alternation *per se* are relevant to the question of memory. In particular, the delay between trials has been varied and, with one pair of trials per day, alternation shows a positively accelerated decline when plotted against delay or log (delay)[4,5].

A positively accelerated decline would be predicted from the following trace decay theory (which owes much to a theory of forgetting put forward by Brown[6]): A trace is laid down when a rat enters a T-maze arm, and this declines in strength with time; following an arm entry there will also, unless the rat is experimentally naïve, be a trace resulting from the last entry to the other arm, and the rat, when it is choosing according to the alternation tendency, will choose the arm with the weaker trace.

Definitions: $\log n_1$ and $\log n_2$ are the strengths, which decline with time, of the traces corresponding to the two arms, $\log n_2$ corresponding to the arm most recently entered. t_1 and t_2 are the respective times since the traces were laid down. $N = \log n_2 - \log n_1 = \log n_2/n_1$. P_a is the probability of alternation, and P_r $(= 1 - P_a)$ is the probability of repetition. The following assumptions are made: (1) when a rat is choosing according to the alternation tendency it will alternate if N is positive, repeat its previous choice if N is negative; (2) N is a random sample from a hypothetical population of values normally distributed about a mean $\bar{N}$ with standard deviation σ_N; (3) $\bar{N}$ is a decreasing function of t_2; (4) σ_N is independent of the value of $\bar{N}$.

From these assumptions it follows that P_r is $\bar{N}/\sigma_N$ expressed as a normal probability integral. Thus if $\bar{N}$ were a linear function of t_2 over some of its range, P_r plotted against t_2 would be part of the lower half of a normal ogive, so that P_a would be positively accelerated over this range. In general, P_a against t_2 will be positively accelerated provided that t_2 is not too large, $\bar{N}$ is sufficiently large when $t_2 = 0$, and $\bar{N}$ is not an extreme negatively accelerated function of t_2.

The following experiment carries the theory a step further: Twelve hooded rats were used; they were highly experienced, having had several hundred rewarded trials in a T-maze. The apparatus was an alley T-maze of aluminium, with the walls of one arm painted grey, the other black; the arms and stem were 2 ft. long, 4 in. wide, and 5·25 in. high. One pair of trials was given per day; the first was forced (access to one arm being prevented by means of a guillotine door), the second free. The delay between forced and free trials was 5 sec, 25 min, 2 h, or 10 h; every day each of these delays was given to three rats, and during the experiment each rat was tested at each delay twelve times. For the 10 h delay the forced trial was in the morning; for the shorter delays pairs of trials were given equally often in the morning and evening. The rats were fed for 2 h every evening, 15 min after all rats had been tested; they were allowed to eat in the maze for 15 sec following every choice.

Since the rats were fed after the evening testing session they were hungrier on evening than morning trials, which might have given rise to a difference in choice behaviour; however, examination of the results for the three shorter delays reveals no significant difference, so morning and evening results were combined. In Fig. 1 mean percentage alteration is plotted against $\log t_2$. The 48 days of the experiment were divided into three blocks of 16 days, and an analysis of variance was performed, using stages of training, individual rats, and delays as the three criteria of classification; the effects due to both delays ($F = 10{\cdot}00$, $P < 0{\cdot}001$) and individual differences ($F = 3{\cdot}21$, $P < 0{\cdot}05$) were significant, but the effect due to stages of training was not ($F = 0{\cdot}82$). The interaction between individuals and stages of training was significant ($F = 1{\cdot}82$, $P < 0{\cdot}05$), which appears to be due to some rats increasing, some decreasing, their general rate of alternation over the course of the experiment. No other interaction approached significance.

The initial flat part of the curve in Fig. 1 would be predicted from the foregoing trace decline theory provided that $\bar{N}/\sigma_N$ is sufficiently large when $t_2 = 0$. However, if $\bar{N}/\sigma_N$ is large, $P_r \simeq 0$, but in fact P_r is initially nearly 0·2. Before trying to reconcile the theory with this finding, let us consider the nature of the individual differences. There are two possibilities: (*a*) Individual differences lie in the function $\bar{N} = f(t_2)$. This possibility may be subdivided further; for example, if $\bar{N} = f(a(t_2 + b))$ we might consider whether the individual differences are in a (which would be the time constant if the function were exponen-

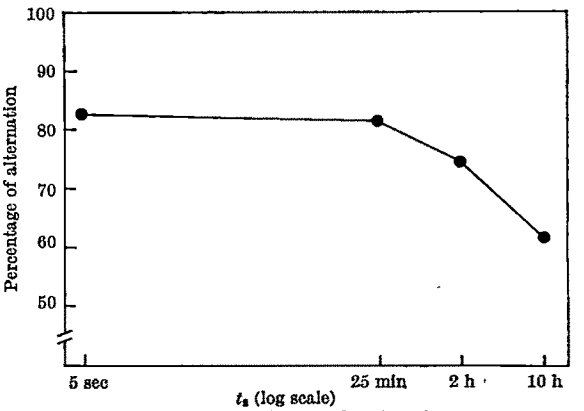


Fig. 1. Alternation as a function of t_2

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trial), or in b (that is, differences in the value of $\bar{N}$ when $t_2=0$). If individual differences were of this form, an interaction between individuals and delays would, in general, be predicted. (*b*) Individual differences are in the probability of choosing according to the alternation tendency. It has been shown[7] that, where all choices are rewarded, rats choose sometimes according to an alternation tendency, sometimes according to a turn preference, and the respective probabilities of these two types of choice might vary among individuals. Thus, independent of the value of N, there might be a certain probability that the choice will not be according to the alternation tendency. Let this probability be q, and let P_a' be the probability that N is positive (that is, $P_a'=P_a$, the probability of alternation, if $q=0$). Then, if the rat is forced equally often to each arm, so that half the choices according to the turn preference will be alternations:

$$P_a=P_a'-\tfrac{1}{2}q\ P_a'+\tfrac{1}{2}q.\ (1-P_a')$$
$$=P_a'\ (1-q)+\tfrac{1}{2}q$$

Now P_a' varies systematically between delays (thus the effect due to delays), and q varies systematically between individuals (thus the effect due to individual differences); but P_a' and q are independent, and therefore there should be no interaction between individuals and delays.

Thus the absence of an interaction in the foregoing experiment supports *B*, that individual differences in alternation are due to differences in q. The existence of q can, in addition, explain why P_a, as estimated from Fig. 1, is not approximately 1 when the curve is flat. We may conclude that it is necessary to introduce q into the theory in order to explain both individual differences, and the absence of 100 per cent alternation at any delay; parameters such as a and b, already defined, cannot, of course, be rejected as potential intervening variables since they may well prove important in other contexts.

In the next experiments an attempt is made to infer certain characteristics of the assumed underlying trace decline (that is, the decline in $\log n_2$ and $\log n_1$). In order to do this two further assumptions are made: (*a*) that the traces corresponding to the two arms decline in strength with time in the same way, and in mutual independence. (*b*) That the general relation between trace strength, $\log n$ and time, t, since the last arm entry may be expressed in the form:

$$n=g(t)$$

Let $n_2/n_1=z\ (=e^N)$, and let $t_d=t_1-t_2$; then:

$$z=g(t_2)/g(t_2+t_d)$$

Regard t_d as a constant and differentiate.

$$\mathrm{d}z/\mathrm{d}t_2=\frac{g'(t_2).\ g(t_2+t_d)-g(t_2).\ g'(t_2+t_d)}{(g(t_2+t_d))^2}$$

Now $g(t_2+t_d)$ and $g(t_2)$ must always be positive (in order for trace strength to be of the form $\log n$), $g'(t_2+t_d)$ and $g'(t_2)$ are negative and $g(t_2)>g(t_2+t_d)$ (since $\log n_2$ declines with time). Furthermore, where the trace decline against t is positively accelerated, $|g'(t_2+t_d)|>|g'(t_2)|$. Thus, if the trace decline is positively accelerated, $\mathrm{d}z/\mathrm{d}t_2$ will be positive, and z (and therefore alternation) will increase as t_2 increases.

Let us suppose, as a working hypothesis, that the underlying trace, n, tends to an asymptote at 0, as $t\to\infty$, and $g(t)$ is either negatively accelerated throughout, or positively accelerated when t is small, and then negatively accelerated. Which of these possibilities is likely to be correct is tested in the following experiments, in which an attempt is made to find out whether or not the underlying trace decline is positively accelerated over the first few minutes.

In both experiments rats were given three trials per day. The first choice was forced to one arm, the second choice to the other arm, and the third choice was free. In the first experiment t_2 and t_d could be either 15 or 45 sec (yielding four possible combinations, each being used equally often); in the second experiment they could be 15 or 90 sec. Details of apparatus and procedure were the same as in the previous experiment. There was significantly less alternation at $t_d=15$ sec, compared with $t_d=45$ or 90 sec. In the first experiment alternation was about the same at $t_2=15$ sec as at $t_2=45$ sec; in the second experiment it was slightly (though not significantly) less at $t_2=15$ sec.

If the underlying trace decline were positively accelerated over the range tested there should be more alternation at $t_2=15$ sec than at the larger values; the failure to find this in the foregoing experiments thus supports the hypothesis that $g(t)$ is negatively accelerated throughout, as against the hypothesis that there is an initial positive acceleration.

The theoretical analysis of this report has been in very general terms, and the theory put forward and its assumptions can only be regarded as mildly strengthened by the demonstration that this analysis is possible. However, with greater precision in the results it should be possible to test between different mathematical formulations of $g(t)$. Consider, for example, a formula suggested by London[8] for memory in general. He supposed that the engram involved in memory is a complex of activated units, and that forgetting occurs through the inactivation of units with time. If it is assumed that the rate of change in the percentage of units still active at time t is proportional to the percentage active, n, out of the number of units initially active, $g(t)$ will be an exponential function. Thus:

$$\mathrm{d}n/\mathrm{d}t=-an$$

therefore

$$n=ce^{-at}\qquad (c \text{ is a constant})$$

Now, when $t=0$, $n=1$, therefore $c=1$. Thus:

$$n=e^{-at}$$

Suppose three trials are given per day as in the foregoing experiments; then at time t_2+t_d after the first trial:

$$n_1=e^{-a(t_2+t_d)}$$
$$n_2=e^{-at_2}$$

therefore

$$z=\frac{e^{-at_2}}{e^{-a(t_2+t_d)}}$$
$$=e^{at_d}$$

Thus z, and therefore alternation, will increase as t_d increases and will be independent of t_2. In the experiments already described there was indeed an increase in alternation with t_d, but also some evidence of a decrease with t_2 (since there was less alternation at $t_2=15$ sec than $t_2=90$ sec); if the latter result is substantiated, we may reject London's theory as an account of the memory trace involved in alternation, provided of course that the assumptions that have led us to this point are justified. These assumptions may be buttressed by finding a mathematical function that does fit the data with precision; or it may be found necessary to modify the assumptions, for example, by allowing some dependence between traces. At present more data are being gathered by means of experiments along the lines of those described, in order to find such a mathematical function, and show its applicability in as many situations as possible.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Meteor Luminosity and Meteor Ionization

In two earlier communications[1,2] I drew attention to the fact that the discrepancy between theory and observation in respect of the light curves of meteors pointed out by Hawkins and Southworth[3] apparently necessitates a modification of the conventional theory of meteor luminosity. There is a better fit between theory and observation if the luminous efficiency factor is assumed to be proportional to the atmospheric density at every point of the meteor trail. However, no physical justification was adduced for such an assumption.

The conventional equation for meteor luminosity/ionization is:

$$I = \frac{1}{2}\left(\frac{dm}{dt}\right) v^2 \tau \qquad (1)$$

where I is the luminous/ionization intensity at any point of the trail, dm/dt is the rate of ablation of the meteor at that point, v is the velocity of the meteor and τ is an efficiency factor that determines what fraction of the kinetic energy of the ablated atoms is converted into luminous/ionization energy. At first sight it is not clear why τ should depend on atmospheric density, although it can have a functional dependence on v.

It is therefore important to enquire whether an explicit dependence of I on ρ in equation (1) can result from the factor dm/dt which is the rate of ablation of the meteor. With a spherical meteor of radius r falling through the atmosphere, the equation for ablation is:

$$L\, dm = \Lambda\, (\pi r^2 dz\, \rho)\, \frac{v^2}{2} \qquad (2)$$

where ρ is the atmospheric density, dm the mass ablated in falling through the height interval dz, L the heat of ablation per unit mass and Λ the heat transfer coefficient.

As has been pointed out by Thomas[4], Λ may be expected to be a function of the speed v and the air density ρ, although in the usual theory it is assumed to be a constant for a given meteor. Let us assume that:

$$\Lambda = \lambda \rho^{\alpha} \qquad (3)$$

where λ and α are constants.

Making use of the hydrostatic equation and the gas equation we readily obtain from equations (2) and (3):

$$dm = -\frac{\lambda}{Lg}\left(\frac{1}{RT}\right)^{\alpha} (\tfrac{1}{2}\pi r^2 v^2)\, p^{\alpha} dp \qquad (4)$$

where g is the acceleration due to gravity, T is the atmospheric temperature, and R the gas constant.

If dr is the decrease in radius of the meteor due to ablation while falling through the height dz, and ρ_m is the density of the meteor, we have:

$$dm = 4\pi r^2 dr\, \rho_m \qquad (5)$$

From equations (4) and (5) we obtain:

$$dr = -Kp^{\alpha} dp \qquad (6)$$

where, to the first approximation, K can be regarded as a constant for a given meteor. From equation (6) we readily obtain the two relations.

$$r = \frac{K}{\beta}(p_e^{\beta} - p^{\beta}) \qquad (7)$$

and:

$$m = K'(p_e^{\beta} - p^{\beta})^3 \qquad (8)$$

Here $\beta = \alpha + 1$ is a constant for a given meteor and p_e is the atmospheric pressure at the end of the meteor trail.

From equation (8) it can be shown that:

$$\frac{dm}{dt} = K_1(1 - \zeta)^2\zeta \qquad (9)$$

where K_1 is a constant for a given meteor to the first approximation, and $\zeta = x^{\beta}$, where $x = p/p_e$.

If H is the atmospheric scale height (assumed constant to the first approximation) at meteor levels, then:

$$x = \frac{p}{p_e} = \exp\left(-\frac{h}{H}\right)$$

where h is the height interval between the instantaneous level of the meteor and the level corresponding to the end of the meteor trail. From equation (9) it is readily seen that dm/dt is a maximum for $\zeta = \frac{1}{3}$ and:

$$\left(\frac{dm}{dt}\right)_{max} = \frac{4}{27} K_1 \qquad (10)$$

If τ is constant, we obtain from equations (1), (9) and (10):

$$I/I_{max} = \frac{27}{4}(1 - \zeta)^2\zeta \qquad (11)$$

This is the equation for the normalized light/ionization curve of the meteor. The corresponding equation expressed as magnitude difference is:

$$\Delta M = M - M_{max} = -2{\cdot}5 \log (I/I_{max})$$
$$= -2{\cdot}5\left[2 \log (1 - \zeta) + \log \zeta + \log\left(\frac{27}{4}\right)\right] \qquad (12)$$

Equation (12) is similar to equation (4) in my earlier note[1] except that ζ now occurs in place of x. Since $\zeta = x^{\beta}$, it follows that for $\beta = 1$, equation (12) becomes identical to equation (4) of the earlier note which is the equation for the light/ionization curve given by the conventional theory. From equation (3) it is seen that in this case Λ is independent of ρ.

When $\beta > 1$, the light curves given by equation (12) and the conventional theory are different. For increasing values of β the ascending and descending branches of the

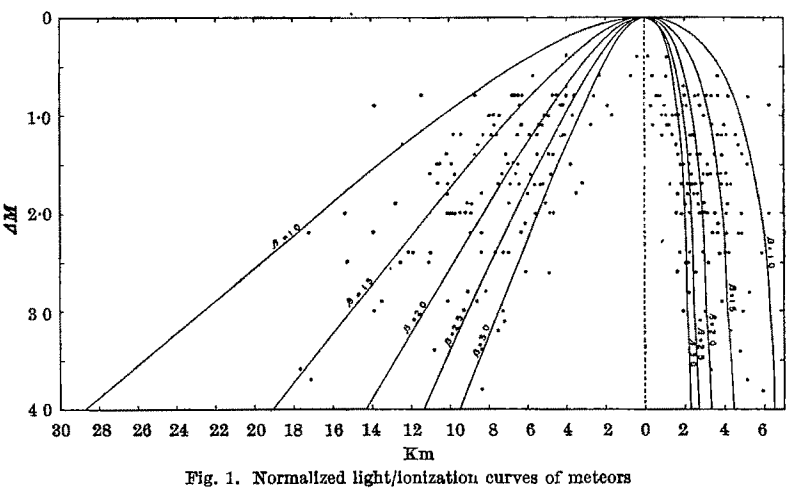


Fig. 1. Normalized light/ionization curves of meteors

light curve become progressively steeper so that the meteor trail becomes shortened. For a given value of ΔM, however, the ratio of the lengths of the ascending and descending branches is independent of β.

Fig. 1 shows the normalized light/ionization curves corresponding to $\beta = 1{\cdot}0$, $1{\cdot}5$, $2{\cdot}0$, $2{\cdot}5$ and $3{\cdot}0$, respectively, assuming[2] a value of 6·38 km for the atmospheric scale height. Fig. 1 also shows the plot of the points corresponding to the beginning and end of the meteor trails in the random sample listed by Hawkins and Southworth[3] after eliminating the majority of the fragmented meteors[2]. There appears to be definite evidence for the dependence of Λ on atmospheric density. The light curve obtained previously, by assuming that τ is proportional to ρ, lies between the light curves corresponding to $\beta = 1{\cdot}5$ and $2{\cdot}0$. This assumption must be rejected because it lacks physical basis. The assumption that the heat transfer coefficient Λ is dependent on the atmospheric density ρ, which is plausible on physical grounds, can explain the major discrepancy between theory and observation in respect of meteor luminosity/ionization.

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PHYSICS

Electric Strength of Compressed Air in a Uniform Field

IN a uniform field, Paschen's law, namely, breakdown voltage $V = f(p.d)$ (where p is the gas pressure and d is the gap distance), holds for values of p up to about 10 atm., beyond which the improvement in electric strength of air becomes progressively less than would be expected from this relationship. Most published data extend only up to some 1,200 lb./in.^{2}, and beyond this pressure information is sparse and predictions of the electric strength, E, are difficult as a result of the lack of experimental data. The cause of this departure from Paschen's law may be attributed to the nature of the electrode surfaces. At high pressures, the electric strength of air is dependent on the shape, disposition and material of the electrodes and also on the degree of smoothness of their surfaces[1-3].

Aluminium electrodes give markedly lower values of electric strength at high pressure than, for example, stainless steel, and the material forming the cathode has greater influence than that of the anode[4,5]. Felici and Marchal suggest that the electric strength of air is dependent on the work function φ of the electrode material, metals with high values of φ giving high values of electric strength. Although φ for stainless steel is not exceptionally large, they attribute the high values of E obtained with this metal to its oxide film, which acts as a barrier to cold emission.

According to Llewellyn-Jones[6], a new ionization process must come into play at high pressure which cannot be any of the 'normal' low-pressure gas-collision processes. Furthermore, this process must be cathode-dependent and the Townsend type of mechanism assisted by field emission has been proposed as this ionization process. Other investigators have attributed deviations from Paschen's law to the action of space charges in the gas. Llewellyn-Jones points out that, if this were true, E would be independent of the nature of the cathode. In addition, he found it difficult to accept the cold emission theory since the Fowler-Nordheim theory requires fields ~ 10 MV/cm to produce significant cold emission.

Assessment of the merits of compressed air insulation at pressures $\geqslant 1{,}200$ lb./in.^{2} is of technological importance. However, more experimental information is necessary and, in particular, it is necessary to know how E varies with change in pressure. If E becomes independent of change in pressure, at what pressure does this take place and how important are the shape, disposition and material of the electrodes?

Recent work in our laboratories has demonstrated that there is, in fact, a continuous upward trend of E up to 1,750 lb./in.^{2}. A few preliminary results will now be discussed.

Stainless steel electrodes, 1 cm in diameter, having profiles suggested by Bruce[7], were used in the tests. Three test runs were made using new electrodes for each run (Fig. 1, curves A, B and C). For tests A and B, the electrode surfaces were carefully machine-polished using successively finer grades of emery paper, then Selvyt cloth impregnated with fine alumina, followed by degreasing, washing and drying. The electrodes used for test run C underwent a slightly inferior process. They were simply hand-polished (after the emery paper process) using metal polish, and then degreased. This treatment is believed to be responsible for the marked difference in curves B and C in Fig. 1 at pressures in excess of 400 lb./in.^{2}.

Air having a moisture content < 200 p.p.m. was passed through a silica-gel drying unit before being admitted to the test chamber. The sequence of tests was from high to low pressure and the vertical lines embrace ten consecutive breakdowns. Determination of the electric strength of gases is usually made after a number of breakdowns to condition the electrode surfaces. This process, referred to as 'conditioning', infers the removal by breakdowns of microscopic irregularities and dust particles. Hence, the number of conditioning breakdowns required reduces with improvement in the electrode surface finish.

In the tests described, the first application of voltage gave a value some 25 per cent lower than the final average value in the conditioned range reached after about five breakdowns. Voltage was raised quickly to about 90 per cent of the breakdown voltage, then very slowly up to break-

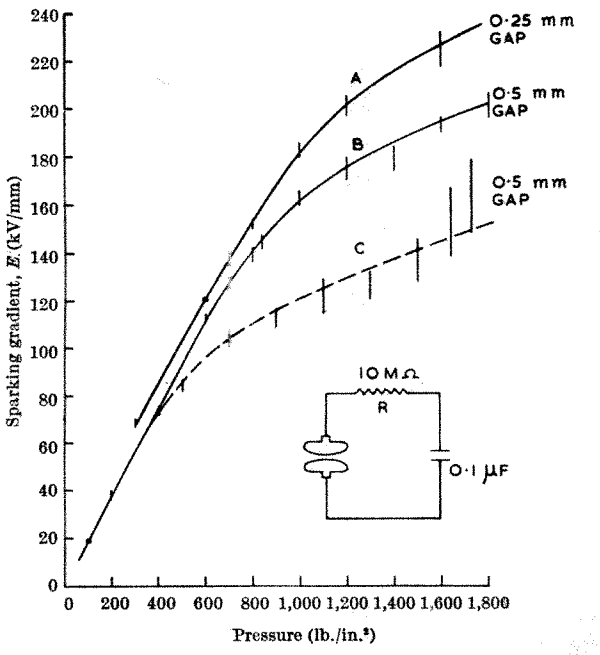
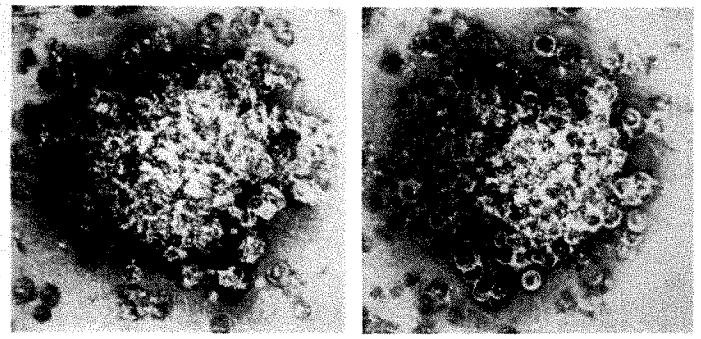


Fig. 1. Direct current sparking gradient of compressed air in a uniform field using stainless steel electrodes (20° C)



Anode Cathode

Fig. 2. Photomicrograph of central area of uniform-field stainless steel electrodes. Test *A*. (× 16)

down. It was found that the 1-min withstand value was within 5 per cent of the minimum breakdown value obtained by raising the voltage slowly. Since conditioning depends partly on the degree of smoothness of the electrode surfaces, it follows that the value of the current-limiting resistance R is also important. To limit damage to the electrodes caused by sparking, a 10 MΩ current-limiting resistance was used. Micrographs of the electrode flats taken after test run *A* are shown in Fig. 2. The pattern of spark roots on each electrode is clearly shown. The peripheries of the cathode roots are more clearly defined than those on the anode.

At pressures in excess of 1,000 lb./in.² other workers appear to have found no appreciable gain in the electric strength of air. Zeir[8] gives values up to 1,760 lb./in.² for gaps up to 0·5 mm and direct voltages up to 100 kV. His results are plotted to a $p.d$ scale and are in general agreement with the present work.

The preliminary results described indicate that if, in fact, there is a levelling-off of electric strength at high pressure, then the critical pressure is well beyond 1,000 lb./in.² for the present test conditions.

We thank Messrs. P. Headley and D. G. McCulloch for advice and assistance in the preparation of the test arrangement.

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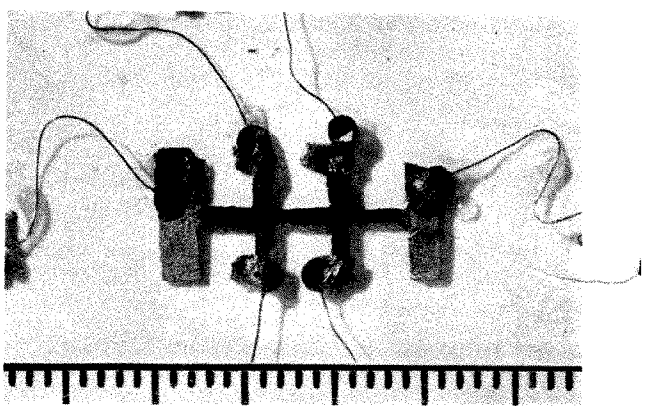
Properties of Synthetic Good Conductors of Electricity

For both theoretical and practical reasons, it is of considerable interest to develop synthetic good conductors of electricity. From a theoretical point of view, these could serve as interesting alternative model substances by which to test various theories of electric current flow in solids. From a practical point of view, synthetic good conductors might for some purposes become useful rivals to the best natural good conductors, such as copper or silver.

One route to synthetic good conductors of electricity lies in the properties of charge transfer compounds, and in particular in the crystal compounds of graphite. As has been known for some time, good conductors of electricity can be formed both by intercalating electron donor atoms such as the alkali metals (n-type) and electron acceptor groups such as bromine or iodine monochloride (p-type)[1]. Electrolytic formation of acid salts, such as the bisulphate[2], the perchlorate[3] and many other acid derivatives[4], likewise leads to electron acceptor compounds that are good conductors of electricity.

However, to use these crystal compounds as model good conductors of electricity, in addition to achieving optimum stoichiometry it is essential to control the defect concentration, which has a powerful effect on scattering charge carriers in the transport of electricity. Considerable advances in this direction were achieved by replacing polycrystalline graphite[1,2] by well-aligned pyrolytic graphite formed by cracking hydrocarbons[3], but for fully informative investigations it is essential to use near-ideal graphite, such as the material formed from pyrolytic graphite by stress-annealing at high temperatures under moderate pressure[5]. Quite large pieces of such near-ideal graphite can now be formed; the best of these materials can be selected by various non-destructive techniques[6].

Fig. 1 illustrates a specimen of near-ideal graphite cut and mounted preparatory to transforming it into various graphite nitrates by exposing it to the vapour of nitric acid under appropriate conditions[7]. With suitable precautions, nitration proceeds smoothly, and can attain the stoichiometry of the different sequences without introducing specimen distortions or additional crystal disorder to any marked extent.



mm

Fig. 1. Specimen of graphite cut and mounted with leads

Resistivities of the resulting specimens of graphite nitrate of the (black) second sequence, and of the (deep blue) first sequence, were measured down to the freezing point of nitrogen. With rising or falling temperatures, electrical resistances were found to be reversible within experimental error, except through the order–disorder transformation at about −20° C (ref. 8). In the lambda region, a hysteresis loop is clearly apparent, particularly for the first sequence compound (Fig. 2). The steep drop in resistance below the lambda point (Fig. 3) is probably due to increased mobility of the charge carriers in the ordered state of this crystal compound.

Temperature coefficients of electrical conductance of different specimens can be compared in a reduced plot of ρ_T/ρ_0. A similar trend is found for all these preparations. A steep drop occurs in all cases on passing through the lambda point. For the ordered crystal compounds below their lambda points, the rate of change with tem-

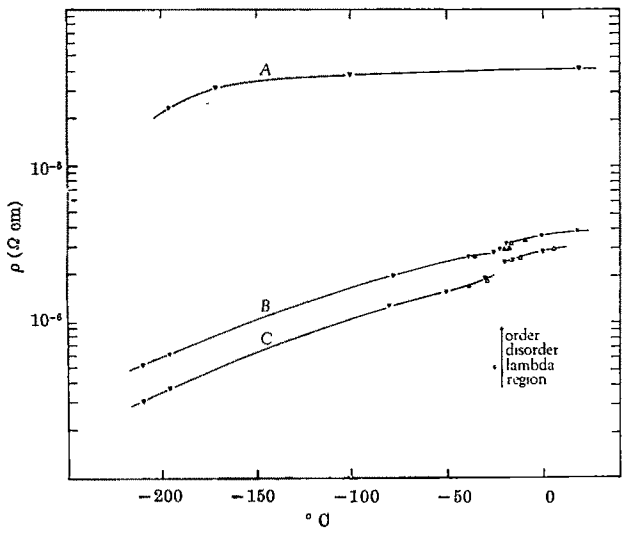


Fig 2. Resistivity of graphite nitrates *A*, Graphite (stress-annealed); *B*, graphite nitrate, second sequence; *C*, graphite nitrate, first sequence. ▼, Descending *T*, △, ascending *T*

perature is comparable with that of natural metallic conductors, such as silver, plotted for comparison in Fig. 3. The influence of residual crystal defects leading to some temperature-independent scattering can, however, be detected in the graphite nitrates, particularly at the lowest temperatures.

On conversion of the original graphite specimen to the nitrate at room temperatures, the crystal expands in the *c*-axis direction but remains unchanged in dimensions parallel to the *a*-axis. Corresponding resistivities for the disordered state, and for the ordered crystals below the lambda point, are recorded for a number of specimens in Table 1. It can be seen that values for the ordered first sequence of graphite nitrate are close to those of copper or silver at comparable temperatures.

Table 1 10^6 RESISTIVITIES (ohm cm)

Graphite specimen	First sequence At 273° K	First sequence At 230° K	Second sequence At 293° K	Second sequence At 230° K
31(2)1	2 84	1·68	3 96	2 57
31(4)5	3 26	1·99	3 68	2·21
31(2)4	3·38	2 02	3·99	2·60
31(4)2	3·45	2·09	3·66	2·32
32(4)2	3·77	2·32	4 06	2·65
9(3)6	3 87	2·51	4·91	3 12

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Black-body Radiation Law deduced from Stochastic Electrodynamics

SOME years ago, one of us developed, in collaboration with M. Spighel and C. Tzara, a stochastic version of Wheeler and Feynman's 'absorber' theory of radiation, with a classical 'zero-point' fluctuating field corresponding to residual interactions of all charged particles[1]. The energy spectrum was derived and found to be proportional to a universal constant—identifiable with Planck's constant *h*. In the framework of this stochastic electrodynamics it was possible to deduce results of a typically quantum flavour, such as the existence of a stationary ground-level for the harmonic oscillator[2]. A weaker but similar result was announced later by T. Marshall[3,4].

Recently, we showed that a new (apart from a very brief suggestion by A. Sokolov and V. Tumanov[5]) but rather natural definition of momentum and energy would dispense with any substrating or 'cut-off' procedures. In this manner further results were obtained in the stochastic model, such as a 'Lamb-like shift' without any *a priori* quantification[6].

Now Planck's energy distribution for thermal radiation within a black-body can be viewed as the energy spectrum of a random electromagnetic field in the body[7]. Therefore it seems natural to look for a derivation of this law based only on the assumptions of our model.

In the search for such a derivation it must be remembered that classical statistical mechanics applied to thermal radiation phenomena lead to an 'ultra-violet catastrophe' as a consequence of the theorem of equipartition

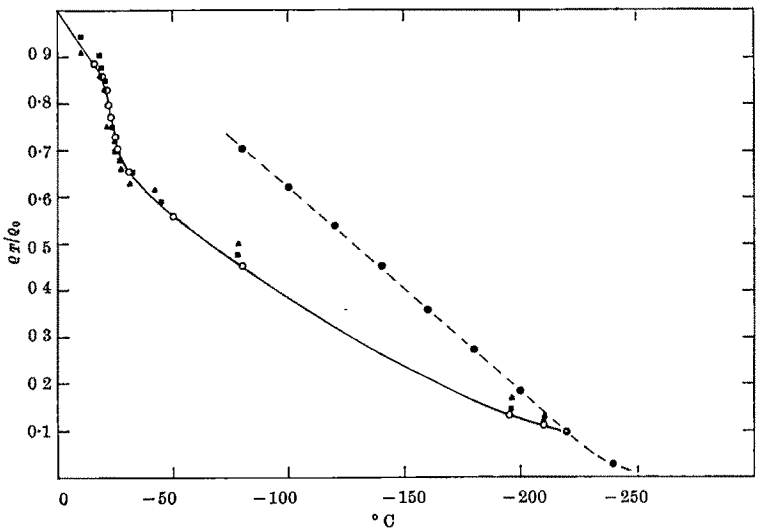


Fig. 3. Resistivity ratio at temperature *T* relative to 0° C First sequence graphite nitrate. Specimen: ○, 31(2)1; ▲, 31(4)5; ■, 31(2)4; ●, silver

of energy. But the theorem itself results from a computation of the average energy which strongly depends, through the canonical distribution, on the classical Hamiltonian. This Hamiltonian is the sum of individual particle Hamiltonians and neglects interactions, while it is understood that a very small interaction still exists and is responsible for the existence of an equilibrium[8].

Now as the black-body to be described here is a system of charged particles in motion, our definitions in ref. 6, equation (5), apply and we can write:

$$P_{\text{electrokinetic}} = m\dot{x} - \frac{2e^2}{3c^3}\ddot{x} + A_{\text{random}} \quad (1)$$

$$E_{\text{electrokinetic}} = (P_{\text{electrokinetic}})^2/2m \quad (2)$$

$$E_{\text{tot}} = E_{\text{electrokinetic}} + E_{\text{pot}} \quad (3)$$

where the symbols have their normal meaning.

The use of equation (3) for the computation of total energy eventually changes the issue of the equipartition theorem. An interaction term which can no more be neglected is to be added to the standard kinetic energy term, namely $kT/2$ per degree of freedom. It is hoped that divergences will be eliminated with the help of our new definition of total energy in the same way as they have been eliminated for the case of an isolated harmonic oscillator.

Therefore our procedure consists of finding a direct evaluation of the interaction energy in the case of a system of linear harmonic oscillators in thermal equilibrium with a black-body at the temperature T. Assuming a body large enough to justify a dipolar approximation, and calling $E_i(\nu)$ and $\overline{E_i}(\nu,T)$ the maximum interaction energy and the average interaction energy, respectively, of an oscillator of natural frequency ν, we have the classical formula:

$$\overline{E_i}(\nu,T) = \frac{c\int_0^{\pi} e^{E_i(\nu)\cos\theta/kT}\, E_i(\nu)\cos\theta\sin\theta\,d\theta}{c\int_0^{\pi} e^{E_i(\nu)\cos\theta/kT}\sin\theta\,d\theta} \quad (4)$$

where θ is the angle of the axis of the oscillator with the direction of the field[9].

Straightforward integration leads to:

$$\overline{E_i}(\nu,T) = E_i(\nu)\, L\,(E_i(\nu)/kT) \quad (5)$$

where:

$$L(x) = \coth x - \frac{1}{x} \quad (6)$$

is Langevin's function, which makes apparent the analogy between this derivation and the classical theory of paramagnetism as already had been suspected in 1913 by Benedicks[10].

If $\overline{E_{\text{tot}}}\ (\nu,T)$ is the average of the total energy for an oscillator, and if one writes:

$$\overline{E_{\text{electrokinetic}}}(\nu,T) = \overline{E_{\text{kinetic}}}(\nu,T) + \overline{E_i}(\nu,T) \quad (7)$$

knowing that, for each degree of freedom:

$$\overline{E_{\text{kin}}}(\nu,T) = \overline{E_{\text{pot}}}(\nu,T) = kT/2 \quad (8)$$

one obtains from (3), (6), (7), (8) applied to (3):

$$\overline{E_{\text{tot}}}(\nu,T) = E_i(\nu)\coth\,(E_i(\nu)/kT)$$
$$= E_i(\nu)\left(\frac{2}{e^{2E_i(\nu)/kT} - 1} + 1\right) \quad (9)$$

A basic property of our model, ref. 6, equation (11), is that to the first approximation:

$$\overline{E_{\text{tot}}}(\nu,0) = \frac{h\nu}{2} \quad (10)$$

On the other hand, we have from equation (9):

$$E_{\text{tot}}(\nu,0) = E_i(\nu) \quad (11)$$

This gives:

$$E_i(\nu) = \frac{h\nu}{2} \quad (12)$$

which, added to equation (9), implies:

$$E_{\text{tot}}(\nu,T) = \frac{h\nu}{2}\left(\frac{2}{e^{h\nu/kT} - 1} + 1\right) \quad (13)$$

The central part played here by $\overline{E_{\text{tot}}}(\nu,0)$, which corresponds to the 'zero-point' field, is noteworthy. This, in addition to the elimination of the ultra-violet divergence, ensures self-consistency to our model, and answers positively a question raised by Einstein and Stern[11].

Finally, equation (13) leads, of course, to the black-body energy distribution law by well-known arguments (see, for example, ref. 12).

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METEOROLOGY

Formation of Depressions in the Indian Seas and Lunar Phase

IT was recently shown[1] that the occurrence of heavy rainfall (10 in. and above in 24 h) is related to lunar phase. It will be evident that such heavy falls are mostly associated with depressions, some of which intensify into cyclones and severe cyclones. A study was therefore undertaken to examine the dates of formation of depressions in the Indian Seas *vis-à-vis* the lunar phase. This communication was prompted by Bradley's article "Tidal Components in Hurricane Development"[2] and is intended to provide some additional information to support his conclusions.

The data for this investigation were taken from a publication issued by the Indian Meteorological Department[3] which catalogues depressions formed in the Indian Seas, and gives the date on which the depression stage was reached and its corresponding position.

The phase of the Moon for each day is given in terms of synodic decimals (hundredths of a synodic month) in the ephemeris by Carpenter[4]. The synodic decimal corresponding to each date was taken from that source and the frequency of formation of depressions corresponding to each synodic decimal was found. Successive ten-unit moving totals of the frequencies were then made, and of the hundred such totals thirty were chosen to correspond to the thirty days of the lunar month. For purposes of comparison the frequencies were expressed as percentages of the mean.

Fig. 1 shows the curve, prepared in this manner, of depressions which formed in the latitude band 19° N–22° N in the Bay of Bengal and Arabian Sea during the period 1877–1960. Fig. 2 shows the frequencies of occurrence of heavy rainfall (10 in. and more in 24 h) along the latitude band 20° N–22° N during the period 1891–1950, and is reproduced from my recent article[1]. A similarity is noticed in the trends of the curves in Figs. 1 and 2; their range is also comparable. The correlation between the two curves is 0·50. The correlation between the curves with 1 day

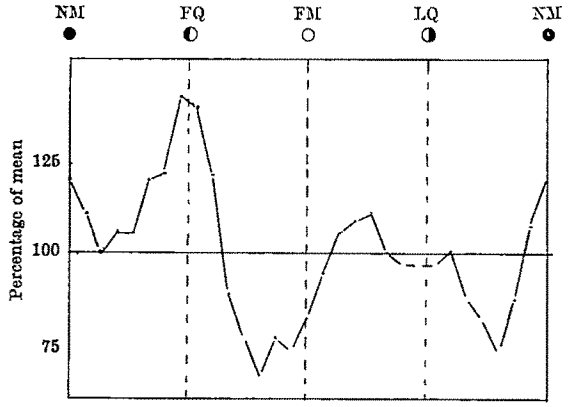


Fig. 1. Frequency of depressions (1877–1960) in the latitude band 19° N–22° N in the Bay of Bengal and Arabian Sea

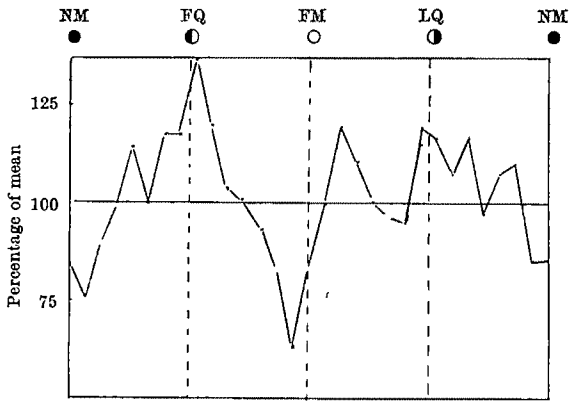


Fig. 2. Frequency of occurrence of heavy rainfall (10 in. and more in 24 h) along latitude band 20° N–22° N, India, 1891–1950

lag (heavy rainfall following depressions) is 0·57 with $\varphi = 0{\cdot}07$. There is a strong indication that the heavy falls were associated with depressions. The tidal influence on the formation of depressions is also indicated, as in both the curves the 14·765 day wave accounts for 48·1 of the total variance and is the largest component.

Any extra-terrestrial effect on meteorological phenomena should be established on a global scale. This calls for an analysis of data pertaining to various parts of the globe. This note, it is hoped, will stimulate interest in further studies of this kind.

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METALLURGY

Some Problems in the Use of Solid-state Galvanic Cells at Low Temperatures: the Determination of the Eutectoid Temperature of the Iron–Oxygen System

THE use of solid-state galvanic cells to obtain thermodynamic information is now well established, and the present work was carried out to obtain experience in the use of zirconia doped with lime, as solid electrolyte for oxygen ions only, at low temperatures. Previous experience with this electrolyte has been above 700° C, below which the resistance of the electrolyte rapidly increases and it becomes correspondingly more difficult to obtain information with useful accuracy. The major cause of inaccuracy at low temperatures is non-attainment of equilibrium in the electrodes. This can be severe where alloys are involved and may limit the lowest temperature at which reliable equilibrated results can be obtained.

In order to test the accuracy of the results obtained within the temperature range 400°–600° C, the eutectoid temperature of the Fe–O system was measured by two methods involving galvanic cells. The first method involved measurements between 400° and 1,000° C, while the second involved measurements between 600° and 1,000° C, and, as will be seen, is inherently more accurate. By comparing the results of both methods some idea can be gained of the accuracy or reliability of the results obtained in the range 400°–600° C.

The electrode systems involved (Ni + NiO, Fe + Fe_3O_4, Fe + FeO) were either so rigidly stoichiometric or of such high diffusion properties that equilibrium was quickly established, only at the lowest temperatures used was the time to reach equilibrium greater than a few hours.

If the standard free energies of formation of FeO and Fe_3O_4 are plotted against temperature, the two curves cross at the eutectoid temperature, that is, the temperature of the Fe–O system at which Fe, FeO and Fe_3O_4 exist together in equilibrium. As may be seen from the phase diagram Fig. 1*A*, the oxides FeO and Fe_3O_4 are stable above and below the eutectoid temperature, respectively, in the simple Fe–O system. Experimentally it is more convenient to consider the free-energy change when nickel oxide is reduced by iron to nickel and iron oxide according to the equations:

(above eutectoid temperature)

$$NiO + Fe = FeO + Ni \qquad \Delta G_1^0 \qquad (1)$$

(below eutectoid temperature)

$$NiO + \tfrac{3}{4}Fe = \tfrac{1}{4}Fe_3O_4 + Ni \quad \Delta G_2^0 \qquad (2)$$

The standard free-energy change of this reaction can be studied following Kiukkola and Wagner[1] by using an electrolytic cell with mixtures of iron and iron oxide, nickel and nickel oxide as electrodes and zirconia doped with 15 mol per cent calcium oxide as solid electrolyte, that is:

(above eutectoid temperature)

$$Ni + NiO \mid ZrO_2 + 0{\cdot}15\ CaO \mid Fe + FeO \qquad (I)$$

(below eutectoid temperature)

$$Ni + NiO \mid ZrO_2 + 0{\cdot}15\ CaO \mid Fe + Fe_3O_4 \qquad (II)$$

On passing 2 farads through these cells the displacement reaction (1) and (2), respectively, is caused to occur and the free energy associated with the reaction may therefore be measured under open circuit conditions as a function of the electromotive force of the cell:

$$\Delta G_1^0 = -2FE_I$$

or

$$\Delta G_2^0 = -2FE_{II}$$

Obviously the eutectoid temperature is just as easily detected if E instead of ΔG were plotted against temperature.

The cells were made up quite conventionally, the electrolyte disks being prepared according to the method proposed by Kuikkola and Wagner[1]. The electrodes were made by pressing pellets of mixtures of metal and metal oxide powders. Spectrographically pure iron, nickel, iron oxide and nickel oxides were used and the experiments were carried out under an atmosphere of purified nitrogen[2].

Below 700° C the initial experiments showed lower values of electromotive force than predicted from known thermodynamics, although the same cells had repeatedly

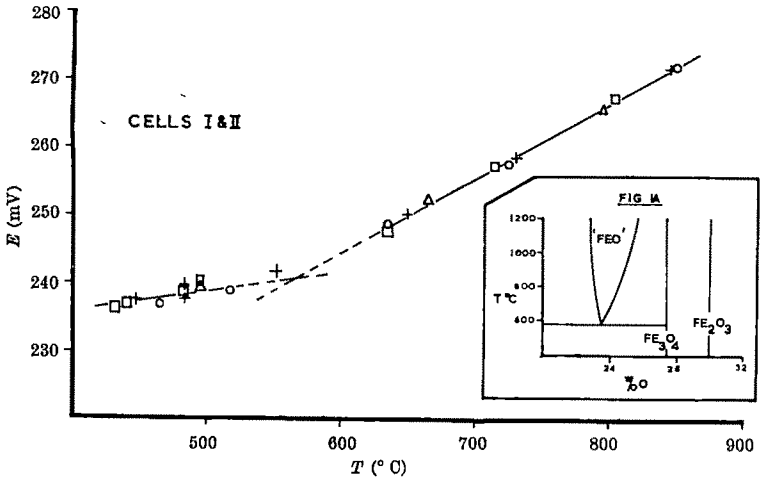


Fig. 1

given correct values at higher temperatures. This result would be expected, however, if the electrolyte were porous, thus allowing oxygen to diffuse as gas from the high oxygen potential electrode. Unless interdiffusion processes are swift enough to maintain equilibrium throughout the electrodes, such permeation of oxygen could cause an oxygen-rich layer to be established at the interface between the electrolyte and the low oxygen potential electrode (Fe + FeO) and, possibly, also an oxygen-depleted zone at the interface between the electrolyte and the high oxygen potential electrode (Ni + NiO). The electromotive force measured under these conditions would be lower than expected from the known thermodynamics—as observed.

This explanation was tested in two ways. First, a new batch of electrolyte was fired to 1,600° C instead of 1,450° C, and this proved to give more reliable results at low temperatures, probably due to more extensive sealing of the pores and consequent elimination of many of the direct migration paths for oxygen gas molecules through the electrolyte. Secondly, the suspected porous electrolyte was used to make a cell of lower electromotive force:

$$Fe + FeO/ZrO_2 + CaO/FeO + Fe_3O_4 \qquad (III)$$

In this case the driving force for diffusion of oxygen through the pores is lower, also the interdiffusion processes in the FeO/Fe_3O_4 electrode are faster than in the Ni/NiO electrode; consequently the results were as predicted from thermodynamics right down to the eutectoid temperature where the electromotive force of cell (III) becomes zero.

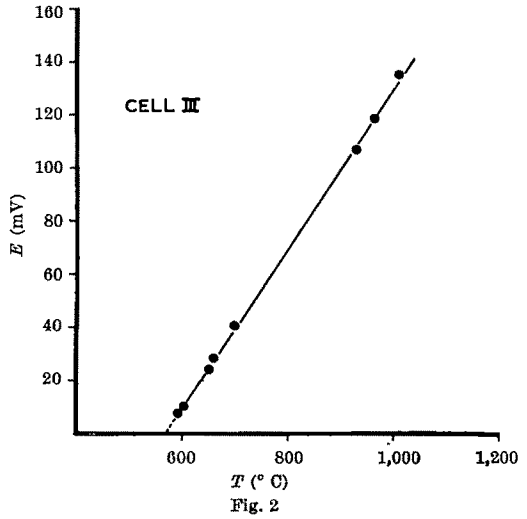


Fig. 2

Subsequent experiments were made using electrolyte tablets about 1 mm thick and 10 mm diameter (giving 10^6 ohms at 400° C), fired at 1,600° C, which produced an electrolyte sufficiently dense for reliable results to be obtained down to about 400° C.

By using a Cambridge 41127/2 galvanometer, readings could be made to within about ±1 mV at 400° C where the cell resistance was about 10^6 ohm.

Fig. 1 shows the results of several runs made using cells (I) and (II), the lines through the points being drawn according to statistical least squares analysis. According to these results the eutectoid temperature lies at 565° C.

The data of Fig. 1 show that reliable results may, with care, be obtained from this type of galvanic cell, at temperatures down to 400° C; however, the plot of Fig. 1 is not ideal for determination of the eutectoid temperature since it involves the extrapolation of two lines (one of which is not very well defined) to meet obliquely. A better method would be to use the cell:

$$Fe + FeO/ZrO_2 + CaO/FeO + Fe_3O_4 \qquad (III)$$

the electromotive force of which is zero below the eutectoid temperature since then both electrodes consist, at equilibrium, of $Fe + Fe_3O_4$. This cell was investigated down to the triple point and the data are plotted in Fig. 2. In this case only a short extrapolation of one well-defined line is required and the intersection with the temperature axis at $E = 0$ gives the eutectoid temperature, which in this case is also given to be 565° C. This is in excellent agreement with 565° C from Fig. 1 and the value of 560° C given by Darken and Gurry[3].

The aim of this report, however, is not simply to provide an alternative estimation of the eutectoid temperature of this system but to emphasize that such determinations may be carried out relatively easily. Using this technique, all that is required is an impervious furnace tube and an inert atmosphere, that is, by removing oxygen and the gases involved in redox systems, such as hydrogen water vapour, carbon monoxide and carbon dioxide, from argon or nitrogen. Under these conditions the galvanic cells used here will operate reliably for days or weeks on end; to obtain results the temperature is simply changed and the system allowed to equilibrate; the time for this process depends on the system under investigation and cannot be improved by alternative methods of measurement.

Most of this work was carried out at the United Steel Companies, Swinden Research Laboratories.

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CHEMISTRY

A New Calcium Silicosulphate

A COMPREHENSIVE investigation is in progress of high-temperature phase equilibria in the system $CaO—SiO_2—SO_3$ which is important in the chemistry of cements. A new compound has been located of molar composition at present best represented as $(2CaO.SiO_2)_2.CaSO_4$. This compound has been prepared from calcium carbonate (99·9 per cent pure), crushed quartz (99·95 per cent pure) and AnalaR $CaSO_4 2H_2O$ by ignition for 150 h at 1,150° C

in platinum boats in a platinum-rhodium resistance furnace. For successful preparation the mixture has to be finely ground to pass a 300-mesh sieve in between periods of heating. The X-ray diffraction pattern of the new compound first became discernible after about 2 h of heating. On continued heating, the X-ray diffraction patterns of the reactants and of dicalcium silicate became progressively weaker until, after 150 h, they reached intensity-levels representative of trace quantities.

The X-ray diffraction pattern of the new compound is given in Table 1. These measurements were made at room temperature using a Guinier-type focusing camera, 114 mm diameter with copper-$K\alpha$ radiation. Relative intensities (I) were estimated visually on an arbitrary scale from 1 (very weak) to 10 (very strong). Reflexions of intensities 2 or less have not been included in Table 1.

Table 1. ROOM TEMPERATURE X-RAY POWDER MEASUREMENTS FOR A NEW CALCIUM SILICOSULPHATE

d	I	d	I	d	I	d	I
8·51	5	2·844	10	2·136	3	1·775	4
7·71	5	2·825	10	2·119	3	1·754	6
5·69	5	2·797	3	2·083	3	1·748	4
5·34	4	2·740	3	2·061	5	1·739	4
4·57	8	2·703	3	2·022	3	1·710	3
4·24	3	2·611	8	2·015	4	1·698	6
4·08	7	2·564	9	1·958	6	1·676	3
3·95	7	2·479	4	1·947	3	1·668	4
3·85	3	2·382	3	1·926	3	1·614	3
3·60	3	2·354	3	1·895	9	1·576	3
3·34	7	2·287	5	1·891	9	1·563	3
3·19	9	2·230	4	1·848	3	1·551	3
3·12	7	2·201	4	1·837	5	1·528	6
3·04	7	2·169	5	1·820	4	1·512	3
2·99	7			1·804	5	1·489	3
2·98	7			1·780	4	1·385	3
						1·367	5

The preparation was in the form of small anhedral equant grains. The refractive index of the material in bulk was 1·635 and of isolated elongated grains slightly lower than 1·635. Birefringence of these crystals was very low and this, together with the very small size of the crystals, has so far prevented a more accurate estimation of optical properties. The density of the new compound is 2·95 g cm^{-3}.

The X-ray diffraction pattern in Table 1 resembles that of a compound detected by Sundius and Peterson[1] as a green mineral which colours the sulphate rings accumulating in cement rotary kilns. Differences between the two patterns include the recognition of doublets in place of single high-intensity lines reported by these authors. The high resolution attainable with the focusing X-ray camera used in the present work may account for this difference. The pattern in Table 1 is also richer than that reported by Sundius and Peterson, although low-intensity reflexions have been excluded from the present report. Sundius and Peterson ascribed to their compound the formula $Ca(Na_2K_2)\ SO_4$: silicate = 1 : 1·75 in which the silicate component was given the formula $Ca(MgFe)O{:}SiO_2 = 2{\cdot}17{:}1$. Their synthetic preparation contained 1·4 per cent MgO, 1·4 per cent alkalis and 0·1 per cent FeO. In the present work the preparation believed to lie in the ternary system $CaO—SiO_2—SO_3$ contained $< 0{\cdot}02$ per cent MgO, $< 0{\cdot}01$ per cent alkalis and 0·01 per cent FeO.

The optical properties of the two compounds are also similar and both materials are coloured green in ordinary light. It is probable that the compound found in the kiln rings is an impure form of the calcium silicosulphate which has been isolated.

The new compound has been detected in a range of compositions in the system $CaO—SiO_2—SO_3$ in preparations of carefully controlled composition which were annealed at approximately 1,100° C in platinum capsules fully sealed by welding. The phase assemblages between $(2CaO.SiO_2)_2.CaSO_4$ and adjoining compounds are being investigated. It has been established that the X-ray diffraction pattern of the new phase is the same in these different compositions in agreement with the proposal that this phase is a compound and not, for example, a solid solution. This calcium silicosulphate has been examined in a high-temperature X-ray camera[2] in a stream of pure nitrogen at successively higher temperatures and its X-ray diffraction pattern recorded up to 1,150° C. The present evidence indicates decomposition not far above this temperature.

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Performance of Oxygen Fuel Cell Cathodes catalysed with Boron Carbide

THE development of suitable inexpensive electrode catalysts is one of the major tasks confronting fuel cell technologists. The platinum group metals have received considerable attention as catalysts, despite their high cost, because of their combination of stability and high catalytic activity. It has been pointed out[1], however, that only a small amount of the world demand for power could be generated by hydrogen/oxygen fuel cells using the entire world production of platinum. The situation is equally unattractive for the other platinum-group metals. Consequently, for any broad-based terrestrial application of fuel cells, it is necessary to find a cheaper and more readily available material. There have been several publications describing some anode[2–6] and cathode[7,8] catalysts that are cheaper than the platinum-group metals.

This report presents some results with boron carbide as a cathode catalyst in alkaline fuel cells. Boron carbide is an electronic conductor, is available inexpensively in powder form, and is resistant to corrosion by fuel cell electrolytes. It also has been reported to be resistant to electrochemical oxidation[9].

The catalyst mixture, containing a 1:1 by weight ratio of boron carbide of 9·5-μ average particle size (obtained from the Norton Company) and 'Teflon' from the *TFE* 3170 dispersion (obtained from E. I. du Pont de Nemours and Co., Inc.), was applied to porous nickel plaques which were afterwards made waterproof by a method similar to that of Niedrach and Alford[10]. The geometric area of the electrode exposed to the 94° ± 2° C 45 per cent potassium hydroxide flowing electrolyte was 48 cm^2. Electrode spacing was 3 mm. Electrode potentials were measured against an external mercuric oxide reference electrode and included a small IR loss of about 40 mV at 100 m.amp/cm^2.

The results of cathodic polarization measurements taken after about 40 h of operation at 50 m.amp/cm^2 are presented in Fig. 1 as open symbols. The closed symbols represent data taken after a minimum of 320 h continuous operation at 50 m.amp/cm^2. Curves A and B are for standard supported precious metal catalysed carbon and uncatalysed carbon electrodes, respectively, and are given for reference purposes. Curve C shows the polarization for boron carbide at three different catalyst loadings—1·5, 4·4, and 9·2 mg/cm^2. It is very unusual that the catalyst loading should have no influence on the polarization. The performance of the electrodes with 1·5 and 9·2 mg/cm^2, when operated at 50 m.amp/cm^2 continuously, however, does diverge at operating times in excess of about 50 h. Curves C and D of Fig. 2 show how these electrodes perform at constant current density as a function of time. The polarization of these two electrodes, measured at times in excess of 320 h, is shown in Fig. 1 by curves E and F. The electrode with the least amount of boron carbide (curve F) changes most with time. Thus it appears that the catalyst loading does influence the electrode performance even though it takes some time for such differences to become evident.

The cause of this decay in performance with time may be due to an impurity in the boron carbide. Consequently,

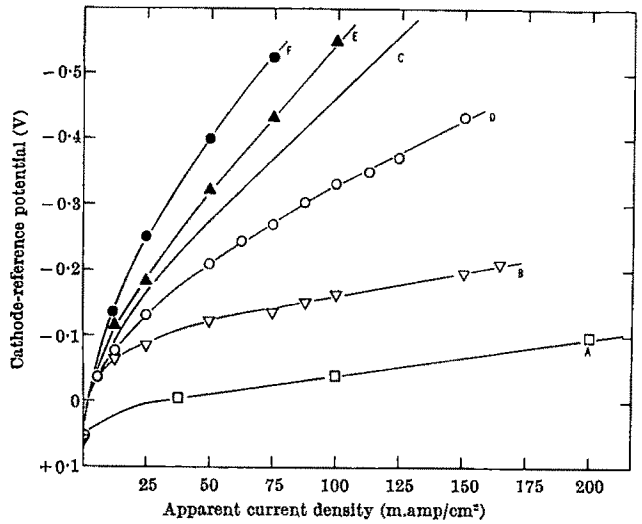


Fig. 1. Cathodic polarization for electrodes in KOH solution, $T = 94 \pm 2°$ C; open symbols, data taken after 40 h at 50 m.amp/cm²; closed symbols, after 320+ h. Curve *A*, catalysed carbon; *B*, uncatalysed carbon; *C*, electrodes containing 1·5, 4·4 and 9·2 mg/cm² boron carbide; *D*, potassium hydroxide washed boron carbide electrodes containing 2·0 and 4·4 mg/cm² catalyst; *E*, electrode containing 9·2 mg/cm² boron carbide after 380 h at 50 m amp/cm²; *F*, electrode containing 1·5 mg/cm² boron carbide after 325 h at 50 m amp/cm²

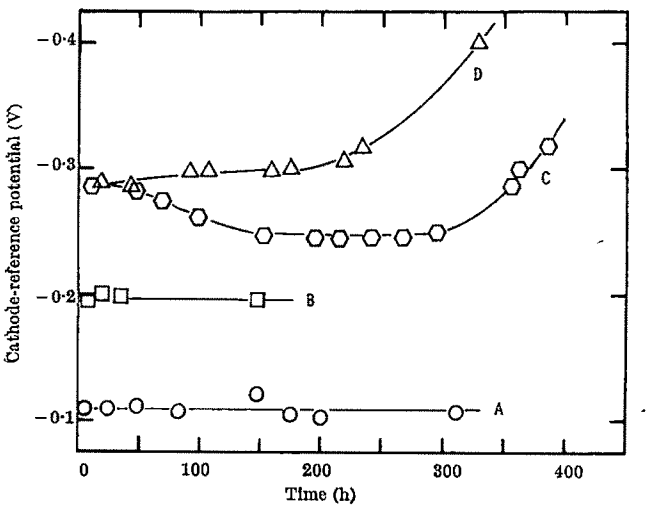


Fig. 2. Cathodic potential-time variation for electrodes operated at 50 m.amp/cm² at $T = 94 \pm 2°$ C, Curve *A*, uncatalysed carbon; *B*, washed boron carbide electrode containing 4·4 mg/cm² catalyst; *C*, unwashed boron carbide electrode containing 9·2 mg/cm² catalyst; *D*, unwashed boron carbide electrode containing 1·5 mg/cm² catalyst

some boron carbide was washed in the following manner before electrode preparation. First, the material was given four 24-h soakings in 6 M potassium hydroxide followed by repeated water rinse to remove potassium hydroxide, and then two 2-h soakings in 30 per cent sulphuric acid followed by several water rinses. It was then dried overnight in a vacuum desiccator. The potassium hydroxide solution, in which the boron carbide powder had been soaked, turned yellow. No attempt was made to analyse this coloured solution. Analyses of the powder, however, revealed that the iron, copper and sulphur content were reduced about an order of magnitude to 0·2 per cent, 0·01 per cent and 0·02 per cent respectively.

The cathodic polarization for electrodes containing 2·0 and 4·4 mg/cm² of the washed boron carbide taken after 40 h of operation at 50 m.amp/cm² is shown in Fig. 1 as curve *D*. Considerable reduction in polarization was achieved by lowering the amount of impurities in the boron carbide sample, but the polarization did not depend on the catalyst loading. A life test of an electrode containing 4·4 mg/cm² of boron carbide was terminated at only 150 h because of the hydrogen anode failure. No change occurred in the performance of the washed boron carbide cathode during that time (curve *B*, Fig. 2). It appears that washing the boron carbide in potassium hydroxide before electrode preparation will improve the initial performance as well as the lifetime of the electrode. Further reduction in the polarization might be possible by further reduction of impurity content.

Optical examination of the boron carbide powder at 400 magnification showed that the sample had many particles in the 0·5 to 2·0μ range as well as some as large as 40μ.

From these data on polarization and lifetime, one would not expect the boron carbide catalyst to replace the usual precious metal catalysts commonly being used. Because of inert electrochemical behaviour, however, boron carbide might be advantageously used as a catalyst support.

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Possible Role of Supercooling in the Spearpoints observed during the Combustion of Zirconium Droplets

RECENTLY, I reported on the spear-like images that appear on photographic time exposures of zirconium droplets burning in free fall[1]. The experiments indicated that the spearpoints coincided with solidification of the incandescent droplets.

I suggested that the sudden intensification at the shoulder of the spearpoint might be caused by a large increase in emissivity as the droplet crystallized. This was supported mainly by the change in the colour at room temperature of droplets quenched in liquid argon slightly above and below the shoulder; those quenched above were black while those quenched below were grey. By comparing the colours with those described in the literature, the composition of the droplets was estimated to be approximately $ZrO_{1.97}$ at the point of solidification.

There is another possible explanation of the spearpoints. The gradual decrease and sudden increase in intensity may be caused by a supercooling of the glowing oxide droplets before they solidify. Rapid release of the heat of crystallization may then raise the temperature of the droplets to the normal freezing point, and yield a more intense thermal emission at this new, higher temperature. A similar process, known as recalescence or the 'blick' effect, occurs when droplets of metal solidify[2,3]. Supercooling is particularly favourable in small droplets because a molten mass is progressively less likely to contain nucleation singularities as its size decreases[3].

This communication reports new experiments involving vibrational nucleation of burning zirconium droplets which suggest that supercooling may participate significantly in the formation of spearpoints.

An attempt to distinguish between intensification by solidification after supercooling and an increased emittance on freezing was made by causing the burning droplets to collide with an aluminium plate at various times during fall. This was accomplished by suspending an aluminium

plate inclined a few degrees from the vertical in the combustion chamber[4]; the plate was arranged so that the burning droplets would strike it at a glancing angle and travel down the chamber with only slight deviations from their original paths. (They did not adhere to the plate at any stage of combustion.) Experiments were performed with 525μ droplets of zone-melted zirconium burning in ultra-pure oxygen at 625 torr as described earlier[1]. The burning droplets were photographed simultaneously with both a still camera and a high-speed ciné camera. With the latter, it was possible to time events to ±0·1 msec.

Without collision, the shoulders of the normal spearpoints formed reproducibly at 217 ± 6 msec after the heating flash. When the droplets struck the plate between 74 and 152 msec after ignition, spearpoints formed at 169 ± 10 msec in twelve out of eighteen experiments; the other six droplets intensified at the normal time. When the collisions occurred between 152 and 200 msec, only three out of thirteen droplets intensified at the normal time. In the remaining ten experiments, the spearpoints were completely suppressed; that is, after collision, the droplets lost intensity smoothly and more slowly until the images disappeared at about the same time as the non-colliding droplets. This is interpreted as indicating that solidification occurred without appreciable supercooling during this time interval. Collision with the plate after 200 msec did not affect the formation of spearpoints.

These results suggest: (1) that nucleation was induced with high probability by deflecting the burning droplets from an aluminium plate; (2) that the droplets had cooled to the true freezing point at 152 msec, the earliest time at which solidification could be induced by mechanical shock.

Assuming that only supercooling was involved in the intensifications, I estimated the amount of supercooling, $\Delta T_- = T(\text{solid}) - T(\text{liquid})$, from the densities of the photographic images. Six separate experiments were performed with 525μ droplets of zone-melted zirconium burning in ultra-pure oxygen at 625 torr (again the same conditions as in ref. 1). The droplets were photographed on Kodak 'Plus-X Pan' sheet film through a 4·01 mm-thick Corning *CS*2-64 filter (transmittance less than 0·5 per cent at wave-lengths less than 620 mμ). The films were calibrated by passing the radiation from a xenon flash lamp through the same filter and a step tablet with known neutral densities. After careful development, the films were scanned 2-mm above and below the shoulders of the spearpoints normal to the direction of fall with a photoelectric densitometer.

The ratio of peak intensities obtained this way, $I(\text{solid})/I(\text{liquid})$, was 3·93 ± 0·26. If it can be assumed that the emittance of the droplets does not change during freezing, then the ratio of absolute temperatures at the shoulder becomes $T(\text{solid})/T(\text{liquid}) = (I(\text{solid})/I(\text{liquid}))^{1/4}$. If T(solid) can be taken to be approximately the melting point of stoichiometric zirconium dioxide, 3,100° K (ref. 5), T(liquid) is approximately 2,200° K. The supercooling, ΔT_-, then, is about 900° K, based purely on radiation intensities. (A rise in temperature of this magnitude is not forbidden from the point of view of energy. Taking the heat of fusion to be 20·8 kcal/mole (ref. 6) and the heat capacity of the melt to be 17·8 cal/mole deg (ref. 6), the temperature rise of molten zirconium dioxide on nucleation could be as large as 1,169° K.)

Using the foregoing assumptions for these combustions, $\Delta T_- \approx 0{\cdot}29\ T(\text{melting})$ (in °K) for the freezing oxide. This value should be compared with the supercooling which holds for the solidification of many metals[3], $\Delta T_- \approx 0{\cdot}18\ T(\text{melting})$.

As yet, it has not been possible to determine the relative importance of either supercooling or changes in emittance on the images of the solidifying droplets. Supercooling, however, is probably the dominant effect. This statement is supported by measurements above 2,200° K on stoichiometric zirconia[7], which indicate that at 6,500 Å, the spectral emittance of the solid is actually somewhat less than that of the liquid. It is also supported by the observation of spearpoints in burning droplets of many other metals as different as strontium[8], barium[8], calcium[8], manganese[8], tantalum[8], iron[8,9] and many ferrous alloys[9]. It thus seems more plausible to attribute spearpoints to supercooling—an effect which should be relatively insensitive to chemical composition—rather than to large emittance changes, which should strongly depend on the chemical nature of the system.

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Heat of Combustion of Benzoic Acid under Thermodynamic Standard Conditions

BENZOIC acid is widely used in thermochemistry as a calorimetric standard substance. This widespread usage as a standard means that benzoic acid is normally combusted in nearly identical bomb calorimeters. As a consequence it has become common practice to quote its heat of combustion under standard bomb conditions (ΔQ_B) as suggested by Washburn[1], rather than under thermodynamic standard conditions ($-\Delta E^\circ$), as this often eliminates the necessity for lengthy thermodynamic conditions. At present the standard bomb conditions for the combustion of benzoic acid are[2]: (*a*) The combustion reaction is referred to 25° C. (*b*) The sample is burned in a bomb of constant volume in pure oxygen at an initial pressure of 30 atm. at 25° C. (*c*) The number of grammes of sample burned is equal to three times the internal volume of the bomb in litres. (*d*) The number of grammes of water placed in the bomb before combustion is equal to three times the internal volume of the bomb in litres. For certain other applications, notably when benzoic acid is used as a starter for the combustion of a less easily combusted material, it is necessary to know its heat of combustion under thermodynamic standard conditions. Such a case arose recently at Harwell in a re-determination of the heats of combustion of natural and artificial graphites.

It is common practice to regard ΔE° as the gain in intrinsic energy on combustion of the benzoic acid in pure oxygen at 1 atm. pressure and 25° C (ref. 3), and as such its numerical value takes a negative sign. This definition is not strictly in accord with current thermodynamic usage which reserves the superscript zero for substances in their modern thermodynamic standard states. For a gas the standard state is defined as the pure substance in the ideal gas state at 1 atm. pressure, that is unit fugacity (internal energy content that of the real gas at zero pressure)[4]. Thus the symbol ΔE° strictly applies to the gain in intrinsic energy on combustion with the products and reactants in their thermodynamic standard states. Numerical values for ΔE° and ΔQ_B are calculated from the experimentally observed heat of combustion by the application of thermodynamic corrections. This method of derivation for ΔQ_B and ΔE°

requires that the difference between them (ΔQ_B – (– $\Delta E°$)) should be a constant since it depends on the thermodynamic corrections applied and not on the experimental values of the heat of combustion. The difference between ΔQ_B and – $\Delta E°$ has been calculated by me by the application of a suitable thermodynamic correction. The method used was a modified Washburn correction as described by Prosen[4] and the calculations were carried out on an I.B.M. 7030 ('*STRETCH*') computer. This calculation yielded a value for (ΔQ_B – (– $\Delta E°$)) of 20·3 J/g.

Values for ΔQ_B, the heat of combustion under standard bomb conditions, are always referred to 25° C, and it is conventional but not obligatory to refer values for $\Delta E°$ to the same temperature. The failure to refer values of $\Delta E°$ to 25° C has been the source of some confusion in the chemical thermodynamic literature. Two estimates of $\Delta E°$ for the combustion of benzoic acid have been published in the literature, one due to Fraser and Prosen[5] of – 26,411·6 J/g (corresponding to ΔQ_B = 26,434·7 J/g) and the other of – 26,418·6 J/g (ΔQ_B = 26,438·1 J/g) due to Gundry and Meetham[3]. These figures apparently lead to markedly different values for the thermodynamic correction (ΔQ_B – (– $\Delta E°$)). A close examination of the paper of Fraser and Prosen shows that their value for $\Delta E°$ is referred to 28° C and not 25° C. Correction of this figure to 25° C yields a value for $\Delta E°$ (25° C) of – 26,414·8 J/g. As discussed here the difference between ΔQ_B and – $\Delta E°$ should be a constant and, in fact, the figures are now seen to be in agreement. Fraser and Prosen find the difference to be 19·9 J/g, Gundry and Meetham 19·5 J/g and I find 20·3 J/g. In addition to these estimates of the Washburn correction, Jessup[6] has calculated it to be 20·0 J/g.

Absolute values for the heat of combustion of benzoic acid are normally quoted in the literature under standard bomb conditions (ΔQ_B). There have lately been a number of accurate determinations of this quantity[4,6–9] and before a value can be assigned to $\Delta E°$ some mean value must be assigned to ΔQ_B. This is necessary because few authors have calculated values for $\Delta E°$ from their experimental results, preferring to present them under standard bomb conditions. At present the best method for obtaining a mean value for ΔQ_B appears to be that advocated by Gundry and Meetham[3], who suggest the use of a weighted mean of the latest determinations. Table 1 is a summary of the latest information on the heat of combustion of benzoic acid under standard bomb conditions. It follows closely the approach of Gundry and Meetham with the following important exceptions: (i) The 1946–48 data of Coops *et al.*[8] have been omitted on account of the acknowledged incompleteness of some of their combustions[10]. (ii) The means were stated by Gundry and Meetham to have been weighted according to their standard errors. A re-examination of the original papers showed that the figures quoted by Gundry and Meetham are not always the standard errors. As a consequence of this the overall mean calculated by them contains an artificial bias. Accordingly, the errors quoted in Table 1 have been recalculated, where necessary, to the common basis of the 'uncertainty interval' as advocated by Rossini[11].

Table 1. HEAT OF COMBUSTION OF BENZOIC ACID

	Benzoic acid batch No.	Heat of combustion (ΔQ_B) J/g	Assigned uncertainty interval
Jessup, 1942 (ref. 6)	*N.B.S.* 39e	26,432·1	2·7*
	39f	26,434·2	2·7*
	"Specially purified"	26,434·7	3·8*
Prosen and Rossini, 1944 (ref. 7)	*N.B.S.* 39e	26,434·7	2·6
Coops and Adriaanse, 1954 (ref. 8)	*N.B.S.* 39f	26,433·9	4·9
	V.U.S.	26,438·1	7·8
Challoner, Gundry and Meetham, 1955 (ref. 9)	*B.D.H.* 692,782	26,436·4	4·4
Gundry and Meetham, 1957 (ref. 3)	*N.B.S.* May, 1949, Calorimetry Conference	26,438·1	5·4

* Calculated by me from data in Jessup's paper.

The overall weighted mean calculated from the data in Table 1 then becomes 26,434·4 J/g with an estimated overall uncertainty of ± 1·25 J/g. In accordance with normal practice the overall uncertainty interval ($2\sigma_m$) is estimated as:

$$\frac{1}{2\sigma_m} = \sqrt{\sum_1^n \frac{1}{(2\sigma_i)^2}}$$

where $2\sigma_i$ is the uncertainty interval for the ith set of observations. Estimation of the error through the foregoing formula suffers from the defect that it fails to distinguish any systematic errors in the means from one set of observations to another. An alternative estimate of the uncertainty interval may be made as:

$$2\sigma_m = 2\sqrt{\frac{\sum_1^n \left(\frac{1}{2\sigma_i}\right)^2 (\bar{x}_i - \bar{x}_m)}{(n-1)\sum_1^n \left(\frac{1}{2\sigma_i}\right)^2}}$$

where $\bar{x}_m$ is the overall mean. This second formula yields a value of ± 1·22 J/g for $2\sigma_m$. The close agreement between these two estimates for the overall uncertainty interval shows that there is no evidence of a significant bias in the data from the various sources.

It is therefore concluded that at present the best estimate for the heat of combustion of benzoic acid under standard bomb conditions (ΔQ_B) is 26,434·4 J/g with an estimated overall uncertainty of ± 1·2 J/g. The corresponding heat of combustion under thermodynamic standard conditions (that is, gain in intrinsic energy on combustion) is 26,414·1 J/g with an overall uncertainty of ± 1·2 J/g.

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π-Bonded Olefines and Olefine Hydrogenation

ALTHOUGH the catalysed hydrogenation of ethylene and other olefines has been a favourite reaction for investigation for many years, it has, in general, proved vastly more complicated than would appear from the simple stoichiometry of the reaction. Continuing investigations are, however, giving more insight into the numerous possible elementary steps involved in the overall reaction. In a recent review[1] Bond has emphasized the role of π-bonded olefines in the metal-catalysed hydrogenation of unsaturated compounds, although he points out that there is no evidence as to whether say π-bonded ethylene will react with hydrogen to give ethane directly, or whether a σ-diadsorbed olefine is a necessary intermediate.

It was therefore thought to be worthwhile to determine whether ethylene platinous chloride $(PtCl_2.C_2H_4)_2$ possessed any catalytic activity for the hydrogenation of ethylene;

it is accepted[2] that in this compound the ethylene is bound to the platinum by σ–π bonding, and might therefore serve as a crude model for ethylene adsorbed on platinum.

Ethylene platinous chloride was prepared according to the method of Anderson[3] from sodium chloroplatinate and ethanol: for ease in subsequent handling the compound was supported on alumina by adsorption from benzene solution. The ethylene used was taken from a cylinder, and was purified by repeated low temperature distillation. Hydrogen was standard tank electrolytic grade, and was passed through a 'Deoxo' unit and a trap at −196° C before use. The reactor was essentially a 'Pyrex' test-tube of approximately 5 ml. capacity, attached to a conventional high-vacuum system by means of a 10/30 standard taper joint.

In a typical run, approximately 0·5 g of the alumina-supported ethylene platinous chloride was weighed accurately into the reactor tube, which was then attached to the vacuum system. The reactor was rapidly pumped down to zero pressure, as measured with a di-*n*-butyl phthalate manometer, the reactor surrounded by an ice bath at 0° C, and ethylene added to a pressure P_E cm of di-*n*-butyl phthalate: hydrogen was then added to a total pressure $(P_E + P_H)$, with the hydrogen pressure P_H always being less than P_E. The change in pressure with time was followed and, in all cases, the pressure decreased by an amount P_H, after which the pressure remained constant. The only hydrogenation product detected by gas chromatography was ethane. During such a run there was no visible colour change in the compound (the alumina-supported material was pale yellow), and on subsequent reweighing no detectable weight change.

If hydrogen alone was added to the reactor under the same conditions rapid decomposition of the ethylene platinous chloride occurred, and black metallic platinum was obtained.

It may be concluded that in at least one case, π-bonded ethylene may react with hydrogen to form ethane without the need or possibility of a σ-diadsorbed intermediate. A quantitative investigation of this and similar reactions is in progress at the present time, and the results will be reported in detail elsewhere.

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BIOCHEMISTRY

Bone Lysozyme: Partial Purification, Properties and Depression of Activity by Parathyroid Extract

DURING an investigation of the metabolism of bone, high levels of lysozyme activity were found which could be markedly decreased by parathyroid extract, an agent known to induce fundamental changes in this tissue[1]. It was also observed that parathyroid extract caused the lysozyme content of other tissues to change. This report details these findings and describes a partial purification of the bone enzyme.

Weanling, fasted, male white rabbits, in groups of 2–4, received a subcutaneous injection of parathyroid extract (Lilly) 18 h before being killed by exsanguination. Similar groups of animals served as controls. The shafts or distal or proximal ends of the femurs were frozen in dry ice and pulverized. The powders were exhaustively extracted with ten volumes of M/15 phosphate buffer (*p*H 6·2) at 3° C. Soft tissues and marrow from the femur shafts were homogenized in the phosphate buffer. Blood was laked by shaking. After centrifugation, the supernatant fluids were assayed for lysozyme[2] with crystalline egg white lysozyme (Sigma) serving as standard. Enzymatic activity was expressed as μg of crystalline enzyme. Deoxyribose was determined by the method of Schneider[3]; in the case of bone, the tissue was first decalcified in 10 per cent trichloroacetic acid. Protein was assayed by the method of Lowry[4], nitrogen by a micro-Kjeldahl procedure[5] and serum calcium by flame photometry.

Depression of lysozyme activity by parathyroid extract. The greatest concentration of lysozyme on a fresh weight basis was found in distal femur and shaft marrow with smaller amounts in the proximal end and shaft (Table 1). The enzyme content of distal femur per μmole of deoxyribose was about five times that of marrow. In parallel studies the concentration of lysozyme per μmole of deoxyribose was equal to that of kidney and spleen and twice and three times that of lung and liver, respectively.

After administration of parathyroid extract, serum calcium increased by 0·5–2·3 m.equiv./l. indicating that the hormone was active. This treatment had no effect on total deoxyribose content, extracted protein and extracted nitrogen of any tissues studied. The dry weight and histological appearance of the bone did not change. The lysozyme activity, on the other hand, decreased from control-levels in every one of twenty experiments in all tissues except lung, where it increased. The greatest decrease in lysozyme activity occurred in proximal femur. On average, for all our experiments, the percentage changes in lysozyme content were as follows: proximal femur, −68; blood, −51; distal femur, −46; liver, −31; spleen, −30; marrow, −16; and lung, +45.

Table 1. LYSOZYME CONTENT OF NORMAL FEMUR*

Tissue	No. of samples	μg lysozyme per g	μg lysozyme per μmole deoxyribose
Distal femur	23	100 ± 25	13 ± 6
Proximal femur	5	53 (33–80)	—
Shaft	2	7·5 (5–10)	—
Shaft marrow	8	81 ± 18	2·7 ± 1·0

* 'g' refers to grams fresh weight of tissue. Standard deviation follows ± symbol; range is shown in brackets.

Depression of lysozyme activity in rabbit tissues induced by the *in vivo* administration of parathyroid extract is similar to the results of Litwack[6], who found that thyroid hormone depressed the level of lysozyme in rat kidney. He reported degrees of depression from 27 to 49 per cent which were generally less than the effects reported herein for bone. One explanation for the change of lysozyme activity by parathyroid extract might be the direct interaction of lysozyme with parathyroid hormone such as has been reported by Litwack[7] for thyroxine. Alternatively, there might be an interaction between the enzyme and products released from the tissue by the action of parathyroid extract. Another possibility is that, in bone at least, the change in lysozyme activity is secondary to an alteration in cellular type and/or a consequent shifting of enzymatic patterns[8].

Partial purification and properties of bone lysozyme. Distal femurs from mongrel rabbits were obtained commercially in frozen form and were extracted as already described. These extracts contained from 4 to 10 μg of lysozyme per mg of protein. The activity did not sediment after 60 min centrifugation at 144,000*g*. Under pressure it diffused through a 'Cellophane' membrane, which retained protein of greater than 30,000 molecular weight, at a rate equal to that of crystalline egg-white lysozyme. The crude extracts were stored at −12° C for more than 2 weeks with no loss of activity; heating at 100° C, *p*H 8, for 2 min destroyed all the activity.

The crude extracts were lyophilized and resuspended in one-tenth volume of distilled water and centrifuged. The pellet was discarded. This procedure resulted in complete recovery of activity with a two- to eight-fold purification. The supernatant solution was then passed through a column of 'Sephadex *G*-75' (10–30 ml. of gel

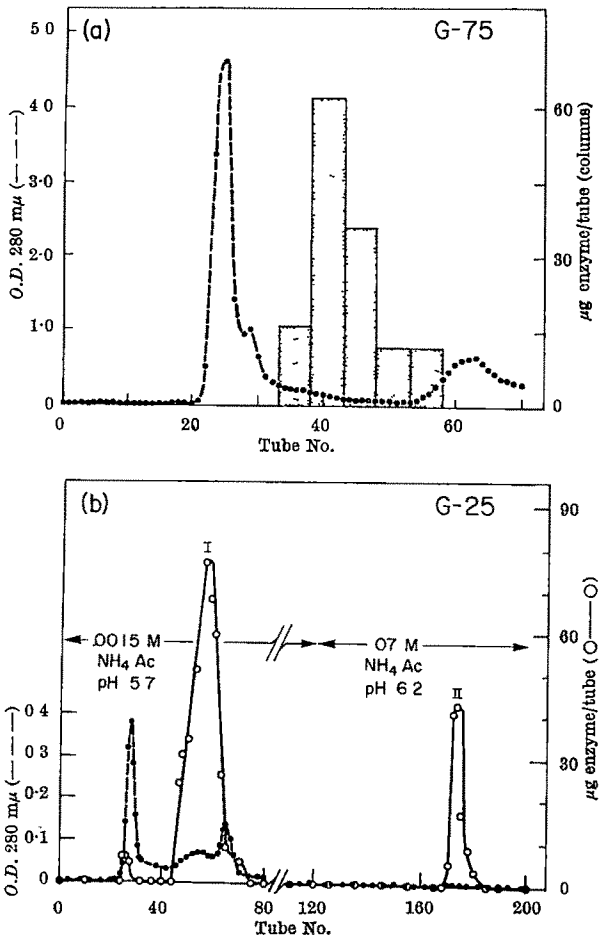


Fig. 1. Purification of bone lysozyme by gel filtration on 'Sephadex *G*-75' and '*G*-25'. The flow rate was 20 ml./h and the fraction size was 5 ml. *a*, Twenty-five ml. of solution containing 1,850 μg of lysozyme with a specific activity of 15·3 μg/mg protein was passed through the gel. The eluted lysozyme fraction contained 105 per cent of the starting activity with a specific activity of 107 μg/mg protein. *b*, Ten ml. of solution containing 1,290 μg of lysozyme with a specific activity of 177 μg/mg protein was applied to the gel. The specific activity of species I was 541 μg/mg protein and that of species II was 1,700 μg/mg protein (minimum)

bed volume per ml. of sample). The gel was eluted with M/15 phosphate buffer, *p*H 6·2, at 3° C. A typical elution pattern is shown in Fig. 1*a*. The enzymatic activity emerged from the column in a broad band following the elution of most of the protein (280 mμ). This procedure led to a fifteen-fold to twenty-five-fold purification with yields from 100 to 150 per cent—suggesting that a lysozyme inhibitor was removed during the procedure.

The pooled fraction, following concentration by lyophilization and a 4–6 h dialysis against distilled water, was applied to a 'Sephadex *G*-25' column and eluted with 0·0015 M ammonium acetate buffer, *p*H 5·7 (Fig. 1*b*). About two-thirds of the activity (species I) emerged from the gel after passage of an effluent volume about twice that of the void volume of the column. The remaining activity (species II) was next eluted with 0·07 M ammonium acetate, *p*H 6·2. This procedure resulted in an essentially complete recovery of activity. Species I was three-fold and species II ten-fold more pure than the starting material for this step. In four such procedures overall recovery, referable to the crude extract, was at least 100 per cent with a purification of about fifty-fold for species I and 200-fold for species II. The specific activities of species I and species II were about one-half and twice that of crystalline egg-white lysozyme, respectively.

Both species of lysozyme lysed *M. lysodeikticus*, *S. lutea* and *B. megaterium*. The optimal *p*H for activity against *M. lysodeikticus* for both was 6·1. That the species were dissimilar was indicated by a marked difference in heat stability. Both species retained full activity after heating at 88° C for 15 min at *p*H 2·5. At *p*H 8·0, however, species II was completely inactivated while species I retained 65 per cent of its activity.

In one investigation the procedure for isolating bone lysozyme was applied to shaft marrow. In elution from 'Sephadex *G*-25' and heat stability, marrow lysozyme resembled species II. Additional studies must be performed, however, before it can be concluded that marrow lysozyme and species II are identical.

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Active Copper Transport in Mammalian Tissues—a Possible Role in Wilson's Disease

IT has long been known that there are two major circulating fractions of serum copper. In the normal person, 95 per cent of the copper is carried by the alpha$_2$-globulin copper oxidase, ceruloplasmin, and the remainder was said to be 'free' or albumin-bound. A previous report[1] demonstrated a third physiologically important dialysable fraction of serum copper bound to amino-acids which facilitated the transport of copper across a semi-permeable membrane *in vitro*. The present investigation shows that there is an active transport mechanism for copper which operates in a metabolically active cell system. The findings lend themselves to interesting speculations with regard to the pathogenesis of Wilson's disease.

The uptake of copper-64 acetate by mammalian tissues was investigated, using fresh liver and kidney cortex slices from decapitated male Wistar rats. Ehrlich mouse ascites carcinoma cells grown in strain *A* mice for 6 days were also studied since they are known to concentrate amino-acids, using an active transport system. The technique of Christensen[2] was followed closely. Groups of liver slices (weighing 200 mg), rat kidney cortex slices (100 mg) or packed ascites cells were pre-incubated for 15 min in a freshly oxygenated Krebs–Ringer bicarbonate (KRB) buffer, *p*H 7·4. A purified 4 g per cent human albumin solution in KRB buffer of *p*H 7·4 was used as basic medium. Labelled cupric acetate was added to the medium in physiological concentration or in excess, with L-amino acids, individually or in combination, in equimolar concentration. The flasks were then incubated in a Dubnoff metabolic shaker, at 37° C, for 30, 60, 90, 120 and 180 min in a 95 per cent oxygen–5 per cent carbon dioxide atmosphere. Similar experiments were done at 20° C and with varying concentrations of oxygen.

Following incubation, the slices were immediately removed, or cells separated, washed three times with 20 ml. ion-free water, blotted, and counted in a Picker scintillation counter.

The ability of amino-acids, particularly histidine, to enhance the uptake of copper into liver slices and Ehrlich

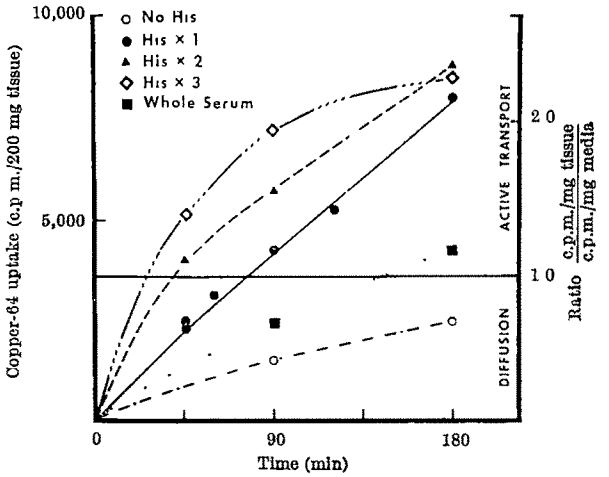


Fig. 1. The effect of histidine on copper-64 uptake into rat liver slices. Histidine (4, 8, and 12 μmoles); copper (10 μmoles); *p*H 7·4, 37° C, 95 per cent oxygen–5 per cent carbon dioxide atmosphere

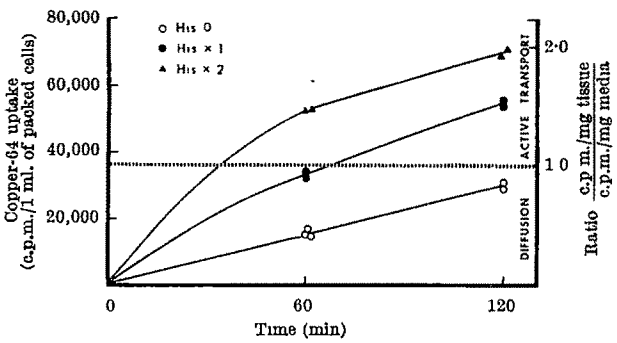


Fig. 2. The effect of histidine on copper-64 uptake into Ehrlich mouse ascites carcinoma cells, *p*H 7·4, 37° C, 95 per cent oxygen–5 per cent carbon dioxide atmosphere. Histidine (4 and 8 μmoles) and copper (10 μmoles) in each medium

mouse ascites tumour cells is shown in Figs. 1, 2, 3 and 4. The ratio of c.p.m. per mg tissue to c.p.m. per mg medium is also shown in each figure. Since a ratio less than unity means simple diffusion and one greater than unity suggests transport against a concentration gradient, the results indicate an active transport mechanism for copper facilitated by amino-acids, especially histidine.

In all experiments the rate of copper-64 uptake was related directly to the initial copper concentration in the medium; the more copper added, the more was taken up by the cells.

4-μM L-histidine significantly increases the uptake of copper-64 into rat liver slices (Fig. 1). Doubling and tripling the histidine concentration resulted in a further proportionate increase of copper-64 uptake until 2 h. However, at 3 h there is a maximal uptake, indicating that the transport system has reached its capacity, which is not related to copper but to the concentration of histidine.

The same results are obtained when Ehrlich mouse ascites cancer cells are used (Fig. 2).

Fig. 3 again shows the marked enhancing effect on copper-64 uptake of histidine (his). Glutamine (glu), threonine (thre), and asparagine (asp) show little effect. The combination of histidine, particularly with glutamine (his + glu) results in greater uptake of copper-64 than the sum of their individual effects. Amino-acid combinations not containing histidine (glu + thre, glu + asp, thre + asp, glu + asp), were relatively ineffective.

The subcellular distribution of copper-64, determined (by the technique of Hogeboom)[3] on liver slice homogenates after 2-h incubation, showed 69 per cent of their activity in the microsomes (38 per cent), mitochondria (25 per cent), and supernatant (6 per cent), confirming that the transported copper-64 was indeed inside the cell.

The transport, as facilitated by histidine and other amino-acids, was found to be dependent on temperature and oxygen. The uptake of copper-64 was significantly reduced at 20° C, and abolished in an anaerobic atmosphere. Atmospheric conditions reduced by 35 per cent the uptake of copper-64 into liver slices, but did not change uptake into the Ehrlich mouse ascites carcinoma cells.

Similar studies indicated that copper-64 was accumulated against a concentration gradient by slices of rat kidney cortex, facilitated only by histidine and not by its combinations, or other amino-acids (Fig. 4).

A cellular transport mechanism for copper has been shown to be linked with amino-acids, especially histidine. It fulfils the criteria and characteristics of an asymmetrical active transport system[4], that is, dependency on temperature, oxygen, concentration, and operation against a chemical concentration gradient.

This transport system appears to be similar in rat liver slices, tumour cells, and rat kidney cortex slices.

In recent years, some doubt has been cast on the aetiological theories for Wilson's disease. The 'ceruloplasmin deficiency theory'[5] fails to explain the disturbances in

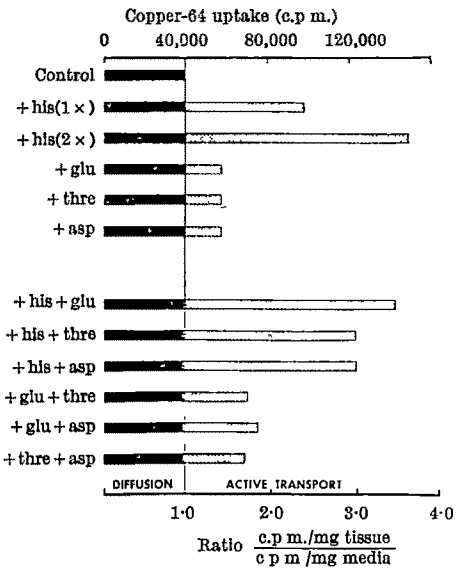


Fig. 3. The effect of different amino-acids and some of their combinations on copper-64 uptake into rat liver slices. All amino-acids are in equimolar (4 μmoles) concentrations. Incubation time was 2 h at *p*H 7·4, 37° C, and 95 per cent oxygen–5 per cent carbon dioxide atmosphere

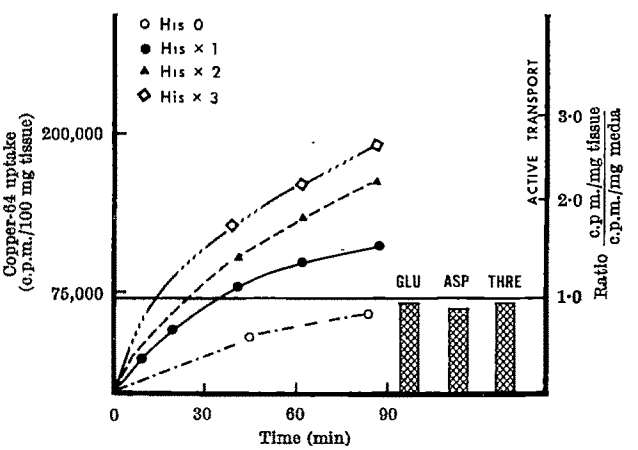


Fig. 4 The uptake of copper-64 into rat kidney cortex slices as facilitated by histidine (4, 8, 12 μmoles). The bars on the right show that glutamine (glu), asparagine (asp), and threonine (thre) are ineffective; *p*H 7·4, 37° C, 95 per cent oxygen–5 per cent carbon dioxide, copper concentration (5 μmoles)

amino-acid metabolism, and the complete lack of correlation between the degree of ceruloplasmin deficiency and the severity of the clinical findings. The failure to determine any genetic relationship in heterozygotes, using ceruloplasmin as the variable, has further weakened this viewpoint. The theory postulating an abnormal tissue protein with a high affinity to copper[6] has had only one proponent, and the findings have never been duplicated[7,8].

The oft-reported[9–11] delayed and diminished uptake of intravenously administered radioactive copper by the liver in patients and heterozygotes, and the elevation (6–20 times normal) of non-ceruloplasmin-bound serum copper[11], suggest that a defective transport mechanism for copper may be the underlying disturbance in patients with Wilson's disease.

The present investigations demonstrate the presence in tissues of an active transport system for copper which is dependent on histidine and perhaps other amino-acids. Impairment of this transport system is postulated as the basic metabolic defect in Wilson's disease. An investigation is now in progress on a patient with Wilson's disease and the related heterozygotes.

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Isolation of Δ^7-Cholesten-3-one from Butterfat

THE direct reaction of butterfat with 2,4-dinitrophenylhydrazine has resulted in the identification of a number of previously undescribed carbonyl compounds[1,2] from dairy products. During the course of those investigations a 2,4-dinitrophenylhydrazone (hereafter abbreviated to DNPhydrazone) was observed with chromatographic properties similar to the aldehyde DNPhydrazones but differing in light absorption properties. Initial studies revealed that the unknown DNPhydrazone gave a positive response to the Liebermann-Burchard reaction. This communication presents some investigations in which the compound has been identified as the DNPhydrazone of Δ^7-cholesten-3-one.

500 g butterfat was dissolved in 1·5 l. of carbonyl-free hexane[3]. The solution was divided and each half was passed through a 10 g 2,4-dinitrophenylhydrazine reaction column[4]. The DNPhydrazone monocarbonyl fraction was isolated from the fat by adsorption chromatography on magnesia ('Seasorb 43') and alumina columns according to the method of Schwartz *et al.*[4]. The saturated aldehyde DNPhydrazone fraction (tan in colour) was isolated from the monocarbonyl DNPhydrazones by adsorption chromatography on a 'Seasorb 43': 'Celite 545' column using 2 per cent methanol in chloroform as the eluting solvent[5]. The DNPhydrazone giving a positive Liebermann-Burchard reaction was separated from the long-chain aldehyde DNPhydrazones on a reverse phase partition chromatographic column prepared from 8 g of dodecane mechanically ground into 15 g of silanized 'HyFlo Super-cel' and packed into the column as a slurry. The eluting solvent was acetonitrile saturated with dodecane. The sample was dissolved in the eluting solvent by gentle heating and placed on the column. Several such columns were necessary to accommodate the quantity of DNPhydrazones obtained from 500 g of milkfat. The major band following the elution of the long-chain aldehyde DNPhydrazones was collected and evaporated to near dryness on a steam bath with the aid of a stream of nitrogen. The residue was taken up in chloroform and the residual dodecane removed from the DNPhydrazone by adsorption of the latter on a 'Seasorb 43': 'Celite 545' (4 g : 8 g) column. The DNPhydrazones adsorbed on the column were washed with 50 ml. of chloroform and the grey band eluted ofrm the column with 2 per cent methanol in chloroform. The effluent containing the Liebermann-Burchard positive DNPhydrazone was evaporated to dryness under nitrogen and the parent compound regenerated from the reagent by allowing a solution of the unknown in 30 ml. acetone containing 1·2 ml. of 5 N sulphuric acid to stand overnight at room temperature. The parent compound was recovered from the acetone solution as follows. Water (5 ml.) was added to the solution, which was then evaporated on a steam bath under nitrogen to approximately 10 ml. The precipitate was recovered by extracting twice with 20 ml. portions of carbonyl-free hexane, and these extracts were combined, dried with sodium sulphate, filtered and evaporated to dryness. The residue containing the unknown was separated from the acetone DNPhydrazone resulting from the renegeration by chromatographing a 5 ml. chloroform solution of the residue on a column containing 2 g of 'Seasorb 43' and 4 g of 'Celite 545' in chloroform. The column was washed with 25 ml. of chloroform and the effluent, containing the steroid, collected. The steroid was further purified by thin-layer chromatography on silica gel *G* using 0·5 per cent methanol in benzene as developing solvent. Development and recovery of the unknown (approx. 1 mg) from the glass plates were accomplished as previously described[6]. The compound crystallized from acetone and water has a melting point of 128°–130° C. Two additional recrystallizations in the same solvent system resulted in fine needles having a melting point of 138°–140° C.

The recrystallized sample was analysed by mass spectrometry. The molecular ion at *m/e* 384 and the peak at *m/e* 271 (loss of alkyl side chain) indicated that the unknown is a mono-unsaturated keto steroid of the cholestane series. Ultra-violet analysis in absolute ethanol revealed a maximum absorption at 202 mμ, which was in keeping with the previous observation that the DNPhydrazone had a maximum absorption at 365 mμ in chloroform indicating the double bond was not conjugated to the carbonyl group.

The steroid gave a 'fast acting' reaction to the Liebermann-Burchard test, a positive Zimmermann test, a negative Salkowski test, positive Tortelli-Jaffé test and a positive selenium dioxide test. These observations were in agreement with those reported and observed for Δ^7-cholesten-3-one prepared and purified according to Busch[7] (m.p. 145° C) (reported: 146°–148°).

Confirmation of the identification of the steroid as Δ^7-cholesten-3-one was obtained from studies of its

Table 1

m/e	Percentage of peak height relative to molecular ion: Authentic Δ^7-cholesten-3-one	Isolated Δ^7-cholesten-3-one
217	12·5	10 6
229	25	25·5
245	16·9	17
247	14·3	16
271	50	47
369	19·5	19 1
384	100	100

infra-red spectra, mass spectra, and melting point. Fig. 1 is a partial reproduction of the infra-red spectra of (*A*) authentic Δ^7-cholesten-3-one and (*B*) the steroid isolated from butterfat. The identical spectra were obtained on a Beckman *IR-5A* infra-red spectrophotometer using micro-potassium bromide disks.

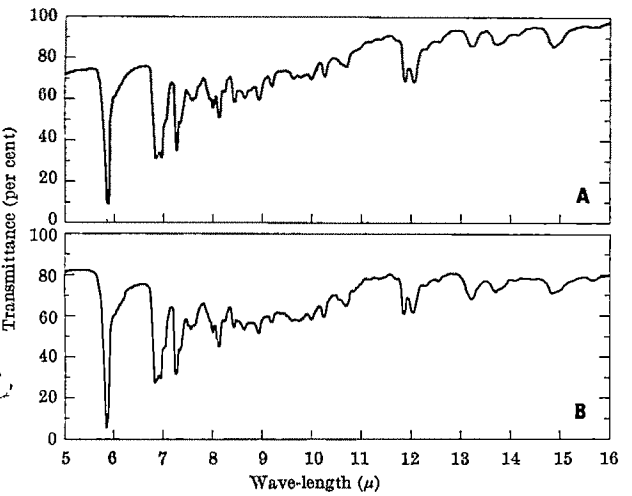


Fig. 1. Infra-red spectra of micro-potassium bromide pellets of (*A*) authentic and (*B*) isolated Δ^7-cholesten-3-one

Table 1 is a comparison of the peak heights of the molecular fragments in the high mass range, relative to the molecular ion, of the authentic and isolated steroid. The spectra were obtained on a Bendix (model 14) 'time of flight' mass spectrometer with a vacuum locked direct inlet system (Bendix Corporation model 843*A*). A temperature of approximately 100° C was used to obtain a good quality spectra.

Recrystallization of the authentic steroid (m.p. 145°) from acetone-water, as was necessary with the isolated steroid because of the minute quantity of sample available, resulted in a lowering of the melting point to 138°–140° C. A mixed melting point with the isolated steroid (m.p. 138°–140° C) resulted in a melt at 138°–141° C. A mixed melting point between the DNPhydrazone of the authentic (m.p. 210°–212° C) and the isolated (210°–213° C) steroid crystallized from acetone–water occurred at 210°–212° C.

The presence of Δ^7-cholesten-3-one in butter fat is, so far as it is known, the only report of its occurrence in nature. Its significance as an intermediate or by-product of cholesterol biosynthesis is speculative.

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Correlation between Resistance to Stem Rust and the Concentration of a Glucoside in Wheat

THE presence of 2-O-glucosyl-2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one in wheat was demonstrated by Virtanen[1] and by ElNaghy and Linko[2]. The latter reported higher concentrations of this glucoside in Khapli and Justin wheats, which are resistant to most races of stem rust, than in Little Club and other susceptible varieties. We have measured the concentrations of the glucoside in the primary leaves of five of the so-called differential varieties of wheat with varying degrees of rust resistance (Little Club, Acme, Kubanka, Mindum and Khapli) and have found that there is a significant negative correlation between glucoside content and the number of races of stem rust to which each variety is susceptible. We have also found that the glucoside content of Kenya 58-Marquis[10] (produced by back-crossing a Kenya 58-Marquis hybrid to Marquis nine times[3]) is correlated with its resistance to race 15*B* of *Puccinia graminis tritici* Erikss. and Henn. This line of wheat carries the temperature sensitive gene *Sr*6 and is resistant to race 15*B* at 62° F and susceptible at 79° F (ref. 4).

Wheat seedlings were grown in the greenhouse or in growth chambers at 62° ± 1° F or at 79° ± 1° F. The glucoside was extracted as described by ElNaghy and Linko[2], chromatographed in two dimensions on paper, using *n*-butanol–methanol–benzene–water (3 : 1 : 1 : 1) as the first solvent and 10 per cent formic acid as the second. It was identified by comparison of its R_F value, ultra-violet spectrum and the spectrum of its colour complex with ferric chloride with those of an authentic sample obtained by one of us (M. A. E.) from Dr. Virtanen. Relative concentrations of the glucoside in the extracts were determined by spraying chromatograms with ferric chloride (2 per cent w/v in acetone containing a few drops of 10 per cent hydrochloric acid) followed by elution of the coloured spots with 80 per cent methanol and measurement of their optical densities at 560 mμ in a Beckman (*DU*) spectrophotometer.

The percentage of rust races to which the varieties used are susceptible was computed from the data in Table 4 of Stakman *et al.*[5]. Reaction types 3 and 4 were scored as susceptible and types 0 to 2 as resistant.

Table 1. RELATIVE CONCENTRATIONS OF GLUCOSIDE IN WHEAT VARIETIES

Variety	Percentage of rust races infecting (*I*)*	Glucoside (*G*)†
Little Club	91	0·030
Acme	65	0 105
Kubanka	48	0·150
Mindum	42	0 230
Khapli	4	0·275
Kenya 58-Marquis[10]		
At 62° F	—	0·250
At 79° F	—	0·155

* Calculated from data in Table 4 of Stakman *et al.* (ref. 5).
† Optical density at 560 mμ of glucoside–$FeCl_3$ complex from 10 g (fresh weight) primary leaves in 4 ml. 80 per cent methanol. $G = 0\ 3045 - 0\ 00293\ I$.

The results, given in Table 1, show that there is a significant negative correlation ($r = -0{\cdot}960$, $P = 0{\cdot}01$) between the percentage of the known races of stem rust that infect a variety and its glucoside content. In addition, the glucoside content of Kenya 58-Marquis[10] was 61 per cent higher when plants were grown at 62° F (resistant) than when grown at 79° F (susceptible). The effect of temperature on glucoside content may be merely fortuitous; it has not been demonstrated whether or not high temperature lowers the glucoside content of varieties in which resistance is unaffected by temperature. We also found, as did ElNaghy and Linko[2], that the aglucone, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one, isolated from crushed primary leaves of Khapli as described by Virtanen and Hietala[6] inhibited the germination of uredospores.

The results do not, of course, prove that enzymatic release of the aglucone from the glucoside plays a part in

resistance. This was postulated by ElNaghy and Linko[2] and from this point of view the aglucone appears to meet several of the criteria which would be required for its classification as a phytoalexin[7]. It is, however, difficult to imagine that variations in the rate and degree of hydrolysis of the glucoside combined with the possible differential sensitivity of different races of the rust fungus to the aglucone do, in fact, account for the pattern of resistance of a single variety of wheat to a number of different races of rust. There are, undoubtedly, many other chemical factors involved. Nevertheless, so far as we are aware, this is the only instance in which it has been possible to demonstrate a correlation between the general level of resistance to rust and the concentration of a single metabolite. The problem therefore deserves a more detailed investigation.

We are grateful to Dr. D. R. Knott for a supply of seed of Kenya 58-Marquis[10]. One of us (M. A. E.) wishes to thank the Government of the United Arab Republic for financial support. The other (M. S.) is grateful to the National Research Council of Canada for a grant.

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Effect of Human Growth Hormone on 'Insulin Basic Protein Complex'

Young and George[1] report that, in their immunoassay system utilizing resin separation, the addition of human growth hormone (HGH) to fasting human serum causes a decrease in 'percentage antibody-bound' of iodine-131 labelled insulin, and they infer that HGH releases free insulin from a serum protein-bound form. We have been unable to confirm this phenomenon in a double-antibody immunoassay system[2,3].

(1) The addition of HGH to fasting human sera incubated with anti-insulin serum and ^{131}I-insulin causes no alteration in the percentage of antibody-bound ^{131}I-insulin (Table 1).

Table 1. ADDITION OF HGH TO FASTING HUMAN SERA CAUSES NO CHANGE IN THE PERCENTAGE OF ^{131}I-INSULIN BOUND BY ANTIBODY
(In double antibody assay system; corresponding standard curve is shown for reference)

Amount HGH added	Bound ^{131}I-insulin (%) (final volume 1·0 ml) Fasting human sera		Buffer solution ± human insulin				
	Serum *A* 0·1 ml.	Serum *B* 0·5 ml.	0 μU	0·6 μU	1·25 μU	2·5 μU	5·0 μU
0	61·0	50·5	66·0	61·4	60·2	55·8	48·1
10 mμg	61·7	—	—	—	—	—	—
33 mμg	—	50·7	66·7	—	—	—	—
100 mμg	61·4	—	—	—	—	—	—
5 μg	61·2	—	—	—	—	—	—

Table 2. ADDITION OF INSULIN TO FASTING HUMAN SERUM CAUSES NO CHANGE IN THE PERCENTAGE OF ^{131}I-HGH BOUND BY ANTIBODY
(Corresponding standard curve shown for reference)

Amount insulin added	Bound ^{131}I-HGH (%) (final volume 0·7 ml.) Fasting human serum 0·1 ml.	Buffer solution ± HGH 0 mμg	0·1 mμg	0·2 mμg	0·4 mμg
0	—*	56	54	51	39
100 μU (human)	56	58	—	—	—
1,000 μU (bovine)	56	59	—	—	—

* No measurable growth hormone in this serum sample in previous assay (percentage bound ^{131}I-HGH in 0 mμg buffer tube = 48 per cent; and in 0·1 ml. serum tube 47 per cent).

(2) The addition of human insulin to human serum, incubated with anti-HGH serum and ^{131}I-HGH, similarly does not impair percentage antibody-bound ^{131}I-HGH (Table 2).

Hence the hypothesis that HGH and insulin can interact at a basic protein binding site cannot be supported. We suggest that Young and George's findings represent an artefact of their anion-exchange resin method. It seems likely that factors in human serum plus the HGH solution alter the binding reaction of the resin, causing a false decrease in 'antibody-bound ^{131}I-insulin'. Such methods require a monitoring device to ensure that complete separation of the antibody-globulin is attained, such as the use of ^{131}I-γ-globulin in control samples[4].

While both hormones are probably bound in serum–insulin to α-1-globulin[5] and HGH to α-2-macroglobulin[6], not only are these different binding proteins, but our data indicate that it is unlikely that either displaces the other from a binding site.

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Gibberellin-like Activity of Helminthosporol and Helminthosporic Acid

HELMINTHOSPOROL (I) was isolated from a culture of *Helminthosporium sativum* by Tamura *et al.*[1], and was shown in this and subsequent studies[2] to stimulate the growth of the second leaf sheath of rice seedlings. However, (I) did not promote the growth of dwarf maize, nor did it give positive results in some other biological tests. Subsequently, Tamura and Sakurai[3] prepared a variety of related compounds and showed that (I) and helminthosporic acid, (II), promoted the elongation of rice seedlings and of lettuce seedling hypocotyls grown in light[4]. Thus, at high dose levels, these substances sometimes showed gibberellin-like activity. Various substances related to (−)-kaurene are probably on the biosynthetic pathway to gibberellic acid, (III), in *Gibberella fujikuroi*[5,6], and probably in higher plants also[7]. Some of these substances give positive results in a number of biological tests for gibberellins[8,9], as does steviol[10]. *Gibberella* converts steviol to an unidentified substance having activity in a wider range of test systems[11].

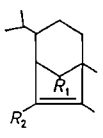
In view of the importance of gibberellic acid to the malting industry[12], and the interest attached to its mode of action, it seemed worthwhile to investigate the ability of (I) and (II) to trigger the release of sugars from deembryonated barley *in vitro*, a release that is catalysed by hydrolytic enzymes synthesized *de novo* in the aleurone layer[13], since this response has always seemed specific for gibberellins. The evident similarity of (I) and (II) to the fused rings *C* and *D* of the kaurene derivatives, the gibberellins and steviol, and the fact that, unlike the other substances mentioned, (I) and (II) have only two rings rather than four, suggest that they may approximate to the minimal chemical structure needed to elicit a gibberellin-like response. In addition, (I) and (II) were tested for their ability to counteract the dwarfing effect of 2-chloroethyl-trimethylammonium chloride (CCC) on the hypocotyls of lettuce seedlings[14]. This compound prevents the synthesis of gibberellic acid by *Gibberella*[15], and so may stop gibberellin synthesis in higher plants. The results, Table 1, show that (I) and (II) are indeed able to reverse dwarfing due to CCC but less well than gibberellic acid itself, a result very similar to that obtained by Tamura and Sakurai[3], using light as the dwarfing agency.

Table 1. LENGTH OF HYPOCOTYLS OF 'ARCTIC KING' LETTUCE SEEDLINGS PREGERMINATED IN DARK, AND SUBSEQUENTLY GROWN FOR 3 DAYS, AT 25° C, IN CONTACT WITH THE TEST SUBSTANCES

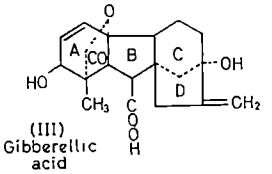
	Length of hypocotyls, cm (each value is the mean of 12–15 measurements)						
μg/Vessel	0·001	0·01	0·1	1·0	10·0	100·0	200
Gibberellic acid	1·07	1·11	1·16	1·15	1·41	1·36	—
Helminthosporol	—	1·08	1·18	1·10	1·09	1·20	1·24
Helminthosporic acid	—	1·09	1·05	1·14	1·12	1·22	1·51

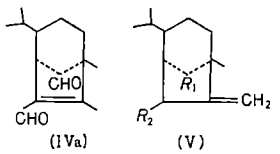
Controls 1·01, 1·03.

Table 2 shows that sugar release from de-embryonated barley is stimulated by (I) and (II), though less well than by gibberellic acid. Several trials show that in six-day incubations the half-maximum sugar release was attained with about 0·002 μg of (III) per flask and 40–55 μg of (I) or (II) per flask. It seemed that neither (I) nor (II) was capable of stimulating such a large sugar release as gibberellic acid, and in several trials neither consistently showed to advantage over the other. At the doses tested, the greater part of sugar release was achieved by (I), (II) and (III) in 4 days. The results were variable, and this may possibly be explained in part by spontaneous oxidation at (I) and (II) by air[3] as also occurs with the related substance helminthosporal (IV)[16]. Paper chromatography of the sugars released by the grain indicated that the pattern of release caused by (I) and (II) was qualitatively the same as that previously reported as being caused by (III)[13]. Briefly, during the initial incubation period maltodextrins and fructosans, accompanied by some sugars with lower molecular weights, appeared in solution and then progressive further release and hydrolysis occurred, until at the end of 7 days' incubation the only carbohydrates readily detected were *iso*-maltose, glucose, fructose and a pentose (probably arabinose). If the stereochemistry of (I) and (II) is similar to that of helminthosporal, (IV), (IV*a*)[17], a toxic substance isolated by de Mayo *et al.*[16], also from a strain of *Helminthosporium*, then the similarity between the ring systems of (I) and (II) and rings *C* and *D* of gibberellic acid (III) is even more marked. The most notable difference is that, on the *D* ring of gibberellins, kaurene and steviol, there is an exomethylene group (or a methyl and hydroxyl attached to the same carbon that should readily yield an exomethylene group by the elimination of water), whereas in (I) and (II) there is a methyl group adjacent to an endocyclic double bond. By analogy with kaurene and *iso*-kaurene[18] (I) and (II) might equilibrate with substances having the skeleton (V) in which case these might be the true effective structures rather than (I) or (II). However, a single attempt to equilibrate helminthosporol with this hypothetical isomer by the use of iodine in benzene[18] destroyed all activity. Interestingly, de Mayo *et al.*[6] have prepared a derivative of helminthosporal (IV) that contains a lactone ring and an exomethylene group derived from one of the aldehyde groups. The marked variations between various gibberellins[19,20] and (I) and (II) in their ability to trigger sugar release must be due to other features of their structure, presumably of rings *A* and *B*. Possibly the gibberellin molecule may be regarded as consisting of the 'effector part'—the *CD* part of the system, and the 'affinity part' that accounts for the binding of the molecule to an active site.



		R_1	R_2
I	Helminthosporol	CH_2OH	CHO
II	Helminthosporic acid	CH_2OH	COOH
IV	Helminthosporal	CHO	CHO





I thank Prof. Saburo Tamura for generous gifts of helminthosporol and helminthosporic acid, and Miss J. Rostance for careful technical assistance.

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Residues from Organic Solvents showing Gibberellin-like Biological Activity

Two years ago, while attempting to measure the gibberellin content of various antibiotic preparations, I was using a purification procedure that had, as its first step, the extraction with ethyl acetate of an acidified solution of the material under test. In control experiments, in which acidified, distilled water was extracted rather than a solution of the biological material under test, evaporation of the final solvent left a residue that had the

Table 2. REDUCING SUGARS RELEASED BY SIX DE-EMBRYONATED BARLEY GRAINS INCUBATED IN 2·5 ML. ACETATE BUFFER (0·5 mM, *p*H 5·3) FOR 6 DAYS AT 25° C, IN A ROTARY SHAKER
Reducing sugars expressed as mg maltose monohydrate

Trial No.	Control	Gibberellic acid		Helminthosporol		Helminthosporic acid	
	Sugars	μg/Flask	Sugars	μg/Flask	Sugars	μg/Flask	Sugars
1	1·0	1	161, 166, 171	200	78, 118, 148	200	167, 172, 177
2	1·4, 1·6, 1·7	10	158, 185, 191	1	<1·2, <1·2, <1·2	1	0·6, 1·5, 2·7
				100	83, 120, 140	100	110, 138, 150
3	0·9, 1·0, 1·3	100	147, 166, 180	5	2·1, 2·4, 3·3	10	4·2, 7·7
				100	93, 112	100	48, 50, 69
4	0·4, 0·5, 1·0	50	180, 186	500	136, 156, 156	100	133, 143, 160

properties of an unstable 'gibberellin' in that it promoted the synthesis of hydrolytic enzymes in de-embryonated barley, resulting in a large increase in the quantities of reducing sugars released into the culture medium[1].

These observations have now been confirmed and extended. Ethyl acetate and, to lesser extents, methyl and *n*-butyl, but not isobutyl acetates, left residues with highly variable biological activity when evaporated using rotation at 20° C, whereas various esters of propionic acid did not. The use of higher temperatures for rotary evaporation leads to even more irregular recoveries of active material. Sodium acetate is not active in this test for gibberellins; indeed, incubations are carried out in dilute acetate buffer (0·5 mM), and at higher concentrations acetate inhibits the response of the test system to gibberellic acid[2]; thus the recent observations on the stimulating effects of low concentrations of acetates on the germination of dormant malting barley cannot be attributed to a 'gibberellin-like' action but rather to some less specific effect as shown by many other substances[3,4]. Thin-layer chromatography shows that there are at least twelve substances in the residue from ethyl acetate, but it has not proved possible to recover active material from these chromatograms, and only traces of active material have been recovered from paper chromatograms. Where detectable, activity occurred between R_F values of 0·2 and 0·5, and most frequently at R_F 0·3 on chromatograms run with *n*-butanol/1·5 N ammonia (3 : 1 by volume, top phase) on Whatman No. 4 paper. Activity was also occasionally detected on the base line, and at R_F 0·9 on these chromatograms. Ethyl acetoacetate is a relatively non-volatile contaminant of ethyl acetate, but neither this nor the pure lithium or sodium salts nor free acetoacetic acid showed biological activity. However, sodium acetoacetate that had been contaminated by precipitation with ethanol did show activity, and so did residues from the rotary evaporation of ethanol ('rectified spirit') and of acetone. At least some samples of rectified spirit yielded more activity per l. evaporated than did any of the ethyl acetate samples tried, but with all solvents there were large variations between batches. Again, thin-layer chromatography showed that the residue from ethanol was an extremely complex mixture. The direct addition of small amounts (up to 50 μl.) of ethanol to the incubation mixture caused sugar release, but the intensity of the response varied with different samples of ethanol and was less than could be obtained with traces of ethanol residues, indicating that a contaminant, rather than ethanol itself, was the active substance. Marginal activity was also detected with *n*-propanol and old (but not freshly distilled) ethyl acetate. 'Tails' from the commercial distillation of rectified spirit were inactive. A commercial preparation of D,L-α-tocopheryl acetate, which was shown to be heterogenous by thin-layer chromatography, showed very marked activity. Many of these results are shown in Table 1.

Attempts to isolate the active material have not been successful, but some observations on their characteristics may be recorded. Shaking ethyl acetate with large volumes (3 × 1/3 volume) but not with small volumes (3 × 1/12 volume) of water apparently reduced the final yield of 'active material', indicating that the active material is at least slightly soluble in water saturated with ethyl acetate. The active material from the residual solid does not sublime under high vacuum from a flask at 60° C to a receiver at −70° C. This result is hard to reconcile with the fact that active residues were obtained by rotary evaporating ethyl acetate that had been washed, dried and distilled through a fractionating column, unless it is assumed that the material appeared in the distilled solvent on standing or changed in the absence of ethyl acetate. 'Spectrosol' ethyl acetate also left active residues after rotary evaporation. However, there were indications that the 'purification' of ethyl acetate reduced the subsequent yield of active material, but that after storage the yield returned to about its previous level. Exposing ethyl acetate to sunlight for a week before rotary evaporation reduced the yield of active material. The active material was soluble in diethyl ether and chloroform, but not petroleum ether. None of these solvents left an active residue after rotary evaporation. Similarly, residues were obtained from freshly purified and redistilled acetone, and from ethanol, and these also showed biological activity.

In view of the repeated failures to isolate the active substance(s), a wide range of compounds that could conceivably be derived from ethanol or ethyl acetate were tested for biological activity (Table 2). At the same time many other substances were tested, some of which show plant growth regulating activities of various kinds. The fact that these tests gave negative results shows clearly that the test using as an index the release of sugar from de-embryonated barley is highly specific for gibberellin-like molecules and substances such as helminthosporol[1,5–7]. It is notable that (−)-kaurene and (−)-kauranol gave negative results in these trials.

Table 1. SUGAR RELEASE FROM DE-EMBRYONATED BARLEY INCUBATED FOR 6 DAYS AT 25° C WITH THE TEST SUBSTANCES

Substance under test (quantity/flask)	Reducing sugars in solution* (as mg maltose monohydrate)
D,L-α-Tocopheryl acetate (25 μl.)	46, 78, 110
Residue methyl acetate (from 350 ml.)	14, 16, 17
Residue ethyl acetate (from 150 ml.)	59, 76
Residue ethyl acetate (from 200 ml.)	120, 130
Residue ethyl acetate (spectrosol, from 350 ml.)	11, 57
Residue *n*-butyl acetate (from 250 ml.)	128, 156
Ethanol, specially purified (25 μl.)	42, 56, 60
Ethanol, commercial sample (50 μl.)	34, 61
Ethanol, redistilled (50 μl.)	22, 44
n-Propanol (50 μl.)	8·7 9·3
Residue ethanol (from 500 ml.)	85, 107
Residue acetone (from 350 ml.)	35, 36
Residue same acetone, redistilled (from 350 ml.)	35, 42

* In the absence of stimulating substances reducing sugars equivalent to 0·3–2·0 mg maltose monohydrate were released, while in the presence of gibberellic acid (about 100 μg) the range was equivalent to 150–180 mg.

Table 2. SUBSTANCES TESTED WHICH GAVE NEGATIVE OR MARGINAL VALUES IN THE DE-EMBRYONATED BARLEY TEST FOR GIBBERELLINS

(a) Substances reported to regulate plant growth or metabolism

Indole acetic acid; benzylaminopurine; 6-furfurylaminopurine (kinetin); 2,4-dichlorophenoxyacetic acid; α-naphthalene acetic acid; 2,3,5-triiodobenzoic acid; 2,4-dichloroanisole; 3-amino-1,2,4-triazole; maltol; kojic acid; digitonin; tigogenin; tomatine; phenyldisodium orthophosphate; *sym*-diphenylurea; thiourea; penicillin *G*; penicillin *V*; 6-amino-penicillanic acid; streptomycin sulphate; aureomycin sulphate; sugar-cane wax, *n*-heptaldehyde; methyl oleate; *n*-octadecanol; phenyl acetic acid; *cis-o*-methylcinnamic acid; coumarin; *o*-coumaric acid; 3-methylcoumarin; 6-methylcoumarin; caffeic acid; ferulic acid; 2(3)-benzoxazolone; ethylene; sebacic acid; 2-chloropyridine; 2-bromopyridine; pyridine-N-oxide, pyridine-2-carbinol; 2-chloro-ethanol.

(b) Vitamins, vitamin concentrates, and related compounds

Yeast extract; ascorbic acid; menaphthone, cholesterol; ergosterol; β-sitosterol; vitamin A acetate; carotene (commercial prep.); linolenic acid; linoleic acid; glucose-*cyclo*-acetoacetate (acid and ethyl ester)

(c) Substances relating to terpenes, or their metabolism

D,L-Mevalonolactone; β-hydroxy-β-methylglutaric acid; *cis*-β-methyl glutaconate; *trans*-β-methyl glutaconate; acetoacetic acid (sodium and lithium salts, ethyl ester); β-hydroxybutyric acid (and ethyl ester); ββ′-dimethylacrylic acid; crotonic acid; squalene; farnesol; citronellol; myrcine; linalool; citral; β-pinene; camphene, santalol; β-ionone; (−)-kaurene; (−)-kauranol.

(d) Substances conceivably derived from ethyl acetate

Diacetyl; acetoin; triacetic lactone; dehydracetic acid; 2,6-dimethylpyrone-3-carboxylic acid; 2,6-dimethylpyrone; acetyl acetone; diacetyl acetone; diacetone alcohol; mesityl oxide; mesitylene; phloroglucinol; acetal; aldol; pinacol; iso-phorone; acetaldehyde; methanol; diethyl acetonedicarboxylate.

(e) Other substances

Cadaverine; D,L-penicillamine; citric acid; abietic acid; morin; phytol; iso-phytol; β-propiolactone; γ-butyrolactone; *cyclo*hexanone; 2-methyl- and 3-methyl-*cyclo*hexanones; iso-propanol; *n*-butanol; iso-butanol; allyl alcohol; propargyl alcohol; 2-methylbutene-3-ol; hept-1-ene-4-ol; 3-methylpent-1-ene-3-ol; *n*-butyl propionate; iso-butyl propionate; ethyl crotonate; sodium vinyl acetate; crotonaldehyde; octene-1; 2,2,4-trimethylpent-1-ene; 2,2,4-trimethylpent-2-ene. Residues from rotary evaporation of diethyl ether, petroleum ether, and chloroform; 3-hydroxypropionic acid; glyoxylic acid; glycollic acid; glycollic aldehyde; ethylene glycol; propane-1 : 2-diol; propane-1 : 3-diol; butane-1 : 3-diol; butane-1 : 4-diol; butane-2 : 3-diol; pentane-1 : 5-diol; hexane-1 : 6-diol.

The discovery that the evaporation of ethanol, ethyl acetate and acetone and some other solvents leaves residues with gibberellin-like activity emphasizes the need for caution in the use of these and other solvents in investigations on gibberellins and the need for adequate 'blank' test procedures. The ethyl acetate used was

manufactured from ethylene via ethanol, and so the active residues must be among the first materials with 'gibberellin-like' activity to be derived from a chemical, rather than a biological, source. It may be remarked that positive results were checked by submitting the 'sugars' released in the test to paper chromatography. This precaution was necessary to exclude the possibility that the substances under test or their breakdown products, rather than sugars from the endosperm, were responsible for the observed reducing power.

I thank the Distillers Company for a gift of ethyl acetate 'tails' and for helpful discussions, Dr. R. H. B. Galt for a gift of (−)-kaurene and (−)-kauranol, and Miss J. Rostance for careful technical assistance. I also thank Dr. A. Whitear for help with many chemical problems.

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pH Dependence of Lactate Dehydrogenase Isozyme Inhibition by Substrate

THE isolated, purified isozymes of lactate dehydrogenase (LDH) differ from one another in a variety of ways. In addition to electrophoretic mobility and immunological reactivity[1], they differ in kinetic behaviour[2], thermal stability[3,4], sub-unit composition[5], and inhibition by lactate[6], pyruvate[7], urea[8], oxalate[9], and α-hydroxybutyrate[10]. This communication describes the finding that the dissimilarity between LDH 1 and LDH 5 in degree of inhibition by lactate can appreciably be reduced by small alterations in *p*H. This dependence of substrate inhibition on *p*H illustrates the need to explore other variables before generalizing from limited *in vitro* to complex *in vivo* conditions.

Human heart and psoas muscle obtained at autopsy were homogenized in barbital buffer, *p*H 8·6. The homogenates were separated by starch-block electrophoresis, as previously described[11]. LDH 1 and LDH 5, recovered by elution from the block, were assayed spectrophotometrically for activity at 340 mμ with NAD (10^{-3} M) as coenzyme and increasing concentrations of the substrate sodium lactate (1×10^{-4}–$3{\cdot}6 \times 10^{-1}$ M). These reactions were measured at *p*H 8·5, 8·3, 8·0, 7·4, 7·0 and 6·6 at 25° C. The coenzyme and phosphate buffer (0·4 M), used to keep a constant cuvette volume of 3·0 ml., were each prepared at the *p*H being investigated. The *p*H of the reaction mixture was measured after the reaction had taken place and it was found that it did not vary from the initial *p*H by more than ± 0·2.

Fig. 1 (bottom) shows that at *p*H 8·3 LDH 5 resists inhibition by increasing lactate concentrations. However, at *p*H 6·6 and *p*H 7·0, after reaching a maximum at low concentrations, LDH 5 activity progressively declines. Variations between the curves at *p*H 6·6 and *p*H 7·0 are probably not significant, and can be attributed in part to differences in the source and preparation of the enzyme. Such small variations occurred in three similar experiments run at identical *p*H's on different enzyme preparations. The curve for LDH 5 at *p*H 8·5 is not shown in Fig. 1; it resembles the curve obtained at *p*H 8·3. Fig. 1 (top) reveals that inhibition of LDH 1 by lactate is appreciable at all *p*H's used. Slight increases in inhibition are observed at lower *p*H. However, even at *p*H 6·6 LDH 5 is inhibited less by lactate than is LDH 1 at *p*H 8·3; the differences are consistently more than 20 per cent. The dependence of LDH inhibition by lactate on *p*H was also observed in LDH 5 obtained from rat liver, as well as in commercial preparations of LDH 1 (pig heart, Boehringer) and LDH 5 (rabbit muscle, Boehringer).

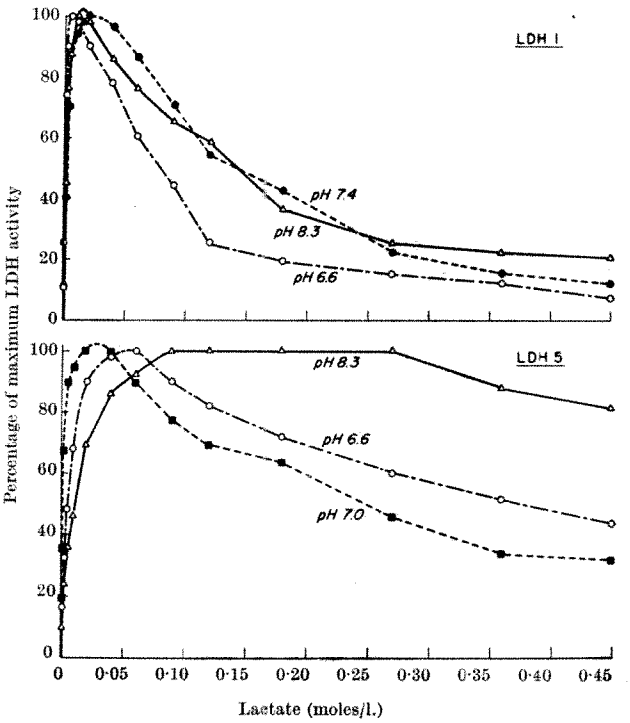


Fig. 1. Reactivities of LDH 1 (top) and LDH 5 (bottom) to increasing concentrations of sodium lactate as modified by alterations in *p*H

At *p*H 8·3, LDH 1 and LDH 5 react with lactate in a manner similar to that with pyruvate; at *p*H 8·3, LDH 5 resists the high lactate concentrations which inhibit LDH 1. Winer and Schwert[12] reported that pyruvate inhibition of a crystalline beef heart preparation containing two LDH isozymes is dependent on *p*H; their results agree with the data presented here for lactate in that pyruvate inhibition of LDH activity increased as the *p*H was lowered[12].

In addition to variations in *p*H, alterations of temperature drastically affect inhibition of the LDH isozymes; thus at 40° C, which more closely resembles physiological conditions than 6° C or 25° C, LDH 1 is more similar to LDH 5 in reactivity to pyruvate[13] than it is at 6° C or 25° C.

Different *in vitro* reactivities of isolated, purified LDH 1 and LDH 5 to pyruvate[7] at temperatures which are not physiological form the basis of a theory which states that LDH 5, because of its resistance to pyruvate, occurs as the predominant isozyme in anaerobically metabolizing tissues where, it is claimed, high levels of pyruvate develop[14]. In comparison, LDH 1, because of its inhibition by pyruvate, occurs as the principal isozyme in aerobically metabolizing tissues where low pyruvate concentrations are found[14]. Evidence for this theory, largely circumstantial, has been drawn from reports of shifts in isozyme pattern toward increased prominence of LDH 5 in such diverse environments as exist in neoplasia[15], growth of cells in tissue culture[16], hormonal stimulation of uterus[17], and phylogenetic evolution of various muscles[18]. Several exceptions to the theory have already been reported, most notably the examples of mature human erythrocytes[11], platelets and bovine lens fibre cells[15], all anaerobic cells which have little LDH 5, but large amounts of LDH 1 (ref. 20). Furthermore, pyruvate and lactate levels of cells shifting toward LDH 5 predominance under the anaerobic environments already mentioned reach the

peak but not the inhibitory regions of the curve (Fig. 1) relating enzyme activity to substrate concentration[21]. Thus, substrate concentrations high enough to inhibit LDH isozymes may be unattainable *in vivo*[21].

Regarding this theory of LDH isozyme distribution among tissues, the present experiments on *p*H dependence of LDH isozyme inhibition by lactate as well as others involving the effect of temperature on the reactivity of LDH 1 and LDH 5 toward pyruvate[21] demonstrate that even the *in vitro* differences between isolated, purified LDH 1 and LDH 5 in reactivity toward substrate may be modified and appreciably diminished by altering certain simple conditions. This investigation, therefore, suggests the dangers of extrapolating from limited *in vitro* to complex *in vivo* conditions when attempting to elucidate different tissue distributions of LDH isozymes.

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Respiratory Activity of Rat Intestinal Mitochondria

THE difficulty of measuring the respiratory activity of intestinal homogenates and of mitochondria isolated from the small-intestinal mucosa of the rat has been recorded by others[1–3]. The inhibition of succinoxidase activity reported by Nakamura *et al.*[1] was later shown by Stanbury[2] to be associated with the release of the unsaturated oleic and linoleic fatty acids. This inhibition was partly overcome by the addition of rat serum[1]. The present study was undertaken to examine more fully the protective effect of serum, as a preliminary to the study of the respiratory activity of the jejunal mitochondria of the rat infected with the nematode *Nippostrongylus brasiliensis*.

In all experiments the mitochondria were isolated by the following procedure. White rats were killed by a blow on the head and two-thirds of the small intestine was then removed, everted, washed in saline, and blotted to remove excess mucus. The mucosa was scraped off with the edge of a glass slide. The mucosae from two rats were pooled and the mitochondria were isolated from a fresh weight of 2–3 g by the method of Schneider[4]. The standard medium for isolation and resuspension of the mitochondria was 0·25 M sucrose + 0·003 M ethylenediamine tetraacetic acid (EDTA). The latter was added to chelate with the calcium expected to be released from parasites homogenized with the intestinal mucosa in subsequent experiments with infected rats[5]. A second medium contained the same concentrations of sucrose and EDTA, but 30 per cent bovine serum albumin (BSA) solution (Armour Pharmaceutical Co.) was added to give a final concentration of 1·5 per cent. The mitochondria were resuspended in the same medium in which they were isolated, so that from 0·3 to 0·6 mg of nitrogen was contained in the 0·5 ml. which was added to each Warburg flask.

The reaction mixture of 3 ml. and the procedure used for manometry were those described earlier[5], except that 1 mg nicotinamide-adenine dinucleotide (NAD) in 0·04 M nicotinamide (final concentration) was added to each flask. It was found that NAD in the presence of all substrates except succinate did not appear materially to affect the activity over the initial 10–20 min, but was better able to sustain the respiratory activity for 50 min. All activities were calculated from the uptake of oxygen over the first 20 min after an equilibration period of 10 min. The results of typical experiments when mitochondria were isolated in the standard medium with and without BSA are shown in Table 1.

Table 1. $QO_2^{(N)}$ OF MITOCHONDRIA OF THE SMALL-INTESTINAL MUCOSA OF THE RAT ISOLATED IN 0·25 M SUCROSE + 0·003 M EDTA WITH AND WITHOUT 1·5 PER CENT BSA

Substrate (10^{-2} M)	Standard medium	
	Without BSA	With 1·5 per cent BSA
Glutamate	≏ Endogenous rate	331
Malate	,, ,,	438
α-Oxogluturate	,, ,,	265
Succinate	211	532

When mitochondria were isolated without BSA, the $QO_2^{(N)}$ for glutamate, malate and α-oxogluturate was no better than the endogenous rates recorded with mitochondria isolated with or without BSA. The initial activity with succinate was relatively high but fell rapidly and was very low 40 min after equilibration. Isolation with 1·5 per cent BSA substantially increased the rate of $QO_2^{(N)}$ for succinate, which was then sustained for a longer period and resulted in a high rate of activity for the other substrates.

When 0·5 per cent BSA was used in the medium, the activities were consistently one-third to one-half those in 1·5 per cent BSA. If the concentration of BSA was increased to 2·5–3·0 per cent, frothing during homogenization interfered with the isolation and no further increase in activity was observed.

Electron micrographs were prepared from mitochondrial pellets fixed in buffered 1 per cent osmium tetroxide and sucrose (45 mg/ml.), embedded in methacrylate and examined in a Siemens Elmiskop I microscope. Mitochondria isolated as described here are shown in Figs. 1 and 2. Without BSA the mitochondria were swollen, vacuolated and the cristae were ill defined. When 1·5 per cent BSA was added, the mitochondria were smaller, non-vacuolated and the cristae were well defined.

Peroxidation may be associated with swelling of mitochondria[6], but there was no evidence of peroxidation when mucosal homogenates or isolated mitochondria were examined by the thiobarbituric acid method of

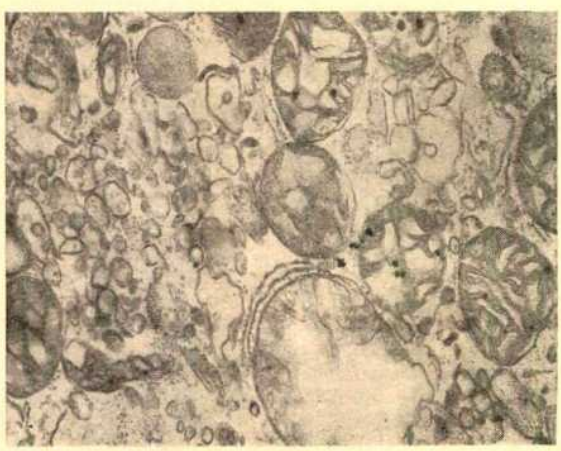


Fig. 1. (×20,000)

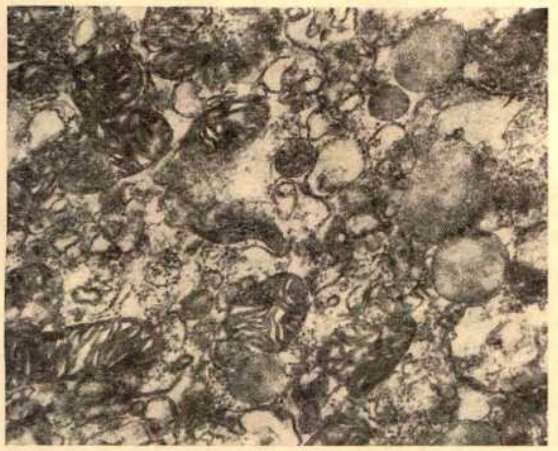


Fig. 2. (×20,000)

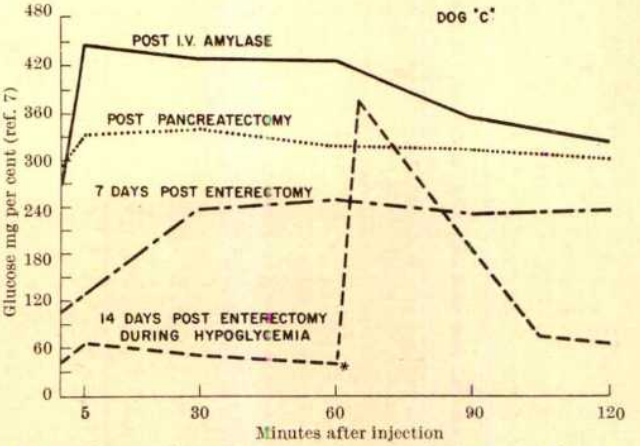


Fig. 1. Glucose tolerance tests. Test dose; 0·3 ml. 50 per cent glucose per kg, intravenous. * 3·0 ml. 50 per cent glucose per kg, intravenous

Tappel and Zalkin[7]. Nakamura *et al.*[1] reported that rat serum added to rat intestinal homogenates increased succinoxidase activity and suggested that the albumin of the serum bound free fatty acids liberated by homogenization. They did not state the concentration of serum used or the stage of isolation at which it was added; nor did they examine the effect of serum on respiratory activity in the presence of the other substrates used in the present experiments. The protective effect of albumin in the presence of fatty acids has been examined recently in more detail by Björntrop *et al.*[8], who confirmed that it bound the fatty acids. It was concluded therefore that 1·5 per cent BSA added to the medium of 0·25 M sucrose and 0·003 M EDTA was sufficient to protect the mitochondria isolated from the small-intestinal mucosa of the rat from the effect of unsaturated fatty acids.

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PHYSIOLOGY

Hypoglycaemia, Elevated Liver Glycogen and Diminished Liver Amylase in Pancreatectomized–Enterectomized Dogs

DURING the course of investigations into the source and role of serum amylase, we made the surprising observation that when depancreatomized diabetic dogs are enterectomized, the typical hyperglycaemia was changed into pronounced hypoglycaemia, associated with high levels of liver and tissue glycogen.

Three dogs were pancreatectomized by the avulsion technique[1]. Biopsies of muscle and liver for glycogen and amylase determination were taken. The dogs were maintained on kennel ration to which was added 5 g of commercial lipase and 1 g of choline per day. Insulin (about 10 U of N.P.H.) was given subcutaneously once a day; a daily urinary glucose excretion of 0·5–1·0 per cent as measured by 'Clinitest' tablets was maintained. In two dogs, several determinations of intravenous glucose tolerance, using 0·3 ml. of 50 per cent glucose per kg body-weight, yielded typical diabetic curves. After 1–2 months, the three pancreatectomized dogs were again operated on. Specimens similar to those obtained at the first operation were again taken, and the entire small intestine was then removed leaving only about 2 cm of ileum. Vagotomy was performed at the same time to protect the remaining ileum and colon from the gastric acid.

Following the second operation, the animals were maintained by the intravenous infusion of 5 per cent glucose in 0·45 per cent sodium chloride to which were added 15 U. of regular insulin and 20 mequiv. of potassium chloride per l., and appropriate amounts of vitamins and antibiotics. On this regimen, the daily urine contained about 1 per cent glucose. The intravenous glucose tolerance test yielded typical diabetic curves. At the second operation, no pancreatic tissue was found in any dog.

Within 7–15 days after the second operation, each of the previously hyperglycaemic animals became hypoglycaemic and remained hypoglycaemic despite the intravenous infusion of large amounts of glucose without insulin. Intravenous glucose tolerance tests carried out during the period of hypoglycaemia yielded flat curves (Fig. 1). Biopsies of the liver obtained at this time from each of the animals showed abnormally high levels of glycogen (about twice the pre-enterectomy values), and low levels of amylase (about a third of the pre-enterectomy levels).

After the hypoglycaemia and negative glucose tolerance tests were demonstrated in one dog, the animal was anaesthetized, and biopsies of the liver, muscle and kidney were taken for glycogen and amylase determination. 2,000 U. per kg body-weight of crystalline hcg amylase were then infused intravenously, and specimens of venous blood for serum amylase and blood glucose were taken at 30 min, and then at hourly intervals for 6 h. At the sixth hour, biopsies of the liver, muscle, and kidney were again obtained, the animal closed, and the intravenous glucose tolerance test repeated.

The infusion of amylase caused a striking increase in the level of blood glucose (Fig. 1), and a fall of the liver glycogen. The glycogen content of the kidney changed little, and that of the muscle rose (Table 1). The amylase levels of the liver, kidney and skeletal muscle rose (Table 1); that of the liver most markedly. With the blood glucose stabilized at the end of 6 h at about three times the pre-infusion level, the repeat glucose tolerance test was typically diabetic (Fig. 1).

Although the series is small, the hypoglycaemia and obliteration of the glucose tolerance curve occurred in three of three animals subjected to delayed enterectomy following pancreatectomy. In all dogs, the liver glycogen

Table 1. SERUM AND TISSUE AMYLASE TISSUE GLYCOGEN

	Serum amylase	Liver Amylase	Liver Glycogen	Kidney Amylase	Kidney Glycogen	Skeletal muscle Amylase	Skeletal muscle Glycogen
Pre-pancreatectomy	8·0	6·9	6·9			0·9	0·82
Pre-enterectomy	4·2 (3·1–5·8)	2·9	6·7			0·4	0·49
During hypoglycaemia	2·1	0·7	13·6	0·26	1·67		0·63
6 h after intravenous infusion of amylase	28·6	48·2	9·1	5·7	1·39	2·3	0·88

Serum amylase; U./ml.; method of Van Loon *et al.* (ref. 3).
Liver amylase; U./g; method of McGeachin *et al.* (ref. 4).
Glycogen; g/100 g; method of Good *et al.* (ref. 5).
Normals; (ref. 6), Liver amylase about 3/4 that of the serum amylase. Liver glycogen; 6–10 per cent. Kidney glycogen; 0·5–0·9 per cent. Skeletal muscle glycogen; 0·5–1 per cent.

was abnormally high during the period of hypoglycaemia. The basic physiological effect seemed to involve difficulty in mobilizing liver glycogen. The intravenous infusion of crystalline hog amylase restored the hyperglycaemia and diabetic glucose tolerance curve. The simultaneous removal of the pancreas and small intestine causes no such effect[2].

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Release of Acetylcholine from the Cerebral Cortex during Stimulation of the Optic Pathway

ELECTRICAL stimulation of a peripheral sensory nerve produces an increase in the rate at which acetylcholine (ACh) is liberated from the surface of the exposed contralateral somatosensory cortex[1]. Under light anaesthesia this procedure also evokes a smaller but more widespread release of ACh from the brain, probably by activating non-specific ascending pathways from the reticular formation[2].

It is not known whether these effects are confined to somatic sensory pathways, and the present experiments examine the release of ACh from the surface of the brain during stimulation of the optic pathway.

Rabbits were anaesthetized with 'Dial Compound' (allobarbitone and urethane, Ciba, Ltd., 0·7–0·9 ml./kg) and ACh was collected from the exposed surface of the cortex using the 'Perspex' cylinder technique introduced by MacIntosh and Oborin[3]. Details of the method and of the biological assay procedure for ACh have been described previously[1]. In the present experiments the fluid in the cylinders contained eserine sulphate ($1·0 \times 10^{-4}$ g/ml.) and the release of ACh was raised by the addition of atropine sulphate ($4·0 \times 10^{-7}$ g/ml.).

The eyes of the rabbits were shielded from light and the resting release of ACh from the primary visual receiving area of the cortex was determined bilaterally for 4–15 collection periods each of 15 min duration. These resting outputs varied considerably from animal to animal and with the depth of anaesthesia, but were relatively constant in any one animal, not varying by more than ± 15 per cent over 2–4 h. The output from lightly anaesthetized animals was 2·2–34·7 ng ACh/15 min/cm² cortex.

In eight lightly anaesthetized rabbits, exposure of the eyes to bright light for 30–60 min was associated with a bilateral increase in the rate of ACh release from the visual cortex (2·2–7·3 times the resting output, Fig. 1*A*). This evoked release from the visual cortex was accompanied by a smaller rise from the motor and somatosensory cortex (1·2–2·5 times the resting output, Fig. 1*B*.)

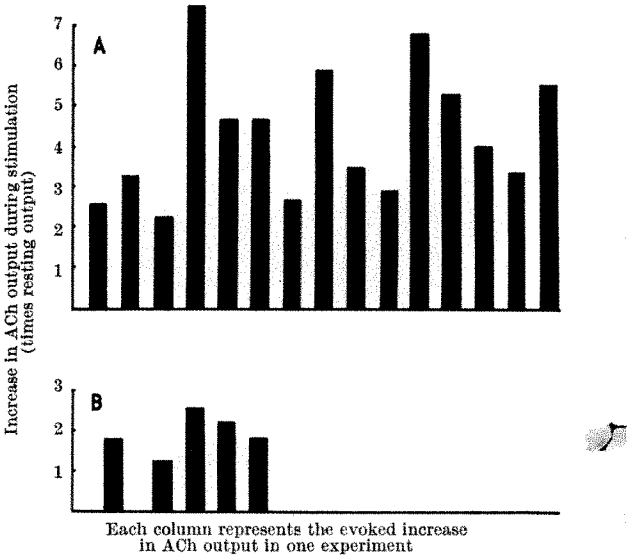


Fig. 1. Summary of individual experiments. The increase in ACh output during bilateral stimulation of the retina by light. *A*, Primary visual receiving areas; *B*, motor and somatosensory areas

In five lightly anaesthetized rabbits direct electrical stimulation (20–60 stimuli/sec, 1·0 msec pulses) of the dorsal nucleus of the lateral geniculate body, using a stereotactically placed coaxial electrode, produced an immediate increase in the rate of ACh release from the ipsilateral primary visual cortex (2·0–4·9 times the resting output, Fig. 2*A*). There was a simultaneous but smaller rise in ACh output from the contralateral primary visual area and from the motor and somatosensory regions of the cortex (1·1–1·9 times the resting output, Fig. 2*B*).

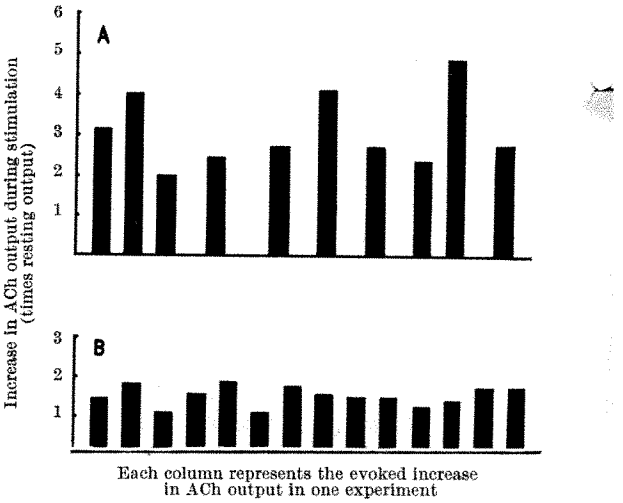


Fig. 2. Summary of individual experiments. The increase in ACh output during stimulation of the lateral geniculate body. *A*, Primary visual receiving area; *B*, motor, somatosensory and contralateral visual cortex

These experiments suggested that stimulation of the optic and of the somatosensory pathways evoked a similar pattern of ACh release from the surface of the brain; a large increase from the primary receiving areas and a widespread but smaller release from other regions of the cortex.

It is likely that the widespread evoked release of ACh is a consequence of the indirect activation of ascending path-

ways from the reticular formation, since Kanai and Szerb[2] have reported that direct stimulation of the mesencephalic tegmentum will produce this effect.

It is not yet clear whether the larger increases in ACh release obtained from the primary receiving areas are due entirely to a more effective activation of reticular pathways to these regions. They may be attributable in part to the liberation of ACh from intracortical nerve endings associated with the fourth order neurone in the direct sensory pathway.

Grants to one of us (J. F. M.) from the Royal Society and the Medical Research Council supported this work.

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Inability of the Electroretinogram to recover after a Brief, High-intensity Light Stimulus

Buckser and Potts[1] observed the recovery of the rat electroretinogram (ERG) after weak light adaptation, and related this to the visual threshold. Buckser[2] proposed a model to relate the recovery of the ERG *a*-wave after partial light adaptation to the level of the light adaptation. This communication reports the failure of the ERG to recover to the dark adapted ERG after exposure to a brief, high-intensity light flash.

Dark adapted albino rats were curarized, artificially respirated, and positioned under a commercial photographic electronic flash unit (Hershey Manufacturing Co.). The light was focused on the cornea by means of appropriate lenses. The preparation of the rat, the recording technique, and the reproducibility of the results are described elsewhere[3]. An a.c. amplification system with low- and high-frequency cut-offs at 0·06 and 1,000 c/s, respectively, was used.

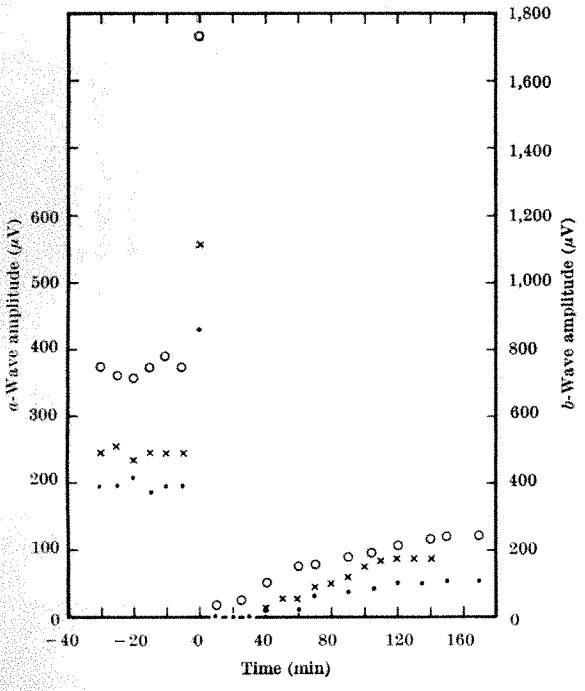


Fig. 1. Recovery of the *a*-wave and *b*-wave amplitudes after a brief high-intensity stimulus. '0' time represents the time the high-intensity stimulus is given. Negative time represents the time before the high-intensity stimulus was given. ● and ×, The *a*-wave amplitudes obtained from two different experiments. ○, The *b*-wave amplitudes obtained from one experiment

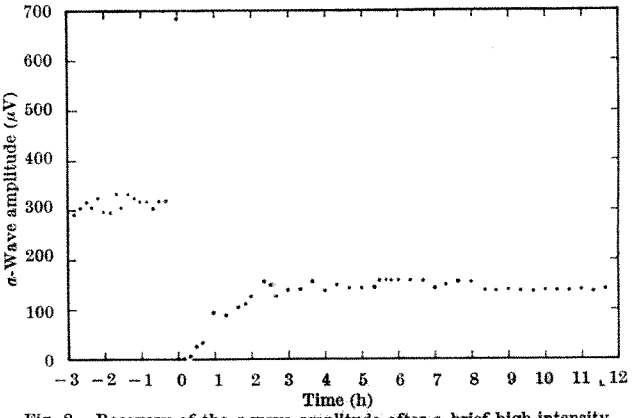


Fig. 2. Recovery of the *a*-wave amplitude after a brief high-intensity stimulus. '0' time represents the time the high-intensity stimulus is given. Negative time represents the time before the high-intensity stimulus was given

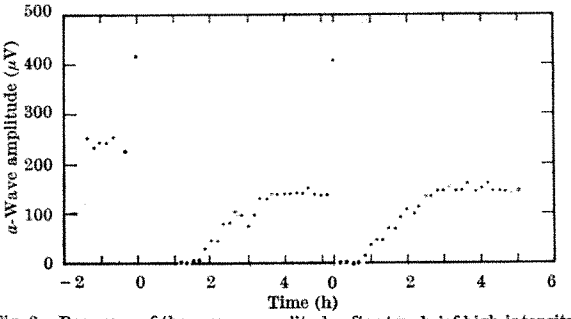


Fig. 3. Recovery of the *a*-wave amplitude after two brief high-intensity flashes. '0' time represents the time the high-intensity stimulus is given. An equilibrium recovery response was attained before the second flash was given. Negative time represents the time before the high-intensity stimulus was given

A series of ERG's were obtained from the dark adapted rat with neutral density filters equal to 5·0 positioned between the flash unit and the eye with an interval between flashes never less than 10 min. The neutral density filters were removed and a single ERG was recorded in response to the entire energy of the flash unit. The filters were replaced, and the ERG's again recorded with the interval between flashes being 10 min or greater.

The *a*-wave amplitude, $E(a)$, obtained from two experiments is shown plotted against time in Fig. 1. In the experiments reported here the $E(a)$'s were approximately constant at 195 and 245 μV when the 5·0 neutral density filters were used prior to the high-intensity flash, and 430 and 555 μV in response to the high-intensity stimulus. Approximately 40 min were required after the high-intensity light before an *a*-wave was detected. The *a*-wave amplitude recovered to approximately 50 and 80 μV 2 h after the light flash and remained constant. The recovery *a*-wave amplitudes after the high-intensity light flash are much lower than the pre-high-intensity amplitudes. The failure of the *b*-wave amplitude to recover to the pre-high-intensity flash amplitude is also shown in Fig. 1.

In Fig. 2, $E(a)$ is shown before, in response to, and after the high-intensity light flash. The *a*-wave amplitude does not recover to the pre-high-intensity flash, even after almost 12 h of dark adaptation.

The eye was exposed to a second high-intensity light flash after an equilibrium response was obtained following the initial bright lights. The ERG recovery was again recorded in response to the 5·0 neutral density filtered light flash. The results are shown in Fig. 3. The *a*-wave amplitudes, after recovery from both high-intensity light stimuli, are considerably lower than the pre-high-intensity stimuli. $E(a)$ was constant at approximately 245 μV prior to the initial high-intensity stimulus, and recovered to 140 and 150 μV following the first and second high-intensity stimuli, respectively. $E(a)$ in response to the two high-intensity stimuli was 415 and 407 μV.

Additional work is being done to determine whether the decrease in the ERG amplitude is related to the pigment concentration, or changes in the structure of the retina and the receptor cells.

I thank Mr. David Buffin for performing many of these experiments.

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PHARMACOLOGY

Adrenergic β-Receptors and Non-shivering Thermogenesis

Sellers, Scott and Thomas[1] demonstrated that after prolonged cold exposure (cold acclimatization) heat production through shivering is gradually replaced by an increase in non-shivering thermogenesis. It is often assumed that muscle tissue is an important site of non-shivering thermogenesis. Recently, Himms-Hagen[2] has suggested that acceleration of the triglyceride cycle of brown adipose tissue is a major mechanism of non-shivering thermogenesis in cold-acclimatized rats. As lipid metabolism of adipose tissue is under β-adrenergic control, a study of the effect of β-adrenergic blockade on electrical muscle activity in rats exposed to about 4° C after acclimatization to 23° C or 4° C would be expected to yield valuable information. This communication presents preliminary data of such an investigation. Cold-acclimatized rats were removed from the cold room, anaesthetized with urethane (1 to 1·25 g/kg)—a drug known to alter only the degree, not the type, of the electrical response of muscle activity[1]—and exposed to a temperature of 4° C. Needle electrodes were inserted into a leg muscle and electrical activity was measured with a Grass model 5 polygraph[3]. Rectal temperature was monitored throughout the experiment. Each rat was tested at least twice, once when given drug treatment, and once when given saline or nothing. The β-adrenergic blocking agent, propranolol (*AY* 64043) (kindly donated by Ayerst, McKenna and Harrison, Ltd., Montreal), was used at doses of 0·3 or 0·9 mg/kg intraperitoneally every 30 min. Fig. 1 shows rectal temperature, duration of exposure to 4° C and the effect of the drug on electrical muscle activity in six cold-acclimatized rats. None of the animals shivered during an experimental period of 2·5 h when treated with saline. A seventh rat showed signs of shivering and was therefore excluded from this series. After treatment with propranolol, all six rats showed a marked increase of electrical muscle activity. Representative tracings of the muscle activity before and after propranolol are shown in Fig. 1: no effort was made to quantitate accurately the observed increase.

It is concluded that β-adrenergic blockade interferes with non-shivering thermogenesis so that heat production, mediated by electrical muscle activity, increased.

In order to determine whether the response was dose-dependent, two dose levels of propranolol were used. As can be seen from Fig. 1, the differences between the effects of the two doses are not obvious, and so this question remains to be settled.

It is not possible to draw any conclusions from these experiments regarding the site of non-shivering thermogenesis (muscle, adipose or other tissues); however, they confirm the importance of β-adrenergic receptors in cold-acclimatization. In other experiments it was found that rats acclimatized to 23° C and exposed to 4° C all shivered and propranolol did not interfere with the shivering.

Further investigations of the mechanisms underlying these observations are in progress. This work was supported by grants from the Defence Research Board and the Medical Research Council of Canada.

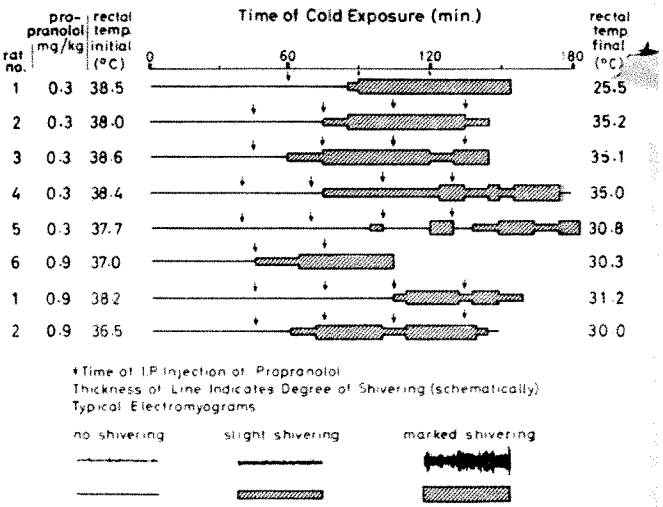


Fig. 1. Effect of propranolol on shivering of cold-acclimatized rats anaesthetized with urethane and re-exposed to 4° C. Note absence of shivering during control period

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Destruction of Cephalosporin C by Ultra-violet Light

WITH the recent development of potent penicillinase-resistant semi-synthetic cephalosporins[1], there is considerable interest in the parent antibiotic, cephalosporin *C* (ref. 2). This antibiotic, which displays a peak of ultra-violet absorption at 260 mμ, is often detected on paper chromatograms by exposure to a source of ultra-violet light. Using this technique during some investigations on the biosynthesis of the antibiotic[3], I noted an extreme lability of cephalosporin *C* to ultra-violet light.

It had been common practice in this laboratory to locate the antibiotic on paper chromatographic strips by placing the strips on the glass filter surface above the ultra-violet light source. After the dark area was circled in pencil, the strip was placed on the surface of an agar medium seeded with either *Escherichia coli* or *Bacillus subtilis*; the plate was incubated at 37° C for 16 h. It was noted that the intensity of the antibiotic activity of the cephalosporin *C* spot varied from experiment to experiment and depended on the type of ultra-violet filter used. (These original observations were made by Robert B. Walton and Richard Burg.) When a red-purple 'Corex *A*' polished filter (No. 9863, Corning Glass Works, Corning, New York) was used, the resulting antibiotic activity was good. On the other hand, use of a clear 'Vycor' glass filter (No. 7910, Corning) resulted in poor antibiotic activity. It was suggested that, because the 'Vycor' filter allows more 260 mμ light to pass, ultra-violet light was being absorbed by the compound on the paper and inactivating it. To investigate this, paper disks (Schleicher and Schuell Co., Keene, New Hampshire) were dipped into a solution containing approximately 100 μg of the sodium salt of cephalosporin *C* per ml. of 0·02 M phosphate buffer (*p*H 7·0). After drying, the

Table 1. INACTIVATION OF CEPHALOSPORIN *C* ON PAPER BY ULTRA-VIOLET LIGHT

Time of exposure (min)	Cephalosporin *C** (μg/ml.)
0	86
0·17	106
0·50	86
1·0	49
2·0	35
4·0	< 35

* Assayed by size of zone of inhibition against *E. coli MB*-208.

disks were exposed for various lengths of time to the ultra-violet light source covered by the 'Vycor' plate. As shown in Table 1, rapid inactivation occurred. When a 'Corex' plate was used, no destruction of cephalosporin *C* occurred in this same time interval although prolonged exposure did cause inactivation. Visible light from a fluorescent lamp did not inactivate the antibiotic. Inactivation by ultra-violet light also occurred when the antibiotic was applied to Whatman No. 1 chromatography paper instead of antibiotic disks.

To determine whether inactivation also occurred in solution, cephalosporin *C* at 400 μg per ml. of buffer was irradiated in a manner usually used for mutation of bacteria. 20 ml. were placed into a dish and the solution was agitated by a magnetic stirrer. Irradiation was from an ultra-violet lamp placed 10 in. above the bottom surface of the dish in a dark room ; no filter separated the light source from the solution. At various times, paper disks were dipped into the solution and were assayed by an agar diffusion assay against *B. subtilis*. As shown in Fig. 1, rapid inactivation occurred in solution. There was no photoreactivation of cephalosporin *C* as determined by exposing the solution to visible light after irradiation. A similar experiment in which the absorbance at 260 mμ was measured in a Beckman spectrophotometer (model *DB*) at various times showed that ultra-violet light also decreased the 260 mμ absorbance of cephalosporin *C* (Fig. 2). It is of interest that in each of the experiments (Table 1 and Figs. 1 and 2), there was an initial increase in activity or absorption before degradation occurred. The significance of this observation has not as yet been ascertained.

The site of action of ultra-violet light on cephalosporin *C* is probably the β-lactam-dihydrothiazine nucleus. No cleavage of the aminodipyl side-chain was detected by a phenylacetylation technique[4]. Removal of the *o*-acetyl group could result in a decrease in antibiotic activity but not in loss of absorption at 260 mμ[5]. The loss of only 17 per cent of the 260 mμ absorption which occurred at the same time as destruction of almost 90 per cent of the antibiotic activity is consistent with the fact that many of the degradation products of the cephalosporin nucleus retain the ability to absorb light of 260 mμ wave-length[6].

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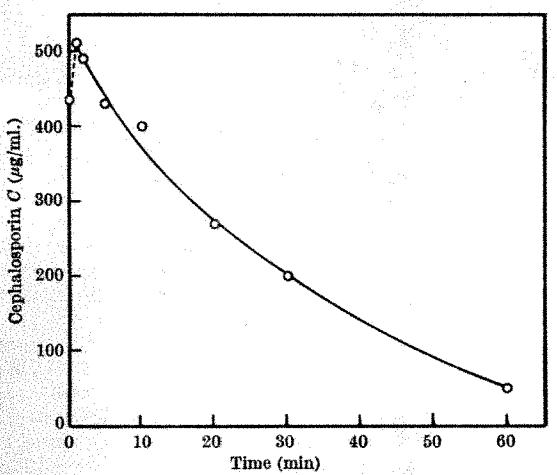


Fig. 1. Destruction of the biological activity of cephalosporin *C* by ultra-violet irradiation—*B. subtilis* used for assay

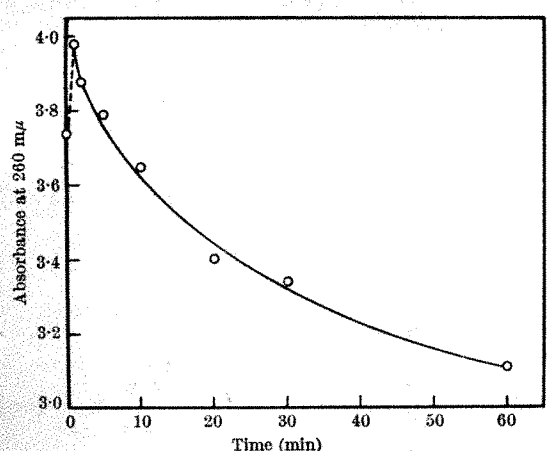


Fig. 2. Decrease in absorbance at 260 mμ of cephalosporin *C* by ultra-violet irradiation

HAEMATOLOGY

Absorption-spectrum of Haemoglobin of Leeches

ALTHOUGH it has been known for nearly a hundred years[1] that the red pigment of the circulatory fluid of non-rhynchobdellid leeches is haemoglobin, there appear to be no records of the absorption-spectrum or of its main features. While removing the blood from the tissue-pigments of leeches I have had occasion to record the absorption-spectra in neutral aqueous solutions of the oxyhaemoglobin of *Hirudo medicinalis* L, *Haemopis sanguisuga* (L) and *Erpobdella testacea* (Savigny). The spectrum for *Hirudo* is shown in Fig. 1, and the positions of the main features in the spectra of all three genera are given in Table 1.

From these records there seems little doubt about Lankester's original identification of the pigment. They show one further point of interest, namely, that the main α- and β-peaks are situated at a longer wave-length than those of most other haemoglobins which have been recorded[2], including those of other annelids. The difference is most marked for the α-peak at 582–4 mμ. This wave-length is even longer than that of the myoglobins which, in general, is longer than that of the recorded haemoglobins—with the exception of *Simocephalus* haemoglobin[2]. Taking into account both the α- and the β-peaks, the haemoglobin of the larva of *Gastrophilus*[3] shows rather a close resemblance to that of the leeches.

The myoglobins have a higher oxygen-affinity than the haemoglobins, and both the *Gastrophilus* larva and Cladocera such as *Simocephalus* are known to develop and use haemoglobin only under anoxic conditions[3,4]. It is there-

Table 1. WAVE-LENGTHS (mμ) OF THE PEAKS (UPPER LINE) AND TROUGHS (LOWER LINE) IN THE ABSORPTION-SPECTRA OF THE OXYHAEMOGLOBINS OF (1) *Hirudo*, (2) *Haemopis* AND (3) *Erpobdella*

(1)	273	(345)	415	547	584
		318		518	568
(2)	274	347	417	544	582
		314	382	514	567
(3)	273	342	418	546	584
		318	372	515	567

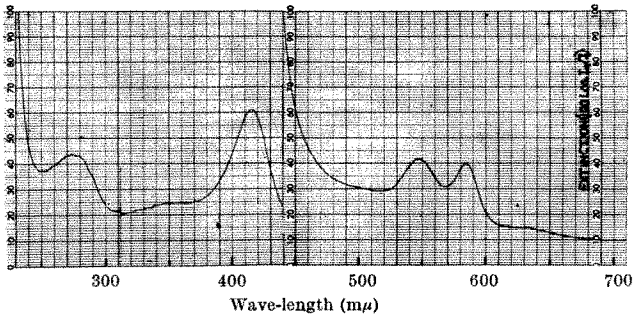


Fig. 1. Absorption-spectrum of aqueous solution of oxyhaemoglobin of *Hirudo*: extinction of light (100 log (I_0/I) as a function of wave-length, in mμ. Scanned from right to left. Solution diluted 4 × for wave-band below 440 mμ. Artefact at 312 mμ marks switch-over from tungsten to hydrogen lamp

fore possible that the haemoglobin of leeches also has an unusually high oxygen-affinity and that this has adaptive value. At the same time there is at present no reason to think that leeches live under conditions as extremely anoxic as the outstanding values of the α-peak of their haemoglobin would imply, and the problem is one of some interest. Internal parasites usually have haemoglobins with a higher oxygen-affinity, and with an α-peak at a longer wave-length, than those of their host[2], but carnivores such as *Haemopis* and *Erpobdella* are not even external parasites. Many leeches tolerate stagnant conditions, but these three genera swim well and are presumably not at the mercy of their surroundings. Conceivably a high oxygen-affinity compensates for shortcomings elsewhere in the oxygenation-system.

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Blood Platelets as a Source of Enzyme Activity in Washed Trypanosome Suspensions

THE standard method used for separating trypanosomes from infected blood involves repeated differential centrifugation of a suspension of trypanosomes and blood elements in a phosphate-buffered saline solution containing glucose. The success of this procedure varies not only from one species to another but also with the degree of parasitaemia. Suspensions of the Liverpool strain of *Trypanosoma rhodesiense* may be obtained free of red blood cells and leucocytes in this way, but with *T. vivax*, *T. congolense*, *T. lewisi* and the '*M*' and '*J*' strains of *T. rhodesiense* other techniques have to be used for complete removal of blood cells and platelets[1,2].

In this laboratory, critical microscopic examination of suspensions of the Liverpool strain of *T. rhodesiense* prepared in the foregoing manner showed that, although red blood cells and leucocytes were absent, a quantity of platelets, varying between 12 and 20 per cent of the trypanosome count, was regularly present. Counts were made with a haemocytometer using an untreated glass pipette and the diluting fluid of Cramer and Bannerman[3]. Several techniques, dependent on the size, adhesive or agglutinating properties of platelets[2,4–8], were tried in order to reduce this contamination. The most successful of these for routine separation was the defibrination technique used by Fulton and Spooner[2]; this reduces the contamination to less than 3 per cent and gives a 95 per cent recovery of trypanosomes.

A knowledge of the extent of contamination from blood platelets is essential for any quantitative enzymatic investigations, since platelets exhibit significant metabolic activity[9,10]. Fig. 1 shows the relative contribution of

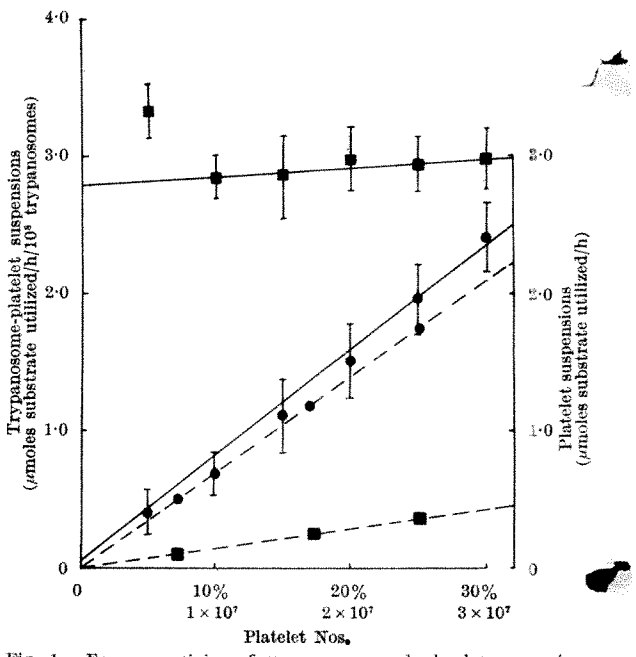


Fig. 1. Enzyme activity of trypanosome and platelet suspensions. ●, Lactic dehydrogenase activity; ■, L-α-glycerophosphate dehydrogenase activity. Each experimental sample (3·0 ml.) contained phosphate buffer, *p*H 7·4 (300 μmoles), NADH (0·5 μmole), and 0·1 ml. of a water lysed trypanosome-platelet (—) or platelet (– – –) suspension. The mixed suspensions contained about 10^9 trypanosomes/ml. (10^8 trypanosomes *c.* 0·5 mg protein by the biuret method). The platelet suspensions contained between 0·5 and 5×10^8 platelets/ml. (1 mg protein *c.* $9{\cdot}3 \times 10^8$ platelets). Sodium pyruvate (10 μmoles) or a solution of mixed triose phosphate esters (3·5 μmoles) was added at the start of the experiment

platelets in mixed trypanosome-platelet preparations to L-α-glycerophosphate dehydrogenase (GPDH) (L-glycerol-3-phosphate : NAD^+ oxidoreductase, *EC* 1.1.1.8) and lactate dehydrogenase (LDH) (L-lactate : NAD^+ oxidoreductase *EC* 1.1.1.27) activity. This activity was determined from the decrease in optical density at 340 mμ as reduced nicotinamide-adenine dinucleotide (NADH) was oxidized. The first of these enzymes is part of the coupled dehydrogenase–oxidase system which accounts for the major part of the respiratory metabolism of the blood forms of *T. rhodesiense*[12,13]. Lactate dehydrogenase activity has been reported or implied in the same species[13–16]. The present results show that whereas platelets contribute little to the GPDH activity of a *T. rhodesiense* suspension, they are almost entirely responsible for the observed LDH activity. In quantitative enzymatic investigations, therefore, whether of blood parasites or of other blood cells, the possibility of error due to contamination by platelets cannot be disregarded.

I thank Dr. I. B. R. Bowman for his advice and Mr. B. Cover for his assistance.

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IMMUNOLOGY

Effect of Immunosuppression on First-generation Isotransplantation of Chemically Induced Tumours in Mice

THE existence of host defence mechanisms which operate against autochthonous cancer has been suspected for many years, and the possibility has been suggested that such resistance might depend on mechanisms similar to those which bring about homograft and heterograft rejection[1,2]. This presupposes that the host is able to recognize its own tumour cells as foreign and to react so as to inhibit them, perhaps by way of specific immunological mechanisms. Several authors have presented evidence from transplantation and serological studies which suggests that tumour cells are antigenically different from normal cells of the tumour-bearing host in which they arise[3].

Suppression of the immunological reactivity of a graft recipient by various forms of chemotherapy or total-body radiotherapy has been shown to delay rejection of homologous (allogeneic) transplants of normal tissues in animals and man, and homologous or heterologous (xenogeneic) transplants of tumour in experimental animals[4–8]. If such immunosuppression were shown similarly to influence growth of syngeneic tumours (auto- or iso-transplants), it would provide yet another kind of evidence that tumour tissue contains antigens foreign to the normal host tissues and that the immunological mechanisms of the host react against these antigens to inhibit tumour growth. It seemed reasonable to test this possibility by studying quantitatively the transplantability of syngeneic tumours under conditions which suppress or enhance immunological responses. Such an investigation has been undertaken in this laboratory and the results obtained with *cyclo*phosphamide ('Cytoxan') are sufficiently interesting and reliable to merit this preliminary report. Experiments with other immunosuppressive agents are in progress.

*Cyclo*phosphamide ('Cytoxan') was chosen for this study because its immunosuppressive effect had previously been demonstrated in a variety of experimental systems[4–6] and because its pharmacological characteristics seem well suited to the objective. This drug, like other polyfunctional alkylating agents of the nitrogen mustard type, acts very rapidly after systemic administration to initiate cytotoxic changes, including destruction of those cells responsible for immunological reactions. These effects progress over a period of several days and persist for two or more weeks, but although cytopathological evidence of this effect is progressive over this considerable period of time, the drug itself is very rapidly hydrolysed to a non-cytotoxic form within a few minutes or hours of injection. Thus, if administration of the drug is stopped 24 h or more before a transplant is injected, it results in a host in which immunosuppression will continue for a considerable time but in which there is no persistence of a cytotoxic drug which might inhibit the tumour transplant.

Tumours were induced in *C57BL*/6 mice by single intramuscular injections in the thigh of 1·0 or 0·5 mg of 20-methylcholanthrene (MC) suspended in 0·1 ml. of olive oil. Fibrosarcomata usually developed at the injection site 4–6 months later.

For a transplantation study one tumour donor was killed and the tumour was promptly removed, minced as finely as possible with scalpels, suspended in an isotonic solution (Puck's saline *A*)[9] containing 0·01 per cent trypsin and 0·02 per cent collagenase and agitated for about 1 h on a magnetic stirrer. The cell suspensions were then washed three times with 10 ml. of saline *A*, counted in haemocytometers, and resuspended in saline *A* at the desired concentration.

Recipients were *C57BL*/6 mice of the same sex as the tumour donor, 7–10 weeks of age (Millerton Research Farms, Millerton, New York). Isogeneity of the *C57BL*/6 line has been demonstrated by acceptance of reciprocal skin grafts.

The cell doses chosen for tumour transplantation were those expected to produce about 50 per cent tumour takes in the control group, as judged by results of preliminary experiments with the same type of tumour. Tumour cell suspensions were injected subcutaneously into the inguinal region of the recipients in a volume of 0·1 ml. Care was taken to mix the suspension thoroughly at frequent intervals before injections in order to assure even cell distribution.

The recipients were palpated at regular intervals to follow tumour development and growth. Tumours were measured by callipers in the longest diameter and were recorded in categories of tumour size in increments of 5 mm of tumour diameter.

Prior to the implantation of the tumour cells, experimental groups of mice were treated by intraperitoneal injections of 'Cytoxan'. Animals in the control group were given intraperitoneal injections of 0·1 ml. of isotonic saline on each day that the experimental group received treatment. In one experiment, treatment consisted of five daily injections of 0·30 mg of 'Cytoxan' per mouse (about 15 mg/kg of body-weight) dissolved in 0·1 ml. of isotonic saline commencing 7 days prior to tumour transplantation. Thus tumour cell inoculation was 72 h after the last *cyclo*phosphamide injection. In all other experiments the treated mice received a single injection of 0·6 mg 'Cytoxan' in 0·1 ml. of isotonic saline (about 30 mg/kg) 4 days prior to challenge followed by three daily injections of 0·30 mg. This gave an interval of 24 h between the last injection of the drug and the inoculation of tumour cell suspension. A fresh solution of 'Cytoxan' was prepared daily. There was no lethality due to the drug at this dosage level.

Several attempted homotransplants of the MC-induced tumours from *C57BL*/6 mice were not accepted by random-bred Swiss mice.

Three experiments were carried out with numbers of mice sufficient for statistical evaluation. Data from preliminary experiments have been omitted. The incidence of tumour takes ranged from 31 to 88 per cent in the control groups of the three experiments presented, while the number of cells inoculated was of the same magnitude in all the tests (Table 1). Other experiments with the same type of tumour indicate that there is an almost 100 per cent acceptance of tumour isotransplants with inocula of a million cells whereas inocula in the magnitude of 100,000 cells gave variable results but seldom give 100 per cent takes. Since a different primary tumour was used in each of the experiments presented, whereas the recipients were syngeneic (isologous), these variations in tumour transplantability presumably reflect differences in aggressiveness inherent to the individual tumours. Wide variation in latent period and in rate of growth was also observed during the induction of primary tumours.

Table 1. FREQUENCY OF TAKES* OF FIRST PASSAGE METHYLCHOLANTHRENE-INDUCED SARCOMATA IN ISOLOGOUS RECIPIENTS (*C57BL*/6 MICE)

	Inoculum of cells per mouse × 10^5	Controls	(%)	Pre-treated	(%)	Duration of exp. days	*P*
Exp. No. 43	2·5	70/99	71	82/98	84	60	< 0·05
Exp. No. 49	1·5	22/74	31	39/72	54	60	≪ 0·01
Exp. No. 53	2·0	85/97	88	77/85	91	40	> 0·60
Total	—	177/270	65	198/255	78		< 0·01

* Fraction indicates the number of tumours over the total number of mice in the group. Tumours that regressed are registered as no-takes.

The data presented in Table 1 show that there was a significantly higher number of acceptances of tumour isotransplants in mice which were pre-treated with 'Cytoxan', as compared with control groups, in two of the three experiments. The probability values (*P*) by χ^2 analysis were $P < 0{\cdot}05$ in experiment number 43 and $P < 0{\cdot}01$ in experiment number 49. This difference was not observed in experiment number 53, but since the

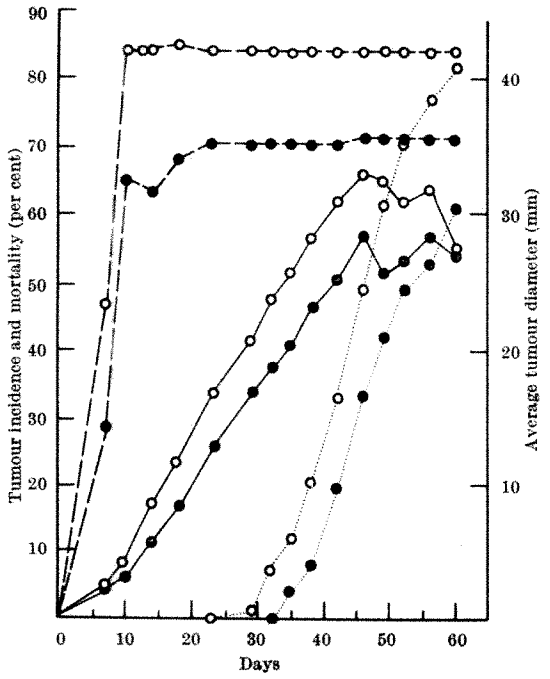


Fig. 1. Experiment No. 43. Comparison of tumour takes and mean tumour diameters in isogeneic recipients pre-treated with 'Cytoxan' and in untreated controls. ○, Controls; ●, experimental animals. – – – –, Incidence as a percentage of the total number of mice per group; ——, average tumour diameters (tumour-bearing mice only) in mm; ·····, mortality due to tumour as a percentage of the total number of mice per group

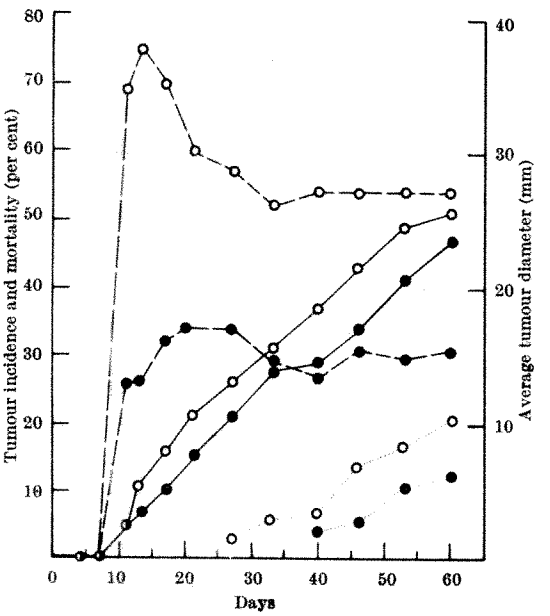


Fig. 2. Experiment No. 49. Same conditions as Fig. 1

incidence of tumour takes was about 90 per cent in the control group of that experiment, a significant increase in the number of takes in the experimental group could not be expected.

The total dose of 'Cytoxan' was the same in all these experiments, but the interval between the last injection of the drug and the inoculation of tumour cells was 72 h in experiment 43, and 24 h in experiment 49. The difference between the experimental group and the controls is greater in the experiment with the 24-h interval. Optimal time relationship as well as optimal dosage of the immunosuppressive medication remains to be determined.

In addition to the increased acceptance of tumour transplants in the pre-treated mice, there was also a slightly shorter period from inoculation to the appearance of palpable tumours, a higher rate of growth, and earlier deaths due to tumours.

Figs. 1 and 2 show the course of two experiments. In experiment 49 (Fig. 1) the peak incidence of palpable tumours in the pre-treated group was on the thirteenth day after inoculation. Afterwards there were a number of regressions until day 33. The period during which the regressions occurred probably coincides with the recovery of the experimental animals from the immunosuppressive effect of 'Cytoxan'. The transplants that were rejected were less than 5 mm in diameter. No significant number of regressions occurred in the other experiments.

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Antibody Inhibition by a Minimal Dose of Antigen and Response to a Sudden Increase of the Dose

WE have already demonstrated[1-3] that continued administration of an optimal level of an antigenic stimulus to an adult animal finally produces a considerable decrease or even a disappearance of circulating antibodies ($\simeq 400$ days). The inhibition applies to complete as well as to incomplete antibodies; it is specific, that is to say, the animal responds normally to another antigenic stimulus, and it is accompanied by a very poor cytological response[3]. As the phenomenon is produced by relatively small doses of antigen, in contrast to 'immunological paralysis', it is appropriate for testing the response of the inhibited animal to a sudden increase in the antigenic dose. Animals inhibited to *Brucella abortus* show a small but significant increase in antibody, after the injection of a large antigenic dose[2,3]. This communication presents the results of our investigations of this phenomenon in the rabbit using human erythrocytes.

We have recently shown that this phenomenon of antibody inhibition by hyperimmunization is produced, not only by an optimal dose of antigen, but also by a minimal dose. Administration of a minimal dose shows a somewhat different evolution, but eventually gives the same considerable decrease in antibody titre[4]. We thought it would be interesting to investigate the response of animals inhibited by this small dose to a dose ten times higher than that which established inhibition.

Eight rabbits (weighing from 2·3 to 2·7 kg) were hyperimmunized by intravenous injection, three times a week, of 1 ml. of 26 per cent suspension of human erythrocytes (group A_1, from the same donor throughout the experiment); the experiment was continued during a period of 480 days. On the last day of this period, 10 ml. of the same erythrocyte suspension was injected. Four animals died within 24 h of this injection, and the remaining four showed a very clear response to this increase of the antigenic stimulus. With this in mind, we have examined only agglutinating titres, determined by the method of two-fold dilutions with two intercalated series, beginning at dilutions of 1/5 and 1/7·5.

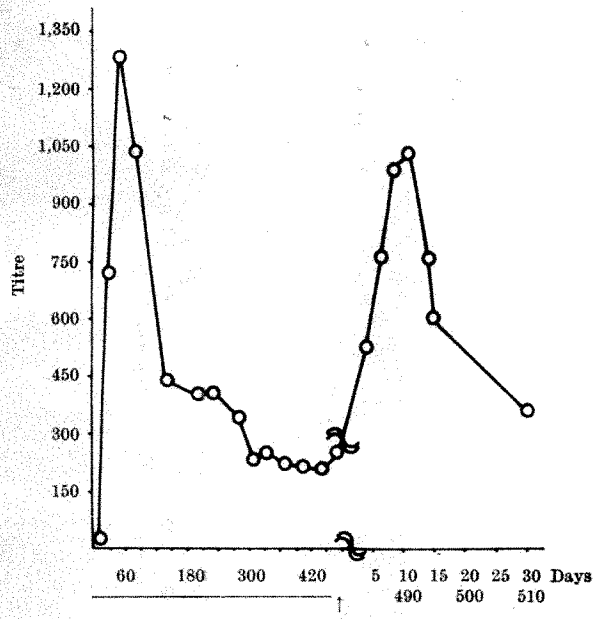


Fig. 1. Response of a batch of four rabbits inhibited by hyperimmunization with human erythrocytes during 480 days, to a single tenfold dose of these erythrocytes

As shown in Fig. 1, these four animals showed a good initial response to hyperimmunization with these doses of antigenic stimulus; the response reached a maximal level at about 60 days (1/1,280), but by the 300th day the titre practically stabilized at about 1/200; this continued for 180 days. They were then injected with a single tenfold dose of antigen and the titre increased rapidly to 1/1,000, which represents a good secondary response, very much greater than that obtained with the same dose injected into a batch of five normal rabbits (Fig. 2).

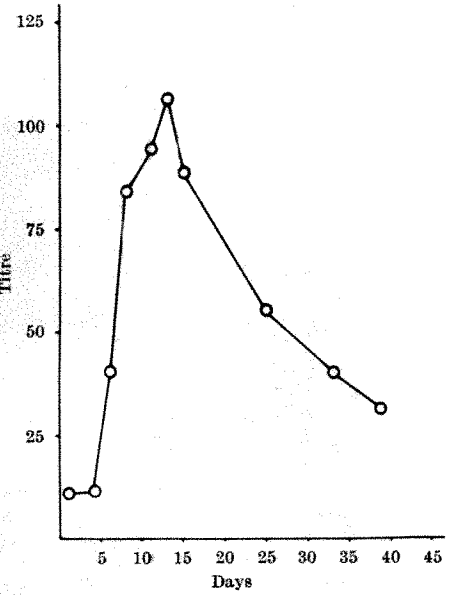


Fig. 2. Response of a batch of five normal rabbits to the same single dose as was received by the rabbits in Fig. 1

This fact confirms our previous observations with *Brucella* that an animal inhibited to an optimal dose of antigen by hyperimmunization responds to a large increase of the antigenic dose with a significant increase in antibody titre. As with the phenomena of 'immunological tolerance' and 'immunological paralysis', this phenomenon of 'inhibition by hyperimmunization' lasts as long as the antigenic stimulation persists. For these reasons we have postulated the existence of an 'immunological equilibrium'[5] between stimulus and response at the intracellular level. This equilibrium can very probably be established at different absolute levels, and, among other possibilities, it can probably be broken by a change in the intensity of the antigenic stimulation, as has been observed in the case of rabbits inhibited by hyperimmunization to small doses of human erythrocytes. This equilibrium must play an important part in the normal non-production of auto-antibodies against the 'self' antigens which are continuously stimulating the immuno-competent system. In some cases this equilibrium with auto-antigens may not be attained at zero level but at a low antibody level, and this is surely the case with normal anti-I cryoagglutinins. We have some experimental data with rabbit cryoagglutinins that seem to favour this explanation. A thorough discussion of this postulated 'immunological equilibrium' between stimulus and response has recently been published[5].

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PATHOLOGY

Centron–Cortex and Cortex–Medullary Cord Junctions in the Rat Lymph Node, as seen in 1μ Paraffin Sections

PREVIOUS communications to *Nature* have shown (*a*) that lymph-borne carbon diffusing into rat lymph node cortex from the subcapsular sinus is deflected by a barrier system around the stromal follicle or 'centron'[1]; (*b*) that lymph-borne iron dextran within the medullary cords is prevented from entering the cortex by a similar type of barrier system[2].

The mechanism of these barrier effects is obscure, but their existence has at least directed attention to the much-neglected histology of the junctional zones between centron, cortex and medullary cord, which is the subject of this communication.

The problem is that ordinary technical methods are inadequate for examining these junctions. Routine haemalum-stained 5μ paraffin sections contain too many artefacts. 1μ sections cut in paraffin-beeswax-stearic acid are necessary[3], and double nucleic acid staining[4], with its more precise delineation of lymphoid nuclei and cytoplasm, is also of value.

Experimental conditions should be strictly specified. The left para-aortic lymph node of the 80–100 g rat is examined 4 days after completion of an antigen course delivered into the corresponding hind foot pad (0·1 ml. Commonwealth Serum Laboratories T.A.B. vaccine twice daily for 5 days).

Under such circumstances, the centron shows a high proportion of blast cells, which may lie immediately adjacent to the lymphocytes of the surrounding cortex without any intervening space. However, a narrow cleft is more usual (Fig. 1), bounded on one side by centron blast cells orientated in a smooth semi-circular line, and on the other by a more irregular wall of cortical lymphocytes. Crossing this cleft can be seen fine protoplasmic processes, which serial sections show are derived from stromal macrophages in the vicinity.

In other areas, instead of a cleft, appears a series of irregular sinuses bounded by fragile macrophage extensions of the same type (Fig. 2). The slightest processing

defect causes their disruption, with the production of a wide peri-centron artefact space.

The cortex–medullary cord junctional area has a rather different morphology. Along some of its length the small lymphocytes of the cortex and the plasma cells of the medullary cord are in direct boundary contact, with few plasma cells penetrating the cortex to any depth.

An intermediate zone composed largely of macrophages can sometimes be seen. In such an area the population of lymphoid cells drops considerably, but never disappears. Large, vesicular macrophage nuclei became relatively more numerous and obvious. The background of macrophage cytoplasm forms a continuous pseudosyncytium, with the boundaries of individual cells very poorly defined. There are few visible spaces or cytoplasmic processes. The width of the macrophage zone is irregular; artefact splitting is seldom seen (Fig. 3).

Although the peri-centron and cortex–medullary cord junctions have features that differ in detail, they both show the importance of stromal macrophages. Around the centron this system of cells forms a thin layer of lymphatic sinuses. Between the cortex and the medullary cord the same type of cell is orientated in a broader and more pavemented pattern.

To be correlated with these appearances are the results obtained by treating unfixed lymph node sections with Indian ink[1], which outlines the processes of stromal macrophages, but shows no obvious change in these processes at either the peri-centron or the cortex–medullary cord zones[1,2]. However, this method uses 10–15μ sections, and would not be capable of showing the fine changes in macrophage orientation which are demonstrated by the 1μ sections under examination.

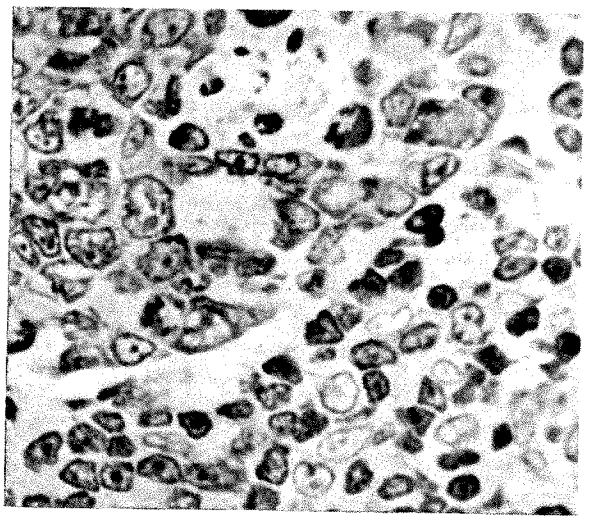


Fig. 1. Portion of lymph node cortex showing, above, the smooth edge of the centron, outlined in blast cells, and, below, cells of the cortex, mainly small lymphocytes. There is a narrow dividing cleft between these areas, running from top right to mid-left. Paraffin-beeswax-stearic acid, cut at 1μ, stained by basic fuchsin-azure *B* (see ref. 4) (× 600)

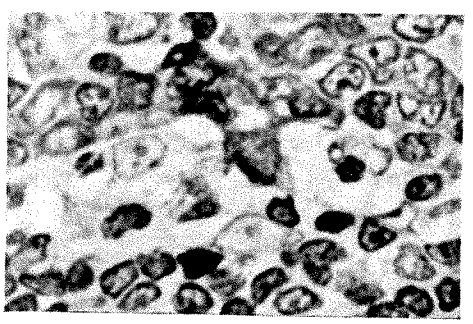


Fig. 2. A view of another portion of the same centron edge as Fig. 1. Note the pale, vesicular macrophage nuclei, with abundant granular cytoplasm throwing out processes, which form the walls of lymphatic sinuses. These processes are specially liable to artefact disruption. Paraffin-beeswax-stearic acid, cut at 1μ, stained by basic fuchsin-azure *B* (× 600)

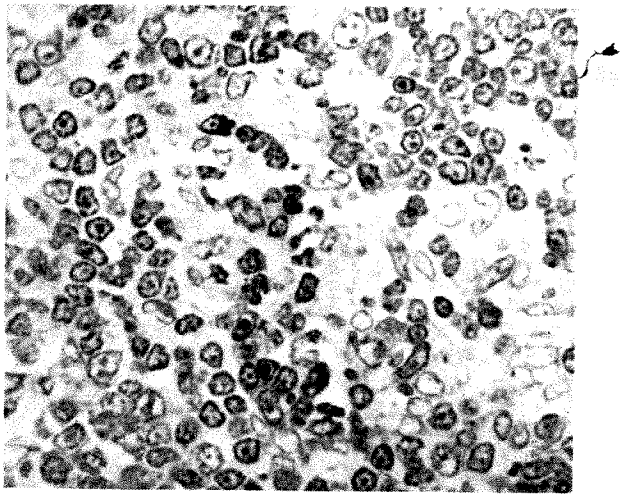


Fig. 3. Junction zone between the cortex and medullary cord of a rat lymph node. Above and right is a group of cortical lymphocytes; below and left are masses of plasma cells in the cord. Note the diagonally placed intermediate area with poorly defined margins, containing a relatively larger number of pale, vesicular macrophage nuclei, and a smaller number of lymphoid cells. Paraffin-beeswax-stearic acid, cut at 2μ, stained by basic fuchsin-azure *B* (× 400)

It is reasonable to assume that peri-centron and corticomedullary particle blockage is in some way a function of the stromal macrophages which are prominent at these sites. How these cells achieve this effect is a matter for further observation and experiment.

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Further Evidence of Enhanced Tumour Destruction with Combined Laser Energy and Chemotherapy

A PREVIOUS article[1] has reported on the significant increase in the tumoricidal effect of laser energy when combined with a course of chemotherapy. In the previous study, multiple pulses of ruby laser (6,943 Å) were used on Cloudman *S*-91 melanomata in CDF_1 mice, and *cyclo*phosphamide was used as the chemotherapeutic agent. This communication reports a similar experiment in which neodymium laser energy (10,600 Å) was used, on the same tumour–host system with the same chemotherapeutic agent. It will be seen that the substitution of a neodymium laser for the ruby laser does not significantly effect the increased oncolytic properties of the combination therapy.

For this experiment the Cloudman *S*-91 melanoma was transplanted into 150 CDF_1 mice by injecting 0·05 c.c. of a tumour cell suspension subcutaneously into the shaved dorsal flank. The cell suspension was prepared by mincing 1 g of freshly excised tumour tissue through a Snell cytosieve with 10 c.c. of sterile saline. When the tumours had reached a size of approximately 6 mm in 126 animals they were randomly divided into four groups of approximately 30 mice each.

*Cyclo*phosphamide in a dose of 25 mg/kg/day was administered subcutaneously daily to two groups. Between the fourth and fifth day of chemotherapy the group of mice receiving laser treatment only, and the group

receiving laser plus chemotherapy had their tumours exposed to between 500 and 1,100 J of laser energy. The dose depended on the size of the tumour as follows: 5–6 mm *D* received 500 J; 7–8 mm *D* received 800 J; 9–10 mm *D* received 500 + 600 J. (Tumour size in this experiment was measured in terms of *D*. Two approximately perpendicular measurements of distance across the tumour were taken, *L* and *W*. *D* is defined to be $\sqrt{LW}$.)

The animals were paired prior to laser treatment so that an equal distribution of the various tumour sizes was present in each of the four groups. The laser used in this experiment has previously been described in detail[2]. It consisted of a neodymium glass rod capable of delivering up to 1,000 J of focused energy on the target with a pulse length of 2 msec. The beam was focused through a double convex lens and the treated tumours were placed in the converging part of the beam, 1 cm from the focal point. A spot size of 3 mm was produced on the mouse skin giving an energy density for an 800-J pulse of 11,428 J/cm². Prior to laser exposure, each animal was anaesthetized with an intraperitoneal injection of pentobarbital and immobilized on an operating board under the laser beam.

A gross tabulation of the outcome of the experiment is presented in Table 1. At the time of application of the laser, the average tumour size in the 'laser plus chemotherapy' group was 7·63 mm, while that in the 'laser only' group was 7·75 mm. The difference in size is attributable to the chemotherapy which slows the growth of the tumours used in this experiment. All tumours in a control group of 34 mice grew rapidly and proved fatal. The animals with tumours that were apparently entirely destroyed have been followed for at least 16 weeks after receiving laser treatment and are continuing to be followed.

Table 1. RESULTS OF THE EXPERIMENT

Outcome	Treatment		
	Laser	Chemotherapy	Laser and chemotherapy
Tumour present at all times	1	33	0
Tumour recurred	26	0	6
Tumour apparently cured	2	0	12
Mouse died without tumour	0	0	12
Totals	29	33	30

It is of interest to compare the time interval between laser treatment and the first evidence of tumour recurrence in the two laser groups. The technician error in estimating the presence of measurable recurrent tumour was approximately 2 days; that is, a measurable tumour may have been present at most 2 days before being reported. The minimum recurrence time after laser was only 2 days, the maximum was 22 days, and the average recurrence time was 8·75 days. Although there were fewer recurrences after combination therapy, the minimum recurrence time was 20 days after exposure to the laser, and the maximum was 110 days. If, in our evaluation of the worth of combination therapy as against laser therapy alone, we allow some weight to the amount of time without measurable tumour, the combination therapy is noticeably superior.

Although no detailed comparison of results using the two wave-lengths of laser energy was undertaken, it is apparent from the data of Table 1 that a course of *cyclo*phosphamide in combination with energy from a neodymium laser is more effective than either *cyclo*phosphamide or laser alone. This is in accord with the results obtained in the previous study[1]. The large number of mice which apparently died of toxic effects during the course of combination therapy is an indication that additional experimentation is necessary to determine the optimal method of combining the two treatments. Further experiments along these lines, and on the effects of other drugs as well as other tumour–host systems, are at present in progress.

We thank James R. Dearman and Norman Cook of the Applied Physics Branch, Redstone Arsenal, Huntsville, Alabama, for their generous assistance and for the use of their laser equipment.

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RADIOBIOLOGY

Radiation Sterilization of Food

I AM constrained to comment on the article entitled "Direct and Indirect Effects of Radiation on Plant Cells: Their Relation to Growth and Growth Induction" by Holsten, Sugii, and Steward[1]. I am impelled to do this mainly because of what I deem to be an irrelevant statement made in the concluding remarks of the article which states: "The work has other and obvious implications for the radiation sterilization of food, especially in those cases in which the material so radiated is relatively rich in sugar".

I feel that Prof. Steward *et al.* have not only drawn false conclusions from inadequate data but indeed have made wild statements, unsubstantiated by their own evidence and contrary to the immense amount of literature in the field of radiation chemistry.

First, let me comment on the latter statement. Data are presented in Table 8 on the ultra-violet absorption spectra of sucrose irradiated and irradiated plus autoclaved. To me it would have been imperative to run the spectra on autoclaved sucrose as well, and to do this at different intensities of heat and with different quantities of irradiation. Some of these experiments have, in fact, been carried out during investigations concerned with the absorption spectra of sugars and the implication of the spectral changes in respect to the non-enzymatic browning of certain food[2,3]. Among the compounds which absorb in the region cited by the authors and about which some information is available in the literature are 5-(hydroxymethyl) furfuraldehyde and levulinic acid. Perhaps these may have been involved. They have been implicated in the sugar breakdown alluded to above. The absorption maximum at 285 mμ corresponds to 5-(hydroxymethyl) furfuraldehyde and that at 265–268 mμ to levulinic acid. Pigman and Goepp[4] have postulated the formation of levulinic acid from 5-(hydroxymethyl) furfuraldehyde. The ultra-violet absorption spectra of levulinic acid and the role of 5-(hydroxymethyl) furfuraldehyde in the discoloration of sugar solutions were published by Singh *et al.*[5].

The second point relates to the question of correlation between carrot cells, *Vicia faba* seedlings and animal systems. Indeed, there are hundreds or thousands of simple compounds that can cause mutations when relatively simple systems, such as the two cited, are exposed thereto. Such effects cannot, however, be compared with defined whole animal systems, such as rats, cats, dogs, chickens and humans, replete with mechanisms for modification, alteration, and digestion of the foods by the alimentary tract and the detoxification and excretion mechanisms of the liver and kidney.

To me it is difficult to conceive how anyone could imply or speculate that the data that are presented on the irradiation of sucrose solutions, coconut milk, etc., and the response of carrot cells and *Vicia faba* seedlings to these have any pertinence, even remotely, to the radiation preservation of foods of any type. The conclusions drawn by Steward *et al.* are immaterial and unwarranted. It is indeed unfortunate that these conclusions have been given wide publicity by the popular press.

The readers of *Nature* should be aware that the wholesomeness of some twenty-one different foods has been studied in a carefully designed and planned country-wide programme, which has extended over some 10 years. This work was planned in concert with the Office of the Surgeon General of the U.S. Army and the U.S. Food and Drug Administration. It has been periodically and carefully reviewed and scrutinized, in depth, by a committee of the National Academy of Sciences–National Research Council. Extensive laboratory data have demonstrated the safety of the process and shown that the foods studied can be consumed with impunity.

Included among the twenty-one different foods which have been studied with respect to their wholesomeness when they are treated with sterilizing doses of ionizing energy are three which are high in sugar, namely: pineapple jam; sweet potatoes immersed in sugar syrup; fruit compote. These have been fed to multiple generations of two species of animals, one being a non-rodent, over a 2-year period. Complete histopathological data and physiological responses on the animals have been obtained. The feeding studies of the animals have been carried out on a 35 per cent food intake level. All this information is available in the open literature, and these data have been, or are being, submitted to the U.S. Food and Drug Administration in order to obtain clearance for their sale.

In addition to the animal studies, investigations of the chemical effects of ionizing energy on the various components of foods, including carbohydrates, have been carried out as part of the U.S. Quartermaster Program and the Atomic Energy Commission Program and are available in the open literature.

Based on the data obtained in these extensive investigations, which go far beyond the level of due prudence which would normally be required for conventional methods of food processing, the U.S. Food and Drug Administration has started to issue regulations permitting the sale of radiation-treated foods in the United States.

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Depletion of Mouse Spermatogonia following Exposure to Polonium-210

LOCALIZATION of polonium-210 (^{210}Po) in the interstitial spaces of the testes of rats has been demonstrated radioautographically[1] and a very slow rate of excretion of ^{210}Po from rat testis has also been shown. ^{210}Po is a naturally occurring alpha-emitting element of the uranium decay chain. A particle energy of 5·3 MeV allows a range for the ^{210}Po alpha particle of about 37μ in tissue. Since this distance is sufficient to penetrate from the interstitial spaces of the testis into the outer cell layers of the seminiferous tubules of the testis where spermatogenesis occurs, it seemed important to ascertain the effect of ^{210}Po on spermatogenesis. The survival of resting primary spermatocytes in stage VII tubules of mouse testis was measured by methods described by Oakberg in studies of the response of the mouse testis to external radiation exposure[3] and later used for the evaluation of tritiated thymidine toxicity[4]. Male Harvard Swiss Wistar mice 2–25 weeks of age were injected peritoneally with 0–2 nc. of ^{210}Po, obtained from the Radiochemical Centre, Amersham, and constituted in 0·05–0·25 ml. of isotonic weakly acid solution prepared by dilution with normal saline. At intervals from 5 days to 10 months after injection, mice were killed by cervical dislocation and their testes removed and fixed in either Bouin's solution or 10 per cent neutral formalin. The tissues were embedded in paraffin and cut into 5μ sections. Prepared slides were stained with periodic acid–Schiff–haematoxylin stains and examined at 1,000 × magnification using an oil immersion objective. Only seminiferous tubules cut in cross-sections so that they appeared round were selected for scoring. Only those tubules in stage VII, as established by maturation of the acrosomes of the spermatids[5], were counted. 15–30 tubules were counted from each slide. Serial adjacent sections were avoided. The results are plotted in Fig. 1.

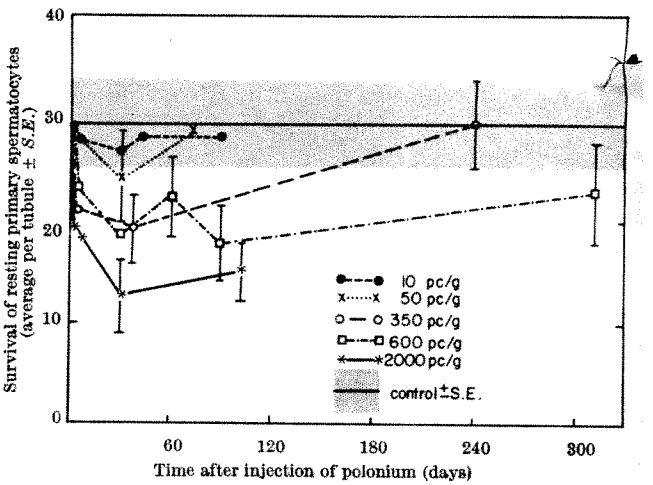


Fig. 1. Survival of resting primary spermatocytes in testes of mice injected with polonium-210 at zero time and killed at varied times thereafter. Amounts injected intraperitoneally indicated by legend. Standard errors indicated by span around points. All points lying outside standard error of control count are significantly different from control value (*t*-test $P < 0{\cdot}01$) except 30-day point on 50 pc./g line (*t*-test $P < 0{\cdot}05$)

These results can be considered in the light of spermatogenesis in the mouse. Resting primary spermatocytes (RPS) in mice are the result of a series of six cell divisions which begin with a dormant type *A* spermatogonium and proceed through mature type *A*, intermediate and type *B* stages to the RPS stage, which is the stage of meiotic prophase, after which mitotic division occurs to form haploid spermatoids[6]. The total time-course of these cell divisions requires about 7 days, so that testes from mice injected with ^{210}Po seven or more days prior to killing have been exposed to the internal radiation from ^{210}Po throughout the total cycle of spermatogonial divisions. Since cell division is known to be highly radiation sensitive, the system just described maximizes radiation exposure during the challenge of a series of cell divisions.

Radiosensitivity of the spermatogonia stages differs considerably from LD_{50} of 20–24 r. for intermediate and type *B* spermatogonia to the LD_{50} of several hundred r. for dormant type *A* cells[7]. The latter cells remain even after large doses of radiation and serve to replenish the cells of the tubule within 60–90 days after an acute radiation exposure. In the present experiments, these cells probably account for the recovery seen after 30 days at almost all doses of ^{210}Po used. At the highest level of exposure, that following administration of 2·0 nc. of ^{210}Po per g body-weight, there was only a slow and slight recovery during the period of observation.

Table 1. RETENTION OF POLONIUM-210 IN MOUSE TESTIS AND KIDNEY FOLLOWING INTRAPERITONEAL INJECTION OF 10 PC. PER G BODY WEIGHT

Days after injection	pc. ^{210}Po per g tissue (corrected for physical decay) Testis	Kidney
30	16·3	55·5
60	11·6	9·8
75	9·2	8·9
90	9·2	15
120	2·5	5·2
140	1·4	4·9

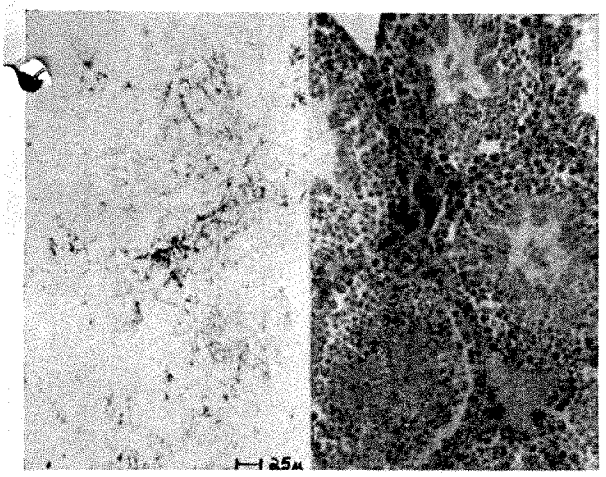


Fig. 2. Autoradiograph of testis from mouse injected intraperitoneally with 2,000 pc./g body weight of polonium-210 and killed 5 days later. Note concentration of radioactivity in interstitial tissue surrounding tubules. Compare the autoradiograph with the stained section of testis (right) taken from a similar animal and photographed at the same magnification. The α tracks, which appear as short streaks, penetrate the basement membranes of the tubules and traverse the spermatogonia in the periphery of the tubules. (Autoradiograph prepared with Kodak '*NTA*' liquid emulsion, exposed four months, developed in Kodak '*D*-19' and photographed unstained)

The most probable reason why regeneration of cellular elements was slow and delayed is that the ^{210}Po remains in the testis and continues to irradiate the spermatogonia. Significant amounts of ^{210}Po persist in the testes even after 90 days, as shown by radiochemical assay of excised testis (Table 1). Autoradiography has confirmed the localization of this ^{210}Po within the intertubular spaces such that the basement membrane of the tubules is exposed to a maintained flux of alpha particles (Fig. 2).

The results of preliminary investigations of the functional effects of this exposure suggest that overall fertility of the mouse is not grossly impaired by ^{210}Po exposure although the systemic effects of larger doses of ^{210}Po impair the general vigour of the animals and appear to depress their reproductive activity. Because of the uncertainties in the effective target and in the regularity of distribution within the tissue, the radiation doses associated with the administration of ^{210}Po cannot be calculated with any accuracy. As an estimate, based on the amounts of ^{210}Po assayed in tissue, the administration of 0·1 nc. of ^{210}Po per g body-weight (the minimum amount effective in producing significant cellular damage to the testis) should deliver at least 300 mrad local radiation dose during the first 10 days after administration. If the relative biological effectiveness of the radiation is 10, this corresponds to a rem dose of 3 r., which is identical to that dose of gamma radiation which produces a similar minimal but significant effect in this system[6].

This work was performed as part of a thesis presented to the faculty of the Harvard School of Public Health in partial fulfilment of the requirements for the degree of doctor of science in hygiene in the field of radiological health, and was supported in part by the Division of Radiological Health, U.S. Public Health Service. I was then on assignment from Training Branch, Division of Radiological Health, when the work described herein was performed.

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BIOLOGY

Clutch Size in the *Sulidae*

FACTORS influencing the evolution of clutch size in birds have received notable attention from Kluijver[1], Lack[2], Wynne-Edwards[3] and others. The family *Sulidae* (order Pelecaniformes) provides a good example of adaptive radiation within a group, different clutch sizes having evolved in a balanced ecological/behaviour system. This problem was studied on the Bass Rock, Scotland (1960–63), the Galapagos Islands (1964) and the Peruvian Guano Island of Guañape Norte (1964–5) for *Sula bassana*, *S. sula*, *S. dactylatra*, *S. nebouxii* and *S. variegata* (*S. leucogaster* data from Murphy[4] and Dorward[5]). Data for *S. abbotti* are lacking, but this species probably closely resembles *S. sula*.

These species show a finely graded series of clutch sizes: *S. bassana* and *S. sula* (1), *S. dactylatra* (1–2), *S. leucogaster* (1–3, usually 2 and very rarely 3), *S. nebouxii* (1–3, usually 2 but often 3) and *S. variegata* (1–4, usually 2 or 3).

The single young of *S. sula* grew irregularly, experiencing frequent and extensive weight losses, took around 19 weeks to reach an average fledging weight of 900 g and had a 2-month period of post-fledging feeding by their parents. 30–60 per cent starved before fledging. All experimentally twinned nests failed completely.

S. dactylatra never reared more than one young per season, though the 5–6 day younger chick survived up to 1 month before succumbing to persecution by the sibling. A fledging weight of 2,500–3,000 g was reached in 16–20 weeks and was followed by a post-fledging feeding period of 7–9 weeks.

S. leucogaster also rarely rears more than one chick. This fledges in about 17 weeks at an average weight of 950 g. Post-fledging feeding occurs for at least 8 weeks (Dorward).

S. nebouxii not infrequently rears two young (three have been recorded) even though these hatch, as in *S. dactylatra*, at five-day intervals. A fledging weight of about 1,750 g was reached in 14–15 weeks and post-fledging feeding lasted for 8 or more weeks.

S. variegata often rears two young; sometimes three and four have been recorded. The fledging weight was not recorded, but the fledging period is probably 12–15 weeks. Post-fledging feeding occurs.

Apart from the obvious importance of the Anchoveta-rich Humbolt Current adjacent to its breeding islands, which favours *S. variegata*, I suggest that these facts can be partly explained by differences in the feeding habits of the species concerned. *S. sula* and *S. dactylatra* forage at considerable distances from the nesting colony and often spend 1–5 days away from the nest. *S. nebouxii* and *S. variegata*, on the other hand, regularly fish close inshore and can complete a successful fishing trip in as little as 30 min from leaving to returning. In possible association with this habit they both, but particularly *S. nebouxii*, show a more pronounced sexual dimorphism in size than do other boobies; males weigh on average only 1,280 g (two-thirds of the average female weight) and apparently tend to specialize in inshore fishing in shallow water, sometimes plunging into less than 2 ft. In the later stages of growth the young are fed solely by the female—probably a unique phenomenon in the *Sulidae*. Because the average duration of fishing trips is shorter in *S. nebouxii* and *S. variegata* the young are less subject to starvation periods in the early, more vulnerable stages. In species where starvation periods are more probable, two or three young would endanger the survival chances of all by dividing the available food. The ability of *S. nebouxii* and *S. variegata* to rear more young than *S. sula* or *S. dactylatra* may thus be directly related to the average duration of their fishing journeys. This may itself result from the tendency of each sulid

species to specialize in particular species of fish. Flying fish *Exocoetus* spp. are commoner in the regurgitations of *S. sula* and *S. dactylatra* than of *S. nebouxii*.

Although *S. leucogaster* seems anomalous, rearing only one chick yet apparently showing a tendency to fish closer inshore than *S. dactylatra*, it is clear from Dorward[5] and others that this species suffers frequent and severe food shortage, which in effect favours the rearing of a single chick.

Although at first sight *S. bassana* seems to support the above relationship by invariably laying a single-egg clutch and also typically fishing at considerable distances from the breeding colony (up to 200 miles or even more), an experiment carried out in 1962 and 1965 on the Bass Rock showed that pairs given one extra young under controlled conditions fed both adequately and so gained a large reproductive advantage over their fellows[6]. Evidence gathered for *S. sula* and *S. dactylatra* showed that rearing young had no effect on the weight of the parents, which suggests that the normal clutch size is unlikely to impose such a strain on the parents that an addition would be highly detrimental. By inference this is likely to be true for *S. bassana*. If clutch size is mainly conditioned by the number of young that the parents can feed adequately[3], *S. bassana*, on the above results, may have been expected to evolve at least a balanced polymorphism with respect to clutch size, the advantages of larger clutches perhaps being partly cancelled by their total failure in certain years. However, the British population of *S. bassana* is at present increasing rapidly and is therefore unstable with respect to its food supply. Also, the gannet's long life (annual adult mortality 6 per cent), deferred maturity and low reproductive rate lead to a slow population turnover and may mean that the one-egg clutch, which was presumably derived from the phylogenetically more primitive polyparous condition, has not yet had time to revert as an adaptation to a favourably changed environment.

The foregoing evidence and hypotheses may partly explain the otherwise puzzling variation in clutch size in a closely related, fish-eating family with basically similar plunge-diving feeding mechanisms.

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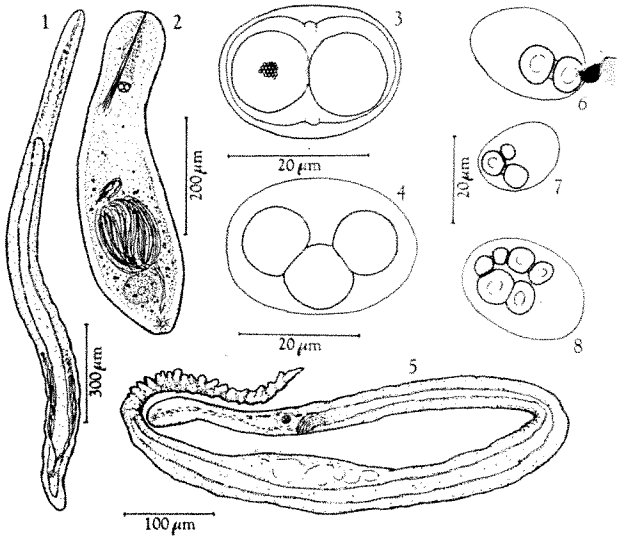
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New Polylithophorous Marine Turbellaria

IN 1930–31 Steinböck[3] described *Nemertoderma bathycola*, a peculiar new turbellarian from deep bottoms near Greenland. He considered the species to be rather primitive. A detailed investigation of a closely related if not identical form from the Scandinavian West Coast was published a few years later by Westblad[7]. In 1949 Westblad described *Meara stichopi* which is parasitic in echinoderms[8]. These forms are characterized mainly by a statocyst carrying two statoliths (occasionally one, three or four[1]; Fig. 4), a neuropilem and very primitive genital apparatus.

During recent investigations of the microfauna of some European coasts[4–6], I found all the previously described Nemertodermatidae including (from deep bottoms off Bergen, Norway) Steinböck's original form. In addition, several undescribed species were encountered mainly from littoral sand. One of these (Figs. 1 and 3), an elongated form characterized by an intense salmon-red longitudinal stripe in the anterior part of the body, was found at Kristineberg on the west coast of Sweden. This species



Figs. 1–8. (1) *Nemertoderma* sp. I from the west coast of Sweden; (2) *Nemertoderma* sp. II from the Adriatic; (3) statocyst of *Nemertoderma* sp. I; (4) statocyst of an aberrant specimen of Westblad's *Nemertoderma*; (5) polylithophorous turbellarian from the west coast of Sweden; (6–8) statocysts of three different specimens of the same as (5)

also occurs in the Adriatic in addition to a more compact species. The latter carries a rather strange broom-like organ of unknown function in the anterior region (Fig. 2). Some new forms, also undoubtedly related to *Nemertoderma*, have been recorded among R. Riedl's collections from the Red Sea[2].

A still more interesting discovery, also in littoral sand from Kristineberg and the Adriatic, is the small elongated turbellarian shown in Figs. 5–8. Although it usually carries only a single statolith, specimens with two, three and even six statoliths have been found. The structure of the statocyst is reminiscent of the statocyst of the nemertean *Ototyphlonemertes*. Furthermore, the animal has a pharynx simplex and a ciliated intestine—characters usually associated with the Catenulida.

The strange characters of this polylithic worm and the great variability of form, colour and glands in *Nemertoderma* lead to problems in the systematics of the new forms. Anatomical study of the material should reveal much additional data and a full account will follow.

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Carbohydrate and Amino-acid Composition of the Egg Capsule of the Whelk *Buccinum undatum* L.

THE characteristic monosaccharide sugar spectrum for glycoproteins of animal origin has, until comparatively recently, been regarded as being limited to the two hexosamines, glucosamine and galactosamine; the neutral hexoses, galactose, mannose and occasionally glucose; and the methyl pentose, fucose. One or other of the neuraminic acids is frequently present. The occurrence, in nature, of such monosaccharides as xylose, arabinose and rhamnose has hitherto been regarded as being confined to plants and micro-organisms. The presence, however, of xylose as a key constituent of acid-mucopolysaccharides[1], and also accompanied by ribose, arabinose, galactose, mannose, fucose, glucosamine, galactosamine and sialic

acid in a mucoprotein isolated from autoclaved elastin[2], has recently been reported. In the present investigation, xylose and rhamnose have been found to occur in conjunction with protein in a material of invertebrate origin.

The eggs of the whelk *Buccinum undatum* are laid enclosed in protective capsules which, after the hatching of the eggs, are frequently found cast up by the sea along the shore line. These capsules have a horny sclerotized appearance and are semi-transparent and brownish in colour. For the present investigation, the capsules were broken up into smaller fragments and washed thoroughly with water before examination. The capsular material was insoluble in water, 0·5 M sodium chloride and 4 N sodium hydroxide. On treatment with sodium sulphide solution (5 per cent w/v) the material exhibited swelling but did not go into solution. Treatment with performic acid at 4° C again produced swelling, but the material did not dissolve. Prolonged treatment with 45 per cent w/v aqueous phenol at 90° C failed to bring about extraction of material into either the aqueous or phenol phases. Treatment with 4 per cent w/v trichloroacetic acid at 60° C was also without effect and no carbohydrate could be detected in the extract. The capsules also exhibited considerable resistance to proteolytic digestion. Crystalline pepsin, trypsin, chymotrypsin and bacterial proteinase ('Novo') were almost completely without action and the powerful proteolytic enzyme 'Pronase' (Kaken Chemical Co., Japan) brought about only a low degree of degradation after incubation with the material for 5 days.

Paper chromatography of acid hydrolysates of the material and colorimetric estimation of the aniline-hydrogen-phthalate stained spots, after elution[3], revealed the presence of the monosaccharides, galactose, glucose, mannose, xylose, fucose and rhamnose, in the approximate molar ratios of 2 : 1 : 3 : 1 : 2 : 1 respectively. These sugars account for some 5–6 per cent of the total weight of the material, as estimated by an orcinol reaction[4]. Approximately 2 per cent w/w hexosamine (Elson–Morgan reaction[5]) was present, but this sugar has not been positively identified although its position on an automatic amino-acid analyser (Technicon) chromatogram suggested that it was neither glucosamine nor galactosamine. No sialic acid could be detected in the material[6]. The resistance of the carbohydrate to extraction by the various procedures described above suggests that it might be covalently bound to the protein present.

The amino-acid composition of the capsule material, estimated by automatic amino-acid analyser after acid hydrolysis, is shown in Table 1. Tyrosine and histidine are completely absent. The low concentration of sulphur-containing amino-acids suggests that the insolubility of the material cannot be attributed to disulphide cross-linking. Amino-acids account for 77·5 per cent of the total weight of the capsule material.

Table 1. AMINO-ACID COMPOSITION OF THE EGG CAPSULE MATERIAL
(*a*) Amino-acids in g/100 g substance. (*b*) Amino-acids in μmoles/100 μmoles total amino-acids

Amino-acid	(*a*)	(*b*)
$CysSO_3H$	0·76	0·75
Asp	10·73	13·49
Thr	4·43	6·23
Ser	5·21	7·90
Glu	15·47	17·58
Pro	1·02	1·49
Gly	2·09	4·66
Ala	4·03	7·56
Val	4·72	6·74
Met	0·26	0·27
Ileu	4·89	6·24
Leu	7·24	9·24
Phe	5·64	5·72
Lys	6·03	7·32
Arg	3·97	3·81

Examination of the infra-red spectra of finely powdered samples of the material, in Nujol mull, confirmed the chemical data. The characteristic amide bands of protein were present at 1550, 1660 and 3300 cm^{-1} while the presence of carbohydrate was indicated by bands in the region 950–1,100 cm^{-1} and 3,400 cm^{-1}. Ester sulphate bands were not present.

The mode of secretion of the egg capsule in the marine mollusca has been the subject of some controversy (reviewed in detail by Fretter[7]). Cunningham[8] reported that in *B. undatum* the egg capsule was formed by a secretion from the sole gland of the foot whereas Fretter[7] suggested that the capsule in this mollusc was secreted by the lower part of the female genital duct and kneaded into shape by a temporary groove along the base of the foot. On the basis of histochemical evidence Ankel[9] and Fretter[7] have suggested that the egg capsules of several molluscan genera might be composed of mixtures of protein (probably conchiolin) and 'mucin'. Drew[10] noted a distension, by a granular secretion, of the hypobranchial gland of the female of *Nuculla delphinodonta* (lamellibranchiata) during the breeding season and suggested that the egg capsules were derived from this material. A similar conclusion was drawn by Gersch[11] from the observation that the hypobranchial gland in *Gibbula tumida* (gastropoda) secretes most actively during the breeding season. In view of these observations it is interesting to note that the composition of the egg capsule in *B. undatum* does not resemble the composition of the animal's hypobranchial secretion, which is composed of a polyglucose sulphate-peptide complex loosely linked to a glycoprotein which contains mannose, galactose, glucose, fucose, glucosamine and galactosamine and in which the amino-acid composition is different from that of the capsule protein[12].

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A Method of preparing Sperm Whale Teeth for Age Determination

PREVIOUS methods of preparing sperm whale teeth for age determination have generally involved the preparation of ground and polished thin sections[1–3], or half teeth[2,4,5], which have been examined under a dissecting microscope[2–7], a surface projector[2], polarized light[2], or after treatment with histological stains, for example carbol fuchsin or haematoxylin[2–4,6].

Since the preparation of thin sections and the use of histological stains are time-consuming, an attempt has been made to develop a quick, less exacting method of acid etching. At the same time a photographic technique has been established to reduce the eye strain involved in using a magnifying glass or dissecting microscope and to eliminate possible errors in counts and measurements caused by examining small fields rather than the tooth as a whole. This technique has been developed in the course of examining large numbers of sperm whale teeth, as part of an investigation into the sperm whale stocks off Western Australia which is now in progress.

Where haematoxylin staining increases the definition of laminations in the dentine by differential absorption of the dye, our method produces a similar effect by differential etching. Light and dark bands in reflected light appear as a sequence of ridges and grooves respectively which photograph extremely well and allow rapid and repeatable counts and measurements to be made. Fig. 1

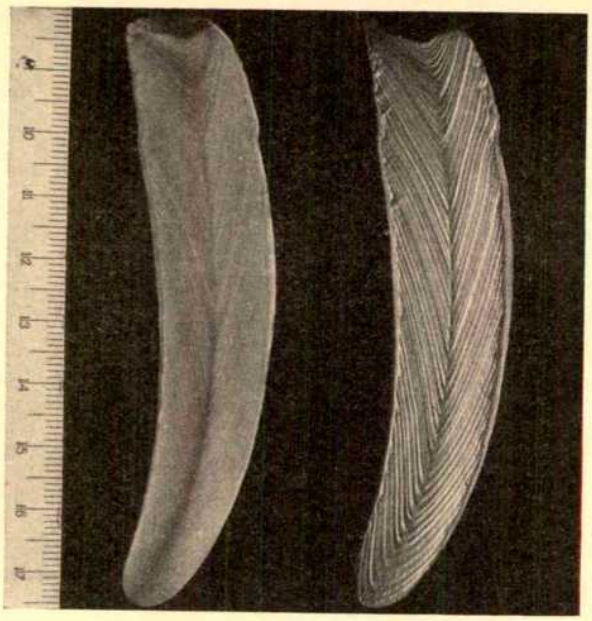


Fig. 1. Longitudinally bisected tooth. Left, before treatment. Right, after treatment. Scale in centimetres

shows a longitudinally bisected tooth from the front of the lower jaw of a female whale 32 ft. in length.

The method of mounting whole teeth for bisection is similar to that described by Sergeant[3]. Using greased 0·25 in. 'Perspex' angle pieces as moulds and laminated five-ply as a base, whole teeth were mounted (with their longitudinal axis horizontal) in Ciba 'Araldite' casting resin (resin *CY*248 and hardeners *HY* 965 and 966). Alternatively Ciba 'Aerosil' can be added to the mixture to obtain a thicker consistency and so reduce the amount of 'Araldite' required. The curing time is about 4 h. Once mounted, teeth were bisected longitudinally with a 12 in. diameter fine-toothed slitting saw. The blade was cooled with a water-soluble oil mixture. When thin sections were required a 'Perspex' plate was glued with 'Araldite' to the cut surface of the mounted half tooth and again cut on the circular saw. With this method, sections about 300-μ thick, which could be ground further with 'Carborundum' abrasive were obtained. Without the 'Perspex' support it was impossible to cut thinner than 500μ as the sections tended to curl and split. The first cut was made slightly short of the centre line to obtain a flat face. The 'Perspex' plate was then glued to the flat face and the next cut made slightly behind the centre line leaving the section attached to the 'Perspex' plate. With teeth which curved in two planes the best cut was sometimes slightly off-centre.

Various concentrations of hydrochloric, nitric and formic acids have been used in an attempt to etch the cut surface of the mounted half tooth. We have found that 5 per cent formic acid in tap water at room temperature (approximately 22° C) for 30 h gives good definition of major zones but little fine detail. The fine dark bands which occur between the major or most prominent dark bands were intensified by an alternative treatment with 20 per cent formic acid at 80° C for 90 min.

It is difficult to determine how etching is proceeding during the process, since the only apparent change is a slight softening and swelling of the dentine due to the effect of acid on collagen, but when the tooth is soaked in water to remove the acid and then allowed to dry at room temperature, the results are quite obvious. The surface instead of being flat is corrugated by a sequence of ridges and grooves. If the corrugation is not sufficiently defined the tooth must be refaced and the whole process repeated.

Because of the monochrome nature of the tooth surface and the reflexion of incident light, difficulty was experienced in counting and measuring the distance between successive ridges. This difficulty was overcome by photographing the etched surface and producing a representation of the almost uniform cream-yellow surface in contrasting tones of grey and black; enlarged prints also facilitated counting and measuring. In order to correct measurements for magnification a scale graduated in millimetres was photographed with each tooth.

A 'Pentax S.L.R.' 35-mm camera body fitted with a 'Takumar' 2·2/55 mm lens and No. 1–9 mm and No. 2–18 mm extension tubes was used together with Ilford 'Pan F' film with a comparatively slow speed (50 A.S.A.) and an extra-fine-grained emulsion.

A satisfactory light source consisted of two 250-W self-reflecting flood lamps (Philips *PF*217*E*/43 S.M.) positioned to give maximum shadow detail and contrast, with two sheets of finely abraded plastic to act as a light-source diffusor.

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Models of Living and Fossil Populations of Animals Generated by a Computer

BIOLOGISTS have long been interested in the dynamics of living populations of animals, but for obvious reasons they have been less concerned with the nature of the resulting dead populations. Recently, palaeontologists[1,2] have begun to investigate the dynamics of living populations and their transformation into fossil communities. It is now possible to complement these studies with mathematical models which define the form of a stable living population and its accumulating fossil counterpart. The processes of recruitment, growth, seasonality and mortality have been programmed for an '*IBM* 7094' computer and the output expressed in terms of age-frequency and size-frequency for the living and resulting dead populations.

Although the programme is specifically designed to simulate the population dynamics of certain marine invertebrates, it can be modified for other groups of animals. The number of recruits is specified for each week of a 48-week year, and the kind of recruitment can be varied from the short burst of summer recruitment occurring in Arctic waters to the more even year-round recruitment common in the Tropics. As many as 100,000 animals may be recruited in one year. The recruits are introduced at a size of one unit and are grown in weekly increments to any desired size, up to a maximum of 999 units. The growth increments may be changed at the end of each four-week period and different coefficients of variation in the growth rate may be specified. The maximum life-span of any animal is limited to 10 years. Seasonal cessation of growth, as, for instance, during the winter months or the period of reproduction, may be introduced for any month or any combination of months, reproduction starting at any given age. Mortality rates may be of any kind or degree (we have used constant, increasing and decreasing rates) and are specified in terms of deaths per thousand animals per week. The mortality rate can be increased by a desired factor (maximum × 10) during periods of no growth.

From this programme a series of graphs have been obtained which clarify some of the causes of the different

forms of size-frequency distributions seen in fossil populations. It is possible that the programme may also be of use to biologists who may wish to determine the population dynamics of a known animal under different physical and biological conditions, and for this purpose we have included in the output figures the size-frequency and age-frequency distributions of the living population at the end of each quarter year. One experiment will serve as an example. Ten thousand animals recruited evenly over a four-week period during the month of June are grown at a constant increment of 2 units per week, which is in turn subjected to a coefficient of variation of 2. The death rate is constant at 10 per 1,000 per week, but growth is stopped annually for three months from December to February. Graphs of the input-data are shown in Fig. 1.

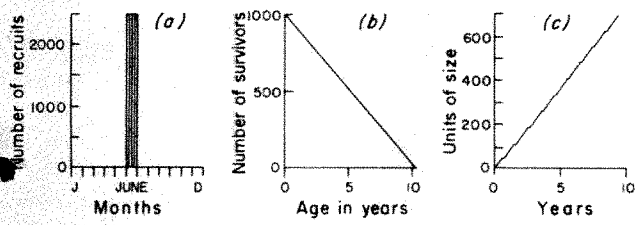


Fig. 1. Graphs of the input data. (*a*) Recruitment of 2,500 animals per week for the four weeks of June. (*b*) Constant death rate of 10 per 1,000 per week. (*c*) Constant growth rate of 2 units per week (growth stopped each year during the three months December–February). This growth rate is subject to a coefficient of variation of 2

The size-frequency distribution of the living population (Fig. 2*a*), as it would be found at the beginning of summer, shows the sharp bursts of yearly recruitment and the effects of mortality. The peaks representing the older recruits gradually merge as a result of the increasing overlap of the size classes. This effect is caused by the coefficient of variation imposed on the growth rate.

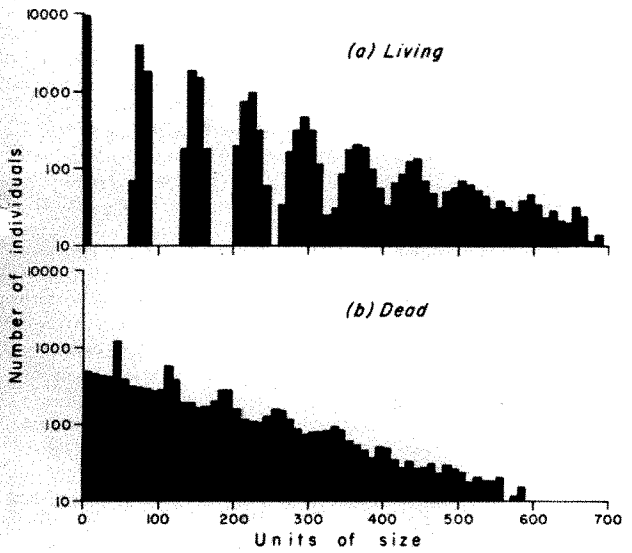


Fig. 2. Size-frequency graphs of (*a*) living and (*b*) resulting dead populations generated by the computer from the information shown in Fig. 1. The irregularity of the largest-size classes in the histograms arises from a 'Monte-Carlo' random-death effect written into the programme. The effect is apparent only with small numbers

The resulting dead population (Fig. 2*b*) also shows a series of peaks, but they represent individuals which died during periods of no growth. These peaks would have been more pronounced had the animals been subjected to increased mortality during the three winter months.

By means of this programme it is possible to generate numerous kinds of living and fossil populations. The number of combinations that may be fed into the computer is obviously very large and we are concerned that our final results should be generally useful and yet compact. If any reader has proposals that may be relevant to the project we would be glad if he would write to one of us.

Access to an '*IBM* 7094' computer without charge has been provided by the Computing Facility of the University of California, Los Angeles.

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Influence of Herbicides on Respiration of Young *Pinus* Seedlings

Field and greenhouse studies with a number of herbicides have demonstrated various degrees of growth inhibition of young gymnosperm seedlings. The effects vary greatly with the chemicals and dosage used, species, age of plants, manner of herbicide application, and environmental conditions[1–4]. Considerable work has been carried out on herbicidal influences on metabolism of herbaceous plants, but very little information is available for the responses of woody plants. Triazine herbicides are generally accepted as inhibitors of photosynthesis[5,6]. Metabolism is probably altered by atrazine in red kidney bean[7] and by simazine in barley roots[8]. The extensive literature on 2,4-*D* and 2,4,5-*T* shows that metabolism varies greatly with species, age of plant, and tissues tested[9–12]. Ingle and Rogers[13] have shown that dalapon may reduce phosphate uptake while it does not alter respiration materially. EPTC causes some increase in respiration of mung beans and corn embryos after relatively long exposure to the herbicide[14]. Experiments have now been extended to evaluate the short-time effects of the active and inert ingredients of commercial formulations of several herbicides on respiration of tissues of woody plants. Herbicides and corresponding commercial formulations were obtained as: 2,4-*D* (esteron 10-10), 2,4,5-*T* (esteron 2,4,5-*OS*), 2,4-DEP (falone 44-*E*), EPTC ('Eptam 6 *E*'), CDEC ('Vegadex'), CDAA ('Randox'), simazine (simazine 50 *W*), atrazine (atrazine 50 *W*), ipazine (ipazine 25 *E*), prometone (prometone 25 *E*), dalapon ('Dowpon'), and sesone (sesone).

Respiration of three-day-old *Pinus resinosa* L. seedlings was determined in a Lardy-type Warburg manometer. The centre well of each vessel contained 0·2 ml. of 20 per cent potassium hydroxide and a piece of filter paper. Each vessel contained 1·0 ml. of 0·1 M acetate buffer (*p*H 4·7) and 1·0 ml. of herbicide suspension. Distilled water was substituted for herbicides in controls. Oxygen uptake was determined at 15-min intervals for 150 min.

At 4,000 p.p.m. of active ingredient, commercial formulations of DCPA, simazine, or sesone had no significant effect on the oxygen uptake of the recently emerged seedlings in a short time period. Dalapon at 4,000 p.p.m. showed a slight inhibitory effect. Atrazine depressed respiration, but it did not show an inhibitory effect with *p*H 6·5 phosphate buffer. Similar results were found with commercial formulations of 2,4-*D* and 2,4,5-*T*. Herbicides provided commercially as oil forms, including ipazine, prometone, EPTC, CDEC, CDAA, 2,4-DEP, 2,4-*D* and 2,4,5-*T*, all depressed respiration at *p*H 4·7.

The foregoing experiments suggested the possibility of blocking of oxygen supply or of gas diffusion in the Warburg vessels. Experiments were therefore continued to evaluate separately the influence of active and inert ingredients contained in commercial formulations of herbicides. Mixtures of total inert ingredients were used.

Two separate experiments were conducted on effects of inert ingredients alone. In the first of these the dosages of inert ingredients, at concentrations adjusted for 4,000

p.p.m. of active ingredient, were as follows: 2,4-*D* (1,700 p.p.m.), 2,4,5-*T* (2,100 p.p.m.) EPTC (1,300 p.p.m.), CDAA (4,500 p.p.m.), CDEC (4,600 p.p.m.), ipazine (12,000 p.p.m.), prometone (12,000 p.p.m.), and 2,4-DEP (5,100 p.p.m.). This experiment showed that the inert ingredients of 2,4-*D*, 2,4,5-*T*, EPTC, CDEC, and CDAA had no significant effects on oxygen uptake. In contrast, the inert ingredients contained in formulations of ipazine, prometone, and 2,4-DEP markedly inhibited respiration. As emphasized earlier, however, the concentrations of inert ingredients of ipazine and prometone in this experiment were very high.

In the second experiment the effect of inert ingredients was tested at concentrations of 4,000 p.p.m. of the inert ingredient. Hence the dosages of inert ingredients of ipazine, prometone, and 2,4-DEP were lower than in the first experiment, while those of 2,4-*D*, 2,4,5-*T* and EPTC were higher. At 4,000 p.p.m. the inert ingredients of prometone and 2,4-DEP still depressed respiration, but the inhibition was lowered. EPTC inert ingredients also significantly decreased respiration at 4,000 p.p.m. Inert ingredients of 2,4-*D* also depressed respiration in short-time experiments, while those of 2,4,5-*T* apparently delayed the effect.

These experiments emphasized that metabolism is altered to varying degrees by either active or inert ingredients of commercial herbicide formulations. In summary, respiration was inhibited primarily by the active ingredients of 2,4-*D*, 2,4,5-*T*, EPTC, CDEC, and CDAA and by the inert ingredients of prometone, and ipazine formulations. There may also have been an interactive effect of inert and active ingredients of CDEC and CDAA. Further experiments are needed to evaluate these possible interactions.

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Effects of Seed Germination-stimulants on the Witchweed *Striga euphrasioides* (Vahl) Benth.

PARASITISM in some species of *Striga* has often been questioned. According to Williams[1] *S. euphrasioides* is one of the unlikely exceptions to the group of obligate parasitic species. It was, therefore, felt desirable to investigate its seed germination and compare it with similar investigations on certain species of *Striga* and *Orobanche* that are recognized as root parasites.

Stock collections of seeds were made from mature dry capsules of several plants. For each experiment seeds were removed from the stock in two batches and one of them was washed in running tap water for 48 h. Both batches of seeds were surface-sterilized in chlorine water for 10 min and sown on agar nutrient media (*p*H 5·8). A modified White's medium[2] containing 4 per cent sucrose and 1 p.p.m. indole-3-acetic acid served as basal medium. The effects of gibberellic acid and kinetin on germination

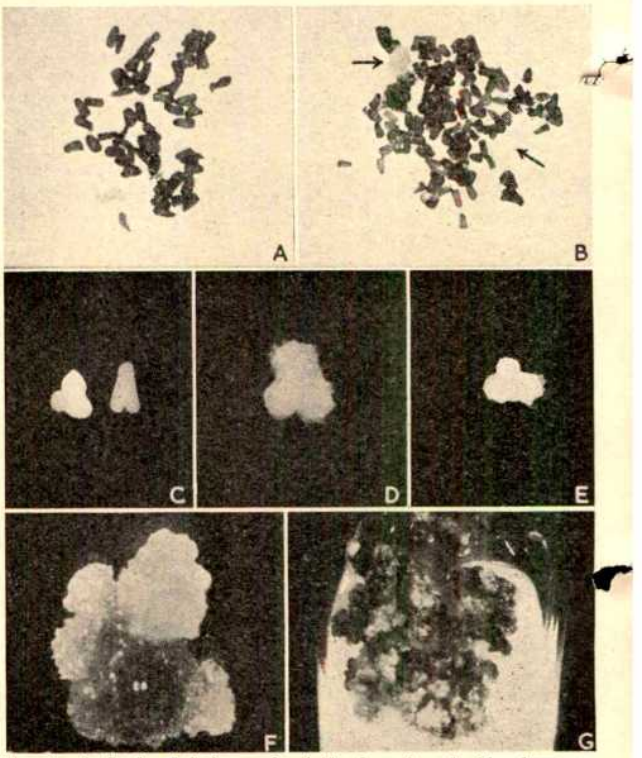


Fig. 1. *A*, Seeds of *Striga euphrasioides* from 3-week-old culture on modified White's medium (WM) (×*c.* 7); *B*, seeds from 2-week-old culture on WM+10 p.p.m. kinetin; two seeds show emergence of embryos (arrow-marked) (×*c.* 5); *C*, embryos removed from 12-day-old culture on WM+10 p.p.m. kinetin (×*c.* 10); *D*, embryo from 24-day-old culture showing proliferation (×*c.* 9); *E*, 5-week-old culture of proliferating embryo on WM supplemented with 10 p.p.m. kinetin (×*c.* 8); *F* and *G*, 5-week-old and 3-month-old cultures of embryo calli on WM supplemented with 400 p.p.m. casein hydrolysate +15 per cent coconut milk (*F*, ×*c.* 8; *G*, ×*c.* 0·9)

were investigated. For each treatment thirty-six cultures were raised in two replicates. Half the number of cultures were grown in diffuse daylight (8–10 ft.-candles) and the other half in the dark.

Unwashed seeds failed to germinate under any experimental conditions. Germination of water-washed seeds was helped by daylight. In subsequent experiments, therefore, only washed seeds were used and incubation in the dark was abandoned.

On the basal medium water-washed seeds lay dormant up to 6 weeks after sowing (Fig. 1*A*). Addition of 5, 10 or 12·5 p.p.m. gibberellic acid to the medium did not help in overcoming dormancy. If, however, the medium was supplemented with 10 p.p.m. kinetin the testa in some of the seeds ruptured within 10–12 days after sowing and the embryos were completely released (Fig. 1*B*). Four days later, root hairs appeared on the elongated radicle. About 18–20 days after sowing the cotyledons formed a callus tissue (Fig. 1*C*, *D*).

With a view to inducing organ formation the proliferated embryos were transferred to media supplemented with one or more of the following substances: kinetin, casein hydrolysate and coconut milk. In treatments with 10 p.p.m. kinetin no organs developed (Fig. 1*E*). Individually as well as in concert, both 400 p.p.m. casein hydrolysate and 15 per cent coconut milk elicited the best growth of the callus tissue. It formed several new mounds along its periphery and grew into a nodulated mass (Fig. 1*F*, *G*). However, shoot-buds did not develop in cultures. A similar response to kinetin treatment was also shown for *Striga asiatica*[3]. Presumably, exogenous factor(s) other than kinetin may be required for shoot morphogenesis. In *Orobanche hederae*, for example, contact with the root of its host, *Hedera helix*, was indispensable for the initiation of shoots[4]. Contrarily, in *Orobanche aegyptiaca* shoot apices differentiated *in vitro* in the absence of any host plants[5].

Briefly, mature dry seeds of *S. euphrasioides* can be caused to germinate by washing in running water (48 h) followed by kinetin treatment. Shoot-buds do not develop in tissue cultures derived from such embryos. Development of shoot-buds may require some factors other than kinetin.

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ENTOMOLOGY

'Paper Factor' as an Inhibitor of the Metamorphosis of the Red Cotton Bug, *Dysdercus koenigii* F.

MATERIALS composed of American paper-pulp have been shown to contain an extractable, heat-stable lipid which exhibits high juvenile hormone activity when topically applied or otherwise brought into contact with the European bug, *Pyrrhocoris apterus* L. (Heteroptera; Pyrrhocoridae[1,2]). The active principle is synthesized by certain species of pulp trees—more particularly, the American balsam fir (*Abies balsamea*). The 'paper factor' (PF) proves to be an extremely potent analogue of the juvenile hormone of *Pyrrhocoris apterus*[3]. Yet, so far, it has proved completely inactive when tested on other laboratory insects including two other species of Heteroptera—*Oncopeltus fasciatus* (Lygaeidae) and *Rhodnius prolixus* (Reduviidae)[1,2].

Pyrrhocoris apterus is a benign species which feeds by its sucking mouthparts on lime (linden) seeds. However, the family Pyrrhocoridae includes some of the world's most troublesome insect pests—notably the 'red cotton bugs' (*Dysdercus* sp.) indigenous to India, Africa, and Australia. Sláma and Williams (p. 414, ref. 1) have considered the possibility that PF "may be effective in the selective destruction of at least certain of these pests, as well as any other insects which show the same hormonal sensitivities as *Pyrrhocoris apterus*".

The purpose of the present communication is to report that the 'red cotton bug', *Dysdercus koenigii*—a considerable pest in India and North Africa—is sensitive to PF.

The experiments were performed on an extract of PF prepared at Harvard University from 250 paper towels ('Scott' Brand 150). The towels were packed into a large glass column (11 cm outside diameter × 115 cm long), soaked overnight in absolute methanol, and then perfused with a total of 5 l. of solvent. The methanolic extract was reduced to dryness to obtain 7·4 g of a viscous sludge from which PF was extracted with 4 × 50 ml. portions of petroleum ether (b.p. 35°–60° C). The latter was filtered and the solvent evaporated to obtain 2·9 g of viscous greenish-yellow oil. Without further purification, this crude preparation of PF was used in the present study.

With the support and encouragement of Prof. B. R. Seshachar, Head of the Zoology Department at the University of Delhi, PF was tested on the same laboratory culture of *Dysdercus koenigii* which has been subjected to detailed investigations in recent years[4]. Individual larvae were isolated within 30 min after moulting to the fifth instar. The insects were provided with water-soaked swabs of cotton wool, but were otherwise starved during the first 28–36 h at 27°–28° C. A measured droplet of an acetone solution of PF was then applied to the mid-dorsal region of each of 10 individuals in each test group. Alternatively, groups of 10 individuals were placed in glass containers in the presence of a 9-cm disk of Whatman No. 1 filter paper impregnated with a known amount of PF. After these manoeuvres, each group was provided with cottonseed and water to provoke development[4].

Table 1. EFFECTS OF PAPER FACTOR ON FIFTH-INSTAR *Dysdercus* LARVAE

PF extract	Percentage of PF-treated individuals showing different effects			
	Imaginal inhibition			Incomplete ecdysis
	Adults	Adultoids	Sixth instar larvae	
Topical application of:				
0·01 μg	30	70	0	0
0·05	20	80	0	0
0·1	0	100	0	0
0·5	0	50	50	0
1·0	0	30	30	40
4·0	0	0	20	80
Contact with paper* impregnated with:				
0·1 mg	0	80	10	10
1·0	0	60	20	20
2·0	0	20	20	60

* A 9 cm disk of Whatman No. 1 filter paper.

The results, summarized in Table 1, show that the PF inhibited the metamorphosis of the insect in two ways: (i) by inhibiting the development of imaginal characters in the sixth instar individuals into which the PF-treated fifth instar larvae moulted, and (ii) by preventing complete extrication of the PF-treated fifth instar larvae from the old larval cuticle during the ecdysis. The degree of imaginal inhibition and failure in the extrication of the moulting individuals was a function of the dose of PF, and a topical application of 4 μg at the outset of the fifth instar caused complete suppression of metamorphosis. Most of the treated larvae failed to extricate themselves from the old cuticle during moulting and died in the process. A small number of individuals, however, moulted into a supernumerary sixth larval instar showing complete inhibition of the imaginal characters.

When the topical application was decreased below 4 μg (Table 1), the number of individuals showing incomplete ecdysis decreased and a progressively larger number of larvae moulted into apparently normal adults, save for a failure of the wings to attain adult size and form. Many of these 'adultoid' individuals lived for weeks without further moulting, and some were capable of mating and laying fertile eggs.

When the topical application was reduced to 0·05 or 0·01 μg, a few larvae were able to transform into normal adults. But even at such low doses, the majority of individuals moulted into 'adultoids' which showed partial inhibition of wing development—a finding which indicates that the wings are far more susceptible to inhibition by PF than are other imaginal characters.

As recorded in the lower part of Table 1, the effects of the topical applications were duplicated when three groups of ten larvae were reared throughout the fifth instar in contact with PF-impregnated filter paper; the experiment was repeated five times with substantially the same results.

The quantitative aspects of the present investigation indicate that low doses of PF have less extensive and less lethal effects on *Dysdercus* than on *Pyrrhocoris apterus*[2]. The difference in the effects between the two species was, however, far less evident at higher dose-levels. Thus, in the case of *Pyrrhocoris*, metamorphosis and sexual maturation were completely blocked by the topical application of 1 μg[2]. This same result was achieved in the present study by 4 μg of a PF extract that was one-third as active as that used in the earlier study.

The ability of PF to block the metamorphosis and selectively to kill an economically important pest such as *Dysdercus koenigii* is of considerable interest. Though the hormonally active principle is known to be present in only trace amounts in these crude extracts[2], the

'starting material' is available on an essentially unlimited scale in the form of American newspapers and other paper products. More highly purified preparations of PF promise to be effective in the selective control of *Dysdercus koenigii* and, presumably, of other economically important Pyrrhocoridae. This proposition is at present being tested in Delhi.

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MICROBIOLOGY

Production of Yeast Cell-wall Lytic Enzymes on a Semi-defined Medium by a *Streptomyces*

THOUGH a great many results on the lysis of bacteria have appeared, little information has yet been presented concerning the organisms which have lytic activity toward the cell walls of the fungal mycelium. Some of these investigations demonstrated the production of microbial enzymes capable of lysing fungi. A knowledge of the action of enzymes on fungal mycelium may, perhaps, give some idea of the composition of some of the cell wall structures.

During the research in our laboratory for the micro-organism causing the lysis of fungal cells, a number of actinomycetes were found to have strong lytic activity toward the cell walls of some fungi[1–3]. Some information was later presented in relation with the formation of the lytic enzymes[4,5] and the mechanism of the action of one of the strepzymes on the cell walls[6].

A wide variety of culture media and growth conditions have been used by us in preliminary investigations in an attempt to define reproducible conditions for the production of the lytic enzymes. Considerable difficulty has been experienced in obtaining consistently good yields of active filtrates, and this problem has apparently been encountered by other investigators. In our previous experiments[1–3], and in those of other workers[7–9], the isolated strains of *Streptomyces* and *Micromonospora* were inoculated in media in which different kinds of living or dead fungal cells were present. The cells employed in these studies were in the form of pressed baker's yeast, that is, dried brewery yeasts or laboratory grown *C. utilis* cells. Normally, the appropriate amount of cells were added to a salt solution and the complete medium was autoclaved before inoculation with the lytic actinomycetes. Cultures were grown either in the laboratory in shaken flasks or in a pilot plant in stirred and aerated tanks: in either case the cultures were grown at 28° for 6–8 days. In most cases after this incubation period the cells could not be detected microscopically. The organism caused the fungal cells to dissolve completely. It was assumed, therefore, and later demonstrated, that the culture fluid contained lytic factors (enzymes). After it was centrifuged or filtered to remove all the cells, the clear solution of the supernatant was used as the crude enzyme preparation.

Various attempts made in our laboratory to produce the lytic enzymes by growing the lytic organism on defined synthetic or semisynthetic media failed. Other workers stated clearly that yeast cell wall lytic enzymes were only formed when the culture medium contained as a substrate the fungal cells[10]. The organisms often grew well on the least complex media, but produced very small amounts of lytic enzymes or even none at all.

The procedure of adding cells of yeast or moulds to the growth media has certain advantages for the consistent production of lytic material and was used in part of the present work. However, in obtaining material for the purification of the enzymes, it was found that the use of complex media caused variation in the formation of the various enzymes usually present in the lytic preparation, and this interfered with many of the investigations under way in our laboratory. Furthermore, any alterations in the type of fungi (brewery or pressed yeast) or in the type of growth of the cells produced in the laboratory used as the substrate for growth and production of the enzyme often led to significant variations in the activity of the crude preparations. These media often gave rise to variable and unpredictable results and a more defined medium was studied in an attempt to obtain consistent results.

The disadvantages of the fungal cell containing media have now been partially eliminated. As a result of some investigations of the chitinase activity of various *Streptomyces* we found that the lytic activity on the cell walls of various yeasts was high, using filtrates of the growth medium of some *Streptomyces violaceus MR*. This is one of the lytic organisms used in our preliminary studies[8] and is characterized by the production of a potent extracellular enzyme system (showing chitinase and proteolytic activity) which can dissolve a variety of organisms; it was grown on a medium of the following composition: chitin (Koch-Light), 5 g; yeast extract (Difco), 0·5 g; sodium nitrate, 1 g; potassium orthophosphate, 2 g; $MgSO_4 \cdot 7H_2O$, 1 g; sodium chloride 1 g; and distilled water, 1 l. Inoculation was made with vegetative cells of the organism previously grown on slants of a medium containing glucose-yeast-malt extracts. The *Streptomyces violaceus* was grown aerobically in Erlenmeyer flasks agitated by a shaking machine at 28° C. 6–8 days after inoculation, the culture fluid was separated centrifugally from the cellular material. The supernatants were collected and the crude lytic enzyme preparation obtained after precipitation with 70 per cent ammonium sulphate and redissolution in one-twentieth of the volume of distilled water. The activity of the best preparations obtained with this medium was comparable to that obtained with more complex media containing fungal cells. This preparation was very stable when stored at −20° C, and able to digest cell walls of a number of yeasts, such as *Saccharomyces fragilis* (Fig. 1), *Saccharomyces cerevisiae* and *Candida utilis* with the formation of protoplasts under stabilized conditions. Preliminary experiments have shown that it is possible to obtain about 100 per cent protoplast formation with the yeast tested in less than 45 min using the conditions previously described[11].

An investigation is now in progress in our laboratory on the method of separation of some of the more important enzymes involved in the digestion of the fungal cell walls, to enable us to make further studies of their specificities.

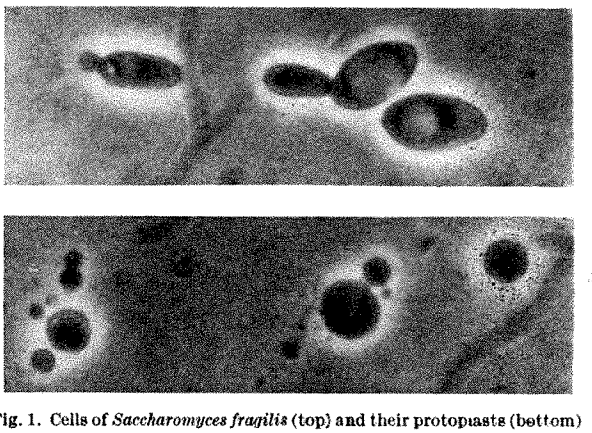


Fig. 1. Cells of *Saccharomyces fragilis* (top) and their protoplasts (bottom)

Results on the enzymatic properties of these will be presented elsewhere.

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VIROLOGY

Neoplasms induced by Rous Sarcoma Virus in New World Monkeys

AT one time, tumour viruses were believed to be species-specific, but tumour induction *in vivo* and cell transformation *in vitro* have made this concept inapplicable to experimental situations. Rous sarcoma virus will not only induce tumours in the original host and other avian species but will also cause *in vivo* and *in vitro* malignant transformation of mammalian cells. The induction of sarcomata in Old World non-human primates by Rous sarcoma virus preparations of the Carr-Zilber and Schmidt-Ruppin strains has been reported by four research groups[1–5]. In most of the *in vivo* experiments with primates 'tissue minces' from Rous sarcoma virus-induced chicken sarcomata were used for inoculation and the published reports gave no clear indication that the tumours were truly invasive and/or metastasizing.

Inoculation of partially purified Rous sarcoma virus of the Schmidt-Ruppin strain (RSV-SR) induced invasive metastasizing immature sarcomata in all of five new-born marmosets, *Saguinus* sp., inoculated during the first 24 h of life. Each animal was injected subcutaneously and intramuscularly with 0·25 ml. of a cell-free inoculum of RSV-SR which had been prepared from material supplied by Dr. E. H. Bernstein of University Laboratories, Inc., Highland Park, New Jersey. University Laboratories used the following method for preparation of their RSV-SR: a 10 per cent extract of chicken tumours induced by RSV-SR was prepared in 0·15 M potassium citrate and 100 mg per cent hyaluronidase by intermittent homogenization for 1 h. The homogenate was centrifuged at 7,600 r.p.m. for 15 min and the supernatant, sealed in ampoules, was shipped to this laboratory in dry ice. Material was either used directly from the ampoules or was further concentrated. For concentration the contents of several ampoules were thawed, pooled, and centrifuged at 30,000 r.p.m. for 30 min; the supernatant fluid was discarded. The sediment was ground together with 'Alundum' with a pestle and mortar and was resuspended in a twentieth or a fifth of the original volume of Hanks's balanced salt solution containing 5 per cent foetal calf serum. The suspensions were centrifuged at 2,000 r.p.m. for 20 min and the top two-thirds of the supernatant was frozen at −65° C. The '20×' and '5×' concentrates were used for inoculation within 1 month of preparation.

The results of inoculation of new-born marmosets are given in Table 1. Tumours developed in all animals within 20–90 days after inoculation of virus, and all died from their malignant disease between 35 and 117 days. Live tumour cells from one of the animals were inoculated into its twin when they were 39 days old, and within 2 weeks this animal developed a large sarcoma at the inoculation site.

Table 1. INOCULATION OF NEW-BORN MARMOSETS WITH PARTIALLY PURIFIED ROUS SARCOMA VIRUS

No. of animals inoculated	RSV-SR inoculum*	Days after inoculation: Tumour first observed	Death
1	Unconcentrated	90	117
1	5×	30	65
3	20×	20,24,25	35,39,53
1	5×10^6 live tumour cells	7	14

* See text for explanation.

All tumours were immature sarcomata showing positive fat staining of most cells, but no clear differentiation between a liposarcoma and an embryonal rhabdomyosarcoma could be made. The tumours infiltrated the surrounding tissues without a clear demarcation and many tumour cell emboli were seen in the lungs. Definite histological evidence was found of tumour invasion of the lung parenchyma from the tumour cell emboli. No other metastases have so far been found. Preliminary electron microscopic studies have not revealed any virus particles in the tumour cells.

Tumour tissue, kidney and normal muscle tissues obtained at autopsy were grown in cell culture; the original outgrowth and cells after several tissue culture passages of both tumours and normal tissues were karyotyped. The chromosomes of all tissues could easily be identified as marmoset chromosomes. A number of inconsistent chromosomal aberrations were observed in sixty-six tumour cells so far investigated, but a specific marker has not appeared. The detailed data of these experiments, the results of recent investigations on the antigenic analysis of the tumour cells, and the presence or absence of virus in the tumours and other tissues will be published later in a more extensive report.

This work provides a laboratory model of virus-induced tumours in primates. The tumours were produced by cell-free virus preparations; they were truly invasive and metastasizing and they ultimately killed the animals. One tumour was successfully transplanted by transfer of living cells to a twin. Similar experiments can be performed by any laboratory equipped with standard animal-house facilities, as maintenance of marmosets does not require either specialized facilities or generous space. The animals are well characterized, as wild-caught marmosets have been maintained and studied both in this and other laboratories for the past few years. Marmosets are available in large numbers and at reasonable cost; they are sufficiently small that large numbers can be kept in a limited space, and at the same time they are large enough for the serial bleedings and other surgical procedures to be performed. The animals breed in captivity and a colony of pairs maintained specifically for this purpose provides a regular supply of new-born animals. The baby marmosets can be handled within a few hours of birth and can be either returned to the parents for rearing or hand-reared.

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Plaque Formation with a Phlebotomus (Sandfly) Fever Virus in Baby Hamster Kidney (*BHK* 21) Cells

BABY hamster kidney cell line *BHK* 21, clone 13 (ref. 1), has been used for a plaque assay method for phlebotomus fever viruses. The technique used was a modification of the method described by Russell[2] for herpes virus.

Cells were grown in Eagle's medium containing double amounts of amino-acids and vitamins, 10 per cent tryptose phosphate broth (Difco) and 10 per cent unheated calf serum (ETC). When a confluent sheet of cells was formed, they were dispersed with equal volumes of 0·02 per cent versene solution and 0·25 per cent trypsin (Difco). The cells were suspended in ETC medium; washing was not necessary. They were then counted and the cell suspension adjusted to contain 10^7 cells per ml. The cells can be used immediately or stored at 4° C for up to a week.

Aliquots of 2×10^6 cells in 0·2 ml. ETC were placed in bijou bottles and 0·2 ml. of virus dilution were added. The cell-virus suspension was then shaken gently at 37° C for 30 min. After shaking, 4·5 ml. of ETC were added to each bottle, the contents mixed and poured into 60 mm glass Petri dishes, which were then incubated in a humidified atmosphere containing 5 per cent carbon dioxide at 37° C.

After 5 h of incubation, the ETC medium was removed and the cells were overlaid with 8 ml. of Eagle's medium containing the sodium salt of carboxymethyl cellulose (CMC) in a final concentration of 0·75 per cent and 10 per cent unheated calf serum (ECMC). The overlay medium (ECMC) was made up as follows: 1·3 times normal strength Eagle's medium, 75 ml.; calf serum, 10 ml.; 5 per cent CMC in distilled water, 15 ml. It was of advantage to dissolve 750 mg of CMC in 15 ml. of distilled water in a 150 ml. bottle and autoclave. The Eagle's medium and calf serum were then added. Thorough shaking was necessary to gain a uniform ECMC solution. It was possible afterwards to use a pipette for distributing the overlay medium.

After the addition of the ECMC overlay, the Petri dishes were incubated as before, care being taken not to move them during incubation. 4–5 days later, the ECMC was removed and the cell sheet was either fixed in methanol for 5 min and stained with 1 : 10 Giemsa for 30 min or stained unfixed with 0·1 per cent crystal violet. The stain was then poured off and the plates were gently washed in a bowl of tap water, left to dry and the plaques were counted.

The phlebotomus fever viruses used in this study were:

Sicilian type: 46th mouse passage
Naples type[3,4]: 58th mouse passage

The Sicilian type of virus produced plaques which were irregular in outline and of 1–2 mm in diameter after 4 days of incubation (Fig. 1). The average titre was 7×1[illegible] P.F.U./ml. as compared with $7{\cdot}4 \times 10^8$ LD_{50} in 2–4-day-old suckling mice inoculated intracerebrally. In a plaque reduction test, 1 ml. of a Sicilian virus dilution containing 300 P.F.U. was mixed with 1 ml. of fourfold dilutions of immune sera prepared in mice against the homologous virus and the Naples virus. The serum–virus mixture was incubated at 37° C for 1 h and then 1 ml. of the mixture was inoculated into *BHK* 21 cells, as already described; two plates were used per serum dilution. The homologous serum gave a 50 per cent plaque reduction titre of 1 : 180, whereas no neutralization was obtained with the Naples serum.

Attempts to produce plaques with the Naples type have so far proved unsuccessful.

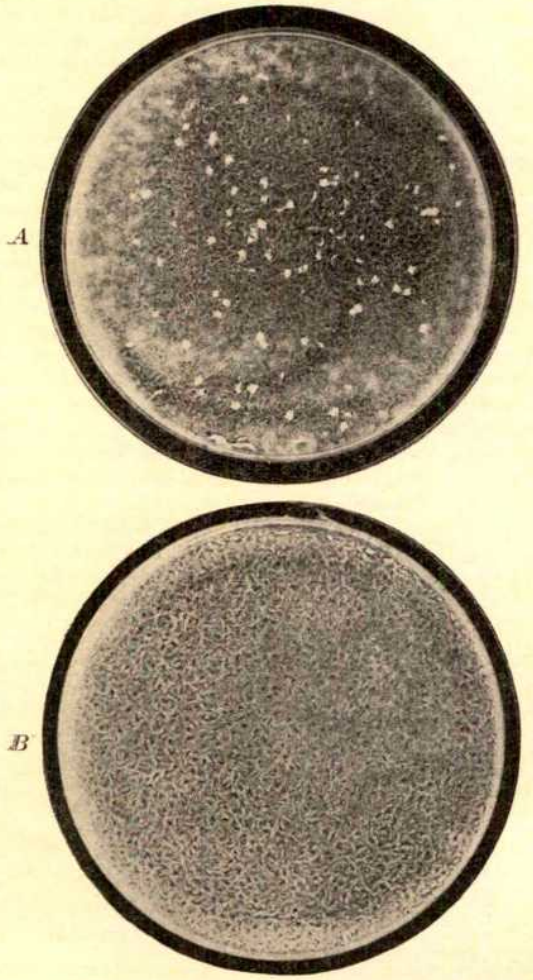


Fig. 1. *A*, Type of plaques produced by Sicilian type of phlebotomus fever virus in *BHK* 21, 10^{-5} dilution of virus, 4 days after inoculation. *B*, Control, cells treated with diluent only

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CYTOLOGY

Cytoplasmic Localization of Phytohaemagglutinin in Peripheral White Cells

SALINE extract of the red kidney bean (*Phaseolus vulgaris*) possesses a factor which increases the rate of cell division in cultures of peripheral white cells[1], and a factor which agglutinates red cells[2]. Reciprocal absorption shows that the two factors are separate and the mitogenic moiety has been further characterized as a mixture of proteins, the predominant one having a molecular weight of about 128,000 (ref. 3). The mitogenic extract therefore lends itself to labelling with fluorescent compounds. Such a marker would be valuable in tracing the mode of action of the mitogen(s) since it is still not known whether the leucocyte agglutination, apparently inseparable from the mitogenic activity[4], is also necessary to the mitogenic activity, nor whether the substance acts extra- or intra-cellularly, or both.

To pursue this question a commercial preparation of phytohaemagglutinin (PHA—Burroughs Wellcome Lot No. K4971) was labelled with fluorescein isothiocyanate[5] (FITC—B.B.L. Batch No. 401670) at a fluor-protein ratio of 1 : 40 and cleared of unconjugated FITC by passing it through a 'Sephadex *G*-25' column. The conjugate was

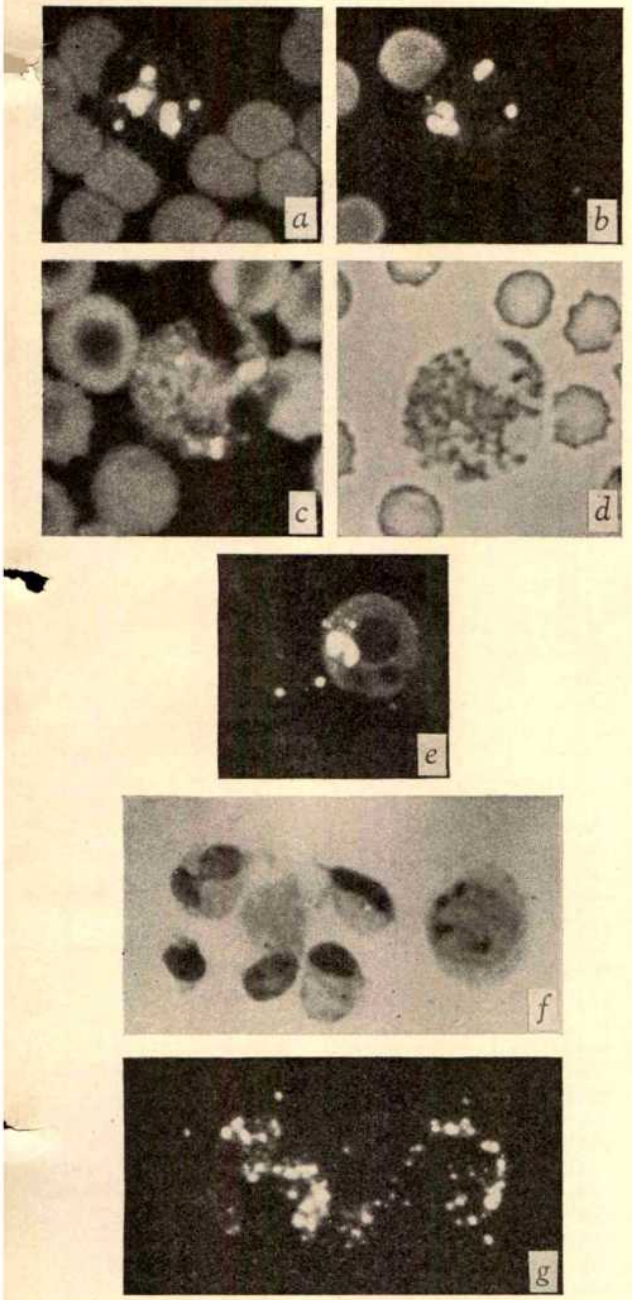


Fig. 1. Fluorescein conjugated phytohaemagglutinin in (*a*) and (*b*) polymorphonuclear neutrophils; (*c*) an eosinophil before and (*d*) after staining to show displacement of the eosinophilic granules by the fluorescent inclusions; (*e*) an early transformed cell; (*f*) and (*g*) a clump of transformed cells (× 440)

added to whole-blood cultures before and after absorption with red cells[6]. Control cultures contained unlabelled PHA, FITC alone and the separate reagents added simultaneously. Those cultures showing mitotic activity with the lowest necessary concentration of conjugate were investigated. It was possible to detect conjugate inside the white cells and, in cultures using unabsorbed PHA, coating the red cells within 15 min after inoculation. At this early stage, white cells of all types (Fig. 1*a–d*) contained fluorescing conjugate. At later stages (Fig. 1*e–g*) PHA could be seen in the characteristic blast-like cell of PHA-stimulated cultures—coarse chromatin with prominent nucleoli and basophilic vacuolated cytoplasm. At all stages fluorescence was confined to the cytoplasm and took the form of spherical inclusions situated apparently at random throughout the cytoplasm, and not associated with nuclear structures or the cytoplasmic membrane. The appearance of the white cell inclusions was the same whether or not red cell-absorbed PHA was used.

It appears, therefore, that the haemagglutinating and mitogenic factors are indeed separate, but that both contain molecules capable of conjugation with FITC. It also appears that the mitogenic fraction is ingested, not only by the small mononuclear cells presumed to be the precursors of PHA cells[7–9], but also by granulocytes. Without isolated or pure populations of the various cell types it is not possible to say whether the presence of the conjugate is followed by morphological changes in all cells; nor whether the well-recognized clumping of white cells in PHA-stimulated cultures is associated with an exchange of materials essential to the blastoid transformation and mitosis[10]. It was noteworthy that the characteristically persistent eosinophils were usually to be found at the later stages singly situated but surrounded by transformed cells. The similarity between immunological reactiveness of round cells[11] and PHA transformation, and the role of eosinophils in the earliest stages of the *in vivo* antigen–antibody response[12], suggest that heterogeneous cellular interaction may form a component of transformation in PHA-stimulated cultures.

The heterogeneous cellular distribution may also simply be a reflexion of the heterogeneity of a population of conjugated proteins not all of which are mitogenic and may be explained as the result of non-specific pinocytosis with no implications as to the necessary future development of the ingesting cell.

Finally, it is noteworthy that a phenomenon most remarkably associated with leucoagglutination and with an increase in mitotic activity should be associated with an initiator exclusively intracytoplasmic in its localization. This suggests an indirect effect of PHA. Increase in the number and size of lysosomes and histochemical changes indicating an increase in anaerobic glycolysis[7] may be the result of an uncoupling of synthetic metabolic pathways similar to those used in immunological and phagocytic[13] functions of white cells which culminate in γ-globulin production and cell enlargement. The passage of a threshold in size and metabolic activity may then lead to mitosis and division.

These possibilities can now be explored further by use of the labelled PHA, and later experiments will investigate the specificity of the reaction using antisera to PHA, the behaviour of agglutinins and mitogens from other sources and the behaviour of the different cell types in isolation and in various combinations before and after treatment with limiting amounts of PHA.

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GENETICS

Synthesis of Ribonucleic Acid by Human Chromosomes and a Possible Mechanism of its Repression

IT has recently been suggested that a genetic basis for cellular differentiation in multicellular organisms might be reflected in the distribution pattern of inactivated deoxyribonucleic acid (DNA) in the chromosomes[1]. Inactivated DNA is characterized by three features, namely, heterochromatinization, late replication at the time of doubling, and inactivity of synthesis of ribonucleic acid (RNA). To substantiate this hypothesis, we are now studying the pattern of DNA and RNA synthesis of chromosomes in human cells of various origin by means of autoradiography. In studying the synthesis of RNA by human chromosomes, we encountered a dilemma. The chromosomes are identifiable only in metaphase as are most somatic cell chromosomes in multicellular organisms. During metaphase and anaphase, however, synthesis of RNA completely ceases in all the cells of multicellular organisms so far investigated[2-7]. It is not, therefore, feasible at present to study the pulse labelling pattern of RNA on metazoan chromosomes except polytenic ones. Previous investigators[6,8] have studied RNA synthesis mainly in meiotic chromosomes as these chromosomes remain visible throughout the meiotic cycle. However, they are not chromosomes of differentiated somatic cells in which we are interested. Hsu reported synthesis of RNA in intermitotic nuclei of mammalian somatic cells[9], in which the Barr body was shown to be inactive in the synthesis of RNA. In general, however, the chromosome is not identifiable as a distinct morphological unit in the intermitotic phase of metazoan cells. To overcome this dilemma, we decided to label the cell just before division and examine the distribution of the label at metaphase which immediately follows the labelling. The interval between the labelling and the examination was chosen so as to be sufficiently short to prevent secondary diffusion of the labelled RNA from the site of the synthesis. If the labelled RNA is not released immediately after its synthesis and does persist during mitosis, it would be expected that the label incorporated at late *G2* and prophase would also remain detectable on the metaphase chromosomes.

Leucocytes, from peripheral blood of a normal woman, were collected after addition of phytohaemagglutinin (Difco). They were cultured for 72 h in a medium (tissue culture medium 199, Difco), with penicillin and streptomycin, and squash preparations were made at the termination of the culture. Uridine-^{3}H or thymidine-^{3}H was introduced (0·5 μc./ml.) 3 or 6 h, respectively, before the cells were fixed. All the cultures were subjected to colchicine treatment (10^{-6} M) during the last 3 h. The specimens were stained with acetic acid-orcein, washed for 10–12 h in running water and autoradiographed by dipping them into Kodak '*NTB*-3' emulsion.

In autoradiographs labelled with thymidine-^{3}H, we found metaphase chromosomes with heavy labelling on one of the *X*-chromosomes, identifying this as the late replicating *X*-chromosome. In contrast, however, in metaphase chromosomes labelled with uridine-^{3}H, the *X*-chromosome was free from radioactivity (Fig. 1) while all the other chromosomes were detectably labelled. Structures other than chromosomes showed little labelling. This finding indicates that (1) RNA that is synthesized on the chromosome just before division is not released from the site of synthesis during mitosis, and (2) one of the *X*-chromosomes is inactive in RNA synthesis. The results not only demonstrate the feasibility of this method for investigating the distribution of genetically active and inactive loci in metazoan chromosomes, but also suggest a possible mechanism of genetic repression. In the autoradiographs, it was shown that RNA attaches



Fig. 1. Autoradiograph of human chromosome, 3 h after introduction of uridine-^{3}H. The cells, derived from the peripheral blood of a woman, were cultured for 72 h. The arrow indicates the genetically inactive *X*-chromosome

firmly to the metaphase chromosomes that are completely repressed in RNA synthesis. Since the turnover rate of RNA synthesis on the chromosome should be regulated both by the rate of RNA synthesis and the rate of removal of the products from the template, failure or suppression of removal of RNA from the template DNA should be followed by repression of RNA synthesis. It is possible to test experimentally the presence of this mechanism in differential inactivation of genes, by introducing a labelled precursor of RNA just before or at the time of gene inactivation and examining at later stages the persistence of the label at the inactivated loci of the chromosomes. If RNA masks the template DNA at the inactivated loci, it should be detectable by means of autoradiography. Experiments are now in progress with the view of substantiating this hypothesis.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, April 25

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion Meeting on "Breakdown of Large Air-gaps and Effect of Voltage Wave Shape" opened by Dr. F. D. A. Boylett and Dr. D. F. Oakeshott.

PLASTICS INSTITUTE, LONDON SECTION, REINFORCED PLASTICS SUB-GROUP (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Mr. P. Lynch: "The Advances of Hot Press Moulding in the U.K.".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Prof. S. Thorarinsson: "An Island is Born".

Tuesday, April 26

ROYAL SOCIETY OF ARTS, COMMONWEALTH SECTION (at John Adam Street, Adelphi, London, W.C.2), at 5.15 p.m.—Prof. J. A. Lauwerys and Dr. Elsie C. Graham: "Basic English as an International Language With Special Reference to Scientific Study in the Commonwealth".

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion Meeting on "Problems in the Design and Installation of Surge-Proof Transistorized Control Equipment" opened by Mr. R. F. Eade, Mr. D. Harrison and Dr B. E. Noltingk.

PARLIAMENTARY AND SCIENTIFIC COMMITTEE, GENERAL COMMITTEE (in Committee Room 11, House of Commons, London, S.W.1), at 5 30 p.m.—Discussion on "Fisheries Research" initiated by Sir Frederick Brundrett, K.C.B., K.B.E., and Dr. H. A. Cole.

SOCIETY FOR ANALYTICAL CHEMISTRY (in the Meeting Room of the Chemical Society, Burlington House, Piccadilly, London, W.1), at 6.30 p.m.—Prof. R. E. Wainerdi (U.S.A.): "Automated Systems for Extra-Terrestrial Nuclear Activation Analysis".

Wednesday, April 27

BIOMETRIC SOCIETY (at the Wellcome Building, Euston Road, London, N.W.1), at 2.30 p.m.—Prof William F Taylor "On the Estimation of the Risk of Foetal Death"; Dr R. M Cormack: "Tests for Equal Catchability".

POLAROGRAPHIC SOCIETY (in the Metallurgy Lecture Theatre, Royal School of Mines, Prince Consort Road, London, S.W.7), at 2 45 p.m.—Dr. M. E. Peover: "Polarographic Characteristics of Organic Compounds in Organic Solvents". 4.45 p.m.—Eleventh Annual General Meeting.

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 3 p.m.—Annual General Meeting.

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Annual General Meeting, followed by Dr. G. D. Robinson: "Another Look at Some Problems of the Air-Sea Interface" (Presidential Address).

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the Railway Engineering Group of the Institution of Mechanical Engineers, at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 5 30 p.m.—Mr. G. Gilbert and Mr. H E. H. Davies, "Pantograph Motion on a Nearly-Uniform Railway Overhead Line"; Mr. T. A. Willetts and Mr. D. R. Edwards: "Dynamic Model Studies of Overhead Equipment for Electrical Traction (Part 1—Simple Catenary Equipment)"; Mr. T. A. Willetts, Mr. C. E. Allen and Mr. D. R. Edwards: "Dynamic Model Studies of Overhead Equipment for Electric Railway Traction (Part 2—Sagged Simple Catenary Equipment)".

CHALLENGER SOCIETY (joint meeting with the Glaciological Society, at the Linnean Society, Burlington House, Piccadilly, London, W.1), at 5.45 p.m.—Dr. Wilford Weeks "Toward an Understanding of the Physical Properties of Sea Ice".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Two papers on "The U.K. 3 Satellite". Mr. W. M. Lovell: "Data Handling and Telemetry Equipment"; Mr. F. P. Campbell: "The Satellite and its Ground Checkout Equipment".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT I.E.R.E.–I.E.E. MEDICAL ELECTRONICS GROUP (at 9 Bedford Square, London, W.C.1), at 6 p.m.—Discussion on "Physiological and Psychological Hazards of High Intensity Sound and Light" opened by Mr. B. Shackel.

Thursday, April 28

BOROUGH POLYTECHNIC (at Borough Road, London, S.E.1), at 10 a.m.—Symposium on "Friction and Wear—Can We Afford It?"

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Prof. A. R Ubbelohde, F.R.S.: "Melting and Freezing of Metals"; Dame Kathleen Lonsdale, F.R.S., Mr. E Nave and Mr. J. F. Stephens: "X-ray Diffraction and Mass Spectrometer Studies of a Chemical Reaction in a Single Crystal".

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1), at 5.15 p.m.—Dr. M. Zelen: "Applications of Exponential Models to Problems in Cancer Research".

INSTITUTE OF PETROLEUM, EXPLORATION AND PRODUCTION GROUP (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. Charles Weinaug (U.S.A.): "The Role of Viscous Fingering in Miscible Fluid Displacement".

INSTITUTION OF CIVIL ENGINEERS, HYDROLOGICAL GROUP (at Great George Street, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Aquifer Field Assessment" introduced by Dr. J. Ineson.

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Sir Harrie Massey, F.R.S.: "Scientific Research in Space" (Fifty-seventh Kelvin Lecture).

Friday, April 29

INSTITUTION OF THE RUBBER INDUSTRY, LONDON SECTION (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 9 15 a.m.—Conference on "Tyres".

UNIVERSITY OF LONDON (at the Royal Veterinary College, Royal College Street, London, N.W.1), at 5 p.m.—Prof. Sir William Weipers: "Lethal Factors in Experimental Intestinal Obstruction".*

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. G. W. Carter: "Distribution of Mechanical Forces in Magnetized Material"; Mr. C. J. Carpenter "A Note on the Distribution of Mechanical Forces in Magnetized Material" (discussion on Prof. Carter's paper); Mr. B. D. Popovic: "Evaluation of Magnetized Energy Density in Magnetized Matter".

TELEVISION SOCIETY (in the Conference Suite, I.T.A., 70 Brompton Road, London, S.W.3), at 7 p.m.—Annual General Meeting.

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. F. Hoyle, F.R.S.: "The Nature of the Quasi-Stellar Objects".

Monday, May 2

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 2.30 p.m. and 5 p.m.—Colloquium on "Current Topics in Tropospheric Propagation".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 5 p.m.—Annual General Meeting.

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Annual General Meeting, followed by Mr. A. Mott. "The Changing Structure of the U.K. Chemical Industry".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Annual General Meeting, followed by Dr. W. T. Norris: "M.H.D. Generation of Electricity".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER (with an honours degree in pharmacology, physiology, biochemistry or zoology and also a higher degree or evidence of original research in an appropriate field) IN PHARMACOLOGY—The Clerk to the Council, School of Pharmacy, University of London, 29/39 Brunswick Square, London, W.C.1 (April 27).

JUNIOR LECTURERS (2) (with an honours degree in physiology with zoology, chemistry or physics, or a medical qualification) IN PHYSIOLOGY—The Registrar, King's College (University of London), Strand, London, W.C.2 (April 29).

LECTURER IN EDUCATION; and a LECTURER IN MATHEMATICS—The Principal, Bede College, Durham (April 29).

ASSISTANT LECTURER IN BIOCHEMISTRY—The Registrar and Secretary, The University, Bristol (April 30).

ASSISTANT LECTURER IN MATHEMATICS (applications are particularly invited from candidates with interests in approximation theory or numerical analysis or probability and statistics, although candidates with interests in other fields will be considered)—The Secretary, University of Lancaster, Bailrigg House, Lancaster (April 30).

ASSISTANT LECTURERS (2) IN BIOCHEMISTRY—The Registrar and Secretary, The University, Bristol (April 30).

LECTURER IN CHEMISTRY (ORGANIC)—The Registrar, The University, Manchester, 13, quoting Ref. 88/66 (April 30).

LECTURERS or ASSISTANT LECTURERS (2) (with a particular interest in political, historical or economic geography, and able to lecture on the regional geography of South East Asia, the Monsoon Lands or Eastern Europe) IN THE DEPARTMENT OF GEOGRAPHY—The Registrar, University College of Wales, Aberystwyth (April 30).

POSTDOCTORAL FELLOW IN THE DEPARTMENT OF CHEMISTRY for work on molecular structures by microwave spectroscopy—Prof. J. Sheridan, Department of Chemistry, University College of North Wales, Bangor, North Wales (April 30).

SENIOR LECTURER IN THE DEPARTMENT OF MOLECULAR BIOLOGY—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (April 30).

LECTURER (medical qualifications not essential, but preference will be given to candidates with special experience in electron microscopy) IN ANATOMY—The Secretary of the University Court, The University, Glasgow (May 1).

CHAIR OF BACTERIOLOGY—The Secretary, University of Edinburgh, Edinburgh (May 3).

LECTURER IN PURE MATHEMATICS—The Registrar, King's College (University of London), Strand, London, W.C.2 (May 6).

READER IN NUMERICAL ANALYSIS IN THE DEPARTMENT OF MATHEMATICS—The Registrar, University of Strathclyde, George Street, Glasgow, C.1 (May 6).

READER (preferably with a special interest in either functional or classical analysis) IN PURE MATHEMATICS—The Registrar, University of Strathclyde, George Street, Glasgow, C.1 (May 6).

LECTURER IN ARCHAEOLOGY—The Secretary of the University Court, The University, Glasgow (May 7).

LECTURER or ASSISTANT LECTURER (Probationary) IN PHYSICS—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (May 8).

CHAIR OF HIGHER EDUCATION—The Registrar, The University, Manchester, 13, quoting Ref. 81/66/Na. (May 9).

CHAIR OF SOCIOLOGY—The Secretary, University of Stirling, Stirling (May 9).

DIRECTOR (highly qualified scientifically with a marked aptitude for administration and negotiation with public bodies and others) OF THE INSTITUTE OF GEOLOGICAL SCIENCES, LONDON—The Natural Environment Research Council, Establishments Division, State House, High Holborn, London, W.C.1 (May 9).

CHAIR OF CELL BIOLOGY—The Secretary of the University Court, The University, Glasgow (May 13).

CHAIR OF SOCIOLOGY—The Secretary of the University Court, The University, Glasgow (May 13).

SENIOR LECTURER or LECTURER (preferably with an interest in either entomology or parasitology) IN THE DEPARTMENT OF ZOOLOGY—The Inter-University Council, 33 Bedford Place, London, W.C.1 (May 13).

ASSISTANT LECTURER or LECTURER (preferably with a special interest in comparative physiology) IN THE DEPARTMENT OF ZOOLOGY—The Registrar, The University of Liverpool, Liverpool 3, quoting Ref. CV/22 (May 14).

CHAIR OF VIBRATION STUDIES IN THE INSTITUTE OF SOUND AND VIBRATION RESEARCH—The Deputy Secretary, The University, Southampton (May 14).

LECTURER IN CHEMICAL ENGINEERING or BIOCHEMICAL ENGINEERING—The Registrar, University College of Swansea, Swansea (May 14).

LECTURER (preferably with research experience in spectroscopy or chemical physics subjects) IN PHYSICAL CHEMISTRY—The Registrar, University of Strathclyde, George Street, Glasgow, C.1 (May 14).

RESEARCH DEMONSTRATORS (with research interests either in organic or physical chemistry) IN CHEMISTRY—The Registrar, University College of Swansea, Swansea (May 14).

LECTURER (non-medical) IN CHEMICAL MICROBIOLOGY—The Registrar, The University, Liverpool, quoting Ref. CV/16 (May 20).

SENIOR LECTURER IN THE DEPARTMENT OF HISTOLOGY—The Registrar, The University, Liverpool, 3, quoting Ref. CV/23 (May 20).

RESEARCH FELLOW IN THE DEPARTMENT OF MATHEMATICAL PHYSICS for work in the field of elementary particle physics (consideration will also be given to candidates with other interests)—The Assistant Registrar (Science and Engineering), The University of Birmingham, Birmingham, 15 (May 21).

RESEARCH FELLOW (with a degree and postgraduate research experience in biochemistry and preferably some knowledge of physiological teaching) IN THE DEPARTMENT OF BIOCHEMISTRY to investigate the mechanism of enzymic adaptation to contractile activity in skeletal muscle under the supervision of Prof. S. V. Perry—The Assistant Registrar (Science and Engineering), The University of Birmingham, Birmingham 15 (May 21).

LECTURER IN INORGANIC CHEMISTRY at Makerere University College, University of East Africa, Uganda—The Inter-University Council, 33 Bedford Place, London, W.C.1 (May 27).

SENIOR FELLOW IN THE DEPARTMENT OF THEORETICAL PHYSICS, Research School of Physical Sciences, Institute of Advanced Studies, Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, May 27).

RESEARCH ASSISTANT (Biochemist or Pharmacologist) to work at Naples and University College, London, on investigations of the nervous transmitter systems of octopus, under the general supervision of Prof. J. Z. Young—The Secretary, University College, Gower Street, London, W.C.1 (May 28).

RESEARCH ASSISTANT (Experimental Psychologist or Zoologist) to work at Naples and University College, London, on investigations of the learning mechanisms of octopus, under the general supervision of Prof. J. Z Young—The Secretary, University College, Gower Street, London, W.C.1 (May 28).

SENIOR LECTURER or LECTURER IN PURE MATHEMATICS at the Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, May 30).

POSTDOCTORAL RESEARCH FELLOW IN SOLID STATE CHEMISTRY or PHYSICS at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, June 1).

LECTURER (with a degree or an equivalent qualification in which emphasis is on mining with adequate supporting geology, and preferably practical experience in the mineral industry) IN MINERAL TECHNOLOGY, University of Otago, Dunedin, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or The Registrar of the University (New Zealand and London, June 15).

SCIENTIFIC OFFICERS (2) (with at least a good honours degree in botany or zoology, and for one post a knowledge of biochemistry an advantage) TO STUDY NEMATODE PARASITES OF PLANTS—The Secretary, Rothamsted Experimental Station, Harpenden, Herts, quoting Ref. 1051/69 (June 30).

ASSISTANT LECTURER or LECTURER IN PSYCHOLOGY—The Registrar, Portsmouth College of Technology, Hampshire Terrace, Portsmouth.

COMPUTER ENGINEER (with experience of large valve computers)—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1.

DEPARTMENT RESEARCH ASSISTANT (Biochemist) to work on metabolism of the eye in relation to disease—Dr A. Pirie, Nuffield Laboratory of Ophthalmology, University of Oxford, Walton Street, Oxford.

GRADUATE to study the kinetics of metabolic processes in relation to the synthesis of plasma lipoproteins—Dr. G. L. Mills, Courtauld Institute, Middlesex Hospital Medical School, London, W.1.

RESEARCH FELLOW (with postgraduate research experience) IN QUANTUM ELECTRONICS for work involving a theoretical and experimental study of the scattering of laser light by the atmosphere—Prof. W. A. Gambling, Department of Electronics, The University, Southampton.

SCIENTIFIC INFORMATION OFFICER (able to write good English, and preferably with a botanical or agricultural degree or a knowledge of or aptitude for foreign languages) for duties which will include preparation of abstracts for the journal *Plant Breeding Abstracts*, editing and classification of biological literature, and dealing with scientific inquiries—The Director Commonwealth Bureau of Plant Breeding and Genetics, School of Agriculture, Cambridge.

SENIOR LECTURER or LECTURER (well qualified academically with proven research and teaching ability, and preferably a knowledge of, or experience in, the chemical industry) IN INORGANIC CHEMISTRY—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 10/G.

VISITING LECTURER IN GEOGRAPHY—Prof. W. B. Johnston, Department of Geography, University of Canterbury, Private Bag, Christchurch, New Zealand.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Agricultural Research Council. Annual Report of the Low Temperature Research Station, Cambridge, 1963–64. Pp. 76. (London: H.M. Stationery Office, 1966) 6*s*. [23

Building Research Station Digest (Second Series), No. 67: Soils and Foundations—3. Pp. 6 (London: H.M. Stationery Office, 1966) 4*d*. [23

Welwyn Hall Research Association. Annual Report, 1964–1965. Pp. ii+19. (Welwyn: Welwyn Hall Research Association, 1965.) [23

University of Birmingham. Report of the Vice-Chancellor and Principal for the calendar year 1965. Pp. 43. University Research Committee—Thirty-sixth Annual Report on Research and Publications (Session 1964–1965). Pp. 188. (Birmingham: The University, 1966.) [23

Ambassade de France, Service de Presse et d'Information. A Survey of French Aid to Developing Countries. Pp. 52. (London: Ambassade de France, Service de Presse et d'Information, 1966) [23

North York Moors. Edited by Dr. Arthur Raistrick. (National Park Guide, No. 4.) Pp. x+113+27 plates. (London. H.M. Stationery Office, 1966.) 6*s*. net. [23

Field Studies Council. Annual Report, 1964–65. Pp 35+6 photographs. (London: Field Studies Council, 1966.) [23

The Year Book of the Royal Society of London 1966 (No. 70). Pp. 351. (London: The Royal Society, 1966.) 21*s*.; 3.15 dollars. [23

Ministry of Aviation: Electronics Research Council. The Educational Role of the Ministry of Aviation Establishments—Working Party Report. Pp. iv+24 (London: H.M. Stationery Office, 1966.) 2*s*. 3*d*. net. [23

Fabian Society. Fabian Tract No. 366: Workshop Bargaining—a New Approach. By Dr. Norman Ross. Pp. 28. (London: Fabian Society, 1966.) 3*s*. 6*d*. [23

International Tin Research Council. Annual Report 1965. Pp. 44. (Greenford, Middx: Tin Research Institute, 1966.) [23

Bulletin of the British Museum (Natural History). Zoology. Vol. 13, No. 9. A Review of the Muridae (Order Rodentia) of Uganda. By M. J. Delany and B. R. Neal. Pp. 295–355. (London: British Museum (Natural History), 1966.) 27*s*. [23

International Nickel Limited. Rhodium. Pp. 21. (London: International Nickel Limited, 1966.) [23

Parliamentary and Scientific Committee. Annual Report 1965. Pp. 26. (London. Parliamentary and Scientific Committee, 1966) [23

Viruses and Cancer. By Prof Albert B. Sabin. (Fourth Marchin Lecture delivered May 28, 1965, at the University of Newcastle upon Tyne.) Pp 18. (Newcastle upon Tyne: The University, 1965.) 5*s*. [23

Ministry of Agriculture, Fisheries and Food. Technical Bulletin No. 13: Soil Phosphorus. (Proceedings of a Conference organized by the Soil Chemists of the National Agricultural Advisory Service, October 4–5, 1962.) Pp v+159. (London: H.M. Stationery Office, 1965.) 37*s*. 6*d*. net. [23

Microbes in Action. By Prof. D. E. Hughes. (An Inaugural Lecture delivered at University College, Cardiff, April 30, 1965.) Pp. 21. (Cardiff: University of Wales Press, 1965) 3*s*. 6*d*. [23

Office of Health Economics. Factors which May Affect Expenditure on Health. Pp. 20. (London: Office of Health Economics, 1966.) [23

Other Countries

Durban Museum Novitates. Vol. 7, Part 10 (November 30, 1965): A Catalogue of Birds of the South African Sub-Region. Part 2: Families Glareolidae—Pittidae. By P. Clancey. Pp. 305–388. 1 50 R. Vol. 8, Part 1 (September 1, 1965): Miscellaneous Taxonomic Notes on African Birds, 23. By P. Clancey. Pp. 1–17. 75 cents. Vol. 8, Part 2 (September 1, 1965): Observations on Some Natal Butterflies. By C. G. C. Dickson. Pp. 19–23. 30 cents. (Durban: Durban Museum, 1965) [

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Entomology, 1964–65. Pp vii+7. (Canberra. Commonwealth Scientific and Industrial Research Organization, 1965.) [12

National Academy of Sciences—National Research Council NAS-NS-3002 (Rev.): Radiochemistry of Arsenic. By Harold C. Beard Revised by J. G. Cuninghame. Pp. v+39. (Washington, D.C.: National Academy of Sciences—National Research Council, 1965. Available from the Clearinghouse for Federal Scientific and Technical Information, N.B.S., U.S. Department of Commerce, Springfield, Virginia) 1 dollar [12

Publications de l'Institut National pour l'Étude Agronomique du Congo. l'Organisation de l'Agriculture dans les pays en voie Développement. Par J. Henry et F. Jurion. Pp. 69. (Bruxelles: Institut National pour l'Étude Agronomique du Congo, 1965.) 100 francs. [12

Commonwealth of Australia: Department of External Affairs. Australian National Antarctic Research Expeditions. ANARE Data Reports, Series D (XV) Meteorology. Publication No. 81: Meteorology: Davis, Macquarie Island, Mawson and Wilkes, 1962. Prepared by the Bureau of Meteorology, Melbourne. Pp. xvii+456. (Melbourne: Antarctic Division, Department of External Affairs, 1965) [12

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Building Research, 1964–1965. Pp. 83. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1965.) [12

National Academy of Sciences—National Research Council. Space Research: Directions for the Future, Part 2. (Report of a Study by the Space Science Board, Woods Hole, Massachusetts, 1965.) Pp. vii+192. (Washington, D.C.: National Academy of Sciences—National Research Council, 1966) [12

United States Department of the Interior: Geological Survey. Bulletin 1197: Bibliography of North American Geology, 1961. Pp. xvii+663. 2 dollars. Water-Supply Paper 1592-B: Cross-Channel Transfer of Linear Momentum in Smooth Rectangular Channels. By R. W. Cruff. Pp. v+26. 15 cents. Water-Supply Paper 1759-F: Water Resources in the Vicinity of Municipalities on the Eastern Mesabi Iron Range, Northeastern Minnesota. By R. D. Cotter, H. L. Young, L. R. Patri and C. H. Prior. Pp. iv+27+plate 1. Water-Supply Paper 1817-B: Identification and Measurement of Chlorinated Organic Pesticides in Water by Electron-Capture Gas Chromatography. By William L. Lamar, Donald F Goerlitz and LeRoy M. Law. Pp iii+12. 15 cents. Geophysical Abstracts, No 227, December 1965. By James W. Clarke, Dorothy B. Vitaliano, Virginia S. Neuschel and others Pp. iii+947–1029. 35 cents. (Washington, D.C.: Government Printing Office, 1965.) [12

United States Department of Agriculture, Index-Catalogue of Medical and Veterinary Zoology. Subjects: Trematoda and Trematode Diseases. Part 4: Subgenera and Genera E-G. By Mildred A. Doss, assisted by Katharine Forsyth Roach and Virginia L. Breen. Pp. v+597–807. (Washington, D.C.. Government Printing Office. 1965.) 60 cents. [12

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THIRTY YEARS AFTER

IT is a source of great pleasure that Prof. Peter Kapitza is about to pay a brief visit to Britain after an absence of thirty years. In a very real sense, he has a claim on the goodwill and even the affection of those who still marvel at what physics accomplished in the first few decades of this century. Those who do not remember the thirties will nevertheless be eager to welcome somebody who worked at the Cavendish in those heroic days. And even those of Kapitza's contemporaries who may have thought that Rutherford was spending too much of the meagre resources of the laboratory on Kapitza's equipment for generating strong magnetic fields will not stint their welcome now. For was not Kapitza the man who ran the informal club at which the Young Turks at the Cavendish plotted the next steps forward—positron, neutron, artificial disintegration and the rest? Was not Kapitza a Fellow of the Royal Society in 1929? And best man at the Chadwicks' wedding? With the passage of time, these are the things that people remember. As Kapitza himself explained to Rutherford in a letter written after he had failed to return from the Soviet Union in the summer of 1935, "after all, we are only small particles of floating matter in a stream which we call Fate. All we can manage is to deflect slightly our track and keep afloat . . .".

Kapitza has been away too long, and he will find that many things have changed. For one thing, the Cavendish is no longer the centre of the Universe, even though some of its buildings may have lasted unreasonably well. It remains a splendid laboratory for research and teaching, but the most lasting monument to what happened there in the time of Rutherford may be the way in which his students have helped to create a dozen comparable laboratories. And physics has grown with such vigour and success as to transcend the boundaries of single laboratories. Even if, in the thirties, Rutherford's strategy on spending money had been different, and if the Cavendish had been quicker off the mark with its first cyclotron, the effect on recent history would only have been transient. It is unthinkable, for example, that different decisions then could have enabled Britain now to build for itself a proton–synchrotron to match the 200-GeV machine soon to be built in the United States.

If the place of the Cavendish has changed, so has that of Britain in the world. The country is no longer the self-contained scientific community of the thirties. The traffic from Britain—and the rest of Europe—to and from the United States is increasingly valuable. Links between European laboratories are already strong and promise rapidly to grow stronger. Most often, the consequences of this growing interdependence are stimulating. The disadvantages are few, if any, for it remains to be demonstrated that even the unplanned emigration of scientists is in the long run a disadvantage.

The changed status of British science accords with the changed status of the British economy. In 1935 Britain was more prosperous than most countries except the United States. Now the country finds it more difficult than most to maintain the standard of prosperity to which Western Europe has become accustomed. Technically, British industry can no longer expect to tackle everything, but must deploy its effort carefully between computers and aircraft, ships and steelworks. So there is a preoccupation with science and technology, and with attempts to harness these forces of innovation to the growth of the economy. Nobody need be surprised that things have gone like this, but nobody need be despondent either. Britain remains a nation of great talent. That has not changed. But Kapitza may notice some of the discomforts of changing status. Support for scientific research is growing, but it should grow faster. Post-graduate education and training are spreading, but not quickly enough. And there is such a concern to turn science into quick prosperity that it is no wonder that British pride in achievement is often tinged with uncertainty.

There is evidence that Kapitza is also worried by these things. Earlier this year, in *Komsomolskaya Pravda*, he made the plain statement that "we must not be afraid to say that in the past few years the gap in science between the Soviet Union and America has not been narrowing". This sentence has been widely quoted, for obvious reasons, but it followed a careful analysis of the reasons why the Soviet Union has not reaped all the industrial and economic benefits that might have been expected from the massive expenditure on science and technology in the past thirty years. Kapitza's conclusion was that "in organizing the process of the assimilation of new technologies by industry, the approach must be purely individual, without any cut and dried rules, and the character of the people concerned and the nature of the external conditions must be taken into account in each particular case. Questions of finance and personnel are, of course, settled by an appropriate decision, but the successful assimilation of new technologies depends on good relations between pupils and teachers and on their common interest in success . . .".

The irony is that this applies almost as well in Britain as in the Soviet Union. In both countries, it would seem, the climate of society is such that scientists and technologists do not contribute all they might to industrial prosperity. The merit of Kapitza's argument is the emphasis it puts on psychological factors, and in particular on the importance that scientists and technologists should somehow be able to believe that their efforts could effect great changes What he is saying is that in the application of science and technology, as in all other creative activity, success depends on the extent to which it is possible to engage the imagination and the enthusiasm of talented people. It appears that it is equally easy to escape from this truth in Britain and in the Soviet Union, though perhaps for different reasons. Is it too much to hope that one benefit of Kapitza's visit will be a resolve to study this common problem from the two very different points of view of Britain and the Soviet Union?

Kapitza's reflexion on the "gap" between the United States and the Soviet Union raises wider issues. Two things need saying. First, the harvest of scientific research in the Soviet Union is indeed much less than might have been expected from the effort devoted to it since the Second World War. There is nothing wrong with the

quality of the Russian work or the character of Russian scientists—far from it—but there could well have been more of it. Secondly, Russian science remains more isolated than it need be and should be from what happens elsewhere in the world, and this is true even though there have been enormous improvements in the past decade in the freedom with which Russian scientists travel abroad or invite colleagues to visit them in the Soviet Union. It is still something of a rarity for research students and their more senior colleagues to spend long periods at each other's laboratories, for example, and it is hard not to link these circumstances together. In other words, may it not be that Russian efforts in recent decades would have been even more successful if it were not for the obstacles which still remain to a free intellectual traffic between East and West? Quite simple experience will suggest that there is good cause to fear something of the kind, and if the Soviet Union is contributing less than it might to the rapid progress of science, everybody loses. This is why another benefit to be sought from Prof. Kapitza's visit is a still further liberalization of the rules for exchanging science and scientists between the Soviet Union and everywhere else.

LUCKY THIS TIME?

THE annual meeting of the British Association is usually a disappointment. Each year for several years, well-wishers have been hoping for the best and have been denied it. Most of the participants, who would obviously not be there if they did not wish to be, must have been puzzled to know why an institution that gave them pleasure should have been denounced as a bore by so many of those not wishing to attend. The trouble has been that the external influence of the meeting has been steadily diminishing. A century has gone since Huxley and Wilberforce fought over the *Origin of Species*. As things are, the association is neither a learned society nor an indispensable channel for the communication of science to the world at large. Over the years, it has grown in on itself. It has lacked a cutting edge.

This is why it is good, and a surprise, to be able to record that things could well be different. Plans for the meeting which begins at Nottingham on August 31 this year include some promising innovations. Briefly, the Association is going to see whether it can somehow arrange for an open discussion of three public issues in which science and technology are deeply engaged—water conservation in Britain, supersonic aircraft and the development and use of drugs. The intention is to confront public policies on these issues with the criticisms of unofficial persons, professional scientists and others. Obviously the success of these meetings will depend on whether their chairmen can avoid capture by received doctrine on the one hand and cranks on the other. On supersonic aircraft, for example, a course will have to be steered between the vested interests of the aircraft manufacturers and the frequently ill-considered arguments of those who denounce all noise produced by agencies other than nightingales. But if things go well, the result could easily be a better understanding of some important public issues. That would be valuable in itself, but the general officers of the Association are after bigger fish. Plainly, they are hoping that they have found a reason for the Association's continued existence. With luck and good management, they may be right.

MANAGEMENT OF INDUSTRIAL RESEARCH

Suits

Speaking of Research. By Dr. C. Guy Suits. Pp. xii+466. (New York and London: John Wiley and Sons, Inc., 1965.) 57*s*.

SPEAKING of Research is a collection of addresses that Dr. Guy Suits has delivered to a variety of institutions in the United States. Although some of these may have been available in that country, their availability in Britain is very limited. Their collection into one volume provides a unique opportunity for Dr. Suits to review the progress, over a period of twenty years, of one of the most outstanding and successful industrial research laboratories in the world. Most scientists are familiar with the pioneering researches of Irving Langman and their effect on the fortunes of the General Electric Company. The more recent achievements of the laboratory are less well known, and it is therefore all the more interesting that some glimpse of the history of these achievements should come from the pen of Dr. Suits. The author claims that his crystal ball may often appear cloudy and crazed because the articles are produced with subsequent amendment and the benefit of hindsight. They are all the better for remaining unaltered, because it does give the reader the benefit of seeing how difficult it is to look ahead in science and technology and confidently to predict the course of events. Some people think this can be done with precision, but the more eminent the leader of a large research enterprise, the more modest he is in claiming unquestioned ability to forecast the course of events ahead.

It is inevitable that some subjects are dealt with in more than one address, but this adds interest to the account, for it gives the reader an opportunity to chart the course of events over a period of years. Many of the chapters deal with the nuclear power topic which General Electric has been engaged on for a very long period. Little has appeared and thus these accounts, though not detailed, give a conspectus of new points of view that must have guided the thinking of General Electric during the past two decades. Maybe the most valuable and illuminating are those essays on the management of scientific research. Dr. Suits comes down in favour of a certain amount of broad planning of basic research. His reasonable thesis is that the interests of General Electric are wide but in spite of diversity are not in fact so wide that wide choice of areas of study should not be defined. The hope expressed is that wise guidance can give the freedom necessary for highly original work of high quality and yet of great potential development if in fact the basic work turns out to be fruitful. Certainly, the more recent record of the laboratory would substantiate this approach to planning. Although it is not the most important, the detailed account, with interesting quotations from laboratory notebooks, of the successful attempts to produce diamonds is one of the most interesting. Here is a case where the conditions for the existence of diamonds were known, but the problem of transforming graphite to diamonds in a finite time was a matter of the utmost difficulty and might easily have eluded those engaged in the work. There is, maybe unfortunately, little reference to the synthesis of borazon and nothing about its potential as an exceptionally hard material.

One of the shorter addresses deals with postgraduate training in physics. For its research laboratory General Electric almost exclusively recruits Ph.D.s, because Bachelors of Science are not highly trained enough for pioneering work in an advanced laboratory. However, Dr. Suits is worried about the length of time needed to obtain the degree of Ph.D. and yet at the same time he deplores the tendency to specialize at the expense of a broad training, including formal instruction. His opinion is that if something has to go it is the amount of origina

research, for he says that the introduction to industrial research is best conducted in the industrial research laboratory itself.

The final address on selectivity in research is packed with thoughts that are of great general interest, not only in industrial research but also in government or government-sponsored research and development. The first basic problem is that in the United States research and development in the industrial field only increase at 10 per cent per year in dollars, whereas research and development for the country as a whole grow at a rate of 20 per cent. The 10 per cent barely copes with the normal increase in costs and in salaries. In these circumstances, this means that an industrial laboratory has to try to become more productive and this can only be done by a much more critical selection of projects. Fashion in research is attacked vigorously and a slavish following of fashion without good reason is reckoned to be one of the best ways of decreasing productivity. For example, Dr. Suits considers research into lasers, and particularly their applications, is a field where far too much research has been done without there being a prior appreciation of the value of the application of research. Another danger is to try to keep a finger in every pie as a kind of insurance policy. Dr. Suits considers this a waste of money and talent. He is surprisingly critical of the attempts to inject science into those industries that have been around virtually for centuries. What can be done in these industries has already been done. The problems involved in the support of big science come in for some scathing criticism too. Bigness is what matters rather than the criterion as to whether it is good science too. Even more surprising is the analysis of the situation where the research venture is completely successful but when this point is reached no one can find an application for the knowledge so acquired. Dr. Suits admits in his speech that his tongue is sometimes in his cheek in making these remarks; nevertheless the questioning is very necessary at present not only in the United States but in Britain as well. This is a volume to be read with great interest by everyone interested in the management of research and development and also in engineering.

HARRY MELVILLE

SOCIAL IMPLICATIONS OF NUCLEAR POWER

A Peril and a Hope

The Scientists' Movement in America, 1945–47. By Alice Kimball Smith. Pp. xiv+591. (Chicago and London: The University of Chicago Press, 1965.) 10 dollars; 72*s*.

A PERIL and a Hope is an excessively long and detailed account of what is described as the scientists' movement in America, 1945–47. It is concerned more especially with the way in which scientists connected with the development of the atomic bomb and with nuclear power awoke to the social and political implications of their work and sought to influence national policy. Its scope is, in time at least, more limited than that of Robert Gilpin, in *American Scientists and Nuclear Weapons Policy*, but it is not limited to questions of the use of nuclear weapons. It is nearer in scope to A. H. Compton's *Atomic Quest*, which at some points it amplifies and corrects. The real interest and value of the book lie in its contribution to the continuing discussion of the influence of science and of scientists on public policy, the limits of that influence and the mechanism by which advice is most effectively tendered. Much of it is strictly relevant to conditions in the United States, and here the excessive detail makes it more difficult to bring out the relevance of the experience described to conditions in Britain. Even so, it should contribute to a clearer understanding of that experience, and the book includes, moreover, as appendixes, the important Jeffries report of November 1944 on the prospects of nucleonics and the French report of June 1945 on nuclear power and nuclear weapons, neither of which appears to be easily accessible in Britain.

The book throws some fresh light on the struggle in the United States to free nuclear energy from military control and to avoid the domination of nuclear research by secrecy or security restrictions. It also illuminates the initial approaches to the international control of nuclear activities, but its chief value seems to lie in pointing out how much is demanded of scientists who are seriously concerned about the social consequences of their work. To influence public policy effectively requires much more than goodwill, more even than clear understanding of the scientific and technical factors and their implications; it demands an understanding of the political process also and a readiness to accept the conditions of effective participation. Mrs. Smith might perhaps have told her story more briefly and effectively, but at least in conclusion she puts the issues and the price demanded succinctly and unmistakably.

R. BRIGHTMAN

PHYSICAL PRINCIPLES OF MAGNETISM

The Physical Principles of Magnetism

By Prof. Allan H. Morrish. (Wiley Series on the Science and Technology of Materials.) Pp. xv+680. (New York and London: John Wiley and Sons, Inc., 1965.) 125*s*.

THE magnetic properties of materials is a field of investigation in which many important and significant developments have taken place during the past 25 years. The unified account of magnetic phenomena in solids, which *The Physical Principles of Magnetism* provides, will therefore be welcomed by the increasing number of physicists and engineers who wish to acquire a comprehensive knowledge of this branch of solid-state physics.

After an introductory chapter on classical electromagnetic theory, the first portion of the book deals mainly with paramagnetic systems. This is followed by a chapter on the magnetic properties of an electron gas, and accounts are given here of such topics as Pauli spin paramagnetism, the de Haas–van Alphen effect and the magnetic properties of superconductors. The remainder of the book is devoted to an extensive discussion of magnetic materials in which the magnetic carriers are strongly coupled by exchange forces. Ferromagnetics, antiferromagnetics and ferrimagnetics are each treated separately and in considerable detail.

The author has dealt with the subject mainly in a theoretical way. Experimental results and data are, however, included to illustrate and compare with the theory, but experimental methods and techniques are rarely described. Wherever possible, phenomenological approaches have been adopted to the topics discussed. Thus, for example, magnetic resonance is explained in terms of a precessing dipole, while the Weiss molecular field is used as a basis for the theory of ferromagnetism. For a complete understanding of a number of sections of the book, however, an acquaintance with solid-state theory and a good working knowledge of quantum mechanics are essential. The subject matter is presented in a clear and coherent manner, and the text is adequately illustrated with well-prepared diagrams. Since this is a book devoted solely to magnetism, the use of c.g.s. units is perhaps appropriate, since it is still fashionable for experimentalists to use these units when quoting their results. For the benefit of readers who are familiar only with the M.K.S. system, a conversion table is provided in an appendix. A useful feature of the book is the inclusion of a

bibliography and a set of problems at the end of each chapter.

The sections of the book describing magnetic resonance phenomena are particularly interesting and, taken together, give a comprehensive account of the way in which this comparatively recent technique has been used to investigate magnetic systems. The discussion of paramagnetic resonance in crystals containing transition metal ions is commendably concise, while the chapter on nuclear magnetic resonance contains all the essential physics of this phenomenon. In view of their applications in wave guide systems, a more detailed description of ferrimagnetic microwave devices could have been justified, while some of the theory in the rather lengthy discussion of resonance in strongly coupled dipole systems could have been condensed.

The chapters dealing with ferromagnetism are well written and merit special mention. A remarkably complete discussion of the theories of ferromagnetism is given, and this includes some of the more recent work that has been done on this difficult topic. The section on spin-waves is very informative. The domain structure of ferromagnetic materials receives ample coverage and a good description is given of the various mechanisms which participate in the magnetization process. There are short, but valuable, sections on permanent magnet materials and ferromagnetic alloys.

Although material published since about the beginning of 1964 is not included, this is a well-worth-while publication on magnetism which can be recommended as a text-book for the theoretician and a useful reference work for the experimentalist. G. D. ADAM

BIOLOGY OF NEUROGLIA

Biology of Neuroglia

Edited by E. D. P. de Robertis and R. Carrea. (Progress in Brain Research, Vol. 15.) Pp. xi+297. (Amsterdam, London and New York: Elsevier Publishing Company, 1965.) 95*s*.

IN Volume 15 of Elsevier's present series on the progress of brain research, Dr. E. D. P. de Robertis and Dr. R. Carrea edit the proceedings of the tenth Latin American Congress of Neurosurgery, which was held at Buenos Aires in 1963 and was devoted to an inter-disciplinary symposium on the "Biology of Neuroglia". The organizers of this symposium selected topics dealing mainly with the ultrastructure and function of neuroglia, particularly in relation to cerebral oedema. It is appropriate that the pioneer morphological studies of Hortega and Cajal are acknowledged in *Biology of Neuroglia* by the particularly elegant demonstration of neuroglial staining methods provided by Dr. M. Polak. Dr. de Robertis points out in his introductory chapter that these earlier morphological investigations of the Spanish school provided no information about the function of neuroglia in the nervous system, whereas this symposium is mainly concerned with those recent advances that have added to our knowledge of the ultrastructural organization and electrolyte-regulating functions of the neuroglia.

The enzyme histochemistry of normal and reactive astrocytes is authoritatively discussed by Dr. R. L. Friede, with particular reference to the role of these cells in pathological processes and their response to sodium ions. Dr. Igor Klatzo and his colleagues demonstrate the leakage of fluorescent-labelled protein from cerebral capillaries in cerebral oedema. Drs. J. Miquel and W. Haymaker discuss the significance of the glycogen accumulation seen in heavily irradiated astrocytes; they attribute this deposition to the effects of radiation-induced anaerobic metabolism. As a future problem they might consider it worthwhile to determine whether the astrocytic activities of uridine diphosphoglucose glycogen synthetase and phosphorylase are altered by irradiation.

Possible routes for the entry or transport of water and ions in the brain are presented by Drs. H. Davson and M. Bradbury, while Dr. A. Lazanksy considers the same problem in the retina. Drs. H. M. Pappius and L. Bakay discuss—in their respective contributions—the intra- or extra-cellular distribution of water in various forms of experimentally induced cerebral oedema. Dr. J. A. Zadunaisky and his colleagues report their ultrastructural and electrolyte investigations on neuroglia in the incubated brain; they conclude that the neuroglial cells are particularly concerned with osmotic and ionic regulation within the brain. The changes in astroglial and oligodendroglial volume after exposure to anoxia or hypothermia are illustrated in an interesting presentation by Drs. H. Collewijn and J. P. Schadé. Amino-acid transport within the brain is discussed by Dr. E. Levin and his associates. The electrical responses of cultured neuroglial cells are described by Dr. I. Tasaki; such stimulation initiates a slow wave of contraction in these cells.

The functional and metabolic symbiosis of neuroglia and neurones is discussed by Dr. Svaetichin and his co-authors and by Dr. R. Galambos. The latter author briefly but comprehensively reviews the functions of neuroglial cells in response to injury, in myelination and demyelination, in synaptic transmission and in Hydén's neuronal-satellite metabolic system.

Clearly it is impossible to include every discipline in a symposium of this sort, but it is to be regretted that Dr. R. M. Torack and his co-workers could not present their recent electron-histochemical studies on neuroglia in this volume.

The production of *Biology of Neuroglia* and the profuse illustrations are in accord with the customary excellent standards of the Elsevier Publishing Company. I have only a minute personal axe to grind: I dislike edited discussions presented in an informal verbatim manner.

This book can be thoroughly recommended as an excellent guide to those interested in the progress of contemporary research on neuroglia. C. W. M. ADAMS

INTRODUCTION TO BIOLOGY

Molecular Biology of the Gene

By Dr. J. D. Watson. (Biology Teaching Monograph Series.) Pp. xxii+494. (New York and Amsterdam: W. A. Benjamin, 1965.) 11 dollars, cloth; 6.50 dollars, paperback.

MOLECULAR Biology of the Gene offers more than the title promises. It is a modern introduction to the 'New Biology' and is, in fact, based on introductory lectures given to students of biology at Harvard. It dwells primarily on the analytical rather than the descriptive side of biology, emphasizing equally the genetic and the biochemical aspects. It begins with a description of the cell theory and cell division which leads on to the Mendelian laws and the concept of evolution. In the next chapter a summary of cell biochemistry is presented, with special reference to *E. coli*, the best understood organism at the molecular level. There follows an extensive discussion of the importance of chemical bonds (especially weak bonds) in the interaction of cell constituents, of biochemical thermodynamics and of the concept of template surfaces which operate in the synthesis of protein molecules.

The following chapters are concerned with the fundamentals of modern genetics, the arrangements of genes and chromosomes, gene structure and function, chromosome mapping, recombination, a description of the biochemistry of DNA, of the transcription of RNA on DNA templates and the role of RNA in protein synthesis. Further chapters deal with the replication of viruses, the genetic code, the regulation of protein synthesis, differentiation, the biochemistry of antibody formation, and the final chapter discusses cancer from the point of view of the geneticist.

The book is an outstanding achievement, written by one of the central participants in the modern development of biology. The presentation is clear, authoritative and vivid. The colourful yet concise style is illustrated by the following headings: "Avery's Bombshell" (referring to the discovery that nucleic acids can carry information); "The Twenty-five Year Loneliness of the Protein Crystallographers" (referring to the long lag period before X-ray crystallography yielded major results); "Much More Must be Learned about Ribosomes" (referring to the lack of information on the basic properties of their structure). The book is further enlivened by excellent diagrammatic illustrations. The account is well balanced; achievements as well as gaps in our knowledge are recorded. The approach is altogether highly original. This makes the book profitable not only for the beginner but also for expert biologists of all descriptions including biochemists and geneticists. It was produced within six months and is therefore as up to date as is possible nowadays.

H. A. KREBS

REFLEX ACTIVITY AND THE CENTRAL NERVOUS SYSTEM

Compensatory Adaptations, Reflex Activity and the Brain

By Ezras A. Asratian. Scientific and Translation Editor, Prof. Samuel A. Corson. (International Series of Monographs on Cerebrovisceral and Behavioural Physiology and Conditioned Reflexes, Vol. 1.) Pp. xv + 194. (London and New York: Pergamon Press Ltd., 1965.) 80*s*. net.

THE basic object of *Compensatory Adaptations, Reflex Activity and the Brain* is to extend the interpretation of Pavlov's work in an attempt to explain reactions in the central nervous system as arising out of conditioned and unconditioned reflexes. Thus, among other subjects, spinal shock, restoration of activity after damage to the spinal cord or the brain, and certain metabolic disturbances associated with central nervous exclusion are considered. Conditioned reflexes are suggested to be not restricted to individual neurological levels, but to have a number of associated extensions to the medulla, mid-brain and the cerebral cortex, together with common afferent and efferent pathways.

There are six chapters in the volume, the first four of which are based on a series of lectures given in British universities during 1957–58, but intimate reference to each chapter is not necessary in this review, for the subject matter is not restricted solely to individual sections.

Spinal shock is the subject of the first chapter, the mechanism of its causation affording the main topic. The evidence proclaimed supports Sherrington's views with some qualification. Related changes in the medulla and the parietal cortex are described, their recognition being the outcome of more modern forms of investigation, for example, electro-encephalography.

The use of terms such as 'irritation' and 'stimulation' rather suggests active factors in related causes of reactionary phenomena, as against a negation of function in the form of release of activities of other structures, where inhibition or suppression reigned beforehand.

The claim that the intensity of spinal shock shows some tendency to vary in accordance with the segment of cord damaged is noteworthy; for example, a greater degree of shock is induced by posterior hemisection than lateral hemisection of the cord. It would appear that proprioceptive disorganization has more bearing than the interruption in conduction of other afferent stimuli.

Compensatory mechanisms in the return of activity following disablement of experimental dogs would not necessarily indicate true recovery, but a process of adaptation. This seems a more likely explanation of the intensification of their positive conditioned reflexes and a decline of their inhibitory conditioned reflexes (p. 61). Enforcing an animal to apply a reconditioned structure, for example, the arm of the monkey (p. 63), surely is to be expected to expedite return of available activity within the limb, when the opposite limb has been put out of action by restrictive measures such as splinting or bandaging, or by amputation. Return of movement is recorded in animals where the following experiments have been undertaken: ambulation, where the forelimb on one side and hind limb on the opposite side have been amputated; cross anastomosis of vagus and median nerves; lateral hemisection of the spinal cord; dorsal hemisection of the spinal cord; longitudinal section of the cord. Moreover, recovery of mobility is abolished should the animal be subjected later to removal of the cerebral cortex or decerebration.

To record that the inability of activity to appear in animals subjected to cortical excision or decerebration could in any way be an interference with reflex action is a most restricted attitude. Generalized loss of cerebral cortex, in terms of cortical functions, is so disabling that a restricted aspect cannot be determined on this premise.

It is noted that attention to endocrine dysfunction is given some consideration, for not only is this mentioned but illustrations of affected glands occur. Changes in the ovary, pituitary and thyroid are to be seen. Yet the endocrine disorders arising out of these effects are not discussed. The introduction of the destruction of the whole cortex as a means of supporting the hypothesis propounded is a strange dismissal of the many functions assignable to that part of the brain.

The work is certainly worthy of perusal, though the English demands a most careful analysis, for often the sentences are too long. At a price of 80*s*., however, I would not recommend it for other than the most objective attention.

HARVEY JACKSON

THE LIFE OF FISHES

The Life of Fishes

By N. B. Marshall. (The Weidenfeld and Nicolson Natural History.) Pp. 402 + 43 plates. (London: Weidenfeld and Nicolson, 1965.) 63*s*. net.

THE Editor of *Nature* invites reviewers to suggest a separate title for their reviews, but in the case of *The Life of Fishes* a more apt title than that of the book itself would be difficult to find. Perhaps it would be more accurate to refer to the "Lives of Fishes", for one result of reading this book is to be impressed by the diverse ways in which fishes have become adapted to deal with the manifold facets of life in water, of parcelling out the environment among themselves, and of the ways in which these animals have become masters of their environment.

The scope of this book is immense, even though the author has limited himself to the short and medium time-scales in the lives of fishes. As if this was not enough, however, Mr. Marshall also treats the reader to a masterly review of the long time-scale as seen in the results of evolution manifest in the morphological diversity of living fishes. His final chapter, devoted to a succinct account of the classes and orders of fishes, will be highly valued by students who would otherwise have to seek widely for their information.

But the essence of the book is its concern with fishes in their environment, and not least of all with interactions between the fishes, as mates, as food or even to form near 'super-organisms'. Since his approach is a dynamic one, the author has reduced, so far as possible (and very successfully), the purely anatomical elements necessary to understand the physiological, behavioural and ecological specializations of fishes. To a large extent this has been achieved by the use of clear figures and diagrams.

The first, and largest, section of the book deals with the day-to-day existence of adult fishes. It gives particular emphasis to basic life processes, and to the prob-

lems of motion and respiration peculiar to a dense and not especially well-oxygenated medium. Here, too, are chapters dealing with that fascinating, multi-purpose organ, the swimbladder, and that equally fascinating but still somewhat mysterious 'seventh sense', the lateral line sensory system. Sound production, hearing, sight, electrogenesis and luminescence are all dealt with in broad but satisfactory detail. Although these different subjects are perforce considered separately, never once is the reader left with the feeling that they are being viewed in isolation. The first section concludes with three chapters treating of the ways in which the lives of fishes are conducted on a broader scale. In these chapters, above all, we see the functional integration of the various anatomical, physiological and sensory systems described in earlier sections. Shoaling, migration, courtship, commensalism, mimicry and coloration are all given particular attention, and woven into the persistent theme, devices for survival.

The second section of the book deals with the many aspects of reproductive biology, and the early life-histories of marine and freshwater fishes. Since less is known about these phases in fishes, it is a relatively short section, but none the less it gives a good coverage of what is known. Again the emphasis is on life and the means of ensuring it, this time more on the strategic than the tactical level.

Finally, the geographical and large-scale ecological background to fish life is described in three chapters covering the deep oceans, the shallow seas, and the freshwater habitats. This is no mere description of the stage and backdrops, for, quite rightly, the actors intrude to demonstrate how they have coped with and often capitalized the vagaries of their world.

Naturally, specialist ichthyologists will feel that some aspects of fish life have received too little attention from the author. Personally, I would have liked to see more space given to the freshwater fishes of Africa and South America, since in many respects these species provide synoptic microcosms of the problems (and their solutions) covered in the book. But such complaints would be churlish. Specialists will also find some errors of fact, and omissions of work which they consider important, but these are infrequent.

No one can fail to profit from this remarkably lucid, integrated and, above all, biological account of the largest and ecologically most complex group of living vertebrates.

P. H. GREENWOOD

REGENERATION AND RELATED SUBJECTS

Regeneration in Animals and Related Problems

Edited by V. Kiortsis and H. A. L. Trampusch. (An International Symposium sponsored by NATO'S Division of Scientific Affairs and held at Athens, Greece, April 1964.) Pp. xxiv+568. (Amsterdam: North-Holland Publishing Company, 1965.) 120*s*.

THE subject of regeneration naturally finds a place in most text-books of embryology which usually contain an account of the phenomenon itself and a brief survey of significant experiments in the field. For the interested student such accounts are altogether too brief and will lead him to the standard larger texts on the subject. Unfortunately, these can be comfortably counted on the fingers of one hand and constitute little more than a weekend's reading. The Lagonissi conference on "Regeneration in Animals and Related Problems" has provided the opportunity to create a useful addition to the literature on regeneration.

It is perhaps questionable whether the proceedings of any conference can be welded into a readable whole. Invariably the contributions will be uneven in length, diverse in style and unequal in their scientific merit. That *Regeneration in Animals and Related Problems*, containing as it does more than forty contributions, has much of the unity of a general text says much for the skill of the organizers of the conference and the care with which the book has been edited. English readers should be warned that about one in four of the articles is in French.

Of the three sections into which the book is divided, the general section is the most disappointing. This rests more in the placing of the articles than in their content. Herland-Meeve's article on asexual reproduction might well have been placed in the invertebrate section, and it would have been convenient for the reader to have had the notable contribution by M. Singer on nervous control of amphibian limb regeneration alongside that by C. S. Thornton on the influence of wound skin on blastema cell aggregation, which is some 300 pages farther on. Of the two major sections the one on invertebrates is more uniform in its subject matter and slightly more scholarly in its presentation. Here, indeed, is a happy mixture of general reviews and original research papers. Less satisfactory, possibly because the subject matter did not lend itself to a tidy classification, is the section on vertebrates and related problems. One or two articles here seem to be rather short for a book of this kind and do not seem to add much to the understanding of the larger and more exciting problems of regeneration. However, many of the major aspects of limb regeneration in amphibians are dealt with and there are, in addition, excellent articles on such apparently diverse topics as the repair of skin wounds in mammals (D. W. James), compensatory renal hyperplasia (R. J. Goss) and the relation between regeneration and tumour growth (F. Seilernaspang and K. Kratochwil).

Apart from a passing reference to the trials of Prometheus, the influence of Athens appears in the use of the words "Antiphon" and "Colophon" to head introductory and concluding sections by A. E. Needham. Non-musical readers may be puzzled but they will not be disappointed if they read on. "Antiphon" is a good guide to the contents of the book for the intending purchaser. "Colophon" puts in a plea for regeneration as a discipline in its own right. This is a somewhat sad note on which to end. The study of regeneration has so much to offer biologists in general that one must hope that articles on the subject will long continue to be available to a wide audience, not only by the publication of proceedings of conferences devoted to regeneration, but also in biological journals covering a wider range of subjects.

As the conference was sponsored by the Division of Scientific Affairs of the North Atlantic Treaty Organization, the absence of contributions from biologists of the U.S.S.R. and other countries outside the Organization is understandable; it is nevertheless regrettable in a symposium claimed to be international.

F. S. BILLETT

QUATERNARY STUDIES IN THE UNITED STATES

The Quaternary of the United States

Edited by H. E. Wright, Jr., and David G. Frey. (A Review Volume for the VII Congress of the International Association for Quaternary Research.) Pp. x+922. (Princeton, N.J.: Princeton University Press; London: Oxford University Press, 1965.) 200*s*. net.

THE adjective 'monumental' is often undeserved, but *The Quaternary of the United States* merits it on several counts—whether it be for its 932 pages of large quarto, its 55 authoritative chapters, or the feat of its two editors in assembling together the work of eighty-eight authors in time to have this volume ready for the Seventh Congress of the International Association for Quaternary Research in Colorado in September 1965.

The book is divided into four sections of which the first and largest comprises twenty accounts of the Pleistocene stratigraphy of natural regions of the United States. This is a storehouse of up-to-date information. If the diligent reader comes to the conclusion that some of the chapters conflict with others in their correlations and the degree of multiplicity which they accord to a multiglacial hypothesis, he should also appreciate that when this state of affairs ceases to exist there will be little need for a book of this sort.

Part 2 is devoted to biogeography and it is to be noted that there are two fundamentally different methods of approach. The chapters devoted to the records of pollen analysis or to Pleistocene mammals exemplify the palaeontologist's concept of using fossils to provide the answers to questions of speciation, extinction, and changes in geographical distribution relative to alteration of climate.

There is another approach: that of the student of living plants and animals, who reconstructs from the disjunct distribution patterns of present-day species and sub-species earlier unbroken areas of occurrence or the existence of biological 'refugia' and puts forward theories of speciation or sub-speciation. The relation of these to the Pleistocene history of glacial advances, pluvial periods and times of aridity obviously must remain speculative in the absence of a fossil record. The chapters on Rocky Mountain plant geography and on insects exemplify the second approach, but there are also several attempts to combine modern distributional studies with the fossil record, such as it is.

Archaeology makes up the third section of the book, understandably the smallest, since America is very much a new world. Claims are made that man may have been for 25,000 years or more in North America, but there is no really firm evidence beyond about 15,000 years. One realizes how important radiocarbon dating has been as a complement to stratigraphy in establishing the antiquity and sequence of cultures, starting with that of the Clovis points and ending in recent history.

The fourth and final group of contributions is "Miscellaneous Studies", and here are discussed a number of fascinating problems the implications of which extend far beyond the United States, as does the choice of evidence used to develop the themes. It is not possible to do more than list the topics, but isotope geochemistry, palaeopedology, volcanic ash chronology, palaeomagnetic stratigraphy, dendrochronology, palaeohydrology, tectonics and changes of sea-level are matters which concern students of the Quaternary the world over.

The book is supplied with abundant references at the end of each chapter, summarized in a final index of about 1,800 authors; illustrations are mainly very good and there are commendably few typographical errors. Its price of £10 in the United Kingdom has only been made possible by generous financial support from the U.S. National Science Foundation and all students of the Quaternary should welcome the chance of acquiring a work which will remain a standard reference for a long time.

F. W. SHOTTON

STATISTICS AND ECONOMICS

Contributions to Statistics

Edited by C. R. Rao. Pp. 528. (New York and London: Pergamon Press; Calcutta: Statistical Publishing Society, 1965.) 120*s*. net.

Essays on Econometrics and Planning

Edited by C. R. Rao. Pp. 354. (New York and London: Pergamon Press; Calcutta: Statistical Publishing Society, 1965.) 100*s*. net.

CONTRIBUTIONS to Statistics and *Essays on Econometrics and Planning* have been produced in honour of the distinguished Indian statistician, Prof. P. C. Mahalanobis, for his seventieth birthday. There are thirty-three papers in the volume on statistics and twenty in that on economics. The contributors consist mainly of people who have worked with Mahalanobis at some time, or who have worked on some of the methods and problems, both theoretical and practical, that Mahalanobis has developed. Some of the papers have been written specially for these volumes, while others are based on papers read at meetings, or have been published elsewhere. The topics cover a wide range, and the standard varies from original research by Kolmogorov to vague generalities by relatively unknown writers.

Contributions to Statistics contains eight papers on experimental design and related topics. These include a paper by Bose on combinatorial properties of partially balanced incomplete block designs, and there are two other papers on this topic; there is an interesting exposition by Finney on screening processes, also a paper on asymptotic regression by workers in Geneva. The eight papers on probability and estimation problems include a paper by Kolmogorov on limit distributions, and one by Rao in which he discusses a new criterion for estimation from large samples. There are also included papers by Linnik and Kallianpur, and one by Tintner on Mahalanobis's generalized distance.

The field to which Mahalanobis has contributed so notably is, of course, in sampling, and this is reflected in the eleven papers on this topic. In a comprehensive paper on the work of Mahalanobis in this field, M. N. Murthy of the Indian Statistical Institute discusses in detail the methods of sampling and reduction and control of error that Mahalanobis devised. The other papers include one by Dalenius on lattice sampling using Lahiri's method, and one by Deming on interpenetrating networks of samples. There are several other papers exemplifying and developing techniques originally conceived by Mahalanobis. There are two papers on sequential quality control and two on fractile analysis, a graphical method for comparing groups developed by Mahalanobis as recently as 1958.

Essays on Econometrics and Planning also contains a varied assortment of papers. It includes a paper by Frisch on a parametric solution of the Hicksian model, and a paper by Stone on his models for demand projections; there is also an interesting paper by Myrdal on underemployment. Georgescu-Roegen discusses the problems of measurement and scale, and there is a note by Wold on the problem of interpretation in multivariate models. Balogh analyses the problem of "Planning in Britain" in relation to the Conservative Government since the Second World War, and Goldsmith has a paper on the "National Balance Sheet of the Soviet Union".

There are six papers in which the particular problems of India are discussed. These include valuable statistical-economic papers by Nair Patel and Sastry, and one by M. Mukherjee, who discusses in detail the particular contributions of Mahalanobis in a paper entitled "Scientific Approach in Planning". The volume also includes several papers of a very general nature and of rather variable quality on philosophy and the purpose of economics and planning.

Both volumes are prefaced by a foreword by C. D. Deshmukh, a former president of the Indian Statistical Institute, and concluded with a note by the editors on the "Scientific Contributions of Mahalanobis", and a bibliography. We have here an impressive outline of the work of Mahalanobis, from some of his early work on meteorology and anthropometry to the founding of the Indian Statistical Institute in 1931, and the journal *Sankhyā* in 1933, his extensive work on the sampling of crops commencing in 1937, and the development of survey techniques, an important part of which was the institution of the National Sample Survey to provide periodic estimates of social and economic factors affecting the economy of India.

M. A. WALKER

Studies on the Piriform Lobe

By F. Valverde. Pp. vii+131. (Cambridge, Mass.: Harvard University Press; London: Oxford University Press, 1965.) 60*s*. net.

WITHIN the term 'piriform lobe', Dr. Valverde includes the olfactory bulb, parolfactory structures, piriform cortex and amygdala, together with their projection systems. The first part of this work consists of several experiments with lesions in various parts of the forebrain and the resulting degeneration stained by the Nauta method. The second part comprises Golgi studies of the relevant areas.

Attempting, as it does, to provide comprehensive coverage of the whole system of central olfactory connexions, Dr. Valverde's monograph achieves considerable unity of purpose, although there are inevitably many places where important topics are only superficially treated. No one familiar with neuroanatomical techniques can fail to recognize the enormous amount of painstaking observation which has gone into both the fibre degeneration experiments and also the Golgi studies, and it is notable that there are few workers in this field who have attempted a combined investigation using these two methods.

In the first part of this work, dealing with the degeneration studies, it must be admitted that a certain amount of coherence is lacking. It is not at all clear why the experiments are presented in the order in which they appear, and the text is made difficult to follow by the somewhat unnecessary fragmentation of the amygdaloid projection, and also by the detailed account of degeneration in unrelated systems.

The second part, comprising the Golgi material, is far more coherent, and the central olfactory connexions are followed serially from the olfactory bulb through the olfactory tubercle and piriform cortex to the amygdala and medial forebrain bundle. The very lucid descriptions are complemented by an outstandingly clear set of illustrations, covering every point mentioned in the text —a comment which applies equally to the first part of the book.

On the whole, *Studies on the Piriform Lobe* clearly summarizes present knowledge on this area, while adding further information at several points. It should be welcome at a time when the attention of research workers in many different fields is being directed once again to the phylogenetically older parts of the forebrain.

G. RAISMAN

Hypnotic Susceptibility

By Ernest R. Hilgard. Pp. xiii+434. (New York: Harcourt, Brace and World, Inc., 1965.) 9.50 dollars.

A LARGE number of studies designed to investigate various hypnotic phenomena have been carried out by Ernest Hilgard and his co-workers on a considerable number of college students during the past eight years. Individual differences in 'hypnotizability' have been a major area of interest and in the course of their investigations several scales were developed for the quantitative assessment of hypnotic susceptibility. This book is the outcome of these investigations, and is primarily concerned with a description of the various scales, together with a discussion of the data obtained from their use.

There are three general purpose scales and a scale for yielding profiles of hypnotic ability. Convincing statistical evidence is given concerning their validity and reliability. The items used in the construction of these scales and the resulting data provide the basis for an excellent and comprehensive discussion of many of the major hypnotic phenomena. Indeed, the whole subject of hypnosis is most carefully examined.

The latter part of the book is concerned with the relation of hypnotic susceptibility to a number of personality variables such as age, sex, intelligence, psychiatric diagnoses, attitudes to hypnosis and responses to various personality inventories. Although some significant correlations do emerge, they are insufficient to characterize the hypnotizable person clearly. One of the final chapters is written by the wife of the author, who makes some very interesting inferences from clinical case studies.

The presentation of these susceptibility scales is the central point of the book. However, it also provides a comprehensive and up-to-date review of present-day knowledge on this ill-understood subject. It is a valuable book not only to those concerned with the more academic aspects of psychology, but also to those who make use of hypnosis in the clinical field. A considerable amount of space is given to statistical procedures, which does tend to make it heavy reading for those not specifically interested in this aspect, but a brief and succinct summary is given at the end of each chapter.

A. L. PARKER

Readings in Animal Behaviour

Edited by Prof. Thomas E. McGill. Pp. x+592. (New York and London: Holt, Rinehart and Winston, 1965.) 96*s*.

THIS book is intended as a survey of research in the broad field of animal behaviour, presented in the form of fifty-five representative research papers and reviews, of which all but three were published within the past ten years. It is intended for advanced students of zoology and postgraduates. The contributions are fairly equally balanced between behavioural genetics; the neural and hormonal control of behaviour; the development of behaviour and imprinting; sensory processes, communication and orientation; learning and motivation; and studies of social behaviour, ethology and evolution. Thus there are some seven or eight articles covering each of these areas of research. Very sensibly, less than a fifth of these papers is theoretical. The student to whom the book is addressed, then, receives a handsome collection of original sources which have been chosen to illustrate not only a number of different approaches to the analysis of animal behaviour, but also several strategies in the dissemination of the results of behavioural research.

Probably this approach is of more value in the United States, where the pressures on library facilities for students are greater than they are in Britain, but the editor is to be congratulated on his happy choice of readings. Reviews by F. A. Beach, R. A. Hinde, W. H. Thorpe, N. Tinbergen and others accompany texts by V. G. Dethier, K. von Frisch, H. F. Harlow, H. Lissman, D. S. Lehrman and T. C. Schneirla, to mention only a few of the selected authors. Although the research which is represented is of varying fundamental importance, almost all of it is of high quality; most comparative psychologists will find it pleasant to have this collection on their shelves.

A. D. BLEST

Polychaeta of the Far Eastern Seas of the U.S.S.R.

By P. V. Ushakov. (Keys to the Fauna of the U.S.S.R., No. 56.) Translated from the Russian by Jean Salkind. Edited by Rae Finesilver. Pp. xxvi+419. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1965.) 121*s*. 6*d*.

THIS is an exact translation of Prof. Ushakov's important book as it appeared in 1955. Most of the text consists of keys and brief diagnoses of the forms, but there are introductory chapters on the biology of the polychaeta and the zoogeography of the Far Eastern forms. Users of the translation would be wise to bear in mind the warning given by Prof. Ushakov in his preface, that his work is an interim report on a rapidly developing field: "All the families inhabiting U.S.S.R. seas should be largely revised in the near future". The latest reference in the extensive bibliography is dated 1953, and valuable studies of Far Eastern polychaeta, including taxonomic revisions with zoogeographical implications, have appeared since then.

G. P. WELLS

TECHNOLOGICAL MANPOWER AND POSTGRADUATE STUDY

By PROF. JAMES GREIG

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FEW subjects which have received as much intensive and sustained study in recent years as has that of scientific and technological manpower in Great Britain can have proved themselves to be more tantalizingly elusive.

Educational aims, professional requirements, recruitment, utilization and national needs are factors which, together with their interrelations, have been investigated and assessed. Many valuable statistical data have been collected and analysed and forecasts have been prepared, but it is evident that inherent uncertainties and ambiguities have rendered much of the work indefinite and inconclusive.

The Committee on Manpower Resources for Science and Technology which is charged with the duty of advising the Government on the matter has recently submitted a report in which the problems to be investigated are restated, the lines of action being followed are described and certain recommendations for immediate action are made*. The scope of the Committee's investigation is described as covering such matters as statistical sources; factors affecting the choice of a scientific or technological career; preparation for employment in the context of a rapidly changing technology; the better utilization of scientific and technological manpower; and the relevance of technical supporting staff.

On the statistical side, a survey is in progress utilizing the data of the Census of Scientific and Technological Qualifications of 1961 which, in due course, will be supplemented by a similar census in the present year. From these data, together with the triennial surveys of the Ministry of Labour which have been carried out since 1956, a general assessment will be made of the supply-and-demand position at the present time and also in relation to employers' forecasts for 1968. Statistics from the University Grants Committee, the education departments and the professional institutions will provide information about the inflow of educationally qualified personnel. The requirements for particular disciplines and technologies will be estimated, and it is pointed out that such estimates must be related not only to employers' forecasts but also to rates of economic expansion suggested by national policy.

The factors influencing the choice of subjects of study at school or in higher education are clearly matters of direct concern to the Committee. In the field, investigations are being made by the Schools Council in relation to England and Wales, and the Council for Scientific Policy has initiated a study of the flow of candidates from schools into scientific and technological courses in higher education.

The fact that so very few schoolmasters had the first-hand knowledge of applied science or technology that would enable them to present these subjects in a stimulating and inspiring way was a very severe handicap and one which means must be devised to overcome.

The relationship of the educational system to the requirements of employers is being investigated from two points of view. Starting with consideration of the problem of stating the requirements of industry in terms relevant to education, it is proposed to enquire into whether, and at what stage, there should be a change of emphasis in university or college of technology courses to match industrial needs more closely. An examination is also being made of the extent to which individuals from different academic disciplines are in practice interchangeable. The report commends the policy of retention by the colleges of advanced technology, as they become universities, of sandwich courses in which periods of industrial training are interleaved with academic studies.

Postgraduate courses are considered to have an important part to play in enabling science graduates to become technologists and in educating graduate technologists in specialist subjects. Appreciative reference is made to the encouragement given to the development of suitable courses by the Awards Committee of the Science Research Council by its allocation of awards to technology.

The findings of the Arthur Report, which is entitled "Enquiry into Longer Term Postgraduate Courses for Engineers and Technologists"†, have been considered by the University Grants Committee, by the Postgraduate Awards Committee of the Science Research Council and by professional institutions concerned with technology, and from this consideration it is clear that industry must formulate its needs for postgraduate courses more clearly if educational institutions are to meet them more satisfactorily.

The scope of the enquiry with which the Arthur Report deals has been limited to full-time courses of at least one month's duration. Basic information as to the number and type of courses being run in the session 1964–65 and in the previous session was obtained from a questionnaire sent to vice-chancellors, principals and heads of technological departments. The questionnaire was comprehensive in that it comprised the nature and duration of courses, the subject coverage, the qualification awarded and staffing, together with personal data on students and observations as to industrial support. Questionnaires were sent to 161 university departments, ten colleges of advanced technology and to thirty other colleges. A full complement of returns was obtained and, from the analysis of these, factual information was derived which is summarized in one chapter of the Report. In the session 1964–65 there were 264 separate postgraduate courses run in 120 university or college departments. Three of the courses were concerned predominantly with management, twenty-seven were conversion courses, while 234 represented advanced studies. The total number of students was 1,966 giving an average of some seven students in each course. Of these, 1,642 were pursuing courses of at least a full session duration and the remaining 324 were following shorter courses —just less than 45 per cent were from overseas.

In the same session there were 2,723 postgraduates engaged in research in some branch of technology. Analysis by type of course showed the larger numbers of students to be as follows:

Mechanical engineering	389	Aeronautical engineering	178
Civil engineering	345	Chemical engineering	166
Electrical engineering	306	Production engineering	143

A table setting out the sources of financial assistance to United Kingdom students showed that of 897 who had supplied information, 274 had awards from the Department of Scientific and Industrial Research, 207 were

* Committee on Manpower Resources for Science and Technology. A Review of the Scope and Problems of Scientific and Technological Manpower Policy. Pp. ii+17. (Cmnd. 2800.) (London: H.M.S O., 1965) 1s. 6d. net.

† *Enquiry into Longer Term Postgraduate Courses for Engineers and Technologists, 1964–65.* By H. Arthur, Department of Education and Science. (London, H.M.S.O.) 4s. 6d. net.

supported by their employers, 163 had scholarships and 121 had local education authority awards.

Two short sections of the Report describe in outline the arrangements for discussions with industrial representatives and with academic staff, and these are followed by a fairly detailed presentation, appropriately sub-divided, of views emerging from the discussions. The author emphasizes that it has been his aim to assemble the wide range of views with some sort of coherent and logical pattern and to present them objectively.

It is clear that relatively few students on the type of postgraduate course under review are sponsored by industry and this may be taken as an indication that industry does not regard existing courses as meeting a real or urgent need.

For industrial staff engaged on production work, management is of primary concern and there is increasing recognition of the need for such a staff to have further education and training in efficiency techniques and general management. Much management training is carried on internally by the larger industrial organizations and this is often supplemented by short courses in universities and colleges. Long postgraduate courses in management, pursued immediately after graduation, are not favoured by industry, as it is believed that such studies can be pursued more effectively after some industrial experience, while at a later stage there are all the problems of releasing men of some seniority.

For staff engaged in research, development and design it might be expected that postgraduate studies dealing with advanced fundamental or specialist topics would be recognized as meeting a real need. The numbers sponsored by industry in such courses are, however, still small.

On the research side the facilities and the fund of expert knowledge in the laboratories of the large organizations are great and sustain the claim that the new graduate can acquire all the specialist knowledge which he requires more effectively within the organization than by attending a postgraduate course in an academic institution. From the industrial side the criticism is made that many postgraduate courses merely constitute extensions of the first-degree course and have little relevance to the needs of industry. Some university engineering departments conceive their main function as the pursuit of fundamental knowledge, and this, too, may be regarded as rather remote from the immediate requirements of industry. Although there are some notable exceptions, industry has not shown any great eagerness to recruit from the output of postgraduate courses. From the academic side it is suggested that industry is just not sufficiently forward looking. The incompatibility of those views and the resulting situation suggest that there is, both in the universities and in industry, a regrettable lack of appreciation and of understanding of the partners' interests and problems.

Although the support for courses of 9 months' duration or longer has been meagre, the demand for shorter, more specific courses has been considerable, and this, in some instances, has led to the organization of a full session's course into a series of short self-contained units for which single-units students could be accepted. The short course may well find increasing application in the form of the periodic refresher which the increasing rate of technological advance will inevitably demand.

With regard to the financing of students on postgraduate courses, it is pointed out that the trend in certain of the newer universities towards a more general type of engineering first-degree course might well accentuate the need for postgraduate studies, and if expansion should occur on a major scale as an integral part of engineering education, there could be little justification for expecting the burden to be borne by industry. The conditions of award for Science Research Council grants and the scale of the awards attracted some criticism from the university side.

Interchange of staff by secondment between industrial organizations and university departments, although accepted as desirable, was practised on only a very small scale.

It was evident that the means for co-ordinating industrial needs and for disseminating information about the availability of courses were inadequate and the provision of some form of clearing-house had been suggested.

One of the most interesting incidental observations in the first of these two documents relates to technical supporting staff. Recognizing the danger of serious neglect of educational development for technicians, the Committee on Manpower Resources welcomes the inclusion in the Manpower Survey of 1965 of questions which will yield information for the United Kingdom as a whole about the utilization and the demand for technicians. The comment is made that when a picture of the national need for technicians has been formed it may be possible for the first time to ascertain how far the present provision for their education and training is sufficient.

The results of this enquiry will be valuable in themselves, but it is important that they should be linked with the more fundamental investigation which is described as the "identification of motivations, character and abilities relevant to science and technology and the factors which govern the choice by individuals of a scientific or technological career". Insufficient attention has been directed in the past to the qualities which go to the making of the first-class technician and to the cultivation of his skills within the educational system. His industrial training, like his function, has been ill defined and there is here an area in which the activities of the Industrial Training Boards could be of especial benefit.

The factual information furnished in the Arthur Report reveals a situation which commands attention. Nearly one hundred courses out of a total of 264 with four or fewer students is a statistical fact calculated to invite the condemnation of the educational administrator. From the discussions there appears to emerge a state of misunderstanding almost amounting to mistrust between industry and the universities, a state of affairs quite unrecognized by many familiar with both sides of the partnership.

Some lack of mutual understanding there certainly is, but it is not of a major character. The Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom convened in December 1965 a joint conference with the Confederation of British Industry under the title "Industry and the Universities—Aspects of Interdependence". In the course of the conference, reference was made to a proposal to try to establish some permanent lines of communication between the Confederation and the universities, and at the conclusion of the meeting the chairman stated that the matter would be further explored. Such a development could facilitate in a most effective way the interchange of ideas and, equally importantly, their interpretation.

When, as at present, there is a persistent shortage of qualified scientific personnel, it is scarcely to be expected that industry will willingly release men for postgraduate studies which are in the nature of a long-term investment. The short *ad hoc* course which is of more immediate application does receive support, but the statement in the Report that "many postgraduate courses offered by academic institutions are criticized by industry as being merely extensions of the first degree course . . ." does demonstrate failure to appreciate the real issues.

The man whose mental calibre and personal qualities give promise of research ability requires, after completing his first degree in engineering, an introduction to modern powerful mathematical techniques and he must acquire a knowledge of the physics of the solid state, of thermodynamics and hydrodynamics, sufficiently advanced and fundamental to enable him to co-operate effectively with mathematicians and physicists. Such rigorous

fundamental knowledge can best be acquired when absorptive powers are at their peak and when mathematical manipulative skill is freshly developed and readily exercised—that is to say, immediately after graduation. A course of postgraduate study dealing with such topics can provide the young research engineer with the most powerful tools of his trade and with the skill to use them.

The cogent observation is made in the Arthur Report that "There is a strong feeling that postgraduate courses seeking to give information and experience of a really advanced nature need to draw on an active and successful research programme. This indeed is the context within which many postgraduate courses are offered by university departments. . . ."

It is precisely for this reason that the maintenance of postgraduate courses of an advanced fundamental character should be encouraged, notwithstanding smallness of numbers in individual schools. Such courses give research specialists the opportunity to engage in advanced teaching and, indirectly, expose the graduate student to the stimulus and the challenge of research. They also often serve as a preliminary requirement to entry on a programme of research leading to the Ph.D. degree.

Courses of a specialist technological character or concerned with advanced design methods fall into an entirely different category. Advanced mathematical techniques will, of course, be involved, together with physical phenomena and physical theories, sometimes of a highly specialized character, but these will always be developed directly in relation to industrial application. It is the introduction of the industrial factors that renders these studies more readily assimilable by students who have had some industrial experience.

The advanced fundamental type of course does not, as a rule, require the appointment of specialist staff or involve the provision of particularly expensive equipment. The specialist technological course may, however, be expensive on both those counts.

Courses which are either introductory to research or which contribute to the more effective pursuit of research, advanced development or design, lie clearly within the area of the Science Research Council's responsibilities, and the furtherance of their development necessarily implies the exercise of some directive influence and control.

The development of postgraduate study was greatly fostered and encouraged by the recognition by the Department of Scientific and Industrial Research from 1957 onwards of many such courses for purposes of postgraduate awards.

For some time prior to the publication of the Arthur Report, the Council has been conducting a review of the courses of postgraduate study to which approval has been given and, following the review, the Council withdrew approval from a number of courses for which the numerical support had been very small. In the interests of the efficient and economic utilization of resources this action, aimed at achieving some measure of rationalization, was fully justified. There does, however, appear to have been some lack of recognition of the important differences between the claims of courses of a fundamental character and those of specialist technological type. The unrestricted multiplication of closely similar courses of specialist technological type, with only small numbers of students, is clearly indefensible on economic grounds. The same considerations do not apply to the fundamental type of course, and any policy of rationalization which fails to take account of the long-term importance of such courses may well prove to be false economy.

CONTROL OF WEATHER AND CLIMATE

By PROF. R. C. SUTCLIFFE

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IN the United States, over the past few years, great expansion has repeatedly been urged in research in atmospheric science, and especially in that part of the science directly relevant to weather and climate. There is a parallel interest also in the U.S.S.R., a point which is not overlooked in American advocacy and, through various organizations, there has been notable initiative in international circles. By its Resolution 1802 (1962), the General Assembly of the United Nations called for the development of world programmes. The World Meteorological Organization as a special agency of the United Nations was called on to look to its parish and bring meteorological services up to date; it has responded by the adoption of the concept of the World Weather Watch, an ambitious and costly scheme of continuous global surveillance of the atmosphere. At the same time the International Council of Scientific Unions, as the non-governmental body representing academic interests, was invited to develop a complementary research programme, which is now taking shape through the rather slow but generally irresistible machinery of international committees. A further pressure comes from the United Nations Economic and Social Council through its Advisory Committee on the Application of Science and Technology to Development. In its Second Report published a year ago (May 1965), "Improved Weather Forecasting" and "Weather Modification and Control" are included in a list of a "limited number of especially important problems of research or application".

In Great Britain, not much has yet been heard outside committees and corridors of a national response to these weighty international developments, but this must come before long as the British position in international programmes becomes defined. It is therefore against this background of world interest in meteorology as a whole that we may examine a valuable report recently submitted by the Committee on Atmospheric Sciences to the United States National Academy of Sciences on the special subject of weather and climate modification*. In Britain the subject has barely crossed the threshold of scepticism to be fathered by respectable scientists. Neither have commercial rainmakers succeeded in selling their services to British interests, private or public, on any significant scale. But the tremendous potential of a capability to control the weather and climate has impressed itself so forcibly in the United States, and in the U.S.S.R., as to make the subject a major plank in the national scientific platform. By contrast, a new brochure on *Research in the Meteorological Office* makes no mention of the subject except as a "distant speculative prospect". This may well be a correct description, but the time is ripe for a national reappraisal.

The United States report is presented in two volumes, the first being a summary with recommendations sufficiently brief to be worth the attention of anyone interested

* *Weather and Climate Modification: Problems and Prospects.* 2 volumes. Pp 28 and 198. (National Academy of Sciences, Washington, D.C., 1966) 5 dollars.

in American thinking on world science. The second volume, a review of research and development, provides within its compass of nearly 200 pages quite the most penetrating and most up-to-date appreciation of many of the central problems of weather and climate that has appeared for some time. Every meteorologist will find profit in reading it, even if the control of the atmosphere presents no appeal.

It is not easy further to summarize this survey, for which Volume 1 is itself already the summary of a summary, but a few salient points may be quoted. First, as a measure of the seriousness of purpose, there are the dollars. In 1965 the support from Federal funds for "Weather Modification Programmes" was almost 5 million dollars out of a total for meteorological research of 84 million dollars, all within a total budget for meteorological services and research of 430 million dollars. Comparable figures for the U.S.S.R. are estimated in the report at 20, 70–80 and 700–800 million dollars, respectively: "The Soviets have assigned a higher priority for weather modification than we [the U.S.A.] have". (In Great Britain the overall effort per head of the population is lower by a factor of ten, and that directed specifically to weather modification is not identifiable.) The formal recommendation on this matter reads: "We recommend that immediate steps be taken by agencies to raise the support [for weather modification] from the 1965 level of $5 million to at least $30 million by 1970". This is large-scale research by any standards.

Among the scientific and technical findings, by far the most significant of the positive conclusions refers to the now much-tried, if not always well-tried, seeding by silver iodide. This stems directly from the pioneer experiments of Schaefer in 1946 when real supercooled clouds were first visibly modified. Since that time there has been much careful research and much more inadequately documented activity, but hitherto, of the many independent national and international bodies to study the evidence, probably none has been more than encouragingly non-committal. Now a definite step—one hopes it is a step forward—has been taken with the words: "We find some evidence for precipitation increases of as much as 10 or even 20 per cent over areas as large as 1,000 square miles over periods ranging from weeks to years". A large number of commercial operations are evaluated, and even these, for all their incompleteness as scientific evidence, are given a guarded but definitely favourable report. The qualifying remarks and critical comments do, however, still give the impression that no decisive scientific step forward has in fact been taken, that the evidence is still confusing and unreliable, and that the favourable verdict was perhaps a little swayed by the feeling that after 20 years an expert body must do something more than sit on the fence.

Setting aside the risk that their scientific opinion may be misquoted to mislead the public into useless expenditure, one feels, however, that their verdict is fair. Uncertain and equivocal as much of the evidence may be, there are far too many positive indications to permit the matter to be dropped if the outcome is as important as is believed. Thus the formal recommendations call for "several carefully designed, randomized seeding experiments". "We recommend that attention be given immediately to careful monitoring and regulation of operational programmes for weather modification."

The report, of course, ranges far wider than the area of cloud seeding for increasing local rain. "Experiments in lightning suppression are beginning to show some promising results." "With respect to both hurricane and tornado modification, no practical success can be expected before the development of adequate theories. . . ." "The dissipation of supercooled fogs and low stratus clouds over limited areas is operationally practicable."

On the modification of world climate, we have the welcome view that "As long as our understanding is thus limited, to embark on a vast experiment in the atmosphere would amount to gross irresponsibility". (This philosophy did not, however, prevent the testing of nuclear devices.) Much weight is given to the promise of theoretical mathematical models for testing the effects of modifications with the conclusion that "In 10 years our computer requirements may grow by two or three orders of magnitude". "We recommend, therefore, that all necessary steps be taken to encourage the computer industry to respond to these prospective requirements."

Among other items for attention come "A comprehensive exploration of hurricane energetics", "A comprehensive investigation of hailstorms", water budgets, boundary-layer exchange processes, theoretical models of condensation and precipitation mechanisms, meteorological effects of atmosphere pollution. . . . The need for the greatest care in statistical evaluation calls for "much more direct and continuing communication between statisticians and experimenters". "The tropics have been grossly neglected." There are numerous other opinions and proposals of far-reaching importance in a report which is as nearly unbiased as any interested group could be expected to be. The National Academy's Panel on Weather and Climate Modification has worked closely with a corresponding official body, the Commission on Weather Modification of the National Science Foundation, which has issued almost simultaneously its own special report†, in addition to the seventh annual report on *Weather Modification* from the Foundation to the President of the United States‡. All these are substantial publications, overlapping in many ways but supporting each other in the general thesis that weather control is a field of study calling for the most serious attention. To quote from the seventh annual report: "In 1965 the key words are no longer 'whether' and 'when': they are 'what', 'how' and 'who' ".

The report of the Special Commission of the National Science Foundation has sections and recommendations on the "Biological Aspects", "Statistical Aspects", "The Human Effects", "Legal and Legislative Effects", as well as "International Relations and Finance", raising questions of public policy of much novelty and interest, going far beyond the scope of this brief review.

Already there have been political repercussions, and in February this year two Bills were introduced in the U.S. Senate calling for the development of large programmes in weather modification. In one of these an expenditure of 155 million dollars in three years is contemplated. There is therefore every reason to be confident that research and experiment on something like the grand scale envisaged will in fact go forward in the United States, and doubtless also in the U.S.S.R.

How is it, one may ask, that these countries can justify an investment of effort towards a defined practical goal on a scale which makes that in many other leading countries seem trivial? There is one consideration which, although certainly not the only one or even the major one, is sufficient. Knowledge on how to improve the climate of one's own country can scarcely fail to imply knowledge on how to impair that elsewhere, and there could be no more potent weapon. Obviously, as the world stands at present, the two major powers cannot afford not to keep abreast of knowledge of this kind. For other countries there will be the usual hard choice of priorities, but the fact that weather control is not at the moment of pressing urgency does not mean that it can safely be ignored.

† *Weather and Climate Modification:* Report of the Special Commission on Weather Modification. Pp. 149. (National Science Foundation Publication 66-3, 1966.)

‡ *Weather Modification:* Seventh Annual Report for Fiscal Year ended June 30, 1965. Pp. 109. (U.S. Government Printing Office, Washington, D.C., 1965.) 40 cents.

RANDOM SCANNING OF TAXONOMIC CHARACTERS

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ONE of the principal impediments to the development of numerical taxonomy[1] is the difficulty biologists have of measuring and recording taxonomic characters at speeds and in quantities commensurate with the ability of modern computers to process these data[2]. A simple possible solution would be to record agreement in visible structures over randomly selected minute areas of the images of pairs of organisms. Such a procedure would be feasible by means of optical scanners applied to photographs, films, microscope slides, or drawings. The following preliminary investigation evaluates the feasibility of such an approach.

Random masks, made from twenty-five IBM cards each perforated with twenty-five randomly chosen holes, were placed over black-and-white drawings of two groups of 'organisms'. One group consisted of twenty-nine 'species' of Caminalcules (Fig. 1), imaginary animals generated by J. H. Camin to study taxonomic and phylogenetic principles[3,4]. The second group comprised published illustrations of pupae of thirty-two species of culicine mosquitoes[5]. Labelling was deleted from these drawings (Fig. 2). The illustrations were enlarged by a third and mounted with morphological reference points aligned in a standard position. Each illustration was overlaid with all masks and each of the 625 holes was scored '1' when a black line appeared through it and '0' if no black showed. Illustrations were compared on the basis of matching scores for corresponding masks and holes. Matrices of distance coefficients were computed and clustered[1].

The resulting phenogram (dendrogram of phenetic relationships[6]) of the Caminalcules is shown in Fig. 3. The 'genera' previously established by orthodox taxonomists and by conventional numerical taxonomy[4] were again classified correctly. An exception is the separation of species 21 from genus *B* which is peculiar to the distance

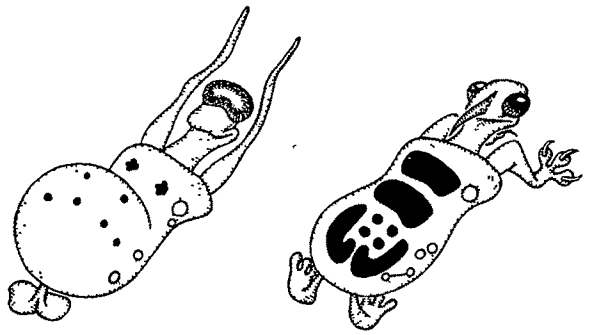


Fig. 1 'Species' 1 and 12 of the Caminalcules, an imaginary group of animals. All twenty-nine 'Recent' species are illustrated elsewhere (ref. 4)

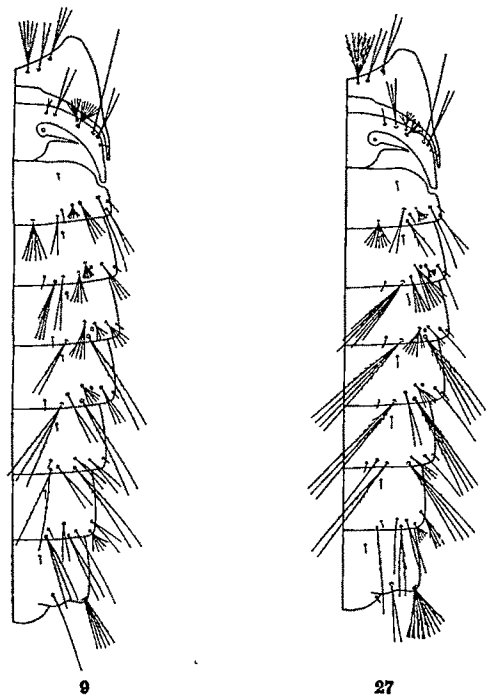


Fig. 2. Pupae of mosquito species 9 [*Aedes* (*Ochlerotatus*) *excrucians*] and 27 [*Culiseta* (*Culiseta*) *inornata*] as modified from Darsie (ref. 5) for random scanning

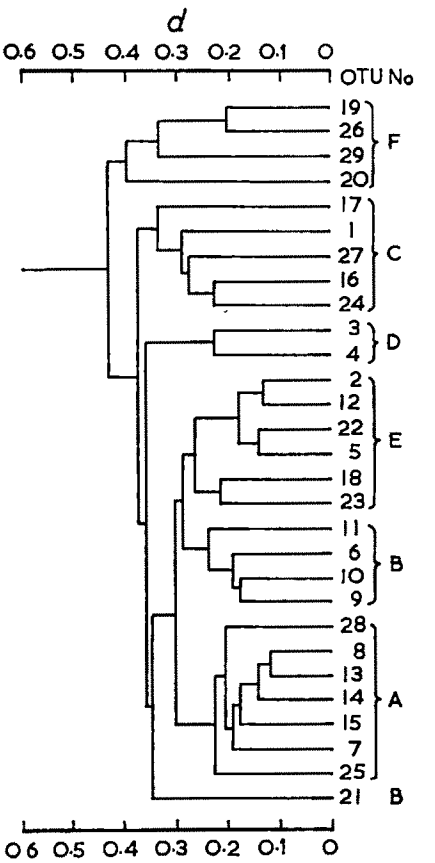


Fig. 3. Phenogram of the Caminalcules computed from a distance matrix based on the random scanning method. Clustering by the unweighted pair-group method using arithmetic averages (ref. 1). The similarity scale is shown as the abscissa. Numbers at the tips of the phenogram correspond to 'species' code numbers. Capital letters to the right of brackets indicate 'generic' groups recognized by teams of orthodox taxonomists and by a conventional numerical taxonomic investigation (ref. 4)

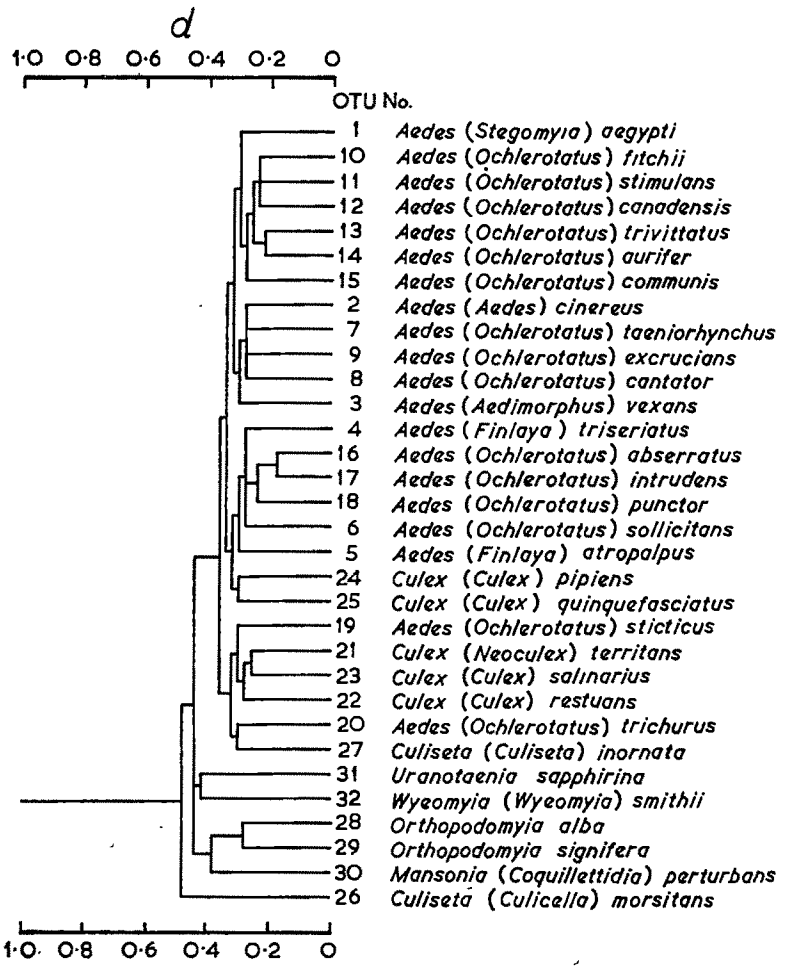


Fig. 4. Phenogram of the images of the pupae of thirty-two species of culicine mosquitoes (ref. 5). This phenogram has been computed from a distance matrix based on the random scanning method. Species code numbers and names are given at the tips of the phenogram. Clustering method and explanatory comments as for Fig. 3

function and is not a consequence of the random scanning[4]. Genus *D* is, however, separated from genus *E* with which previous orthodox and numerical classifications had placed it. Closer study revealed that group *D* had 'evolved' an elongation in which the body grew anteriorly, obliterating the neck. Since the drawings were positioned by the 'shoulders' for reference, species in groups *E* and *D* did not expose 'homologous' structures to the mask. Programmed transformation of the images, stretching some parts and compressing others to increase overall similarity, might have overcome this problem.

In the mosquito study the genera emerged quite satisfactorily, except that species 24 and 25 (*Culex*) are placed with *Aedes*, while species 19 and 20 (*Aedes*) are placed with *Culex*. A study by conventional numerical taxonomy[4] showed better assortment of the genera *Aedes* and *Culex*, but split up the genus *Orthopodomyia* which was retained by the random scanning method. Species 31 (*Uranotaenia*) was more distant in the conventional numerical study than by random scanning. On the whole, the relations between the classifications arrived at by random scanning and conventional numerical taxonomy are as good as can be expected by separate numerical taxonomic investigations[7].

The random scanning method clearly yields meaningful classifications. Whenever phenetic taxonomies are acceptable[6], the method described here may provide a rapid and reliable approach. Problems of size and orientation of organisms remain to be worked out, but should not present insuperable technical difficulties. The implications of the success of the method are that experience and insight into the presumed biological and phylogenetic significance of characters may be less important for obtaining satisfactory classifications than had been generally supposed.

This investigation was supported by a grant and also in part by a U.S. Public Health Service research career programme award, both from the National Institute of General Medical Sciences, to R. R. S. The computations were carried out on an IBM 7040 digital computer at the University of Kansas Computation Center using the *NTSYS* programmes developed by F. J. R. We thank Prof. C. D. Michener and Prof. J. H. Camin for their advice, and William G. Hanis for technical assistance.

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[7] Rohlf, F. J., *Taxon*, **14**, 262 (1965).

A REVIEW OF THE OLDOWAN CULTURE FROM OLDUVAI GORGE, TANZANIA

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THE discovery of a primitive stone industry in Bed I at Olduvai Gorge was first recorded in *Nature* on December 26, 1931. The name Oldowan was later given to this culture, which had been shown to precede the so-called 'Chellean'[1]. Various authors have since used the terms 'Pebble Culture' or '*Civilisation à galets aménagés*' for analogous industries. But these names are misleading, for, as my husband pointed out when describing Oldowan choppers, "specimens occur which have been made from nodules of chert and others from rough irregular lumps of quartz and quartzite, and there seems little doubt that sites containing this culture might be found when tools made from water-worn pebbles are entirely absent"[2]. The aforementioned terms are primarily concerned with the nature of the materials often used to make choppers, rather than stressing the dominant tool types, and their use has led to misconceptions regarding the Oldowan cultural assemblage, taken as a whole.

During the recent Wenner-Gren symposium on "The Systematic Investigation of the African later Tertiary and Quaternary", held at Burg Wartenstein, the question of alternative terminology for the Oldowan was debated

at some length. The term was considered by some to be inapplicable to areas outside East Africa, particularly to North Africa, where the tools so far recorded consist only of choppers, and where a number of Oldowan cultural elements such as small flake tools appear to be absent. It should be borne in mind, however, that although the existence of small tools in the Oldowan was tentatively recorded during the pioneer work at Olduvai, choppers were then considered to be the essential characteristic of the industry. This conception persisted until systematic exploration of sealed living sites was undertaken. When comparable sites are found and excavated in other areas, it is likely that additional tool forms will be found, similar to those of the Oldowan from Olduvai Gorge.

In the description of the Oldowan published in 1951[3], it was stated that a total of seventy-three tools were obtained *in situ* from four different levels in Bed I, one of which is described as a boulder bed. This level is now known to be within Bed II and to contain an industry rather more evolved than the typical Oldowan. It was also noted that small rough end-scrapers and utilized flakes probably formed part of the Oldowan assemblage. This has been fully confirmed and it has also become evident that there is a considerable diversity of tool types and close conformity within certain groups of tools, indicating that tool-making had already attained a measure of standardization, even at the period represented by the lower levels of Bed I, which can now be dated in the region of 1·75 million years with some degree of confidence[4].

In order that the cultural assemblage may be more generally understood, and because some months must still elapse before the full report on the excavations can be completed, it seems desirable to issue a preliminary report on the additional elements which have come to light as a result of detailed study of the assemblages recovered from sealed living sites in Bed I and the base of Bed II during the excavations of the past few years.

The cultural material discussed in the present article extends in time span from the earliest known occupation level in Bed I to a horizon a few feet below the aeolian tuff[5] in the lower part of Bed II. This represents a period of desiccation, and coincides with a break of some magnitude in the cultural and faunal sequence. Whereas there are no appreciable differences to be seen in the stone industry from the material at present under review, analysis of material from higher levels in middle and upper Bed II, now in progress, indicates that a change in cultural tradition took place at a time corresponding to the deposition of the aeolian tuff. Similarly, study of the faunal remains has revealed that a number of mammalian genera commonly found in Bed I do not occur in levels above the aeolian tuff, while other species and genera appear in these deposits for the first time[6].

The present re-evaluation of the cultural material is based on assemblages obtained from four excavated sites, which occur in the following stratigraphic sequence:

HWK East, level 1	Base of Bed II
FLK North, levels 1–6	Top of Bed I
FLK I, level 22 (*Zinjanthropus*)	20 ft. below the top of Bed I
DK I, levels 1–3	Immediately overlying the basalt (formerly regarded as the base of Bed I)

The following artefacts were obtained from these sites:

	HWK East, level 1	*FLK* North, levels 1–2	3	4	5	6	*FLK* I, '*Zinjanthropus*' level	*DK* I, levels 1–2	Total
Tools	51	134	28	19	31	6	60	155	484
Utilized material	65	196	49	27	32	22	145	205	741
Unmodified flakes, etc., including waste	32	516	94	20	92	95	2,358	889	4,096
									5,321

Each site represents an undisturbed living floor where occupational remains were found *in situ*, sealed in by subsequent deposits. The artefacts were invariably associated with a considerable number of broken mammalian bones and with natural, unmodified cobbles and other stones imported to the living sites by artificial means, but lacking evidence of utilization. These have been termed 'manuports'.

The assemblages recovered from all the sites excavated in Bed I and the base of Bed II are remarkably homogeneous. Such changes as do occur, within the period, do not seem to be of sufficient importance to justify any subdivision of the Oldowan. Variations exist, but these appear to have been caused by differences in the raw materials used, rather than by alterations in techniques or in preferred tool types. There is, however, considerable variability in the proportions of the components of the assemblages, not only at different sites and at different stratigraphic horizons, but also within different areas of a single living site.

This zoning of material is particularly evident at *FLK* I, on the occupation surface where the skull of *Australopithecus* (*Zinjanthropus*) *boisei* was found. Here, a densely concentrated mass of flakes, chips and small flake tools, together with smashed animal bones, occurred within a roughly circular area approximately 20 ft. in diameter. On the marginal areas of the site, the stone tools were generally of heavy-duty dimensions and were associated with a number of manuports, while the mammalian remains, consisting largely of non-marrow containing bones, were noticeably less broken. (There is usually a clear distinction in size and weight between tools classed as 'heavy' or 'light' duty. To ensure consistency in classification of the few borderline specimens, however, those in which the mean diameter exceeds 50 mm have been classed as heavy duty, and those in which it is 50 mm or less, as light duty.)

The characteristic elements of the Oldowan, as now re-defined, are as follows: (1) *Tools*—Choppers (side, end, bilateral, pointed, chisel-edged, polyhedral and discoidal); spheroids and sub-spheroids; proto-bifaces; scrapers, heavy and light duty; proto-burins. (2) *Utilized material*—'Anvils'; hammerstones; cobblestones; nodules and blocks; light-duty flakes, etc. (3) *Unmodified flakes and other fragments* (including waste). (4) *Unmodified cobblestones, nodules and blocks* imported to the living sites by human agency, but lacking evidence of utilization ('manuports').

Characteristics of the Industry

Tools

(*A*) *Choppers.*

Side. Usually made on cobblestones, the butt being formed by natural cortex, or else on blocks and nodules with a relatively thick butt. Flaking is usually bifacial, forming a jagged edge. Chipping and blunting from utilization are also generally present on both faces. The maximum measurement is bilateral and exceeds the length from the working edge to the butt.

End. As above, but the maximum length is from the working edge to the butt.

Bilateral. Oblong specimens with truncated, blunt ends; the lateral edges are bifacially flaked, to form two roughly parallel cutting edges.

Pointed. Usually side choppers, in which there is a well-defined medial point on the working edge, formed by the intersection of two deeply indented flake scars.

Chisel-edged. May be either side or end; the working edge is relatively narrow and is rotated so that it lies burin-wise, at right angles to the upper and lower faces.

All the aforementioned may be combined with hammerstone utilization on the butts.

Polyhedral. Angular implements with three or more cutting edges, usually intersecting. In fresh specimens, the edges project considerably, but when extensively utilized, they may be so blunt and reduced that the implement resembles a sub-spheroid. The edges are jagged and the utilization is generally bifacial. Whereas the majority of the choppers in the first five categories

are made on well-rounded cobblestones, the polyhedral variety is more often made on irregular-shaped nodules, in which advantage has been taken of natural projections in order to make a number of relatively short cutting edges.

Discoidal. A bifacially flaked jagged edge is present either on the whole or on the greater part of the circumference. These tools are generally made on parts of cobblestones, which appear to have been split in two and the halves then radially flaked. The majority retain part of the cortex surface on one face, while the opposite face is flaked over the whole surface and is markedly less convex.

(*B*) *Spheroids.* Stone balls, either faceted or pounded smooth on the exterior. The earliest recorded examples are from *FLK* North, in the top of Bed I.

(*C*) *Sub-spheroids.* Usually irregular and sub-spherical in general form. The entire surface is battered, with vestiges of ridges still visible. In some specimens the present form may have resulted either from intentional shaping or from excessive utilization of polyhedral choppers.

(*D*) *Proto-bifaces.* These are usually very crude. They are bifacially flaked along both lateral edges, in addition to the trimming at the tip. Some are high-backed with a flat undersurface and plano-convex cross-section at the working end. The butts are thick and are usually formed by the rounded surface of a cobblestone.

(*E*) *Scrapers.*

Heavy-duty. Generally made on cores with relatively steep one-directional trimming of the working edge. The lower face is flat or slightly concave and is formed by one or more negative flake scars or by a natural cleavage plane. The working edge is usually curved and relatively even, not jagged as in choppers. Utilization, when present, occurs predominantly on the convex face.

Light-duty. End and side scrapers are relatively common at certain levels. The working edges are generally rounded and are trimmed by means of fine, regular retouch, which may be either shallow or steep, depending on the thickness of the specimen. A number of hollow scrapers also occur. One or more notches may be present, generally trimmed from one direction only. They are variable in size, but none are recorded which exceed 25 mm in width or 5 mm in depth.

(*F*) *Proto-burins.* Characterized by a thick-set, relatively short working edge, at right angles to the main plane of the implement. Usually made on triangular fragments of tabular material, with the working edge at one apex. Retouch and utilization of the chisel edge is usually bifacial. These tools vary from small well-made specimens which could be termed true burins, to large crude examples with working edges up to 30 mm in width.

Utilized Material

(*A*) *'Anvils' or utilized angular blocks.* Angular, cuboid blocks or parts of cobblestones having one or more edges approximating a right angle. These edges are usually formed by natural cleavage planes or cortex surfaces, but a few specimens show a minimum of crude preparatory shaping. The particular type of utilization has caused shattering of the edges, with plunging flake scars on one or both sides. Incipient cones of percussion are sometimes evident on the flat surfaces. A small flat-based variety with a dorsal cone also occurs, particularly at the *Zinjanthropus* level at *FLK* I. The term 'anvil' is used as a convenient name for these tools, but does not necessarily imply that they were used exclusively for this purpose: some may also have been used manually.

(*B*) *Cobblestones, nodules and blocks.* Many nodules, mostly of the local vesicular basalt, together with some cobblestones and blocks, show battering and crude flaking from one or two directions, on natural sharp edges and projections. In all specimens the amount of flaking is negligible, with the greater part of the surface consisting of natural cortex. In most cases the flaking present seems to be entirely due to utilization, but there are a few specimens in which one or more flakes may have been intentionally detached in order to obtain crude, sharp edges.

(*C*) *Light-duty flakes, etc.* Flakes and other small fragments showing chipping and utilization from one or more edges, but no deliberate retouch. They may be divided into three categories: (*a*) with straight edges; (*b*) with concave or notched edges; (*c*) with convex edges. Specimens with straight or convex edges usually show chipping on both sides of the edge, while in notched specimens the chipping is generally from one direction only.

Unmodified Flakes, etc. (including Waste)

All the flakes are irregular. In a relatively high proportion of the lava specimens the cortex surface of the cobblestones from which they were struck is present on the dorsal surface. In quartz flakes there is a tendency for the striking platforms to be shattered; in other specimens the platforms are extremely variable, both in angle, form and dimensions.

It is possible to distinguish three types of flakes, as follows: (*a*) *Divergent.* Splayed outwards from the striking platform; either end or side struck. This is the most common type. (*b*) *Convergent.* Having the greatest width at the striking platform, either end or side struck. (*c*) *Approximately parallel-sided.* The platforms are generally of the same width as the body of the flake, end struck.

Resharpening flakes. These are relatively scarce and are almost certainly derived from resharpening the working edges of choppers.

Broken flakes and chips. The highest proportion of debitage from all sites consists of chips, broken flakes and other small fragments.

Core fragments. Small blocks and angular fragments apparently not derived from flakes but rather from shattering blocks of raw material.

Unmodified Stones

Manuports. Cobblestones and weathered nodules of lava, together with some blocks of quartz and other basement complex rocks, on which there is no evidence of utilization.

The salient features of the Oldowan may be summarized as follows:

The majority of choppers and other heavy-duty tools are made from lava, while the light-duty tools, such as small scrapers, are generally of quartz. At certain sites, particularly at *FLK* and *FLK* North, lava flakes are scarce, and are far fewer than the number that would have occurred had the lava tools found at these sites been made on the spot. In contrast, quartz flakes and other fragments are usually greatly in excess of the number which could have been struck from the associated quartz tools. It seems probable, therefore, that many of the quartz flakes were made expressly to be used as light-duty cutting tools, and that even those that retain undamaged edges may have been used for this purpose.

This interpretation is supported by evidence from an isolated group of artefacts found scattered around the skeleton of an elephant at *FLK* I North. These were not on a living floor and must represent the tools used for the specific purpose of cutting meat off the carcass. Quartz flakes were again disproportionately numerous, while the choppers and other larger tools were almost entirely made from lava. (It may be mentioned that experimental use of freshly struck quartz flakes for cutting meat from a carcass left little or no trace of utilization after the skin and soft tissues had been cut through. Chipping of the edges seldom occurred unless the flakes came into contact with bone.)

There is no indication that bone was deliberately worked during Oldowan times, although a few utilized bones do

occur. These have merely been abraded and polished through use and are not comparable, in any way, with the deliberately chipped and flaked bone tools which occur at sites in middle and upper Bed II.

The characteristic features of the Oldowan from Bed I and the base of Bed II may be briefly summarized as follows: More than 75 per cent of the total assemblage consists of unmodified flakes and other fragments—in which waste is included. Utilized material is normally rather more plentiful than tools, but not to a marked extent.

Choppers of various types are the dominant tools. In the case of side and end choppers, both of which were generally made on oblong cobblestones or nodules, a preference for the longer working edge obtainable on a side chopper may be inferred, since these are always greatly in excess of end choppers. The occurrence of polyhedral specimens appears to be largely controlled by the type of raw material available: they were particularly common at *DK* I when a high proportion of irregular-shaped weathered nodules were used, and scarce at *FLK* I North, where the raw material used for making choppers consisted almost entirely of smoothly rounded cobblestones. Discoidal specimens, however, indicate the beginning of a more specialized technique, in which the 'core' on which the tool was to be made was first prepared by breaking a cobblestone in two. Chisel-edged and pointed specimens are relatively scarce, but can be recognized as clearly defined types.

The average ratio of the working edge to the circumference in side and end choppers remains generally constant throughout the Oldowan, but there is considerable variation in the size of the specimens obtained from any particular level. The average mean diameter for the majority of all forms of choppers is 65 mm, but a few diminutive examples also occur, particularly at *DK* I, in which the mean diameter is 35 mm or less, and which weigh under 2 oz.

Proto-bifaces are extremely crude and the specimens show considerable diversity, both in technique and in morphology. They appear to represent some of the earliest and least skilful attempts to make bifaces, in which the methods used were still in an experimental stage, although the value of a pointed tool, with a cutting edge on the circumference, had begun to be appreciated. It is probably significant that these tools do not occur in the lower part of Bed I, and become increasingly common in the higher levels and in the basal levels of Bed II.

There are a few specimens from the top of Bed I (*FLK* I North) which can be classed as true spheroids, or artificially prepared stone balls, such as occur commonly in Bed II. Examples from the lower levels, however, are sub-spherical and, although extensively battered on the exterior, still retain quite prominent ridges.

Both heavy- and light-duty scrapers are relatively common. The former are frequently made on cobblestones and resemble choppers in overall size and weight, but may be distinguished by the nature of the working edges and of the secondary flake scars. Light-duty scrapers, which are particularly plentiful at *FLK* I, where they amount to 30 per cent of the tools, are generally made on flakes or small pieces of tabular quartz. Many are remarkable for their small size and excellent workmanship.

A number of the tools, which have been classed as proto-burins, bear such a close resemblance to the burin of more evolved industries that it has been suggested that the prefix 'proto' should be omitted. The series as a whole, however, includes a proportion of technically crude specimens which would be difficult to class as burins, without some qualification.

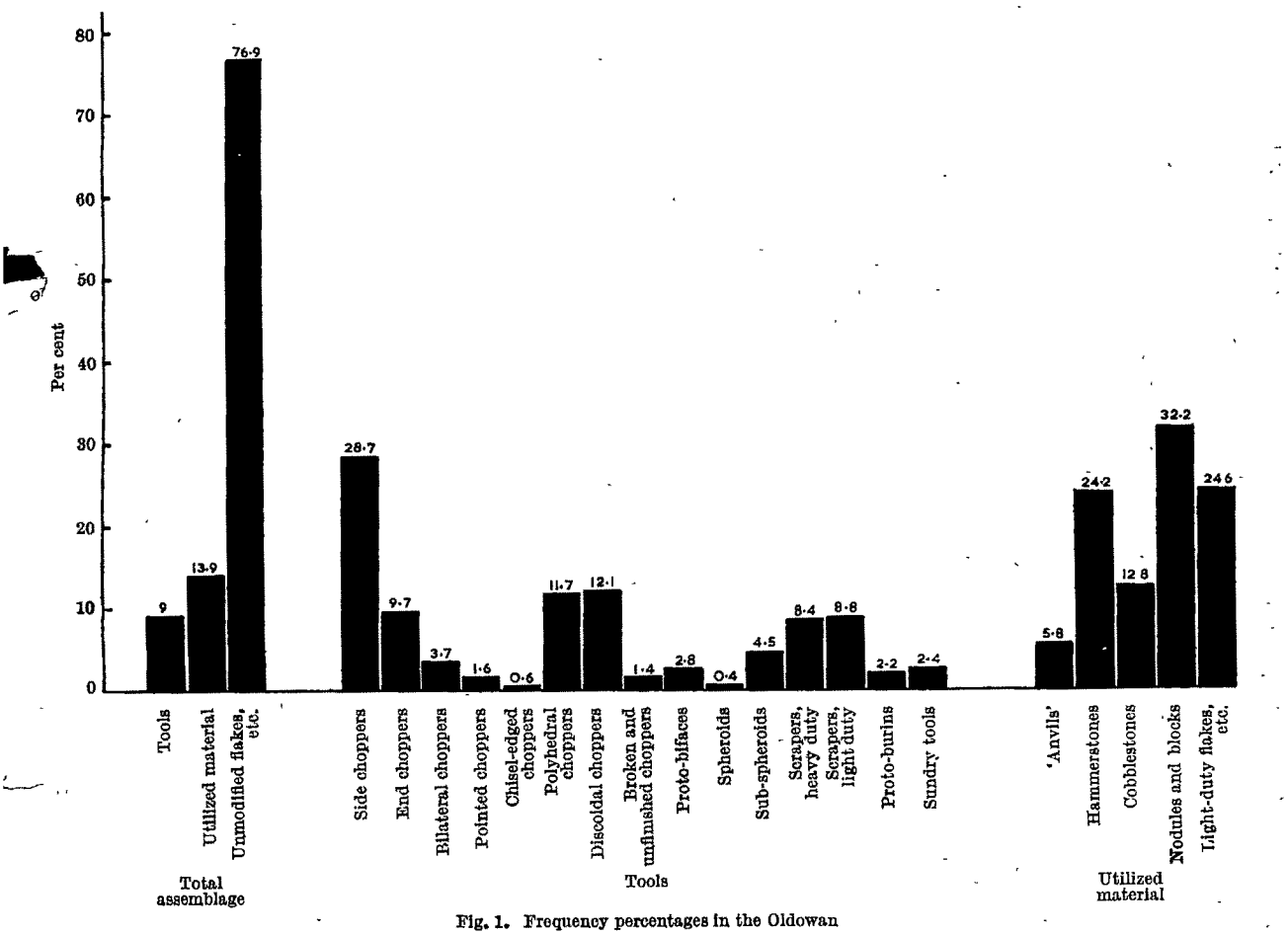


Fig. 1. Frequency percentages in the Oldowan

A statistical analysis of the material reviewed in this article is given in Fig. 1.

Finally, it must be stated that it is not possible to identify positively any one of the fossil skulls found in Bed I, or the base of Bed II, as representing the makers of the Oldowan. Hominid remains have been found on living floors, directly associated with cultural material, and also at places where there were no artefacts, although they occur elsewhere at the same horizon. Furthermore, at *FLK* I, *Homo habilis* remains appear to have come from the same occupation level on which the australopithecine '*Zinjanthropus*' was found[7]. It seems highly improbable that the two types of early hominids co-existed at the same camp, and it must be assumed that one represents the occupant of the site and the other an intruder or victim.

The debris on the *FLK* I floor and at other sites indicates that a substantial amount of meat was consumed, and in this respect the australopithecine, with a dentition evidencing a largely vegetarian diet, is unlikely to have been responsible. It appears even more improbable that a non-meat-eating creature would have required the variety of stone tools which are found in the Oldowan, or would have had the ability to make them, whereas the inception of hunting, even by the most primitive methods, would necessarily create a need for a variety of cutting tools and weapons.

Although the australopithecine may well have made simple 'tools' to meet his requirements, I am of the opinion that the larger-brained *Homo habilis*, whose hand bones also indicate a degree of manual dexterity, is most likely to have been responsible for the Oldowan.

[1] Leakey, L. S. B., *Olduvai Gorge* (Cambridge University Press, 1951).
[2] Leakey, L. S. B., *Olduvai Gorge* (Cambridge University Press, 1951).
[3] Leakey, L. S. B., *Olduvai Gorge* (Cambridge University Press, 1951).
[4] Leakey, L. S. B., Evernden, J. F., and Curtis, G. H., *Nature*, **191**, 478 (1961). Fleischer *et al.*, *Science*, **148**, 72 (1965).
[5] Hay, R. L., *Science*, **139**, 829 (1963).
[6] Leakey, L. S. B., *Olduvai Gorge* 1951–61 (Cambridge University Press, 1965).
[7] Tobias, P. V., Leakey, L. S. B., Leakey, M. D., and Napier, J. R., *Nature* 202, 3 (1964).

NEWS AND VIEWS

Electronics Research and Development (Telecommunications) in the Ministry of Aviation : Mr. J. S. Shayler

MR. J. S. SHAYLER has recently been promoted to deputy chief scientific officer and appointed as director of electronics research and development (telecommunications) in the Ministry of Aviation. He was educated at William Hulme Grammar School, Manchester, and the University of Manchester, where he took an honours degree in electrical engineering in 1938. He then joined the Royal Naval Scientific Service at H.M. Signals School, Portsmouth (which later became the Admiralty Signals Establishment and then the Admiralty Signals and Radar Establishment), where he stayed until 1949, developing high-power shipborne metre-wave radar transmitters and radar displays including early work on data handling. From 1949 until 1951 he worked on search-type asdics with rapid all-round cover at H.M. Underwater Detection Establishment, Portland. He then moved to the Services Electronic Research Laboratories, Baldock, where he worked on the application of millimetre waves, particularly 8-mm waves, and was responsible for building a ground-based lock-follow 8-mm continuous wave radar, which proved the feasibility of the system and was used to measure the spectrum of aircraft returns. From 1955, Mr. Shayler worked on the project studies of the problems of operating new naval aircraft from aircraft carriers at the Naval Air Department, Royal Aircraft Establishment, Bedford, until in 1958 he became deputy head of the Blind Landing Experiment Unit at the Establishment. He was appointed head of the Unit in 1961 and continued as such until his present appointment. At the Unit he was responsible for the research and development of all aspects of landing aircraft in reduced visibility, but was particularly involved with the application of automatic landing to civil aviation.

Physics in the Queen's University, Belfast: Prof. K. G. Emeleus

PROF. KARL GEORGE EMELEUS will retire this year from the chair of physics at the Queen's University, Belfast. Emeleus studied in the University of Cambridge and stayed there for doctorate work in Rutherford's laboratory under the supervision of Prof. Chadwick. After a short time spent in Prof. Appleton's department in King's College, London, he moved in 1933 to Belfast. Prof. Emeleus has devoted his research to the field of "gaseous electronics" embracing plasma physics and electronics. At the time when he started his investigations, this field was little understood. As a consequence, Prof. Emeleus has pioneered in many subjects. His major interests included work on the particle counters of the Wilson chamber; investigations on the application and development of Langmuir probe technique; analysis of the hot and cold cathode glow discharges by means of probes; investigation of the conditions under which forbidden particle transitions can be produced in the laboratory; the introduction of the concept of the subnormal discharge and its experimental and theoretical analysis; the theory of cathode sputtering and chemical reactions in glow discharges; and, particularly, during the past twenty-five years, his thorough investigations in the field of oscillations and plasma turbulence. He was one of the first to recognize the importance of these two phenomena which are now so widely investigated. In addition to nearly one hundred original papers, Prof. Emeleus has written summarizing articles and in particular a book on the conduction of electricity through gases which, although it was first published about twenty-five years ago, is still a valuable introduction to the subject. The pleasant personality of Prof. Emeleus made it a pleasure to work with him. His sincere scientific desire and his able understanding have given the spark of scientific excitement to many of his students.

Prof. D. J. Bradley

DR. D. J. BRADLEY, at present reader in physics at the Royal Holloway College, University of London, has been elected to the chair of physics in the Queen's University, Belfast, in succession to Prof. K. G. Emeleus. Dr. Bradley is a native of Northern Ireland, where he received his grammar school education, and after a teacher training college course (1945–47) he taught in primary schools in Londonderry from 1947 to 1953. He then moved to England and, while teaching in a grammar school at Hitchin and in the London County Council Teaching Service, he took a University of London external B.Sc. (Special) in mathematics by part-time study at Birkbeck College. He followed this with an internal B.Sc. (Special) in physics with first class honours, also at Birkbeck, in 1957. In 1957 he was appointed assistant lecturer at Royal Holloway College where, under the influence of Prof. S. Tolansky, he acquired a

keen interest and skill in spectroscopy and interferometry. As a result of his research on "An Oscillating Fabry–Perot Interferometer" he took his Ph.D. degree. In 1960 he was appointed lecturer in physics in the Section of Applied Physics at the Imperial College of Science and Technology, where he extended his research interest into photoelectronics and space research. Then in 1964 he returned to the Royal Holloway College as reader in physics from where, after only two years, he will transfer to the chair of physics at Belfast. Thus, Dr. Bradley's career began in school teaching and he came into academic physics the hard way. However, this may partly be responsible for his great success as a teacher. Though beginning rather late in university teaching and research in physics, his enthusiasm and ability have carried him a very long way in a short time. He is a very skilful experimenter with a genial nature that engages the confidence of both undergraduate and postgraduate students and makes him a very congenial colleague. All who have known him will feel confident that he will be very successful in his new post.

Low-energy Electron Diffraction

ONE of the first demonstrations in Britain of the technique of low-energy diffraction was recently provided at the Sunbury Research Centre of the British Petroleum Company by Dr. R. Szostak of the Fritz Haber Institute, West Berlin. The technique is being used at a number of laboratories for the study of the structure of the few layers near the surface of a solid crystal which can be penetrated by a beam of electrons with an energy of the order of a hundred volts or less. Dr. Szostak's lecture and demonstration were concerned with the application of the technique to whiskers of iron. Dr. Szostak emphasized that iron is a difficult specimen for single crystal studies because of its $\alpha \rightarrow \gamma$ transition near 900° C and because of impurities, both inherent and induced by cutting and orientating, in massive single crystals. He has found, however, that naturally grown and well-established side faces of whiskers, formed by the reduction of iron (II) chloride in hydrogen near 800° C, offer a successful specimen. Faces exhibited have (100) and (110) orientation. Very bright diffraction patterns were achieved from targets only 0·01 in. broad and with beam energies as low as 5 electron-volts.

After heating up to 850° C the (100) faces exhibited a structure similar to the well-known domain structure of ordered alloys. The pattern expected from clean iron (100) surface was also observed after a long period of ion bombardment and annealing, but this interpretation should be treated with some caution. The addition of oxygen to this surface led to ordered oxide structures with a periodicity equal to a small integral multiple of the unit cell dimensions.

The (110) faces exhibited a great variety of more complicated super-structures and a 'clean' surface could not be obtained without a high background intensity.

Dr. Szostak said that his study of this system was only a preliminary, and that future work would involve more detailed investigation of specific aspects of these interactions.

Advances in Fluorine Chemistry

VOLUME 5 of *Advances in Fluorine Chemistry* covers only two broad topics: oxyfluorides of nitrogen and fluorides of phosphorus (edited by Prof. M. Stacey, Prof. J. C. Tatlow and Dr. A. G. Sharpe. Pp. 288. London: Butterworth and Co. (Publishers), Ltd., 1965. 75*s*.). The first topic is a thorough review of the preparations, reactions and properties of nitrosyl fluoride, nitryl fluoride, and nitroxy fluoride or fluorine nitrate. The thirty pages and 135 references cover a period from 1905 to 1963. The author of the chapter is Dr. C. Woolf, of the Allied Chemical Corporation. The presentation and format follow closely the tradition established by other contributors to prior volumes of this series. The second topic is by Dr. R. Schmutzler, of the E. I. du Pont and de Nemours Company, and is a monumental coverage of almost every known compound which has at least one fluorine atom bonded to phosphorus. The chapter covers more than 230 pages of text involving 771 references in the field. There are more than sixty tables of data which help to bring the topical material into sharp focus for the reader, and apparently no figurative stone has been left unturned to cover the available information and present it in Teutonic detail. Among the topics treated are trivalent and pentavalent phosphorus fluorides, mixed halides, oxyhalides and thiohalides; fluorophosphanes, fluorides of phosphinic and phosphonic acids and their ester and amide derivatives; the fluoroacids of phosphorus and the fluorophosphazenes. There is also a very important section on infra-red, and fluoride-19 and phosphorus-31 nuclear magnetic resonance spectroscopy with chemical shift and coupling constant data made available for literally hundreds of chemical species. Considerable emphasis has been paid to fluorophosphorus compounds as co-ordinating ligands in complexes so that this chapter, which is probably the most thorough one in the series, will also be of interest to co-ordination chemists as well as organic and inorganic fluorine chemists.

More Technical Training

SUGGESTIONS for making fuller use of technical colleges in Britain have been put forward to the Secretary of State for Education and Science by the Committee on Technical Resources—a sub-committee of the National Advisory Council on Education for Industry and Commerce. The recommendations have been warmly welcomed by the Minister, who promises vigorous action in pursuit of a number of detailed recommendations. Clearly the British Government has been overjoyed—and perhaps even surprised—to be told that at least one part of the educational system must be thought of as containing "a very considerable reserve of resources".

According to the statistics in the report of the committee, roughly 1,700,000 students—some full-time and some part-time—now attend technical colleges and other establishments of further education. Investment in these institutions since the Second World War exceeds £200 millions. More recently, the pace of growth, in buildings and in numbers of students, has been particularly quick, and the committee looks forward to even faster growth once the full impact is felt of the work of the industrial training boards now being established, and which are intended to ensure uniform standards of technical training. The same development may well make more funds available to the technical colleges.

The committee's confidence that productivity can be dramatically increased in this frequently neglected part of the educational system rests on its discovery that the sizes of classes now being taught in technical colleges and other institutes of further education are frequently much smaller than they need to be. Among some 86,000 classes of all kinds, the committee found that no fewer than 63,500 were constituted of between eleven and fifteen students. These figures compare favourably with the sizes of senior classes in secondary schools, where the character of the teaching is often very similar. In the circumstances, not even teachers in technical colleges will be surprised that the committee recommends a less generous ratio of staff to students, and a fuller use of educational plant thereby. It is less easy to understand that much of the difficulty about using technical colleges to the full should apparently stem from a misinterpretation of a government policy first laid down in 1957, and which was intended to ensure that public funds could not be spent on technical college courses unless fifteen students enrolled in the first year. Frequently, it seems, this

minimum has been interpreted as a recommended norm, with the result that large classes have been actively discouraged.

Dedijer on Sociology and Science

THE Second Annual Lecture of the Science of Science Foundation was delivered in London on April 28 by Dr. Stevan Dedijer, of the Department of Sociology in the University of Lund. Among other things, Dr. Dedijer was at pains to blame his own specalty for what he described as its neglect of studies of the link between prosperity and the investment of money in research and development, the link between economic growth and the effectiveness of research and, indeed, the whole field of what Dr. Dedijer and his sponsors call the science of science. He was able to assemble an impressive list of official reports from the United States, the Soviet Union, the United Kingdom (where both the Heyworth Report and the earlier document from the Tavistock Institute were found blameworthy) and from the Organization for European Co-operation and Development. "The social and behavioural sciences as a whole have so far neglected to identify and study the major problems and causes of the current scientific and technological revolution."

Among the causes of this neglect, Dr. Dedijer singled out the lack of time for the need to be recognized. Among the cures he recommended, the most tangible was the creation of institutes for "the science of science" in countries of all kinds. He asked that these should be concerned with the collection of information on research and development, the development of national research programmes in the science of science, the infiltration of other branches of the social sciences by the science of science, the creation of specialized units to study difficult problems, and liaison with similar institutes abroad.

Leptospirosis in Man and Animals in Australia

UNTIL recently, leptospirosis was thought not to occur in man and animals in South Australia to any significant degree. In 1953–54, Dr. J. E. McCartney, medical bacteriologist to the South Australian Institute of Medical and Veterinary Science, examined 627 sera for the presence of leptospiral agglutins, but no positive results were obtained. For many years, however, medical authorities concerned with the health of abattoir workers have noticed that febrile illnesses frequently occur which do not appear to correspond with any of the well-recognized ailments commonly seen in these workers—*Q* fever or brucellosis.

In April 1962 serum samples from 120 employees of the metropolitan abattoirs were sent for testing. Leptospiral agglutination tests were performed on these specimens and the results showed that forty-five cases had unequivocal evidence of previous infection with leptospiral organisms. It was therefore decided to set up a routine diagnostic unit at the Institute, and an immediate and comprehensive survey of abattoir workers was organized. In this survey, sera which agglutinated at a dilution of 1 : 100 or more were regarded as significant. Of 894 specimens examined, sixty-five showed leptospiral agglutinins. The groups with the highest incidence of antibodies were meat inspectors, beef slaughterers and workers in the pig hall and offal floor.

Of the clinical cases so far, sera from 140 patients have been examined. There have been eight confirmed cases of leptospirosis, all males and almost exclusively abattoir workers. One hundred and four sera from Aborigines in a variety of areas in South Australia were also examined in the course of other serological tests. No positive results were obtained, thereby confirming the validity of the testing procedure and the occupational incidence of the disease.

Evidence was also collected to determine the incidence of leptospiral disease in animals in South Australia. Animal sera which showed leptospiral agglutinins at a dilution of 1 : 300 were regarded as positive. Forty-five cattle sera were submitted by the Division of Veterinary Pathology. Of these, twelve were positive. Forty-one sera were examined, and twenty-seven were positive. The results obtained so far clearly show that both doctors and veterinary surgeons need to be constantly aware of leptospirosis as a cause of hitherto undiagnosed symptoms in man and animals. Further information is to be found in the twenty-sixth report of the Institute of Medical and Veterinary Science, South Australia (Adelaide: Government Printer, 1965).

Announcements

OPEN days will be held at the National Engineering Laboratory, East Kilbride, during May 4–6. Further information can be obtained from the Director, National Engineering Laboratory, East Kilbride.

THE third national conference of the National Conference of Standards Laboratories will be held at the National Bureau of Standards, Gaithersburg, Maryland, during May 9–12. Further information can be obtained from W. R. Tilley, National Bureau of Standards, Washington, D.C.

A BIONICS Symposium, sponsored by the Aerospace Medical Division and the Research and Technology Division of the U.S. Air Force Systems Command, will be held in Dayton, Ohio, during May 3–5. Further information can be obtained from Dr. H. L. Oestreicher, Aerospace Medical Research Laboratories Wright Patterson Air Force Base, Ohio.

AN international symposium on "Some Aspects of Strontium Metabolism", under the auspices of the Medical Research Council and the United Kingdom Atomic Energy Authority, will be held at Chapelcross during May 5–6. Further information can be obtained from Dr. J. H. Martin, United Kingdom Atomic Energy Authority, Chapelcross Works, Annan, Dumfriesshire.

A CARBON and Graphite Group of the Institute of Physics and the Physical Society has now been created by the Council of the Institute and Society. The inaugural meeting of the Group will be held in the University of Sheffield on May 5. It will take the form of a discussion meeting on "Methods of Characterizing the Pore Structure of Carbons". Further information can be obtained from the Meetings Officer, Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

A SERIES of one-day courses in applied social research will be held at the Tavistock Institute of Human Relations during May. The following courses will take place: stress and conflict in industry (May 2); business organization (May 3); management development (May 4); management selection (May 5); consumer research (May 9); theories of working groups (May 11); personal and executive counselling (May 12); management training (May 13). Further information can be obtained from the Course Secretary, Tavistock Institute of Human Relations, 3 Devonshire Street, London, W.1.

CORRIGENDUM. In the article entitled "Efficiency of Ion Transport Mechanisms", which was published on page 398 of the January 22 issue of *Nature*, the expression $\frac{c_{Na}'}{c_{Na}''}$ in equation (4) should be inverted to read $\frac{c_{Na}''}{c_{Na}'}$.

CORRIGENDUM. Dr. A. Gajdos has written to the Editor of *Nature* stating that the last sentence in the communication entitled "Mechanism of the Inhibitory Effect of Adenine Nucleotides on Porphyrin Synthesis by *Rhodopseudomonas spheroides*", which appeared on p. 640 of the August 7, 1965, issue of *Nature*, should read "These findings do not invalidate the conclusions of our earlier observations".

SCIENTIFIC AND INDUSTRIAL RESEARCH IN INDIA

THE fourth Five Year Plan of the Council of Scientific and Industrial Research, India, is presented in two parts*. Part I outlines the approach adopted to planning and the methods adopted by the Working Group appointed in May 1963 by the Council at the request of the Planning Commission. The salient features of the proposals are then summarized, together with the recommendations regarding co-operative research, research grants and facilities, amenities for research and research staff, co-ordination of research between the Council, universities and ministries, the supply of scientific instruments and the utilization of research results. The Working Group was to make a critical review of the progress of the programmes and schemes of the third Plan period, assess the position likely to be reached at the end of that period and formulate proposals for the fourth Plan in the perspective, where possible, of a 15-year period. The desirability of paying more attention to annual phasing was emphasized.

Four broad principles have been followed in working out the Plan and the requirements of scientific policy under the Council for Scientific and Industrial Research. (1) The national requirements have been established in terms of technological problems and research programmes drafted based on the identification of these problems. (2) After evaluating progress made so far in research, a policy has been worked out for supporting those areas which have shown promise or significance to science or for the national economy. (3) Attention has been given to broadening and diversifying the base of science and technology, for example, through the Council's pool of scientists, fellowships, scholarships, research grants, research centres and co-operative research associations. (4) Attention has been given to certain gaps in earlier programmes, for example, by strengthening research activities in some geographical areas or in certain branches of science or technology, or augmenting and creating central facilities, including library and documentation services, or again in the sphere of effective organization.

In order to put its proposals on firm ground, the Working Group held discussions at various laboratories. Besides critical studies of the laboratories over the past 10 years, it had at its disposal critical studies on their growth undertaken by the Unit for the Survey and Planning of Scientific Research. As regards the established laboratories, features of the proposals are the emphasis on project orientation of research, the time targets of the schedules and the allocation of priorities to projects—high priority being given to those projects which are vital to the national economy. Emphasis is also placed on pilot plant and development work and on the replacement of obsolete equipment. It was noted that gaps occurred in the national laboratories at three levels: in multi-purpose laboratories to meet the requirements of an area or to develop and utilize natural resources; in certain areas of research in which India possesses considerable experience and expertise but establishment of well-defined institutions is necessary to organize research properly to utilize fully available resources; in areas where research is needed but sufficient trained personnel are not available.

It is to meet these three requirements that the Plan envisages first the establishment of five new institutes: a Fire Research Station; a National Institute of Oceanography; a National Science Library; a National Institute for Research in Electrical Engineering; and an Institute for the Study of Social Relations in Science. Five new Regional Research Laboratories are proposed, at Uttar Pradesh, Madhya Pradesh, Kerala, Rajasthan, and Punjab. Eight fields are specifically proposed for new projects: birth control; pesticides, insecticides and agricultural chemicals; marine engineering; automation; meat and fish technology; packaging; substitute materials for electrical appliances; and materials research.

The Plan also provides for the formation of co-operative research associations for tea and cement, and a larger allocation is recommended in the Scientists' Pool to bring back young scientists at present in advanced countries. Besides increased grants to research schemes, the establishment of centres of research in universities (in consultation with the University Grants Commission) is proposed, and funds are to be allocated for sending exceptionally brilliant scientists for training abroad in various specialized fields. Definite provision is to be made for the creation and expansion—both centrally and regionally—of facilities such as libraries, documentation services, service units for routine analysis, and facilities for maintenance and repair of equipment, instruments and calibration. The India National Scientific Documentation Centre and the Publications and Information Directorate are to be further developed, the third Museum of Science and Technology, initiated at Bombay during the third Plan, will be completed during the fourth Plan, and the engineering and design unit will be developed as a fully fledged institution. The Industrial Liaison and Extension Unit at the Council's headquarters is to be reorganized and resources provided for the evolution of an effective programme of international collaboration. Provision is also included for medical help for research staff, accommodation and other facilities.

In 1963–64 the Council provided 24·7 lakhs of rupees for 623 university research schemes and 31·3 lakhs of rupees for 1,428 fellowships, as well as sanctioning block grants of 190,000 rupees to the School of Earthquakes, Roorkee University, 25,000 rupees to the School of Dyestuffs, Bombay University, and 508,000 rupees to the International Meteorological Centre. Proposals are detailed for fifteen different stages to increase collaboration between the Council and the universities, and co-ordinating units on the lines of that already established for defence are to be set up for the Ministries of Health, Industry, Steel and Mines, Commerce, Works, and Civil Aviation. Other proposals relate to the Central Scientific Instruments Organization, and include the establishment in each National Laboratory of a strong instrument division.

Of the total expenditure of Rs. 204·69 crores proposed for the fourth Plan, Rs. 60·69 crores is for the committed expenditure of the Council of Scientific and Industrial Research over the five years. The revised estimates provided for expenditure of 339 million rupees on established institutes and laboratories; 336 million rupees on those started in the second or third Plans; 19 million rupees on science museums; 35 million rupees on co-operative research associations; 74 million on the Scientists' Pool, research grants and fellowships; 40 million on research centres; 146·5 million on central facilities; 24 million on scientific and technical units at headquarters; 21 million on international science collaboration; 91 million on new institutes; 87·5 million on new projects; 184 million on amenities; miscellaneous items bring the total to 1,444 million rupees.

* India· Council of Scientific and Industrial Research. Fourth Five Year Plan, Part I Pp v+74, (New Delhi. Council of Scientific and Industrial Research, 1965.)

BROADCASTING POLICY AND A UNIVERSITY OF THE AIR

ALTHOUGH the White Paper *A University of the Air* was mentioned in the House of Commons in the debate on broadcasting policy on March 3, that debate was concerned with broadcasting policy generally. Of special interest in the debate was the reply of the Postmaster-General, Mr. A. Wedgwood Benn, who stressed the central problem of the finances of the British Broadcasting Corporation. Mr. Benn pointed out that the Corporation could no longer rely on increased revenue from a growing number of television licences. Because the 1954 Act left the Corporation dependent on a fixed licence to compete with programme contractors granted a monopoly of advertising revenue and for whom expansion was highly profitable, it looked as if the Corporation would require a further increase of £1 in the licence in 1966 and another in the next 4 years. Furthermore, for a comprehensive system of local broadcasting a further 5*s*. would be required—it was estimated that licence evasion already amounted to the equivalent of an extra 10*s*. licence. After referring to such alternatives as increased borrowing or an Exchequer grant, Mr. Benn said that the Corporation had now put before the Government proposals which it believed could or might stabilize the licence fee for another 2 years or more without cutting its services; while considering these proposals, the Government was dealing further with the evasion of licences. He also affirmed the Government's intention to introduce legislation to deal with pirate radio companies. On hours of broadcasting, a final decision would have to wait until further discussions had been held with the Corporation.

Mr. Benn stated that he thought that the role of publishing in broadcasting, making outlets available for independent experimental education programmes, was important—it was here that he mentioned the University of the Air. He indicated that he was discussing with the Independent Television Authority the possibility of opening up outlets, under a provision in the 1964 Act, for universities such as Strathclyde, and independent institutions and units which at present had no access to the television screen. Mr. Benn welcomed the development of closed-circuit television and regarded the awakening of public understanding of the possibilities of local radio as a most significant development. Besides the B.B.C., the commercial radio companies, and genuine community stations, including local authorities, were interested in local stations, and the White Paper on a University of the Air also referred to the part which local stations might play. As to a fourth channel, Mr. Benn estimated that this could involve a capital cost of about £30 million and many million pounds a year to run. Here was a question of priorities. On colour television, the Government had accepted the advice of the Television Advisory Committee to use the PAL system of transmission on the 625-line standard. Subject to acceptance at the Oslo Conference in June, colour television on B.B.C. 2 would start with about 4 hours a week towards the end of 1967; the cost was estimated at £1–7 million a year. The cost of sets was estimated at £250 each initially with a consumer expenditure over the first 3 or 4 years of £100 million and a net gain of £10 million in exports.

The White Paper itself* argues that an open university of the air, if it were imaginatively to use new teaching techniques and teacher/student relations, would contribute to the improvement of educational, cultural and professional standards generally, by making available scholarship of a high order to those who look and listen. As is pointed out, it will enable some to accept the full discipline of study and acquire degrees and other qualifications; and it will benefit students in many other parts of the world as well as those studying in the United Kingdom. It is suggested that it will help to meet the urgent need in the developing countries for a highly trained corps of men and women, equipped to provide leadership in national life. At a time when capital resources are scarce, it would provide higher and further education for many without involving vast expenditure on buildings. The proposals are based on the report of an Advisory Committee of which Miss Jennie Lee, Joint Parliamentary Under-Secretary of State, Department of Education and Science, was chairman.

The University would require its own administration centre but would make full use of existing agencies, such as the extra-mural departments of universities, the Workers' Educational Association and local education authorities. It would primarily offer general courses leading to a degree which would be conferred by the university of the air in its own right. A working party should be established to examine the possibility of courses leading to degrees and other qualifications with a technological element. The presentation of courses would involve a combination of television, radio (particularly from local stations), correspondence courses, programmed instruction, tutorials and practicals, short residential courses, and study and discussions at community viewing or study centres. The project requires peak viewing on a television service with national coverage, estimated at least two hours on five evenings a week Enrolment would be open to everyone on payment of a registration fee and no formal entrance requirement would be imposed. The university, which would have its own vice-chancellor and governing body or council, would require a professional staff of about 40–50 and would arrange for regular surveys to assess the effectiveness of courses, and the size and type of audiences following them.

* A University of the Air. Pp 8. (Cmnd. 2922) (London: H.M.S O., 1966.) 1*s*. 6*d*.

LATEX

A MEETING of the Phytochemical Group on "The Distribution and Biochemistry of Latex in Plants" was held on January 6 at the School of Pharmacy, University of London. The opening paper on the distribution of latex in the plant kingdom was given by Dr. C. R. Metcalfe (Royal Botanic Gardens, Kew). Latex, produced in specialized laticiferous cells, is found in about 12,000 species, but these are restricted to about twenty scattered families of angiosperms. With the aid of an extensive series of photomicrographs of sections of laticiferous plants, Dr. Metcalfe explained the differences between articulated and non-articulated laticifers and showed examples of their distribution in the plant. Although many articulated laticifers frequently occur in the phloem, non-articulated laticifers are sometimes dispersed throughout all plant tissues including the xylem.

The most important commercial laticiferous plant is *Hevea*, and the next four papers dealt with aspects of the

structure and biochemical properties of *Hevea* latex. The ultra-structure of *Hevea* latex as gleaned from electron-microscope investigations was described by Dr. P. B. Dickenson (National Rubber Producers Research Association, Welwyn). Three characteristic features found are rubber particles, lutoid particles and Frey-Wyssling particles. The rubber particles are globular or bean-shaped and show a black boundary which is an adsorbed phospholipid-protein layer. Lutoid particles in young latex vessels contain bundles of long microfibrils with a hollow core; these microfibrils, which are protein, cannot be seen in lutoids from more mature latex vessels. The Frey-Wyssling particles which are inclusions in a much larger organelle are large lipid globules and contain tubules with spiral thickening in a complicated internal structure.

Dr. B. G. Audley (National Rubber Producers Research Association) described some recent biochemical investigations of rubber latex. The glycolytic conversion of glucose to either lactate or ethanol requires both the white fraction and the bottom fraction of latex; the function of the latter fraction probably being to supply reduced co-enzymes. However, the bottom fraction could not be replaced by $NADPH_2$ in a latex system which converts β-hydroxymethylglutaryl co-enzyme *A* to rubber, and in this case bottom fraction appears to stimulate rubber synthesis by oxidizing inhibitory unesterified co-enzyme *A* through a phenolase mediated reaction.

The conversion of mevalonate to rubber by *Hevea* latex was reviewed by Dr. B. L. Archer (National Rubber Producers Research Association). Mevalonate is converted to isopentenyl pyrophosphate (IPP) in the aqueous phase which is then incorporated into rubber at the surface of the rubber particles. Dimethylallyl pyrophosphate (DMAPP) stimulates the incorporation of IPP into rubber by a suspension of washed rubber particles. The inhibitory effect of DMAPP on IPP incorporation by latex is explained by the reaction of these two compounds, which is catalysed by enzymes in the latex, to form farnesyl pyrophosphate, an inhibitor of rubber biosynthesis.

Dr. J. F. Pennock (Department of Biochemistry, University of Liverpool) described the isolation of α, γ- and δ-tocotrienols both in the free form and as esters from *Hevea* latex. Only the free tocotrienols and not the esters had previously been found in other tissues. In contrast, the tocopherol fraction of leaves of *Hevea* did not contain any tocotrienols but only the saturated compounds, α-, β-, γ- and δ-tocopherols. Both latex and leaf lipids were found to contain plastochromanol, a cyclic compound related to plastoquinone. It was suggested that the tocotrienols were possibly intermediates in the biosynthesis of tocopherols.

Proteolytic enzymes in latex were discussed by Dr. P. Dunnill (Department of Botany, University College, London). Although plant and animal proteases have similar specificities for artificial substrates, they differ in the amino-acid sequences at the active sites; however, ficin and papain show close similarities in their amino-acid compositions. When proteases are present in laticiferous plants they are markedly concentrated in fruits and seeds. Since laticiferous vessels do occur in cotyledons, it seems that the hydrolysis of storage protein for conversion to other protein at great speed at critical stages in the life-cycle represents the most important role and may account for the very high protease concentration in latices.

The latex of *Papaver somniferum* contains the alkaloids thebaine, codeine and morphine. Results of recent experiments on the inter-relationships of these compounds were discussed by Prof. J. W. Fairbairn (School of Pharmacy, University of London). Analysis of whole plants showed variations over short time periods in the alkaloidal content, and the total of thebaine plus codeine alternately rose and fell, but instead of a corresponding accumulation of morphine there was an apparent disappearance. These results were confirmed by experiments in which [U-^{14}C]-tyrosine was incubated with isolated poppy latex. It was concluded that there was an additional non-tyrosine route to the alkaloids and that morphine is transformed to a non-alkaloidal molecule in the latex itself.

Copies of the paper "Distribution of Latex in the Plant Kingdom" on which Dr. Metcalfe's talk was based can be obtained from Dr. Metcalfe at the Jodrell Laboratory, Royal Botanic Gardens, Kew. J. FRIEND

DELAYED CYCLOTRON PULSE GENERATION IN THE TOPSIDE IONOSPHERE DEDUCED FROM *Alouette* I DATA

By D. B. MULDREW

Radio Physics Laboratory, Defence Research Telecommunications Establishment, Ottawa

THIS article describes a physical process in which the passage of an electromagnetic pulse through the Earth's ionosphere can result in the delayed generation of a second pulse. The initial pulse must have right-hand polarization. The delayed pulse is generated in a height region where the angular frequency ω of the pulse is approximately equal to twice the angular electron gyrofrequency $2\omega_H$. The computed delay time of the delayed pulse is found to be consistent with a 'remote resonance' trace[1] observed on *Alouette* I and *Alouette* II ionograms.

A well-defined remote resonance trace is shown in Fig. 1. From the characteristics associated with such traces it may be inferred that the energy responsible for producing the remote resonance propagates along an ionospheric field-aligned wave guide[2]. In such a guide there would be a gradient in the electric field of the wave in a direction normal to the direction of the Earth's magnetic field. The production of a remote resonance trace is made possible by this gradient.

Owing to the gradient in the electric field there can be a net energy transfer per cycle between the electromag-

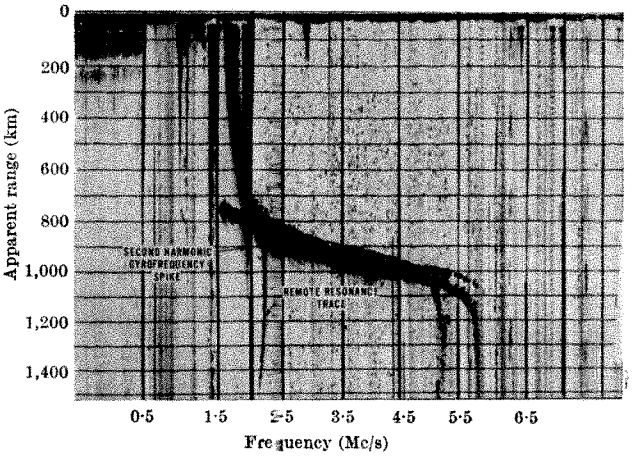


Fig. 1. *Alouette* I ionogram, showing remote resonance trace, recorded September 16, 1963, 1715 U.T. Satellite location 43° N., 65° W., at a height of 1,025 km

netic pulse and the gyrating electrons and it can be shown that this transfer is maximum when $\omega = 2\omega_H$. The position of each electron in its gyrating orbit may be specified in terms of a phase angle, measured from a fixed direction in the plane normal to the direction of the Earth's magnetic field. Before the passage of the pulse, the phases of the electrons are randomly distributed with respect to the thermal energy of the electrons. Energy transfer between the pulse and the electrons is a function of phase and results in a non-random distribution of phase with electron energy that persists for a considerable time after the passage of the pulse. This non-random distribution of electron energies with respect to phase is referred to here as 'energy bunching'. It is convenient to consider the behaviour of all the electrons which, immediately following the passage of the pulse, have a particular phase shift relative to those electrons which gain the maximum amount of energy from the pulse; the phase of these electrons is a function of height, r, measured from the centre of the Earth, and time t measured from the passage of a downward propagating pulse through the height r_0 at which $\omega = 2\omega_H$. These specific electrons will be called 'tagged' electrons. The duration of the initial pulse is assumed to be negligible. The phase of a tagged electron at time t and height r may be written in the form $\varphi(t, r)$; $\varphi^-(t, r)$ will be used to represent the phase distribution with respect to t and r resulting from a right-hand polarized downward propagating pulse at $t = 0$ and $\varphi^+(\tau, r)$ will be used to represent the phase distribution with respect to r at some time τ, resulting from a right-hand polarized upward propagating pulse at $t = \tau$.

For $\omega = 2\omega_H$ the pulse completes two cycles by the time the electron completes one at the gyrofrequency. Electrons which are separated by π radians in phase thus experience the same variation of the field of the pulse and hence there are two possible phase groups for the tagged electrons. It follows that if the phase of one of these groups is considered at a particular time, as a function of height, it will go through one cycle in a height equal to two mean wave-lengths of the pulse. Thus if the mean wave number of the pulse is k, the phase of the tagged electrons at $t = 0$ for a downward propagating wave is

$$\varphi^-(0, r) = \varphi(0, r_0) + \frac{k}{2}(r - r_0) \quad (1)$$

Similarly, in the height region where $\omega \simeq 2\omega_H$, the phase of the tagged electrons at time τ when a pulse propagates upward through the region is

$$\varphi^+(\tau, r) = \varphi(\tau, r_0) - \frac{k}{2}(r - r_0) \quad (2)$$

If it is assumed that there is no component of thermal motion of the electrons parallel to the Earth's magnetic field, then from (1)

$$\varphi^-(t, r) = \varphi(0, r_0) + \frac{k}{2}(r - r_0) + \omega_H t \quad (3)$$

where ω_H, the gyrofrequency at height r, is a function of r. The strength of the Earth's magnetic field (and hence the magnitude of the gyrofrequency) varies as the inverse cube of the distance from the centre of the Earth. Using a Taylor expansion for the gyrofrequency, and retaining the first two terms, we get

$$\omega_H = \omega_{H0} - \frac{3\omega_{H0}}{r_0}(r - r_0) \quad (4)$$

where ω_{H0} is the gyrofrequency at r_0. Substituting (4) into (3) yields

$$\varphi^-(t, r) = \varphi(0, r_0) + \omega_{H0} t + \left(\frac{k}{2} - \frac{3\omega_{H0}}{r_0} t\right)(r - r_0) \quad (5)$$

Since it has been assumed that there is no thermal electron motion along the field, the phase of the tagged electrons at r_0 at time τ, $\varphi(\tau, r_0) = \varphi(0, r_0) + \omega_{H0}\tau$. Hence from (2) and (5) the variation of the phase of the tagged electrons with height, initiated by a downward propagating pulse at $t = 0$, becomes identical with the phase distribution corresponding to an upward propagating pulse at time τ, where τ is given by

$$-\frac{k}{2} = \frac{k}{2} - \frac{3\omega_{H0}}{r_0}\tau \quad (6)$$

$$\tau = \frac{kr_0}{3\omega_{H0}} = \frac{\omega n}{c}\frac{r_0}{3\omega_{H0}} \quad (7)$$

where c is the velocity of light and n is the index of refraction of the pulse of angular frequency ω at the height r_0. At r_0, $\omega = 2\omega_{H0}$; hence from (7)

$$\tau = \frac{2}{3}\frac{r_0}{c} n \quad (8)$$

Any particular phase could have been chosen for the tagged electrons so that the above expression is applicable for all the electrons affected by the pulse near $r = r_0$. Since an electromagnetic pulse propagating in an ionospheric wave guide can produce energy-bunched electrons it might be expected that the reverse process may also occur; that is, that energy-bunched electrons with the correct phase distribution with height could produce an electromagnetic pulse. The evidence that a pulse is produced having the requisite delay time is given by Muldrew and Hagg[2].

It should be noted that if the pulse propagating through the height r_0 at $t = 0$ were an upward propagating pulse (that is, one propagating toward decreasing magnetic field strength), then τ would turn out to be negative. That is, a remote resonance pulse is only generated if the initial pulse propagates downward into increasing magnetic field strength.

The mode of propagation (that is, the path taken by the radio energy) involved in the generation of resonance traces observed by the *Alouette* satellites is then as illustrated in Fig. 2. A pulse of angular frequency ω transmitted from the satellite propagates downward, along an ionospheric wave guide, through the height A in the ionosphere where $\omega = 2\omega_H$. After a time delay τ, the distribution of the phase with height of the energy-bunched electrons at A becomes identical to the distribution that would be produced by an upward propagating extraordinary wave pulse of angular frequency ω. At this time an upward propagating pulse is apparently generated, and it is this delayed pulse that produces the resonance trace on the ionogram. The total time delay of the generated pulse may be calculated by adding to τ twice the time required by the extraordinary wave to propagate from the satellite to height A.

Energy bunching of the electrons at a given height can be affected by random thermal motion of the electrons

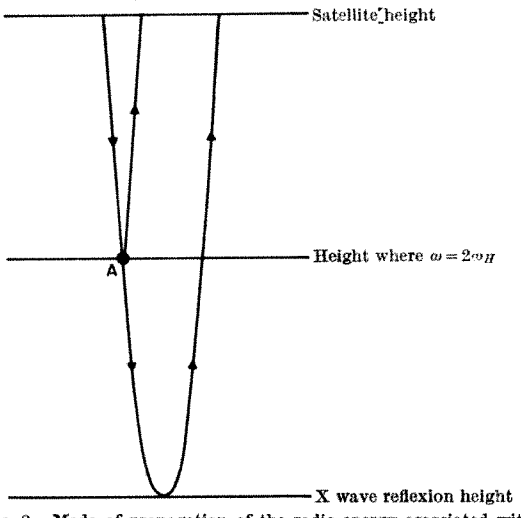


Fig. 2. Mode of propagation of the radio energy associated with the remote resonance trace

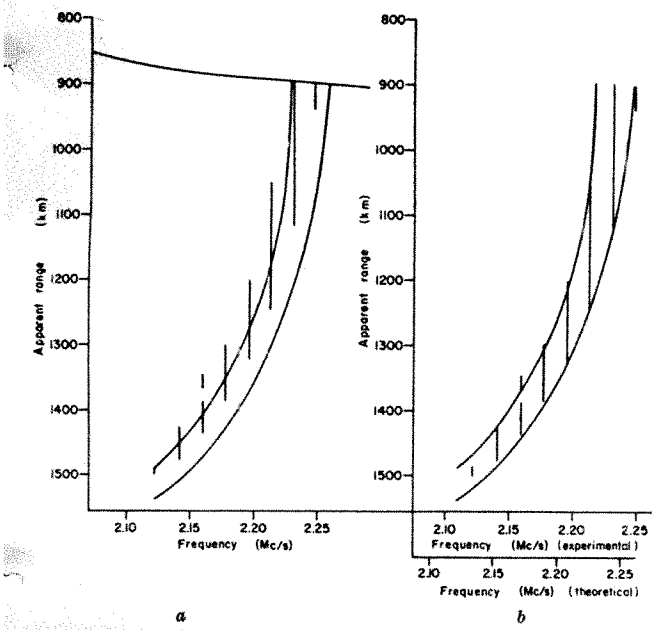


Fig. 3. Comparison of observed (vertical bars) and theoretically computed (solid curves) remote resonance echoes. (*a*) Theoretical curves as computed, (*b*) theoretical curves shifted 12 kc/s for best fit

parallel to the Earth's magnetic field. For a downward propagating pulse initially (at $t=0$) electrons at a given height have two energy bunches; at later times energy bunched electrons arriving at the given height from nearby heights will mix and there will no longer be well-defined energy bunching. However, it can be shown[3] that at the time τ, due to the initial phase distribution of the electrons set up by the pulse and to the gradient in the Earth's magnetic field strength, the electrons at the given height once again become energy bunched and they become bunched in exactly the way that they would have been bunched at τ if there were no thermal electron motion along the field. Thus, neglecting thermal motion along the field does not seriously affect the results of the presentation given above.

The above study does not treat the variation of the index of refraction n with height, electron-ion Coulomb interactions, pulse length, and the finite propagation time of the pulse through the height region near r_0. A consideration of these effects[2] enables the time delay and duration of the remote resonance echoes to be calculated accurately. The results are shown in Fig. 3*a*. The vertical bars show the upper and lower apparent range limits of the observed remote resonance echoes obtained from the ionogram of Fig. 1. The solid curves show the theoretically calculated upper and lower apparent range limits. In Fig. 3*b* the theoretically calculated curves are shifted 12 kc/s to higher frequencies with respect to the experimental data to obtain the best fit. This shift is within the estimated limits of accuracy. The observed and theoretically calculated traces are thus found to be in good agreement.

I thank Dr. R. E. Barrington and Mr. E. L. Hagg for helpful discussions.

[1] Hagg, E. L., *Nature* (in the press).
[2] Muldrew, D. B., and Hagg, E. L., *Canad. J. Phys.* (in the press, 1966).
[3] Muldrew, D. B., to be published.

RADIATION MEASUREMENTS IN COMMERCIAL AIRCRAFT USING A SINGLE COUNTER

By PROF. V. D. HOPPER and W. R. RAWLINSON
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THE opportunity was taken to make cosmic-ray measurements in aircraft during a recent visit by one of us to the United States and the United Kingdom.

Three types of readings were taken using a single Geiger–Müller counter: first, the count rate due to cosmic-rays with the unshielded Geiger tube, secondly the cosmic-ray count rate with the tube shielded by 2·12 g cm^{-2} of lead, and finally measurements of radioactive sources in the aircraft themselves.

The equipment consisted of a single *MX* 120/01 thick-walled halogen-quenched Geiger tube connected to a ratemeter unit giving a direct reading in counts per second (c.p.s.). The lead shield consisted of a cylinder of lead that could be slid on and off the Geiger tube.

The results obtained on each section of the trip are plotted against time (G.M.T.) in Figs. 1*a*, *b* and *c*, together with the altitude record of each flight. Each point in the level parts of the flights consists of the average of between ten and thirty-two spot readings of count rate taken at 10-sec intervals. Few readings were taken on short flights across the United States due to the short times at constant altitudes.

There are two contributions to the experimental error of the results obtained. The first is the statistical fluctuations depending on the smoothing time constant of the ratemeter circuit, the number of readings averaged for each point (usually about twenty), and the count rate concerned. The second source of error is the accuracy of the readings of pressure altitude supplied by the crews of the aircraft. These were generally quoted in thousands of feet, and so an error of ± 500 ft. must be assumed. There may occasionally have been a deviation larger than this. For example, the dip in the results from Sydney to Nandi on August 16, 1965, between 1100 and 1130 h G.M.T. could possibly be due to an altitude drop of 1,000–2,000 ft. The overall accuracy of the results obtained is of the order of ± 1 c.p.s.

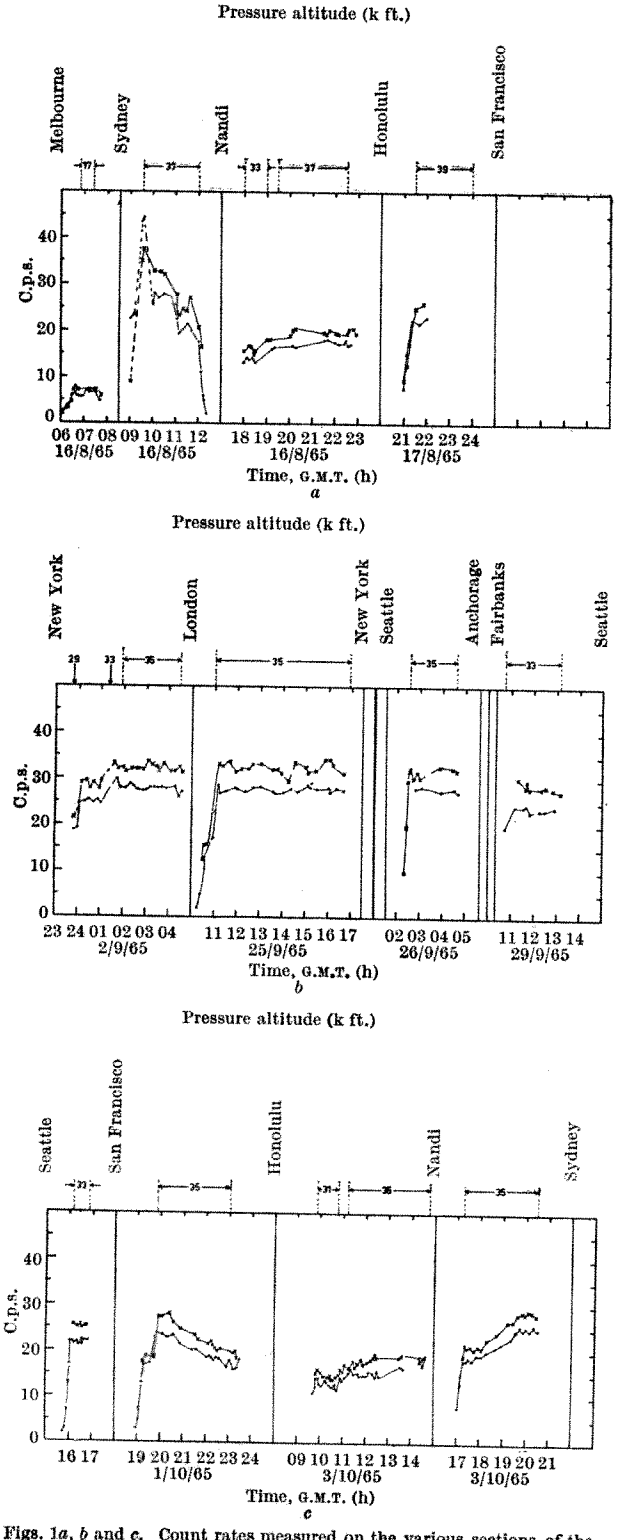
There is a general decrease in count rate as the aircraft approached the equator. The sections Nandi to Honolulu and vice versa include the crossing of the equator and have the lowest overall count rates of the whole trip. This effect is caused by the deflexion of low-energy particles by the magnetic field of the Earth, the intensity reaching a minimum at the geomagnetic latitude[1] with all readings corrected to an altitude of 35,000 ft. using profiles of count rate against altitude at various latitudes obtained in previous work. The points plotted in the region 50° S.–70° S. are taken from the southerly flight on June 3, 1965, already described[2].

From this plot it is seen that the minimum count rate occurred at the geomagnetic equator with a slow increase to 10°, and a steep increase between 25° and 40°. Between 40° and 50° there is a sudden drop in the slope of the curve, giving the so-called 'knee', and from 55° upwards the increase is very slow. Both curves are also symmetrical about the geomagnetic equator within the statistical limits of the points obtained.

At all times (with one exception—see later) on the level parts of the flights the apparatus gave a higher count rate with the lead shielding covering the tube. This is due to the production of secondary particles as the cosmic-ray particles pass through the lead. Also, due to their

energy spectrum, only a small proportion of the cosmic-ray particles are stopped by this thickness of lead (2·12 g cm^{-2}).

Two processes are involved in the increase in count rate with shielding. The first is cascade production where the increase is proportional to Z^2 (Z is the atomic number of the shielding material), and the second is star production where the increase is proportional to Z. Thus for high atomic numbers (for lead, Z is 82) the first process, cascade production, will dominate. From Fig. 2 it is seen that the fractional increase in count rate with 2·12 g cm^{-2} of lead



Figs. 1*a*, *b* and *c*. Count rates measured on the various sections of the trip, plotted against time (G.M.T.). ×, With lead; ●, unshielded

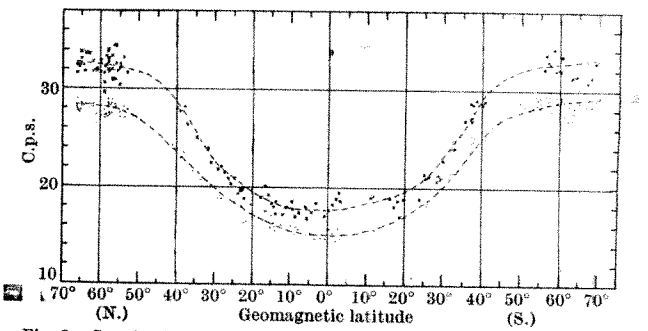


Fig. 2. Count rates corrected to 35,000 ft. plotted against geomagnetic latitude. ×, With lead; ●, unshielded

over the value without shielding at 35,000 ft. is 15 ± 1 per cent for all latitudes covered.

At various times during the trip, measurements were made of radioactive sources in the aircraft. It was found that on two occasions radioactive sources were being carried, and that the 'Exit' notices were also radioactive. In the first part of the trip from Melbourne to Sydney, measurements were made of the radioactivity of the instrument panel in the pilot's compartment. The various readings obtained are listed in Table 1.

Table 1

Date and flight	Position in aircraft	Count rate
August 16; TAA 418	6 in. from instrument panel	40 c.p.s.
	Pilot's seat	12
August 16; Qantas 530	Seat 16*E*: at seat-level	22
		8 (with lead shield)
	at floor-level	60
August 17; Qantas 586	6 in. from 'Exit' notice	20
	6·5 ft. away	0·5
August 25; BOAC 501	Maximum read in 'economy class' compartment	10
October 1; Qantas 597	6 in. from 'Exit' notice	60
October 3; Qantas 587	6 in. from 'Exit' notice	60

Table 2. FLIGHTS ON WHICH MEASUREMENTS WERE TAKEN

Date	Trip	Flight No.	Airline
16.8.65	Melbourne to Sydney	418	TAA
16.8.65	Sydney to Nandi	530	Qantas
16.8.65	Nandi to Honolulu	530	Qantas
17.8.65	Honolulu to San Francisco	586	Qantas
2.9.65	New York to London	2002	Pan American
25.9.65	London to New York	501	BOAC
26.9.65	Seattle to Anchorage	007	North-Western Airlines
29.9.65	Fairbanks to Seattle	902	Pan American
	Seattle to San Francisco	631	Western Airlines
1.10.65	San Francisco to Honolulu	597	Qantas
3.10.65	Honolulu to Nandi	587	Qantas
3.10.65	Nandi to Sydney	587	Qantas

The readings on Qantas Flight 530 and BOAC Flight 501 are due to radioactive isotopes being carried in the aircraft. This is the reason for the count rate with shielding being lower than the count rate without shielding during the first hour of the trip from Sydney to Nandi on August 16. The lead shield reduced the count number of particles from the radioactive sources carried in the aircraft. It was necessary to change seats during this flight so that cosmic-ray readings could be made without measurable interference. It was also necessary to avoid sitting too near to an 'Exit' notice which was radioactive.

A check calibration showed a reading of 10 c.p.s. could be produced by a radium source giving a dosage in the counter of 0·1 mr./h. Thus the maximum dosage observable during the trip of 60 c.p.m. is equivalent to 0·6 mr./h and so for 24 h a dosage of 14 mr. is possible. This may be compared with the acceptable rate of 60 mr./day. The total equivalent γ-ray dosage on a flight from the North to South Pole with a modern jet aircraft is estimated to be less than 5 mr. The cosmic radiation is not the same as γ-radiation from radium in that it penetrates to a greater extent, but the dosage at any part of the body would be well within acceptable limits. A new feature will arise in studying effects above 70,000 ft. where the heavy primary radiation exists, but the simple technique used has produced a valuable record of the cosmic and other radiation levels in the present-day jet airliner.

We thank the crews of the Qantas, Pan American, BOAC, TAA, Western Airlines and North-Western Airlines for allowing these measurements to be made and providing the flight records. The assistance of the Department of Civil Aviation in providing the letter of introduction to these airlines is acknowledged, and also in providing some funds towards the cost of an air fare. The help of the technicians at Naval Observatory at China Lake and at the University of Minnesota in repairing and testing the equipment is appreciated. This work is supported by an Air Force and a Radio Research Board grant.

[1] *World Maps of F2 Critical Frequencies and Maximum Usable Frequency Factors: Table of Geomagnetic Latitude of Points on the Earth in Various Geographic Latitudes and Longitudes* (U.S. National Bureau of Standards).

[2] Hopper, V. D., and Rawlinson, W. R., *Academy Res. Rep.* No. 10. *Cosmic Radiation in Aircraft using a Single Counter.*

CHROMIUM TRIOXIDE AEROSOL FROM HEATED 80:20 NICKEL–CHROMIUM WIRE

By P. GOLDSMITH, F. G. MAY and R. D. WIFFEN

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WHEN wires are heated to temperatures of about 1,000° C they give off a considerable number of very small particles. In laboratory experiments on the behaviour of sub-micron particles it is often convenient to produce the test aerosol by passing air over an electrically heated wire of such metal as 80 : 20 nickel–chromium alloy, platinum or silver. Since most laboratory work has been concerned with the physical characteristics of the sub-micron particles such as their diffusion, deposition and coagulation properties, very little work has been done on the chemical nature of the particles produced from hot wires. The only direct investigation on the chemical composition of the particles was done by Megaw and Wiffen[1] using neutron activation techniques. They concluded that the particles from heated nickel–chromium wires consisted mainly of chromium with about 2 per cent copper from impurities in the metal wire. There was no evidence of the presence of nickel in the particles. The aerosol production from a heated nickel–chromium wire is also dependent on the presence of oxygen in the surrounding gas, and this makes it reasonable to assume that the particles are composed of the stable oxide of chromium, chromium sesquioxide, Cr_2O_3. However, during recent work on the behaviour of sub-micron particles in gradients of temperature and in the presence of diffusing water vapour[2], it was concluded that the particles produced from a heated nickel–chromium wire were deliquescent. To account for this property it was speculated that some chromium trioxide, CrO_3, must be present in the particles. This has since been substantiated by direct chemical tests.

In these experiments the aerosol was produced by passing air over a nickel–chromium wire (electrical resistance 190 ohms/m) 30 cm in length and 0·0083 cm in diameter. The wire was mounted vertically down the centre of a 'Pyrex' glass tube 5 cm in diameter, the walls of which were water cooled. Air was passed through the tube at flow rates of between 4 and 10 l./min. The wire was maintained at a temperature of about 1,000° C by means of a stabilized mains a.c. supply. The aerosol, produced in this way, was stored in either a bag made of aluminized 'Milar', or a water-sealed steel gas holder of about 500 l. capacity. Under these conditions, a very high concentration ($> 10^6$/cm^3) of small particles ($< 0{\cdot}003$ μm radius) was produced, which initially coagulated very rapidly. After storage for about 18 h the concentration was about 5,000/cm^3 with a mean size of 0·05–0·1 μm radius as deduced from their diffusion characteristics.

The particles were found to have the following properties: (*a*) When the gas was supersaturated with water vapour the particles increased in size at a supersaturation lower than that expected from their physical size, assuming them to be insoluble. This increase in size was detected by measurements of their gravitational settling velocity. (*b*) When allowed to coagulate at low humidities in the 'Milar' bag (~10 per cent relative humidity), they formed chain-like aggregates; Megaw and Wiffen had also observed this[1]. Fig. 1 shows an electron microphotograph of such particles. Coagulation at high humidities (> 85 per cent) in the gas holder resulted in the formation of near-spherical particles. Fig. 2 shows the appropriate electron microphotograph. (*c*) After coagulation in the dry state to form chains, the particle shape could be modified to become more spherical by increasing the

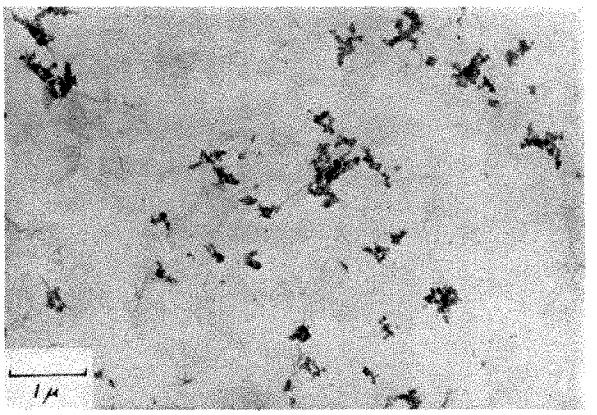


Fig. 1. An electron microphotograph of the particles produced by a hot nickel–chromium wire and allowed to coagulate for 18 h under dry conditions to form chain-like aggregates

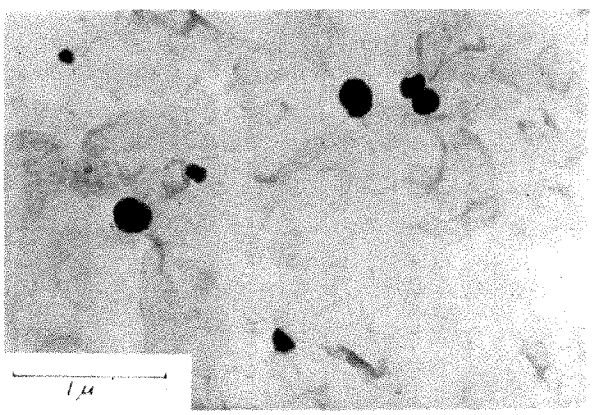
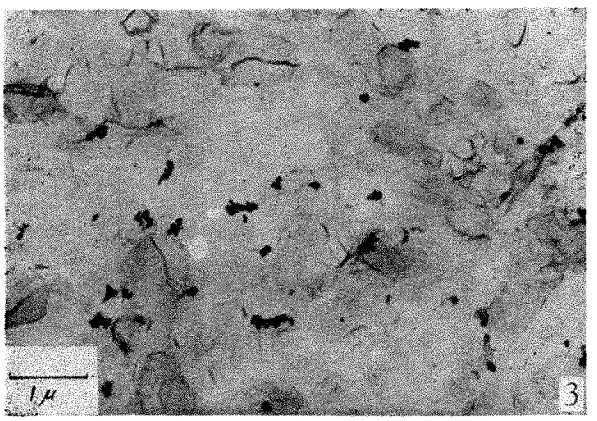
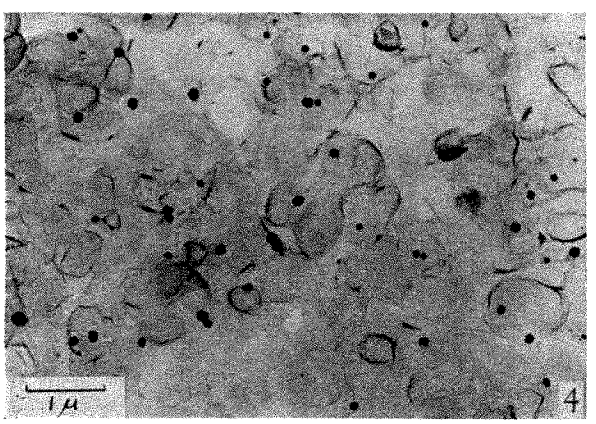


Fig. 2. An electron microphotograph of particles produced under similar conditions to those shown in Fig. 1, but allowed to coagulate under humid conditions. The deliquescent nature of the particles results in droplet-like coagulation to form spherical aggregates

humidity. The aerosol particles before and after humidifying are shown in Figs. 3 and 4. These three properties are all explained if the particles were deliquescent. That these properties are peculiar to particles from nickel–chromium was demonstrated by similar tests with an aerosol produced by a heated platinum wire. These particles coagulated to form chains even in a saturated atmosphere (Fig. 5). The stable oxide, Cr_2O_3, is, however, insoluble and so could not give rise to the deliquescent character of the particles. The presence of some chromium trioxide (CrO_3) on the particles would satisfactorily account for the deliquescence since the relative humidity over a saturated solution of chromium trioxide is 35 per cent. The presence of chromium trioxide in the aerosol deposited on the glass walls of the production chamber was confirmed by spot tests for the chromate ion using barium chloride and silver nitrate, and a blue colour was obtained with hydrogen peroxide which extracted into ether.

The production of chromium trioxide from heated nickel–chromium wires is at first sight surprising, if only because of the fact that this oxide of chromium decomposes at high temperature and the wire is at about 1,000° C. However, it may be that its production is an important step in the liberation and formation of the aerosol. Preliminary results on the relative proportions of the aerosol consisting of chromium VI and chromium III indicate that as much as half the aerosol may be in the form of chromium trioxide shortly after production from a wire at 1,250° C. It is not certain whether all the chromium leaves the surface as chromium trioxide vapour, some of which afterwards breaks down to chromium sesquioxide before leaving the hot zone around the wire, or whether some of the sesquioxide is released by quite a different process.





Figs. 3 and 4. Particles of the nickel–chromium aerosol before (Fig. 3) and after (Fig. 4) being passed into a humid atmosphere after initial coagulation in the dry state. The modification in shape is due to the surface tension of the water film on the deliquescent particles in the humid atmosphere

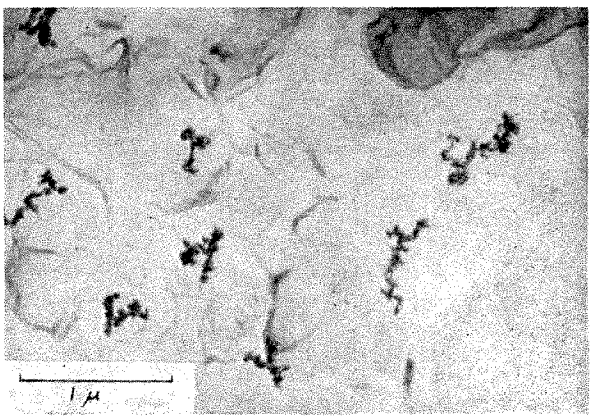


Fig. 5. An electron microphotograph of particles from a hot platinum wire which were allowed to coagulate under humid conditions. The insolubility of these particles results in a chain structure even at high humidities

The results of studies of the corrosion of heated nickel–chromium alloys are not at variance with the formation of chromium trioxide. In tests on the failure of 80 nickel–20 chromium alloy wires, Holler[3] found that the percentage of chromium in the metal fell from 20 per cent to 6·4 per cent when the wire was heated in air at a temperature of 1,100° C for 172 h. Holler suggested that the failure of electrical heaters was due to non-uniformity in the loss of chromium producing local changes in the electrical resistance which gives rise to 'hot-spots' in which loss of chromium is accelerated. Failure of the wire then occurs in these regions.

Work on the oxidation rate of nickel–chromium alloys at temperatures between 500° and 900° C by Gulbransen and Andrews[4] demonstrated the formation of a protective oxide coating which was predominantly chromium sesquioxide. The oxidation rate was dependent on minor constituents such as manganese and silicon as well as the temperature. Oxidation at temperatures between 1,000° and 1,300° C (ref. 5) showed three regions in the neighbourhood of the alloy surface. The innermost layer consisted of granules of chromium sesquioxide dispersed in pure nickel. The intermediate region consisted of granules of nickel chromite, $NiCr_2O_4$, dispersed in nickel oxide, NiO. The layer immediately in contact with the air was composed of pure nickel oxide.

The threshold temperature at which particles are produced by electrically heated nichrome wires is about 850° C. This suggests that the chromium leaves the surface at temperatures above 850° C to produce the sub-micron particles and that this temperature coincides with that at which the oxidation process changes from one type to the other. A study of the aerosol particles emitted by hot surfaces may be a neglected source of information on oxidation–corrosion mechanisms.

The identification of chromium trioxide as a constituent of the particles emitted by hot nickel–chromium wires has led to a consideration of the possibility of an associated hazard to health, as chromium trioxide, unlike chromium sesquioxide, is toxic[6]. It is possible from our results to make some rather crude estimates of the concentration of chromium trioxide in a room heated by a radiant electric fire.

The concentration of particles containing chromium trioxide in a room is a balance between the rate of production and the various loss processes. These processes are the deposition of the particles on the walls and other surfaces of the room, destruction of the chromium trioxide by chemical interactions with minor constituents in the air, and the ventilation rate of the air from the room. For sub-micron particles of chromium trioxide, the ventilation rate will be the controlling loss mechanism. Preliminary results so far obtained on the production of chromium

aerosol from 80 : 20 nickel–chromium wire as a function of temperature show that the production rate in dry air varies from 28 μg/h/cm² of wire surface at a temperature of 1,200° C to 0·14 μg/h/cm² at 950° C. These results were obtained by collecting the particles emitted from a short length (9 cm) of neutron activated nickel–chromium ribbon (0·063 cm × 0·015 cm cross-section) over periods of from 10 to 200 min and assaying the total amount of chromium in the particles from the radioactivity due to the neutron induced chromium-51.

If the rate of production of chromium is only dependent on the surface area of the wire and its temperature, then the production rate of chromium trioxide from a 1-kW electric fire element made of 26 s.w.g. nickel–chromium wire can be calculated, assuming in accordance with our findings that chromium trioxide makes up about half the chromium in the particles. Considering a room of 30 m³ in volume and allowing a ventilation rate of three air changes per hour, it can be shown, for a typical fire temperature of 950° C, that the average chromium trioxide concentration of the air in the room is about 10^{-3} of the 'maximum allowable concentration' of 0·1 mg/m³ given by the Ministry of Labour's *Safety, Health and Welfare Booklet* No. 8 (ref. 7).

This conclusion relates strictly to the particular batch of commercial wire (Nichrome V) used in these experiments, but it is of the type used in many domestic heating appliances. However, it may be expected that the chromium trioxide production rate will also vary with the minor constituents of the alloy, the length of time heated and the type of wire mounting used.

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VARIATION IN SOLAR INSOLATION TO THE SOUTH POLAR REGION AS A TRIGGER WHICH INDUCES INSTABILITY IN THE ANTARCTIC ICE SHEET

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WHEN considering the physics of past glacial advances it is necessary to divide glacial or potential glacial areas of the Earth into two regions: (*a*) regions where glacial ice now melts producing water which flows away from the snout of the glacier; (*b*) regions such as the polar regions where there is very little melting and where the ultimate fate of the glacial ice is either to sublime or to flow as ice on to the sea and be calved as icebergs

In the former case, for example, the Swiss Alps, Scandinavia and the temperate Alpine regions, a lowering of the mean annual temperature will lower the 0° C isotherm so that precipitation which flowed away as water will remain as ice and snow. If the cooling should be sufficient for the ice to spread on to the flat lowlands, the glacial advance will produce ice sheets. The effect is to some extent self-perpetuating in that large areas of ice and snow will cause local cooling.

In the second case, for example, Antarctica, where, because of the low temperatures, glacial melting is already negligible, further lowering of the mean annual temperature does not lead to a glacial advance. In fact, in these regions a lowering of temperature would lead to more extensive cover of the surrounding oceans by sea ice which would decrease the precipitation to the land. This would lead, in turn, to a retreat of the glaciers.

There is considerable evidence[1–3] that Antarctica was more extensively glaciated at times in the past than it is at present. It is frequently assumed (for example, refs. 3, 4 and 5) that the last of these glaciations occurred during the last northern glaciation. From the arguments already stated it can be seen that this is physically unlikely, and that it is more reasonable to expect Antarctica to have been more glaciated during the northern interglacial periods. Thus, it would be expected that periods of growth and recession in the Antarctic ice cap would be out of phase with the glacial phases of the more temperate regions.

Recent work[6–8] on heat transfer between northern and southern hemispheres makes it appear unlikely that temperate regions of one hemisphere could be in a glacial period while those of the other were in an interglacial period. This means that the Milankovitch theory[9], which is based on variations in solar insolation at 65° N. causing glaciations in the northern hemisphere, needs some drastic revision. As Zeuner[10] and others have pointed out, the variations in solar insolation calculated by Milankovitch are in themselves inadequate to produce large-scale glaciation. It has generally been felt that some additional mechanism was required. I suggested[11] that this mechanism was the inherent instability of the Antarctic ice sheet and that the instability might be triggered

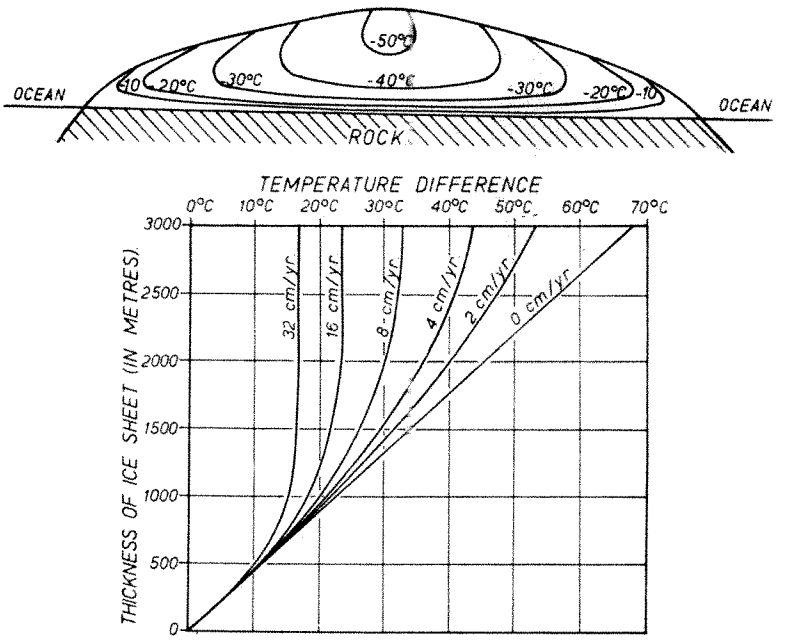


Fig. 1. Schematic diagram of the temperature distribution in a simplified ice sheet and a plot of the temperature difference between the base and other levels near the centre of such an ice sheet (after ref. 14)

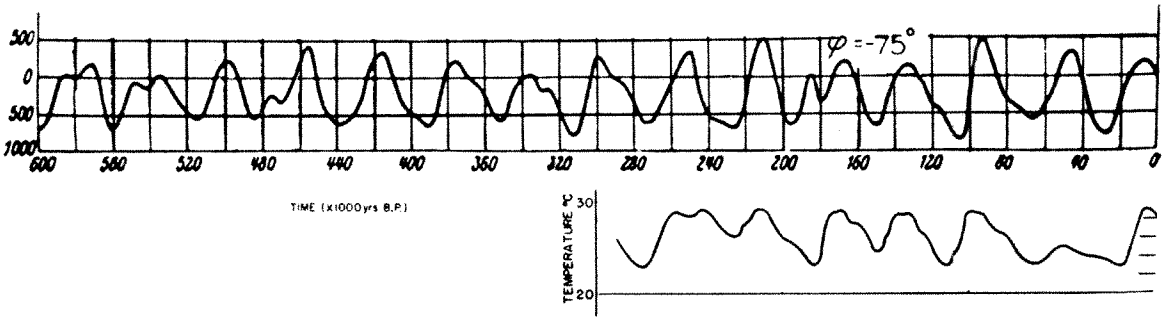


Fig. 2. Top curve: plot of the solar insolation for latitude 75° S. (after ref. 9) compared on the same time scale with the bottom curve, which is a plot of the generalized temperature curve for tropical surface waters (after ref. 13). Note correlation of curve minima

by a minimum in the solar insolation curve. E. P. J. Van den Heuval has, quite rightly, pointed out[12] that it is the variation in insolation to the polar regions that is of particular importance. I would like to enlarge further on this aspect of the theory.

As Emiliani[13] has pointed out, the palaeotemperature record of deep-sea cores seems best interpreted in terms of the periodicity of about 40,000 years. It is interesting that this corresponds to the period of oscillations of the obliquity of the Earth's ecliptic (usually indicated by ε), that is, variations in the angle of inclination of the Earth's axis to a line perpendicular to the plane of the Earth's orbit. This angle is at present 23·5°, but varies periodically between 24·5° and 21·5°. Minima in this value appear to correspond to minima in the temperature curve for tropical surface waters (as measured by the variations in the oxygen-16 : oxygen-18 ratio in the carbonate of foram shells in deep sea cores[13]).

Maxima in ε would correspond to the polar regions being more inclined towards the Sun in summer giving warmer summers in polar regions. Conversely, minima in ε would correspond to cooler summers in the polar regions. During the period of minimum ε the sea ice, which now forms a belt about 300–1,000 miles wide around the Antarctic continent in winter, would be more extensive and would persist farther north for a greater fraction of the summer. Since this would have the effect of moving the Antarctic ice cap farther from the ocean, the precipitation on the surface of the Antarctic sheet would decrease.

It can be seen, therefore, that the obliquity of the Earth's axis affects the insolation to the South Polar regions, which in turn determines the extent and persistence of the sea ice. This controls the effective distance of the ice cap to the open ocean and thus the precipitation on the ice cap. If the precipitation falls below a certain critical value (see Fig. 1) the temperature at the bottom of the ice cap rises above the pressure melting-point. This leads to a catastrophic instability which generates an ice shelf around Antarctica, as I have already proposed[11]. This ice shelf acts as a giant reflector which lowers the heat input to the Earth and drives the temperate regions of the Earth into an ice age.

The net accumulation of snow at the South Pole is 7·4 g/cm², and our recent work[15] has shown that this represents half the total precipitation. The total precipitation at the South Pole is therefore 15 g/cm². If, for example, precipitation were to be reduced by 30 per cent to 10 g/cm² and if the sublimation were to remain constant, the net accumulation would be only 2·5 g/cm²; the ice sheet in the model shown in Fig. 1 would become unstable. If the polar plateau were higher in the past than at present, as is suggested by glaciated nunataks[1–3], the percentage reduction in precipitation required to induce instability would be even less (see Fig. 1).

Thus it can be seen that a decrease of solar insolation in the South Polar regions due to cyclic reductions in ε would offer an attractive mechanism for explaining how the Antarctic ice sheet becomes unstable and thus triggers a catastrophic advance. Fig. 2 shows a comparison of the solar insolation curve in the South Polar regions calculated by Milankovitch[9] as compared with that for Emiliani's oxygen-16/oxygen-18 temperature curves for the surface ocean waters at low latitude[13]. As can be seen, the agreement is remarkably good.

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'DATING' GROUND WATERS OF AGES YOUNGER THAN 1,000–1,500 YEARS USING NATURAL SILICON-32

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FOR the determination of the characteristics of ground waters with respect to their rate of recharge from the surface by percolation of rain/river waters, two techniques based on natural radioactivities have so far been explored. These are based[1–3] on two radioisotopes produced by cosmic rays; namely, tritium (half-life, 12·3 yr.) and carbon-14 (half-life, 5,730 yr.); in recent waters appreciable amounts of these activities arise owing to their injections into the atmosphere during the testing of nuclear weapons. The principle involved in the application of these activities to investigations of ground water characteristics lies in the estimation of fractional decrease due to radioactive decay and thus obtaining the apparent 'age': corrections have to be made for any loss of activity

by other means, for example, chemical losses. The corrections are minimal for tritium, which closely follows the motion of bulk water molecules except for a small isotopic fractionation. In the case of carbon-14, an appreciable and variable loss occurs in its specific activity in the ground waters due to chemical exchange between the bicarbonate in solution and carbonate in limestone minerals present in soil or the water-bearing rocks[2,3]. The errors in the apparent carbon-14 'ages' due to this are estimated to be ± 1,000 yr., thus making the method useful only for dating waters older than 2,000 yr. The tritium method, on the other hand, is limited to determination of ages of water younger than 50 yr.—due to its short half-life.

There is thus a need for a suitable method of dating waters which are 50–2,000 yr. old. This article presents the results of our exploratory analyses carried out to study the possibility of using silicon-32, a radioactive isotope with a half-life of about 500 yr., which is continuously produced in the atmosphere by spallation of argon by the primary and secondary particles of cosmic rays[4]. Its activity has been determined in wet precipitations (rain/snow) and ocean water[5–7] and adequate information also exists on its production by cosmic rays[8]. It also seems certain that all silicon-32 on Earth has originated from cosmic ray action and that so far no measurable amounts have been added by nuclear weapon trials[6].

Silicon-32 decays by β-emission to phosphorus-32, the half-life of which is 14·3 days. The activity of silicon-32 is thus conveniently determined by 'milking' phosphorus-32 activity. We extracted silica from several surface and sub-surface waters by scavenging it with ferric-hydroxide. The extract was chemically purified and allowed to equilibrate with its daughter nuclide, phosphorus-32. The activity of phosphorus-32 was counted using highly sensitive β-counters (background 2–3 counts per hour, with a counting efficiency of about 40 per cent). The radiochemical and counting procedures have already been described in detail[6].

The results of determinations of silicon-32 activity in several surface and sub-surface waters are presented in Table 1. The sampling sites are marked in Fig. 1.

Using a few thousand litres of water, the net phosphorus-32 activities obtained are in the region of a few counts per hour. These small signals can nevertheless be measured with a high precision, because the decay of

Table 1. SILICON-32 CONCENTRATIONS IN SURFACE AND SUB-SURFACE WATERS

Code No.	Nature of sample, date of collection	Volume of water* (10^3 l.)	Observed net phosphorus-32 activity (c.p.h.)	D.p.m. silicon-32 per 10^3 l. water
T.F. 51	River Ganges at Hardwar (U.P.), 1/3/1964	8·3	14·0 ± 0·7	0·14 ± 0·01
T.F. 50	River Ganges at Allahabad (U.P.), 12/3/1964	6·5	6·5 ± 0·6	0·08 ± 0·01
T.F. 53	River Godavari at Nasik (Maharashtra), 26/5/1964	7·8	9·0 ± 0·09	0·11 ± 0·01
T.F. 49	River Godavari at Rajahmundry (Andhra), 20/1/1964	8·2	8·6 ± 0·09	0·08 ± 0·01
T.F. 27	Lake Tansa at Bombay (Maharashtra), 31/5/1963	4·8	6·0 ± 0·6	0·21 ± 0·02
T.F. 20	Tap water from Lake Tansa at Bombay (Maharashtra), 5/4/1963	2·5	3·1 ± 0·8	0·20 ± 0·05
T.F. 28	Tap water from Lake Tansa at Bombay (Maharashtra), 14/6/1963	8·0	14·5 ± 1·0	0·20 ± 0·02
T.F. 60	Tube-well at Lathi (Rajasthan), 190 m depth, 14/11/1964	4·3	1·6 ± 0·64	0·03 ± 0·01
T.F. 61	Tube-well at Dabla (Rajasthan), 220 m depth, 11/11/1964	4·6	2·1 ± 0·73	0·03 ± 0·01
T.F. 47	Tube-well at Vijapur (Gujarat), 40 m depth, 2/12/1963	4·7	2·6 ± 0·65	0·04 ± 0·01
T.F. 46	Tube-well at Pilvai (Gujarat), 140 m depth, 6/12/1963	4·4	1·3 ± 0·65	0·017 ± 0·008
T.F. 59	Tube-well at Banaras (U.P.), 100 m depth, 21/10/1964	2·8	0·5 ± 0·5	0·015 ± 0·015
T.F. 44	Open well at Vile Parle (Bombay), 21/11/1963	7·8	3·7 ± 1·2	0·04 ± 0·015

* This represents the equivalent volume of water from which silicon-32 was extracted: the actual volume taken was 15–30 per cent larger.

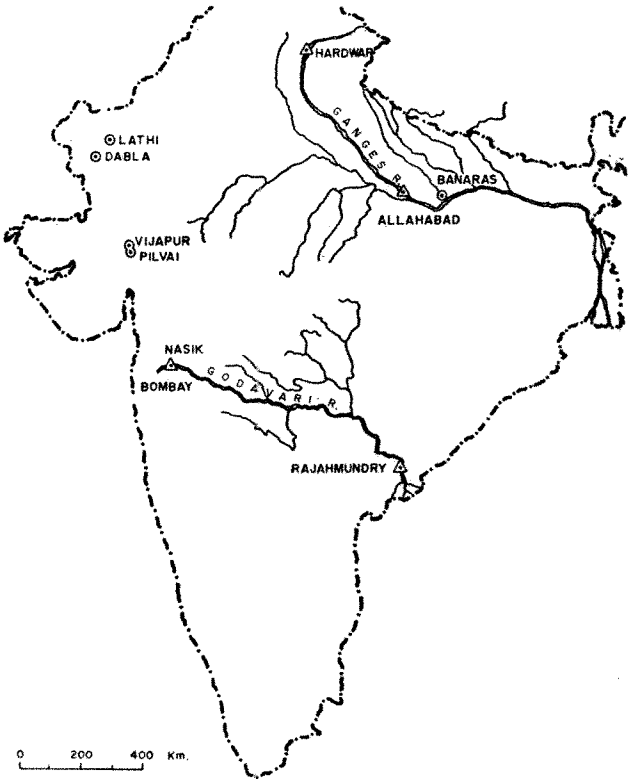


Fig. 1. Sampling sites of surface and sub-surface waters. △, River water samples; ○, tube-well samples

phosphorus-32 activities is followed over periods of 2–3 months. Fig. 2 shows some typical decay curves. Experience based on 'remilking' phosphorus-32-activities from weak silicon-32 activities has shown that we can detect a net counting rate due to phosphorus-32 if it exceeds 0·5 c.p.h., and a high precision in measurement is possible for signals exceeding 5 c.p.h.

The concentrations of silicon-32 in river samples (*T.F.* 51, 50, 53, 49) are in the range of about 0·1 d.p.m./10^3 l. The concentrations in tap water and Lake Tansa, which supplies water to the city of Bombay, are higher by about a factor of two. The tube-well samples from the semi-arid and arid regions of Gujarat and Rajasthan, respectively, have activities which are lower than those in rivers. The results for the Banaras tube-well, which is situated in the Gangetic plains, are not precise enough, as the volume of water processed was small.

The concentrations[6] of silicon-32 in wet precipitations in India average out at about 0·45 d.p.m./10^3 l.—a value which is considerably higher than that even observed for river water samples. In order to understand the geochemical behaviour of dissolved silica in rock/soil environments we carried out several tests, the results of which will now be summarized.

(1) The content of dissolved silica in rain water is small —of the order of 0·2–1·5 p.p.m. Water flowing over rock terrains, or in contact with soil, quickly attains large concentration values, 5–30 p.p.m., as are usually found in surface and sub-surface waters, depending on the rock/soil types. The chemical form of silicon in solution at *p*H less than 9, which, in the case for waters under question, is believed to be monomeric silicic acid[9], and the amorphous species of silica present in soil are supposed to undergo dissolution.

(2) If, to a sample of water containing 20 p.p.m. silica in solution, some soil is added for which the equilibrium silica solubility value is in the region of about 5 p.p.m., the concentration of dissolved silica rapidly drops to 5 p.p.m.

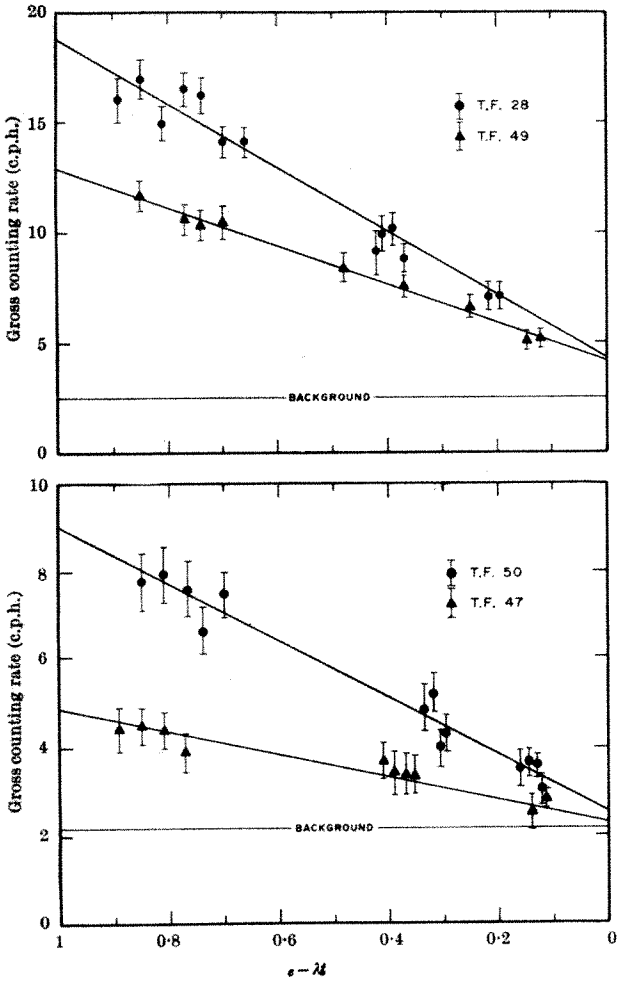


Fig. 2. Observed decay of phosphorus-32 activities 'milked' from silicon-32

(3) The total amount of silica which can be dissolved out of soil by contact with water is quite large—typical values are of the order of 1 per cent.

(4) If the concentration of dissolved silica is increased in a sample of water containing silicon-32 from nearly zero concentrations (0·5 p.p.m.) to about 10 p.p.m., no measurable decrease in silicon-32 activity occurs. (This result is based on an experiment carried out on a sample of rain water (5×10^3 l.). Half the water was processed directly for its silicon-32 concentration. 80 kg of red soil were added to the other half. The soil was stirred occasionally over a period of a month, and silicon-32 activity was then determined in the water.)

From the results already discussed here, and also from the fact that detectable amounts of silicon-32 are found in ground waters, it seems clear that the loss of silicon-32 by chemical exchange in a particular environment is unimportant. Appreciable losses would occur only when the environments alter so that an inorganic precipitation may occur. We ignore in our present discussions the role of biological processes for the removal of dissolved silicon, which seems important only in the case of marine environments or special cases of 'closed' water bodies.

Thus, depletions by chemical exchange between the dissolved silicon and silicon minerals of the water-bearing rocks seem to be unimportant. However, as the rock/soil types may change considerably along the migratory routes of water, significant losses may occur. The magnitude of losses can possibly be ascertained by measuring the concentrations of dissolved silicon at different regions. Analysis of water samples collected from several regions of Gujarat and Rajasthan area showed that these waters have similar silicon concentrations in the two regions (within ±5 per cent) and there was little variability in the sub-surface soil-gravel type. In these cases, it is probable that inorganic precipitation is unimportant.

The present results do not offer any simple explanation of why the Ganges and Godavari river waters near the points of their origin (Hardwar and Nasik, respectively) are already depleted in their silicon-32 concentrations by a factor of about four, whereas subsequent traversals of a thousand kilometres or more do not appreciably change the concentration. The concentration values for Lake Tansa lie between those of river and rain water; about half its total water is annually charged by wet precipitations.

At present, even crude 'age' estimates are not available from the hydrological data for the various tube-well samples. We could not detect any tritium activity in the Rajasthan or Gujarat wells (less than 0·5 T.U.). Detectable amounts of tritium were only found in the Gujarat region, close to the expected recharge zone. Extrapolation of these results indicates that the Gujarat waters may be older than 100 yr. The water supply in this region is rapidly depleting and deeper aquifer-beds have to be tapped. The aquifer in the Rajasthan area is believed to be confined, with a possible recharge from the south. The lack of sufficient rainfall in this area makes it likely that these deep-seated waters are of considerable age.

If a value of 0·08 d.p.m./10^3 l. is assumed for the waters feeding the ground, based on river water data, it is possible to estimate the apparent ages for the water in these tube-wells. Vijapur water would then be estimated to be the youngest of the four tube-wells sampled, *T.F.* 60, 61, 47 and 46, which is in good agreement with expectations considering the recharge direction. It can also be stated with some certainty that none of these waters is older than about 1,000 yr., which is quite significant in considering the hydrological problems encountered in the two areas.

There is a distinct advantage in measuring both tritium and silicon-32 for any study of the characteristics of ground waters. Usually it is not known whether or not the water tapped from an aquifer is isolated from an input system. If a small continuous input occurs, measurable amounts of tritium can be detected, as surface waters are now very rich in 'artificial' tritium. In such cases this will result in wrong apparent 'ages'. A small recent recharge, however, will not alter by any appreciable amount the silicon-32 ages.

Experiments are now being planned to evaluate the feasibility of using silicon-32 for precise 'dating' of ground waters; its validity in determining upper limits in the region of a few hundred years, for which no other method is at present available, seems to be established.

We thank the Exploratory Tube Well Organization of the Indian Government and the Deputy Engineer, Tube Well Maintenance Department, Government of Gujarat, for their help in collecting sub-surface waters for this work. We also thank Mr. P. G. Adyalkar and Mr. R. N. Athavale for several helpful discussions.

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AMINO-ACID COMPONENTS IN FOSSIL CALCIFIED TISSUES

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ABELSON[1] has shown qualitatively, using paper chromatography, the presence of aspartic acid, glutamic acid, glycine, proline, alanine, valine and isoleucine in fossil bone of Devonian age (380 million years old). Later, Wyckoff *et al.*[2], in similar investigations on more recent bone from the Pleistocene period (1 million years), found most of the amino-acids demonstrated by Abelson, and also hydroxyproline, the latter being virtually specific to the collagen group of proteins. In 1962, Little *et al.*[3], using electron microscopical methods, found collagen-like fibrillar fragments present in bone of Miocene age (25 million years old), and in the following year Isaacs *et al.*[4] also gave evidence for the presence of collagen in bone of Devonian and Triassic (200 million years) age, based on X-ray and electron diffraction investigations. Shackleford and Wyckoff[5] investigated bone of Miocene (and Pleistocene[2]) age by means of the electron microscope, and not only observed a fibrillar pattern, but also bandings similar to that characteristically associated with collagen fibrils. More recently Ho[6] has published quantitative data on the amino-acid residues in Late Pleistocene bone, the recovery pattern of which, while showing similarities to collagen composition, also indicated that differential rates of breakdown had occurred through geological time, some amino-acids, such as hydroxyproline, being very rapidly lost or destroyed.

In similar quantitative studies at the Royal Dental School, London, on considerably older specimens ranging in age from Jurassic (150 million years) to Devonian, the following were selected for preliminary analyses: (1) conodonts from the Genesee Group of the Upper Devonian from Erie County, New York; (2) a pliosaur tooth from the Kimeridge Clay of the Upper Jurassic of Ely; (3) a pliosaur ischium of the same age. The separation and assay of the amino-acids present in hydrolysates of the fossil specimens were made by means of a 'Technicon' automatic amino-acid analyser, and the results are shown in Table 1 and Fig. 1.

The results clearly confirm that micro quantities of many amino-acid components are to be found in fossil calcified tissues of considerable antiquity, but a comparison of them with a typical analysis of modern collagen provides only little evidence of affinity on compositional grounds. The amino-acids hydroxylysine and hydroxyproline, which are almost unique to the collagen group, are noticeably absent from the fossil material, though it does contain relatively large amounts of glycine, alanine and glutamic acid as in collagen. These two fossil tissues do show compositional similarities to each other and, from the work of Ho[6], it is conceivable that the amino-acid compositional profiles of the fossil material represent extrapolations of differential rates of loss and destruction,

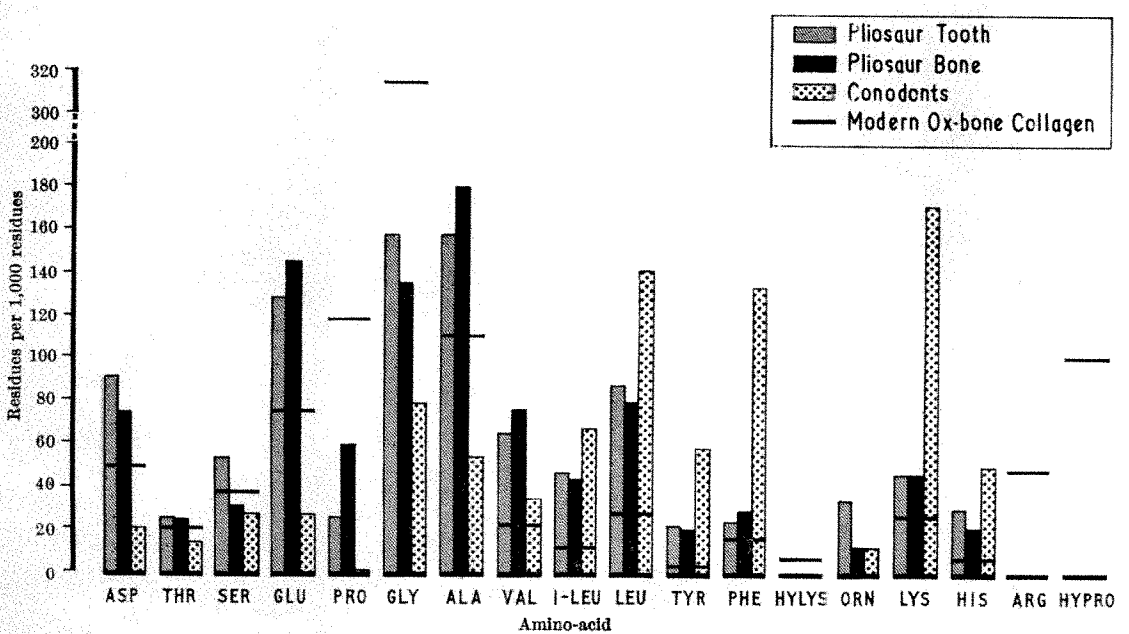


Fig. 1. Amino-acids in fossil calcified tissues expressed as residues per 1,000 residues

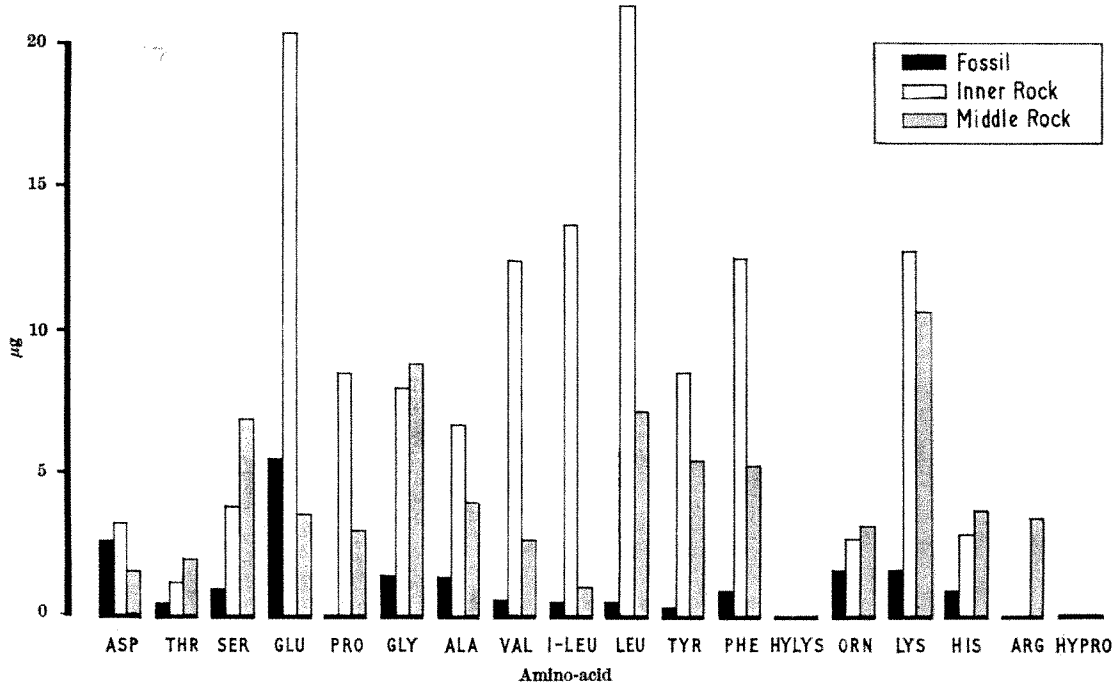


Fig. 2. Amino-acid per 5 g fossil or rock sample

although confirmation of this hypothesis requires further investigation. A comparison of the amino-acid pattern obtained from the conodonts with those of the two pliosaur fossil calcified tissues reveals little evidence of any affinity, the conodont hydrolysate showing only a trace of proline, relatively low concentrations of glycine, alanine and glutamic acid, and significantly large amounts of leucine, phenylalanine and lysine. Such a result could lead to the suggestion that the organic material in conodonts showed little similarity to fossil vertebrate calcified tissues, thus denying a possible vertebrate affinity for the conodonts as previously postulated by Tarlo[7].

However, arguments such as the foregoing can only be valid if, as some workers have suggested, the amino-acid components found in fossil material are derived solely from the remains of the organic matrices of the original animal tissues. With the observations of Erdman, Marlett and Hanson[8] that the same amino-acids found by Abelson in fossil bone are also present in both recent and ancient sediments, it was considered important that quantitative amino-acid analyses of the sedimentary rocks surrounding fossil specimens be carried out simultaneously with analyses of the fossil material itself. In consequence, a preliminary analysis has been made of ichthyosaur bone of Oxfordian (Upper Jurassic) age, together with sediments both immediately surrounding the fossil (inner rock) and sediments from a zone between 0·5 cm and 2 cm away from the fossil (middle rock). The quantitative results of these investigations are shown in Fig. 2.

Table 1. MICROGRAMS AMINO-ACID PER 5 g FOSSIL SPECIMEN

Amino-acid		Pliosaur tooth	Pliosaur bone	Conodonts
Aspartic acid	(ASP)	13·84	10·12	2·00
Threonine	(THR)	3·38	2·98	1·19
Serine	(SER)	6·54	3·36	2·21
Glutamic acid	(GLU)	21·54	21·92	3·09
Proline	(PRO)	3·28	7·14	trace
Glycine	(GLY)	13·62	10·43	4·50
Alanine	(ALA)	16·12	16·57	3·65
Valine	(VAL)	8·58	9·02	3·05
Iso-leucine	(I-LEU)	7·34	5·90	6·82
Leucine	(LEU)	13·10	10·89	14·00
Tyrosine	(TYR)	4·66	3·80	8·15
Phenylalanine	(PHE)	4·50	4·97	16·68
Hydroxylysine	(HYLYS)	0	0	0
Ornithine	(ORN)	5·08	1·19	3·44
Lysine	(LYS)	7·52	7·16	19·00
Histidine	(HIS)	5·12	3·26	5·90
Arginine	(ARG)	0	0	0
Hydroxyproline	(HYPRO)	0	0	0
Unknowns*		1·24	4·07	0

* Calculated as *nor*-leucine equivalents.

These results show that all the amino-acids detected in the actual fossil are to be found in considerably greater concentration in the surrounding rock. Certain amino-acids (glutamic acid, valine, isoleucine, tyrosine, phenylalanine and lysine) are found in the highest concentration in the inner rock zone, and this could infer that these amino-acid components had been leached from the bone at some stage, to become concentrated in the region immediately surrounding the fossil, with a subsequent slow diffusion into the surrounding sediment (middle rock). In contrast, the amino-acids threonine, serine, ornithine and histidine, which are found in higher concentration in the middle rock region, would have diffused from the surrounding sediment into the fossil, having originated from other organic debris.

Whether or not the foregoing hypotheses and explanations prove valid, it is apparent that the processes of differential breakdown and diffusion of organic substances involved in fossilization are far more complex than has previously been realized. For the present, caution must be exercised in drawing too obvious conclusions from the mere demonstration of organic components in fossil material without prior reference to the surrounding sediment. Further work is in progress on this topic in an attempt to clarify some of the many problems raised by these results.

We thank Prof. F. H. T. Rhodes for the gift of conodonts and the Medical Research Council for financial assistance enabling one of us (W. G. A.) to purchase apparatus.

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EVIDENCE FOR DEPOLARIZATION OF THE INTERNAL MEMBRANE SYSTEM IN ACTIVATION OF FROG SEMITENDINOSUS MUSCLE

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THE first direct evidence that the internal membrane system of skeletal muscle is involved in transverse activation was the demonstration by Huxley and Taylor[1] that application of current to certain sites of the frog muscle membrane produced reversible contractions which spread towards the centre of the muscle fibre without an equivalent longitudinal spread. Sensitive sites were confined to the level of the *Z* line, and, since a characteristic triad arrangement of internal membranes was found at this level of the sarcomere, they suggested that a component of these triads was associated with inward conduction of the 'influence' of surface depolarization. Since this report, the internal membrane system of skeletal muscle has been extensively investigated[2,3], and in most instances has been shown to consist of two discrete membrane-limited spaces, transversely oriented tubules (the *T* system), and longitudinally oriented sacs which envelop the myofibrils (the sarcoplasmic reticulum or SR). In frog twitch fibres, the membranes of the SR fuse to form cisterns (terminal sacs) at each end of the sarcomere which, together with a central transverse tubular element, constitute the triads. The *T* system appears to be directly continuous with the surface membrane[4,5] and also in close apposition to the terminal sacs of the SR[4,6,7]. Since these sacs appear to be specific regions for calcium accumulation[8], and since much evidence exists that the final step in activation is an elevation of the intracellular calcium ion concentration[9–12], it seems possible that depolarization beginning at the surface membrane could spread into the depth of the fibre by way of the *T* system to influence the release of calcium from the SR, and thus initiate contraction. As yet, however, there is no direct evidence for potential changes within either the *T* system or the SR as a step in the activation process.

Electrical activation. Both Csapo[13] and Natori[14] have reported that muscle fibres in which the sarcolemma had been removed could be activated electrically. This phenomenon was reinvestigated to see whether it could be attributed to current flow across the internal membrane system. A photomicrograph of the experimental preparation is shown in Fig. 1. A bundle of six to ten fibres was removed from a frog semitendinosus muscle in a sucrose Ringer's[15] or a normal Ringer's solution, blotted lightly, and covered with paraffin oil on a microscope slide. A short segment of one fibre was dissected free and the sarcolemma removed manually from about half its length. The procedure involved dissecting the sarcolemma and a superficial layer of myofibrils from the bulk of the fibre in one region and teasing this along the fibre until a length of about 1 mm had been skinned[16]; the sarcolemma can be removed in this way much as a sleeve is rolled up. Following dissection, the preparation was placed on a microscope stage, permitting simultaneous visual observation and cinephotographic recording. The preparation was illuminated by ordinary light with the condenser aperture stopped down until striations were readily visible.

To study the effects of current flow, silver–silver chloride wire electrodes were positioned in the two bundles of muscle tissue at the ends of the single fibre (Fig. 1*A*). This arrangement provided a large surface area of electrode contact relatively far from the skinned portion of the fibre, so that the irreversible and presumably unphysiological contractures seen at the site of contact between electrode and preparation[14] could be avoided in the skinned portion.

Passage of direct current pulses (200–1,000 msec in duration) between the electrodes resulted in reversible contractions in the skinned portion of the fibre; typical responses are seen in Figs. 1*C* and 1*E*, 2*B* and 3*B*. The contractions were confined to discrete regions, usually less than 50μ in longitudinal extent, and a contractile region as extensive as that seen in Fig. 2*B* was encountered only rarely. In all cases, only a small portion of the sarcomeres in a responsive region contracted, usually in groups of two to five adjacent sarcomeres with apparently relaxed sarcomeres between.

If the direction of current flow was reversed, variable results were obtained. In many instances, no response was elicited until the current was increased, while in others, a more vigorous response resulted from reversal of electrode polarity. Although the same general region of fibre usually contracted with reversed current flow, the distribution of contracting sarcomeres within this region was quite different and bore no apparent relation to the initial distribution. The lowest current that elicited contraction was 1·5 μamp in a fibre of 100μ diameter, a mean longitudinal current density of about 20 m.amp/cm². Thresholds were variable, however, and a current of 4–5 μamp was usually needed to elicit visible contraction.

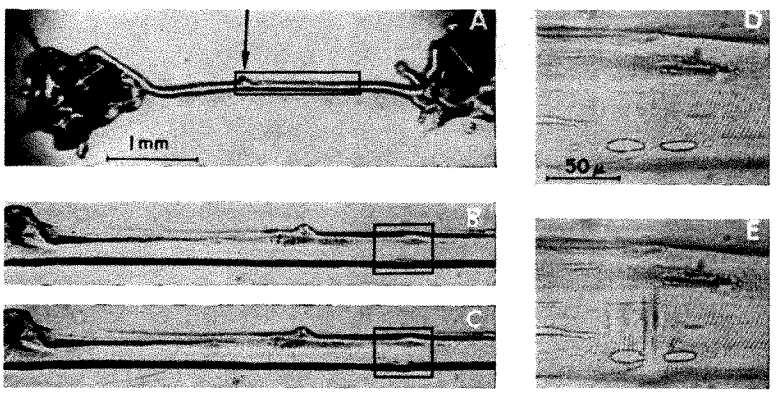


Fig. 1. Electrical activation of skinned muscle fibre. (*A*) Low-power photomicrograph of experimental preparation. Single fibre segment isolated between two multifibre bundles; the sarcolemma has been removed from the junction with the muscle bundle on the right to the point in the centre of the preparation indicated by the black arrow. The silver–silver chloride wire electrodes in the two bundles of muscle tissue at the ends of the single fibre are indicated by white arrows. The electrode in the muscle bundle on the right is not clearly seen. (*B*) and (*C*) Higher magnification of the region outlined in panel *A*. *B*, Before stimulation; *C*, peak of contraction during a 1-sec direct current pulse of 5 μamp. (*D*) and (*E*) Higher magnification of the region outlined in panels *B* and *C*. *D*, Before stimulation; *E*, peak of contraction during 500 msec direct current pulse of 10 μamp. The magnitude of the contraction to 5 μamp had decreased between *C* and *E*

Somewhat larger currents were required with pulse durations less than 100 msec; the threshold for brief pulses was not examined extensively, but, in one instance, a 1 msec pulse of 10 μamp elicited contraction. A few preparations were totally unresponsive, a finding more frequent when the dissection was hindered by the presence of large amounts of connective tissue within the muscle. Although the response disappeared with repeated stimulation, it was sometimes possible to stimulate at a rate of 1/sec for up to 5 min. Following disappearance of the response at one site, an increase in stimulating current usually produced contractions in different regions of the skinned fibre, but it was never possible, even with currents one hundred times threshold, to contract the entire skinned region. Similar responses were not seen in the unskinned portion of the fibre. 'Break' responses, brief localized contractions which followed the cessation of direct current pulses of 200 msec or longer, were readily obtained in both the skinned and unskinned portions, but were not investigated in detail. Large-tipped micropipettes (25–100μ in diameter) filled with 140 mM potassium methyl sulphate agar were occasionally used as electrodes with similar results.

Chloride activation. To test the possibility that longitudinal current acts by producing voltage changes across part of the internal membrane system, an attempt was made to mimic its effect by changing the ionic environment of the membranes. Since the surface membrane of the fibres had been removed under paraffin oil, the composition of the medium bathing the intracellular membrane system in these preparations was essentially that of the normal sarcoplasm and would be expected to have a relatively low concentration of diffusible anions[17]. An increase in the intracellular concentration of a diffusible anion would tend to drive the potential of the myofibrillar space positive with respect to the spaces within the internal membrane system; the magnitude of this potential change would depend on the anionic permeability of the internal membranes. Accordingly, the effect of elevation of the 'intracellular' chloride and methylsulphate ion concentration was compared by application to the surface of the skinned fibre of 0·2–1·2-mμl. droplets of 140 mM sodium chloride, potassium chloride, *tris* hydrochloride (*tris*(hydroxymethyl)aminomethane titrated to *p*H 7 with hydrochloric acid), or potassium methyl sulphate. Alternate droplets containing Cl^- or $CH_3SO_4^-$ were applied every 250μ along the skinned portion. Reversible contractions lasting 1–2 sec were seen in about half the trials the first time a region was tested with the chloride solutions; if a second chloride droplet was applied to the same region, the response was either markedly reduced or absent. The distribution of the chloride-induced contraction appeared to be determined by the inherent responsiveness of the fibre itself. In the experiment shown in Fig. 2, for example, the applied droplet remained well circumscribed, and, presumably, diffusion into the bulk of the fibre produced a symmetrical elevation of the chloride ion concentration about the site of application; nevertheless, a markedly asymmetrical contraction resulted. Sodium chloride, potassium chloride and *tris* hydrochloride were apparently equally effective in eliciting contractions, while the fibre was not responsive to similar droplets of potassium methyl sulphate; the contractions thus seemed to result from the elevation of intracellular chloride.

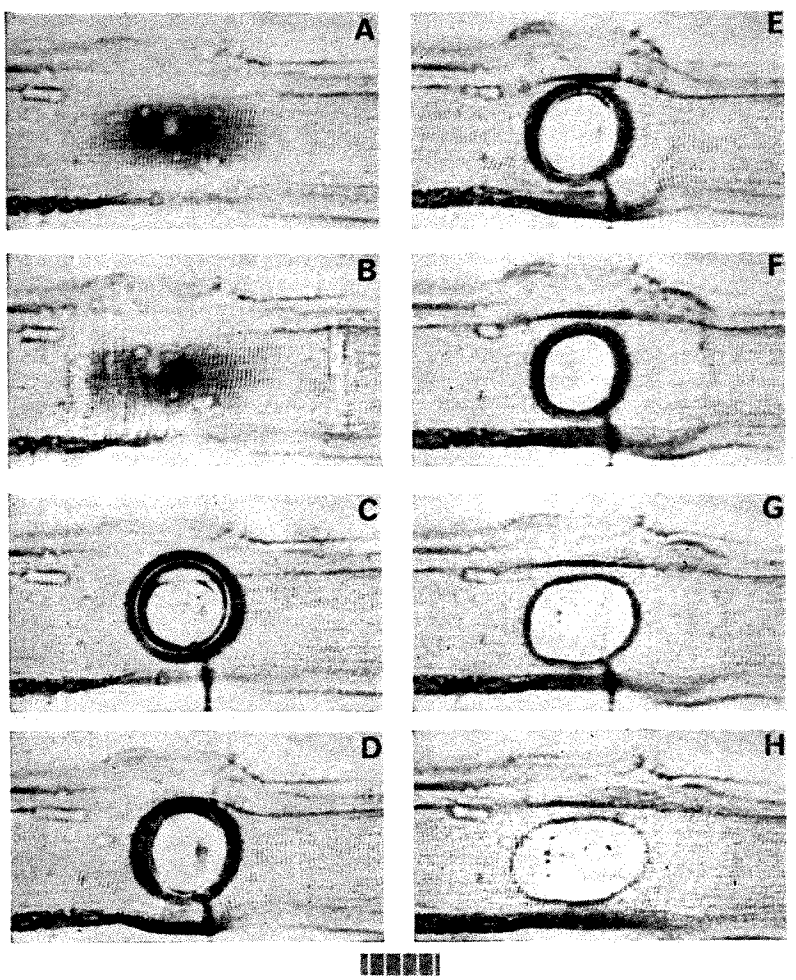


Fig. 2. Response of skinned muscle fibre to current flow and potassium chloride droplet. (*A*) Segment of skinned fibre. (*B*) Same region at peak of contraction during 1 sec direct current pulse of 8·3 μamp. The shadow in the centre of panels *A* and *B* is a potassium chloride droplet out of focus above the preparation. (*C*) Same region at moment of contact of 0·3 mμl. droplet of 140 mM potassium chloride with surface of skinned fibre. (*D*)–(*H*) 0·5, 1, 2, 3 and 4 sec following droplet contact. Note the asymmetrical distribution of the contraction about the region of application of the chloride solution. Grid spacing, 10μ

Over the range of droplet sizes from 0·2 to 1·2 mμl., the extent of the chloride-induced response was not correlated with droplet size. With much smaller droplets (less than 0·01 mμl.), however, chloride did not elicit contractions, presumably because the probability of producing an appreciable increase in chloride ion concentration in a responsive region was much less with the smaller droplets.

The results of chloride application differ markedly from the response of the skinned fibre to calcium application[18]. The calcium-activated contractions are invariably confined to the immediate site of application, and, when the fibre surface is explored, all regions are found to be responsive. In further contrast to chloride-induced contractions, the calcium-activated response can be elicited repeatedly at the same site, and the magnitude and duration of the contraction depend on the amount of added calcium. It is apparent that the mode of action of added chloride is quite different from that of added calcium.

Electrical and chloride activation. If the mechanism of activation by electric stimulation and chloride application were the same, it should be possible to activate the same regions of the skinned fibre with either stimulus. Since chloride application markedly reduced the response of a region to subsequent electrical stimulation, it was necessary to locate an active region first by electrical stimulation and then to

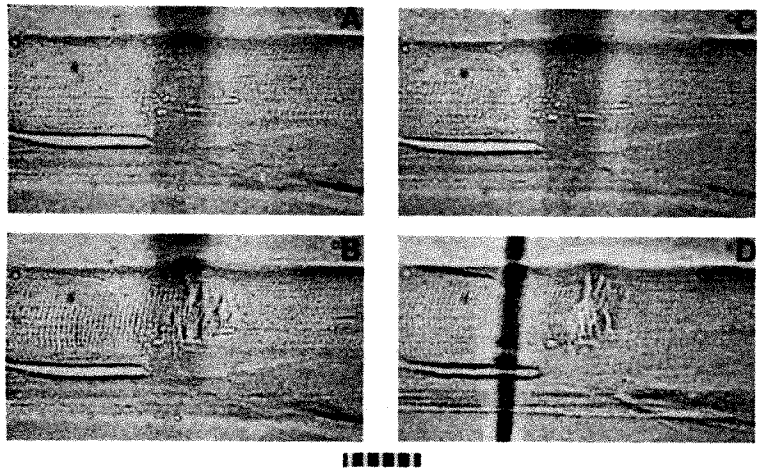


Fig. 3. Experiment showing similar response of skinned muscle fibre to electrical and chloride activation. (*A*) Segment of skinned fibre. (*B*) Same region at peak of contraction during 1 sec direct current pulse of 6·7 μamp. (*C*) Same region following cessation of electric stimulation. The shadow in the centre of the records *A*–*C* is a micro-pipette which is out of focus above the preparation. (*D*) Same region at the peak of contraction about 1 sec after application of 1·2 mμl. of 0·14 M potassium chloride to the preparation. The pipette had been lowered to apply the solution and is more clearly seen. Grid spacing, 10μ

test its response to chloride application; such an experiment is shown in Fig. 3. Fig. 3*B* was taken at the peak of a contraction induced by a 1-sec pulse of 6·7 μamp. A 1·2 mμl. droplet of 0·14 M potassium chloride was then applied to the slide immediately adjacent to the electrically responsive region of the fibre. The applied volume spread rapidly over the fibre surface and elicited the contraction seen in Fig. 3*D*. The distribution of this response is similar to the distribution of the electrical response (Fig. 3*B*), which suggests that both contractions were due to stimulation of the same specialized structure within the fibre. In other experiments chloride application and electric current usually stimulated the same general regions, but the chloride-induced contraction was often more extensive (Fig. 2) and currents many times threshold were able to stimulate only a part of the chloride-responsive regions.

These experiments strongly suggest that chloride application and electrical stimulation activate the skinned muscle fibre by a common mechanism. The spotty distribution of responsive regions indicates that these stimuli do not act on the contractile mechanism itself which, as demonstrated by calcium application, is uniformly responsive in the skinned fibre. It is, of course, possible that the dissection procedure damages the internal membrane system in some fashion so that the contractions induced by chloride application and current flow occur by a totally unphysiological process. Electron microscopic investigations, however, show an apparently intact sarcotubular system through the bulk of the preparation[8], and the finding that those fibres which were dissected with difficulty were less responsive to both current flow and chloride application also argues against this view. Since both stimuli can produce potential changes in the surface membrane, the most direct explanation for the present findings is that they produce similar changes in elements of the internal membrane system and that this leads to activation of the contractile mechanism.

The absence of response of the unskinned portion to current flow is not surprising; electrical activation in intact muscle is abolished when the membrane potential falls below 20–30 mV (ref. 19), and this is presumably the situation in the unskinned portion of the fibre. The responsiveness of the skinned portion to current flow implies that the dissection procedure serves to re-establish excitability in some way. One possibility is that, when the sarcolemma is dissected away, a potential difference can be maintained between the myofibrillar space and the interior of the internal membrane system. This could occur, for example, if the transverse tubules were to seal following disruption of continuity with the surface membrane. Although we have no direct evidence for such a mechanism, it is consistent with the finding that seemingly intact triadic structures are found even in isolated vesicle preparations[20].

If we accept the hypothesis that a potential change across the internal membranes is the mechanism for activation in the present experiments, the question then arises as to whether the primary change occurs across membranes of the *T* system or the SR. Since elements of both membrane systems course continuously in a transverse direction for long distances and seem to be confined longitudinally to one or two sarcomeres[3,5], the predominantly transverse distribution of most of the contractions as seen in Figs. 1, 2*B*, and 3 favours neither alternative. The current strength required to elicit contraction does, however, provide some evidence for excluding the *T* system from a primary role in this phenomenon. Using Sten-Knudsen's analysis[21] of the effect of a longitudinal electric field on the *T* system, the potential across the tubular membrane would change by only a fraction of a millivolt with the current strengths employed in the present experiments. In this case, it would seem more likely that a displacement of the potential across the membranes of the SR itself is the mechanism for activation by externally applied current. (The following values were used in the calculation: the specific resistivity of the SR and *T*-tubule membrane was taken to be that of the surface membrane: 3,000 Ω-cm² (ref. 22). The specific resistivity of the interior of the SR and *T*-tubule was taken to be that of normal Ringer's fluid: 80 Ω-cm. The specific resistivity of the sarcoplasm was taken as 200 Ω-cm[22]. The diameter of the *T*-tubule was taken as 350 Å (ref. 5). The longitudinal stimulating current was taken as 50 m.amp/cm² (4 μamp in a fibre 100μ in diameter), and the current density was assumed to be uniform through the cross-section of the fibre. It should be noted that the calculated potential change across the *T*-tubule is essentially the same for values of tubular membrane and tubular lumen resistance which are an order of magnitude different from those used here.)

Any estimate of voltage changes across the SR membranes involves considerable uncertainty because of the complex geometry of the SR, but, taking a space constant of 60μ and considering the SR as composed of discrete cylinders 400 Å in diameter and 3μ long, the present currents would produce a 1·5-mV displacement of membrane potential at each end of the sarcomere (see earlier). If specialized junctions with a low electrical resistance exist between adjacent terminal sacs by way of the *T* system[7], the effective length of the SR would be increased and the change of membrane potential in a longitudinal field would be greater. In this situation, current could enter or leave the sacs over more than one sarcomere and this might account for the observation that the electrical responses usually involved a few adjacent sarcomeres even when chloride application produced a rather extensive response (Fig. 2).

The present experiments demonstrate that the contractile mechanism of the skinned muscle fibre can be activated by stimuli which are known to depolarize the surface membrane. The possibility that these stimuli act directly on the SR, considered with the localization of calcium to the terminal sacs of the SR[8], suggests that translocation of calcium from the terminal sacs to the myofilament space can be produced by appropriate potential changes across the SR membranes. If this is the case, the normal sequence of excitation-contraction

coupling could involve a sequential depolarization of the surface membrane, T system and, by a mechanism which is still unclear, subsequent depolarization of the terminal sacs of the SR.

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A PHYSIOLOGICAL METHOD FOR DETERMINING THE DEGREE OF METEOROLOGICAL COOLING

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THE great importance of cooling, both for general physiological and clinical studies, explains the many research studies carried out in the past to develop a simple method for determining this cooling factor. However, it is generally known that it is very difficult to determine accurately the degree of atmospheric cooling with a meteorological instrument. Hill's katathermometer[1], the Frigorimeters of Dorno[2], Thilenius[3], Wierzejewski[4], Henneberger and Mörikofer[5] and other similar instruments give only an approximate picture of the physiological influences due to the combined effect of temperature, air movement and humidity known as the 'cooling index'.

Bedford[6], Siple[7], Court[8] and Thomas and Boyd[9] suggested an empirical formula for calculating the cooling index: $h = \Delta t\ (9{\cdot}0 + 10{\cdot}9\ \sqrt{v} - v)$ in which h is the heat loss or wind chill in kcal/m²/h; Δt is the difference in temperature between the surface of the human body and the environment in °C; v is the speed of the wind in m/sec.

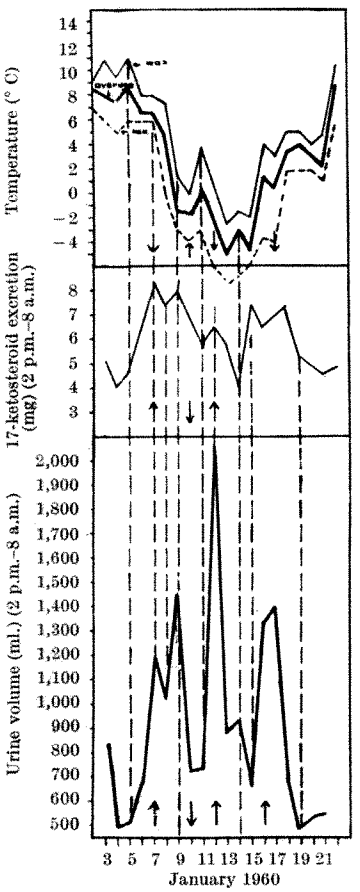


Fig. 1. Urine volume, 17-ketosteroid excretion and air temperature (——, maximum; - - -, minimum; ——, average) during the period January 3–22, 1960

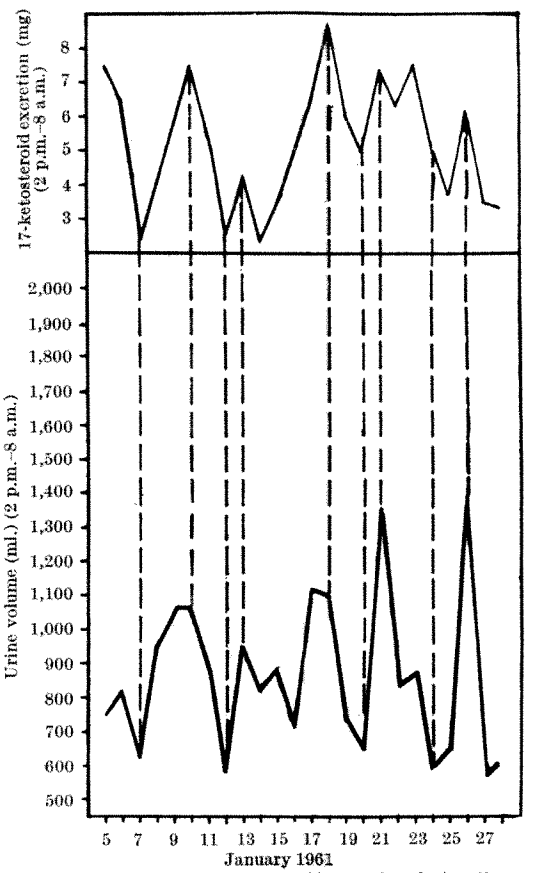


Fig. 2. Urine volume and 17-ketosteroid excretion during the period January 5–28, 1961

A very useful formula was also suggested by Thom[10] at the U.S. Weather Bureau:

The discomfort or temperature–humidity index (THI) = $Td - (0{\cdot}55\text{–}0{\cdot}55\ R) \times (Td - 58)$

in which Td is the dry bulb air temperature in °F, R is the percentage of relative humidity (65 per cent, indicated as 0·65). However, in this latter formula the effect of air movement was neglected, which restricts the field of application to indoor conditions.

In some earlier investigations I found[11] that in subjects with an efficient thermoregulation mechanism[12,13] a num-

ber of characteristic physiological changes occur, as a result of cooling, which seem to give a better picture of the degree of atmospheric cooling from a biological point of view. The changes observed can be summarized as follows: (1) During periods of strong atmospheric cooling a sharp increase of the daily urinary volume occurs within 12 h after a subject has been exposed to the open air for an hour (or even less). With a slight delay in time, the 17-ketosteroid excretion in the urine and the alkalinity increased. These processes are also accompanied by a decreased excretion of hexosamines, of chlorides, sodium and urea. Potassium usually decreased, but due to dietary conditions an increase was sometimes noted. Examples are given in Figs. 1–4. (2) Depending on the degree of cooling, the daily changes of these different physiological parameters may fluctuate as follows: Urinary volume between 500 and 2,000 c.c. (in the adult male subjects studied during our experiments), 17-ketosteroids may fluctuate up to 300 per cent (for example, 9–3 mg/24 h), hexosamines between 20 and > 70 μg/100 c.c., chlorides between 60 and 190 m.equiv./l., sodium between 110 and 170 m.equiv./l., urea between 600 and 2,200 mg/100 ml., the *p*H of urine (at 6 p.m. before dinner) between 4·9 and 6·9 (or more).

In subjects with an efficient thermoregulation mechanism this group of 7 curves (partly parallel, partly inverse) is

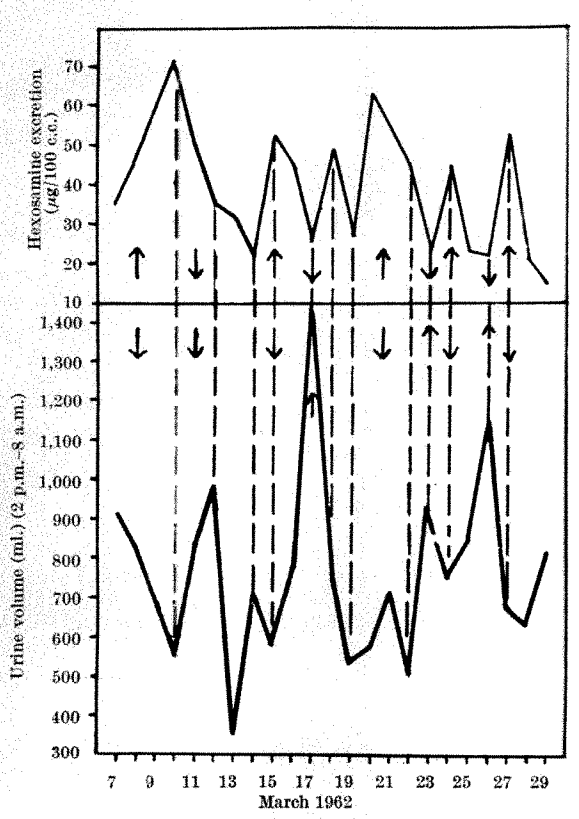


Fig. 3. Urine volume and excretion of hexosamines during the period March 7–29, 1962

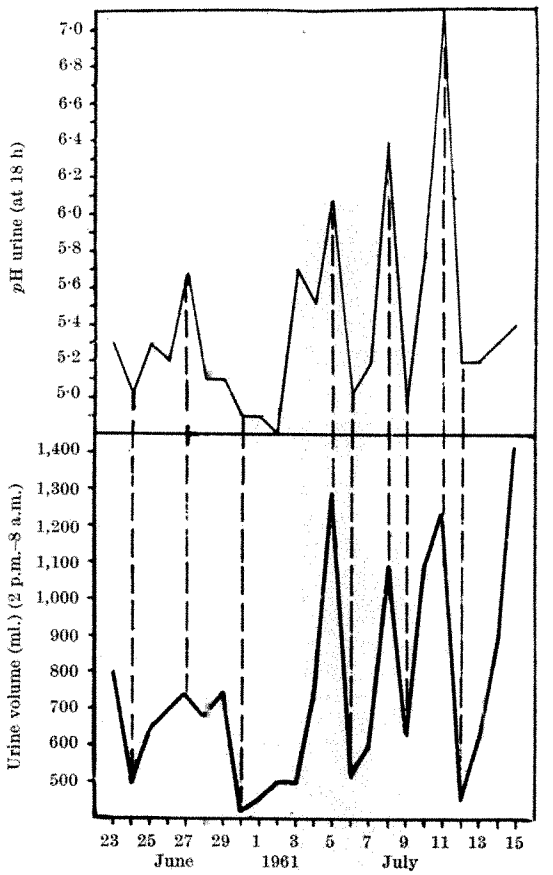


Fig. 4. Urine volume and *p*H during the period June 23–July 15, 1961

so characteristic and indicates so clearly the degree of atmospheric cooling that both for biological and clinical studies it is strongly recommended that this physiological method be used in conjunction with the existing meteorological instruments already mentioned.

As cooling is primarily a biological concept, based on certain human experiences as a result of changes in the thermal environment, it also seems logical to express the cooling index mainly in biological units.

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ERYTHROCYTE PHOSPHOGLUCONATE DEHYDROGENASE POLYMORPHISM

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VARIOUS autosomally inherited differences in electrophoretic character[1–4] and quantitative enzymatic activity[3,5–7] of human red cell phosphogluconate dehydrogenase are known. Fig. 1 is a composite photograph showing eight different phenotypes, after starch-gel electrophoresis of haemolysates and specific staining for this enzyme. Each of the types has been encountered at least once during an electrophoretic survey of 4,558

unrelated individuals from among the patients, students and staff at the London Hospital and Medical College, and during family studies.

About 95 per cent of unrelated subjects were found to possess the single sharply defined electrophoretic band *A* shown in Fig. 1*a*. This 'normal' phenotype is a homozygous state for the enzyme, for which the genotype $PGD^{\alpha}PGD^{\alpha}$ has been proposed[2,4]. Two faint 'satellite' bands (not illustrated), which run ahead of the *A* band, are sometimes seen when the gels are overstained.

Haemolysates from certain related individuals were found to give a single *A* band with substantially reduced intensity of staining (Fig. 1*b*). When assayed, these haemolysates showed only 50–60 per cent of the normal activity. This 'half activity' trait is inherited autosomally and is a heterozygous condition[3,5–7] with one usual gene, PGD^{α}, and one almost completely inactive gene, PGD^{0}. It is not at present certain whether the gene determining this quantitative variant is an allele of the genes determining variations in electrophoretic character but, for reasons given below, it is provisionally regarded as such, and given the symbol PGD^{0}.

Half activity phenotypes can easily be missed during routine electrophoretic investigations and are best discovered by enzymatic assay and checked by family studies. We have recently begun to examine our local population by spectrophotometric assay, for this trait, and as yet do not have a good estimate of its occurrence, but would agree with Brewer and Dern[5], who are studying this condition in the United States, that the frequency is low, and probably less than 1 per cent.

Fig. 1*c* shows a haemolysate electrophoretic pattern with three bands which has been found in 188 individuals (an incidence of 1 in 24). The fastest of these bands is an *A* band; the middle band, *B*, is about as intense as the *A* band; and slowest and faintest of all is the *C* band, sited slightly closer to the middle band than is the *A* band. This triplet pattern was first reported as two bands[1], but as the electrophoretic technique improved it was realized that the third faint *C* band was always present[2]. This phenotype, described as the 'Common Variant', has been shown by family investigations[1,4] to be autosomally inherited and to be a heterozygous condition: it has one usual gene, PGD^{α}, and a structurally modified allele, PGD^{β}. When subjected to mild stress in various ways (by incubation of haemolysate with 0·2 M *tris* buffer at *p*H 8·0 and 37° C for 15 min, or by incubation at 25° C with 1·5 M urea or 0·01 M iodoacetate for similar short periods), common variant haemolysates lose significantly more activity than do normals[8].

Electrophoretic examinations of blood collected in Brazil (35 samples), Nigeria (100 samples) and Uganda (109 samples) revealed three, twelve and twelve instances respectively of the common variant, suggesting that the incidence of the PGD^{β} gene may be greater in these parts of the world than in London.

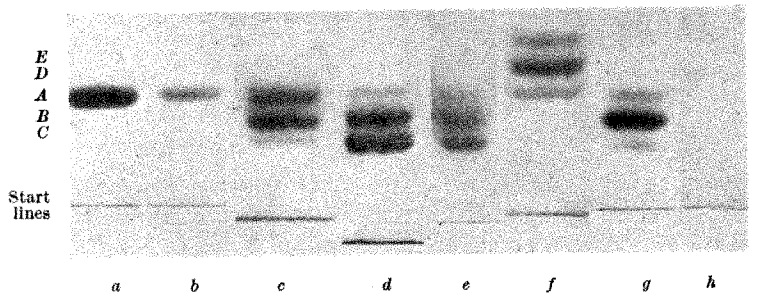


Fig. 1. Composite photograph showing eight different electrophoretic patterns found for human red cell phosphogluconate dehydrogenase after starch-gel electrophoresis of haemolysates. The *A* bands have been placed in line. Since the conditions varied somewhat from experiment to experiment, the start lines do not always coincide. Haemoglobin moves towards the cathode, on the other side of the start line, and does not show. Electrophoretic conditions and staining procedure for the enzyme were essentially as previously described (ref. 1), but potential gradients of 12–24 V/cm were used for periods of 4–6 h

The opportunity occasionally arose to examine extracts of human white cells and other body tissues in addition to red cell haemolysates. Individuals with a normal type of phosphogluconate dehydrogenase in their red cells were always found to have a normal type in other body tissues, whereas when the common variant phenotype was found in the erythrocytes, this was reflected by the presence of the common variant triplet in the extracts from other body tissues, although the relative intensities of the bands sometimes varied. Thus it appears that for this enzyme an inherited variation detected in the red cells is indicative of the presence of that same variation in all other body tissues.

The electrophoretic pattern shown in Fig. 1*d* was given by haemolysates from two subjects in the survey. The same three bands are present as in the common variant, but they have different intensities: the *C* band is the most intense, the *B* band is intermediate in intensity and the *A* band is faint. Normal or near normal activity (70–90 per cent) is found on assay. This phenotype is described as the 'Canning' variant, after the Canning Town district of London where the first subject discovered with this phenotype resides. White cell extracts from these individuals showed a strong *C* band only. Family studies[4] indicated that the Canning variant is the homozygous state for the atypical allele PGD^{β}. If the greater lability to mild physical and chemical stresses of the common variant (genotype $PGD^{\alpha}PGD^{\beta}$) when compared with the normal (genotype $PGD^{\alpha}PGD^{\alpha}$) is to be attributed to the PGD^{β} gene, then the $PGD^{\beta}PGD^{\beta}$ genotype of the Canning variant would be expected to be even more labile, and this was found to be so experimentally[8].

Two brothers gave the electrophoretic pattern of the Canning variant, but the bands stained only weakly (Fig. 1*e*) and the haemolysates showed something less than half activity (40–50 per cent of normal)[3,7]. The seven children of these two brothers segregated into either half activity variants (5 children) or into common variants (two children). The wife of one of the brothers was demonstrated to be of normal phenotype and it is likely that the wife of the other brother was also. Thus it seems that these 'half activity Canning' variants must be of genotype $PGD^{\beta}PGD^{0}$. The segregation of the children into either half activity or common variant phenotypes is noteworthy, and indicated that in this family the genes for quantitative variation and for variation in electrophoretic character are separate from one another. It thus appears likely that these two abnormal genes are alleles of one another (and of a single normal gene).

Fig. 1*f* shows a 'Richmond' triplet, which has occurred twice at random in our survey. The slowest band of this triplet is an *A* band, and the middle band, *D*, is symmetrically placed and has substantially greater intensity than the bands on either side of it[3,4]. Like the other phosphogluconate dehydrogenase variants, the Richmond phenotype has been shown by a family study to be a heterozygous condition, autosomally inherited, and it has been given the genotype $PGD^{\alpha}PGD^{\gamma}$.

Another rare triplet pattern, the 'Hackney' variant, is illustrated in Fig. 1*g*. The slower two bands, although in approximately the same positions as the *B* and *C* bands of the common variant, do not appear to coincide exactly with them, and for this reason are referred to as the *B′* and *C′* bands. The *A* and *C′* bands have approximately similar activities while the symmetrically placed middle *B′* band is substantially more intense. Genetic studies have indicated that this triplet is also a heterozygous condition, autosomally inherited, and the genotype $PGD^{\alpha}PGD^{\delta}$ has been proposed[4].

A brother and sister were discovered who gave no staining reaction at all for erythrocyte phosphogluconate dehydrogen-

Table 1. HUMAN RED CELL PHOSPHOGLUCONATE DEHYDROGENASE PHENOTYPES

Position in Fig. 1	Phenotype No.	Description of phenotype	Approximate activity (as a percentage of normal)	Proposed genotype	Incidence in non-related individuals in London (percentage)
a	I	Normal	100	$PGD^{\alpha}PGD^{\alpha}$	95
b	VI	Half activity	50–60	$PGD^{\alpha}PGD^{\varepsilon}$	(say) 1
c	II	Common variant	80–100	$PGD^{\alpha}PGD^{\beta}$	4
d	III	Canning	70–90	$PGD^{\beta}PGD^{\beta}$	0·05
e	VII	Half activity Canning	40–50	$PGD^{\beta}PGD^{\varepsilon}$	0·02
f	IV	Richmond	100	$PGD^{\alpha}PGD^{\gamma}$	0·05
g	V	Hackney	100	$PGD^{\alpha}PGD^{\delta}$	0·02
h	VIII	Fully deficient	0–10	?	0·02

ase after electrophoresis (Fig. 1*h*), and haemolysates showed about 5 per cent of the activity of normals when assayed spectrophotometrically[7].

The properties of the eight known phosphogluconate dehydrogenase phenotypes in the human are summarized in Table 1. Seven of them can be regarded as resulting from combination of the gene products of identical or non-identical gene pairs selected from five alleles at a single autosomal structural locus.

Three different autosomally inherited phenotypes of phosphogluconate dehydrogenase have also been shown to be present in the red cells of the rat, and starch-gel patterns for these are shown in Fig. 2. The single 'slow' band (Fig. 2*a*) is given by haemolysates from laboratory rats (it is considerably faster than the *A* band of the human). However, this type, plus two additional phenotypes, one with a single 'fast' band (Fig. 2*c*) and one with a triplet of bands comprising both fast and slow bands and also an intermediate band of substantially greater intensity, were obtained when a number of wild rats trapped in the London area were examined. These observations suggested that rats with the slow band are homozygous for a gene PGD^{s}, that rats with the fast band are homozygous for a gene PGD*f*, and that animals with the triplet bands are heterozygous with the genotype $PGD^{s}PGDf$. A possible genetic mechanism is set out in Table 2. These

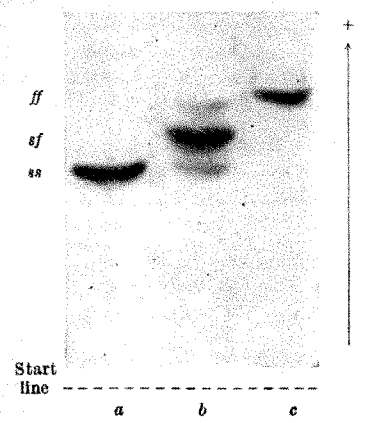


Fig. 2. Three starch-gel electrophoretic patterns given by rat red cell haemolysates when developed for phosphogluconate dehydrogenase activity

Table 2. FORMATION OF PHOSPHOGLUCONATE DEHYDROGENASE ISOENZYMES BY ALLELIC GENES PGD^{s} AND PGD*f*

Genotype	Enzyme sub-units (gene products) Type	Amount (per cent)	Isoenzymes (formed by random association of sub-units in pairs) Structure	Rate of migration	Amount (per cent)
$PGD^{s}PGD^{s}$	*s*	100	*ss*	Slow	100
$PGD^{s}PGDf$	*s*	50	*ss*	Slow	25
	f	50	*sf*	Intermediate	50
			ff	Fast	
PGD*f*/PGD*f*	*f*	100	*ff*	Fast	100

suggestions are supported by examination of the progeny in a breeding programme using rats of known phosphogluconate dehydrogenase phenotypes[9]. Workers in Baltimore[10] have recently found similar slow, fast and triplet bands of phosphogluconate dehydrogenase to be present in pigeons, and they have also concluded that the enzyme is autosomally inherited and that the triplet phenotype is a heterozygous state.

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STRUCTURAL STUDIES OF PIG HEART MALATE DEHYDROGENASE

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COMPARATIVE structural studies of pig heart malate dehydrogenases (*E.C.* 1.1.1.37) have been initiated in this laboratory. The mitochondrial enzyme (*m*-MDH), obtained from Seravac Laboratories (Pty.), Ltd., was submitted to final purification by *CG*-50 column chromatography using a modification of the method of Thorne[1]. Supernatant malate dehydrogenase (*s*-MDH) from the same source was used without further purification.

Comparative Fingerprint Analysis of *m*-MDH and *s*-MDH

Both enzymes were labelled with ^{14}C-iodoacetate after denaturation in 8 M urea, and tryptic hydrolysates were prepared. Two-dimensional 'fingerprints' were made using a double buffer system[2] with pyridine acetate buffer, *p*H 6·5, between the origin and the cathode, and pyridine acetate, *p*H 5·0, between the origin and the anode in one dimension. Formic acid–acetic acid buffer, *p*H 1·9, was used in the second dimension. The fingerprints of *s*-MDH and *m*-MDH are shown in Fig. 1.

Approximately 36–38 peptide spots appeared on fingerprints made from the tryptic digest of *m*-MDH. Six of these peptide spots were positive when tested with the Pauli reagent and five of the spots reacted with the Sakaguchi reagent. Initially five peptides (one neutral and four acidic) containing ^{14}C-*S*-carboxymethyl cysteine (CMC) were located by autoradiography. Later two additional radioactive CMC-peptides were resolved. The first of these was recognized when, during purification, the neutral peptide (*T*-*N*) was separated into two components by paper chromatography. The second 'addi-

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tional' peptide was found when the most acidic peptide (*T*-6) was resolved into two components (*T*-6*a* and *T*-6*b*) by paper electrophoresis. Ultimately these two neutral peptides were found to be overlapping homologous fragments. There appear to be, therefore, six different SH-peptides which can be isolated from tryptic hydrolysates of *m*-MDH.

The probable presence in *m*-MDH of identical, or very similar, sub-units with a molecular weight of about 35,000 is consistent with the following observations: the number of tryptic peptides observed in the *m*-MDH fingerprint is approximately half that expected on the basis of basic amino-acid composition[3]. Moreover, Pauli-positive and Sakaguchi-positive spots number somewhat less than half that predicted on the basis of the amino-acid composition of the protein, assuming a molecular weight of 65,000–70,000. Finally, the number of SH-peptides identified is half the number of cysteine residues present in the (mol. wt. ~ 65,000) molecule.

The number of peptide spots on *s*-MDH fingerprints is considerably more than half that expected on the basis of the amino-acid composition. Moreover, detailed comparison of the fingerprints of *s*-MDH and *m*-MDH reveals apparent similarity only to the extent of several basic peptide spots. The radioactive CMC-peptides in particular were entirely dissimilar by this criterion. It is tentatively concluded, therefore, that any similarity in the two enzymes must be restricted to structural features which remain obscure under these experimental conditions, and that duplicity in the sub-unit structure of *s*-MDH seems to be contra-indicated.

The Structure of Mitochondrial MDH SH-peptides

The radioactive peptides from a tryptic hydrolysate were purified using 'Biogel' filtration and paper electrophoresis. One neutral (T-N_2) and five acidic peptides (T-2, T-3, T-4, T-6*a* and T-6*b*) so obtained were further purified by electrophoresis at *p*H 1·9 and by descending paper chromatography using butanol–acetic acid–water (120 : 30 : 50). The peptide T-N_2 was resolved into two components, T-N_2a and T-N_2b in this solvent system. The amino-acid composition of these six peptides is given in Table 1.

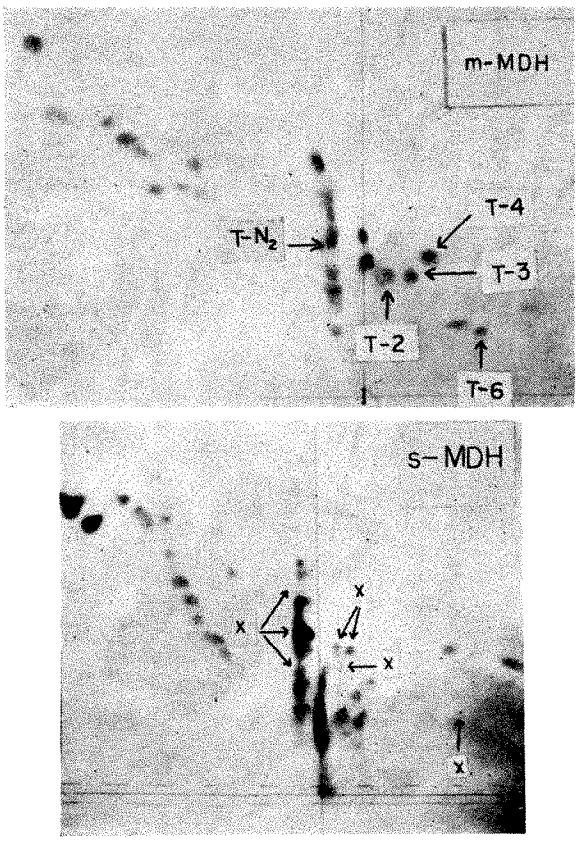


Fig. 1. Ninhydrin-sprayed 'fingerprints' of tryptic hydrolysates from malate dehydrogenases. *m*-MDH, mitochondrial; *s*-MDH, supernatant enzyme. The labelled peptides were detected through autoradiography and are indicated by T-N_2, T-2, . . . in *m*-MDH and by (*x*) in *s*-MDH. See text for experimental conditions

Table 1. AMINO-ACID COMPOSITIONS OF THE RADIOACTIVE PEPTIDES ISOLATED FROM *m*-MDH*

Amino-acid	T-N_2a	T-N_2b	T-2	T-3	T-4	T-6*a*	T-6*b*
Lys	1	2	1	1	1	—	—
His	1	1	—	—	—	—	—
Arg	—	—	—	—	—	—	—
CMC	1	1	1	1	1	1	1
Asp	2	2	1	—	—	—	—
Thr	2	2	1	—	—	—	—
Ser	1	1	—	—	—	—	—
Glu	2	3	3	2	2	1	1
Pro	2	3	—	2	—	1	1
Gly	1	1	1	2	1	—	—
Ala	2	2	1	—	—	—	—
Val	1	1	2	—	3	—	—
Ile	2	3	1	—	—	—	—
Leu	1	1	—	2	—	1	—
Tyr	—	—	—	—	—	—	—
Phe	—	—	—	—	1	—	—
Total	18	22	11	11	9	5	4

* Amino-acid analysis with a Beckman model 120 *B* analyser according to Spackman, Stein, and Moore (ref. 4).

Peptides T-N_2a and T-N_2b. Treatment of each of these peptides with subtilisin (in 0·1 M ammonium carbonate at *p*H 8·2 for 4 h at 35° C) gave hydrolysates which afterwards were subjected to paper electrophoresis at *p*H 1·9. Autoradiography of the electropherogram revealed that each peptide produced two electrophoretically similar radioactive peptides, which indicates that two were overlapping peptides. The composition of the two radioactive peptides was determined to be:

S-N_2-1^x H_2N-Ile*(Glu,CMC)

S-N_2-2^x (CMC,Ala,Pro)Lys-OH

Ile.Glu.CMC.Ala.Pro.Lys-OH

The *N*-terminal isoleucine (*) was determined using the dansyl method[5]. The sequence - - - - Ala.Pro - - - - was assumed because of the known resistance of prolyl peptide bonds to subtilisin.

Peptide T-N_2b produced two radioactive peptides on treatment with chymotrypsin (4 h at 37° C in 0·1 M ammonium carbonate, *p*H 8·2). One of these (C-N_2b-1) was identical with the original peptide in amino-acid content. The second peptide (C-N_2b-2) was a nonapeptide with the following amino-acid composition: (Asp,Thr,Ser, Glu,Pro-Ala-CMC,Ile)Lys.

From these data the remainder of the peptide T-N_2b consists of the following fragments:

(Ile,Glu,Pro)Lys-OH (T-$N_2b - T$-N_2a)

(His,Thr,Glu,Pro,Gly,Ala,Val,Ile,Leu) (T-$N_2a - C$-N_2b-2)

(Asp,Thr,Ser) (C-N_2b-2) − (S-N_2-1 + S-N_2-2)

Peptide T-2. Three radioactive (*x*) peptides as well as one unlabelled peptide were obtained by electrophoresis after subtilisin (see here) treatment of the peptide T-2. The composition of these peptides was as follows:

S-2-1^x NH_2-Ile*(Glu_2,Ala,CMC)

S-2-2^x (CMC,Thr,Glu,Gly)

S-2-3^x CMC

S-2*a* (Glu,Gly,Val_2)Lys-OH

Ile*(Glu_2,Ala(CMC.Thr)Glu,Gly,Val_2)Lys-OH

The N-terminal isoleucine (*) was determined using the dansyl method[5].

Peptide T-4. Treatment of peptide T-4 with chymotrypsin produced one radioactive peptide with the amino-acid composition (Glu_2,CMC,Gly,Val_2)Phe-OH. Treat-

ment of peptide *T*-4 with subtilisin resulted in three radioactive peptides as follows:

S-4-a^x	(Gly,CMC,Gly,Val,Phe)
S-4-b^x	(Glu,CMC,Gly,Val)
S-4-e^x	CMC

The partial structure of this peptide is: (Glu_2,CMC,Gly, Val_2)-Phe.Val.Lys-OH with valine as a probable *N*-terminal residue.

Peptides T-6a and T-6b. A probable explanation for the lack of basic amino-acids in these peptides is that they represent the C-terminal fragment of the enzyme. Attempts to obtain chemical evidence in support of this explanation have, however, been unsuccessful. The carboxypeptidase and hydrazinolysis[6,7] procedures were both ineffective when applied to native and acetone denatured *m*-MDH. Both these procedures gave completely satisfactory results on insulin, β-lactoglobulin and the synthetic tripeptide Gly.Phe.Ala. The purified peptides have not been tested directly for the C-terminal residue structure. If indeed peptides *T*-6*a* and *T*-6*b* represent the C-terminal peptides of *m*-MDH, the possibility of minor structural differences between the two polypeptide chains (or between components resolved by gel-electrophoresis) is suggested.

Comparison of the amino-acid compositions and the partial sequences of the isolated SH-peptides from *m*-MDH with the published structures of SH-peptides from glyceraldehyde-3-phosphate dehydrogenase[8], two alcohol dehydrogenases[9], and lactate dehydrogenase[10] indicates that there are no similarities in the primary structure. Because particular SH-peptides were determined in the last-mentioned four enzymes, it is not possible to compare all the SH-peptides in these enzymes with those from *m*-MDH.

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EFFECT OF NITROUS ACID ON POLYOMA VIRUS

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RECENT reports have indicated that nitrous acid has a mutagenic effect on animal viruses[1,2]. We have previously reported the isolation from *SE* polyoma virus of two variants which differed in their biological and oncogenic properties[3]. Similar polyoma virus variants were isolated by other investigators[4–6].

It was thought of interest to investigate the mutagenic effect of nitrous acid on these variants, especially to discover whether chemical mutagenesis would be effective in raising the rate of spontaneous mutation and/or inducing changes in the properties of these variants. Special attention was given to the effect of nitrous acid on the oncogenic properties of the variants. In addition to the action of nitrous acid on the intact viruses, its effect on their DNA extracts was also studied. The media, cell cultures, virus assay and animal tests which were used in this investigation were as previously described[3].

The procedure for DNA preparation used was that of Gierer and Schramm[7], with the modification that the virus suspension was shaken twice with 80 per cent phenol for 20 min at 60° C. To determine the infectivity of the extracted material, mouse embryo cell monolayers (MECC) in Petri dishes and tubes were inoculated with 0·2 ml. of the sample material diluted in 0·03 M *tris* ('Sigma 7–9') buffered saline. After 1 h of adsorption the fluid was removed and 5 ml. of Eagle's medium supplemented with 5 per cent horse serum were added to each plate and 2 ml. to each tube. The supernatant from the infected tubes and plates was collected and examined for virus content.

Treatment of intact virus with nitrous acid was carried out at 26° C. The reaction mixture contained 2·0 M sodium nitrite; 0·25 M acetate buffer, 0·6 sodium chloride; 0·02 M sodium orthophosphate at *p*H 4·2 or *p*H 5·2. Samples were removed after 1 and 2 h and immediately assayed for surviving virus. After 7–10 days incubation of the inoculated mouse embryo cell monolayer tubes the supernatant was transferred to new mouse embryo cell monolayers and only then could a cytopathological effect (CPE) caused by virus be observed. Every positive sample was identified for the type of virus by *in vitro* markers: haemagglutinating antibody (HA), CPE and plaque size. Virus samples isolated from the individual tubes which differed from the parent strain were also tested for their oncogenic properties by inoculation of 1-day-old mice or 1–3-day-old hamsters. Treatment of the phenol extracted material by nitrous acid was carried out at *p*H 5·2 at 4° C by the method described previously for intact virus. The controls of untreated virus or DNA extract were carried out in acetate buffer saline without sodium nitrite at the same hydrogen ion concentrations.

Experiments on the inactivation of polyoma virus strains treated with nitrous acid carried out at *p*H 4·2 and *p*H 5·2 showed a decrease of infectivity of both strains. No difference in the kinetics of inactivation of the two *SE* polyoma strain variants was noted. Treatment of DNA extracts with nitrous acid at *p*H 4·2 caused rapid inactivation and subsequent experiments were therefore carried out at *p*H 5·2. No inactivation whatsoever was observed in the controls when virus was exposed to acetate buffer at the same *p*H without nitrous acid.

Infectious DNA was extracted from the two polyoma variants and examined for the type of their viral progeny. The yield of infectious nucleic acid was low, and infectious virus was usually obtained from undiluted material. It can be seen from Table 1 that the virus progeny isolated from C^+H^- small plaque and C^-H^+ large plaque variant extracts showed no change from the parent strain in their HA titres, CPE, plaque size and tumour induction in mice.

When 5 mg/ml. DNase were added to the extracts and allowed to act for 10 min, the extracts completely lost their infectivity. Even three consecutive subcultures of the DNase-treated material in mouse embryo cell monolayers remained non-infective.

The two polyoma virus strains were treated with nitrous acid by the technique described previously. Each sample was inoculated into ten to thirty mouse embryo cell monolayer tubes. A control of untreated virus in acetate buffer saline without nitrous acid was inoculated in the same way at limiting dilutions. Uninoculated mouse embryo cell culture tubes were also set up. After 10–14 days of incubation, the tubes were examined for the development of CPE or HA, and 0·2 ml. of the supernatant from each negative tube was passed to freshly

prepared mouse embryo cell monolayer and the procedure was repeated three times for inoculated and control uninoculated tubes.

The data summarized in Table 2 show that the C^+H^- variant treated with nitrous acid yielded the parent-type virus only. Of 77 positive tubes obtained out of 380 inoculated all showed the C^+H^- type markers. In the control untreated titration of the same virus inoculated at limiting dilution, 74 positive tubes were obtained out of 300 inoculated, and none showed conversion to another virus type. On the other hand, when the C^-H^+ virus variant was treated with nitrous acid and inoculated into 299 mouse embryo cell monolayer tubes immediately after treatment, two out of 103 positive tubes resembled the C^+H^- type variant. One of these two viruses was isolated in the first passage and the other in the second subculture. At the same time, in control untreated titrations of C^-H^+ virus at limiting dilution, no C^+H^- virus type could be detected in 72 positive tubes out of 287 tubes inoculated and examined. Eighty-four uninoculated mouse embryo fibroblast cell culture tubes which were included in the test and passaged three times did not show any contamination by latent virus particles.

The DNA extracts of the two virus variants were also treated with nitrous acid. It can be seen from Table 3 that the virus isolated from the C^+H^- variant extract had the same properties as the parent strain. However, treatment of the DNA extract of C^-H^+ variant yielded viruses which were similar to the C^+H^- strain; these showed changes in their haemagglutinin, cytopathogenic, plaque size and oncogenic properties. The HA titre was not more than 1 : 20. The cytopathogenic effect as measured in mouse embryo cell monolayers was very strong, and when they were plated out small distinct plaques appeared. The tumour-inducing capacity in mice decreased significantly. About 10 per cent of baby mice inoculated with undiluted virus with a titre of $10^{6.9}$ in a concentration of 0·2 ml. developed tumours. The few tumours which appeared after inoculation with this strain were subcutaneous sarcomata and mammary gland tumours. Paralysis was also sometimes observed. No parotid gland tumours were seen. When these newly isolated strains were inoculated in baby hamsters (Table 4) a decrease in tumour-producing capacity was noted as compared to the parent C^-H^+ or even C^+H^- strains[8]. These strains were also passaged six times in tissue culture and no change in their properties was observed.

The control virus and DNA samples treated with acetate buffer saline without nitrous acid did not cause changes in the virus type as tested by CPE. HA, plaque size and tumour induction.

The C^+H^- virus or its DNA extract which were treated with nitrous acid did not yield virus different from the parent strain. On the other hand, the C^-H^+ intact virus or its DNA extract which were treated with nitrous acid yielded virus which was not identical with the parent strain but resembled the C^+H^- virus type.

It was shown that cells persistently infected by *SE* polyoma virus yielded virus which has characteristics in common with the small plaque type variant[8–10]. It seems that the small plaque variant becomes predominant whenever conditions for selection or mutation exist. In our case the biological examination of the newly isolated virus strains after treatment of the C^-H^+ variant with nitrous acid shows that they are similar to but not identical with the C^+H^- strain type. They were less variable in the H character; they did not give or only occasionally gave HA titres of 1 : 20. They produced strong cytopathic effect and small plaques, and were less tumorigenic for baby mice and baby hamsters. They yielded tumours in about 10 per cent of mice and hamsters inoculated with virus diluted 1 : 100. These strains were assayed for susceptibility to nitrous acid. The inactivation curves were compared with curves obtained with the parent strains. All the curves were found to fall within the same range of variation.

From our preliminary experiments it became evident that the first inoculation of mouse embryo cell monolayers was not sufficient for isolation of polyoma virus from single particles or from residual virus after partial inactivation[11]. Similar results were reported by Rowe *et al.*[12]. Therefore, three successive passages were carried out from each

Table 1. CHARACTERISTICS OF POLYOMA VIRUS DERIVED FROM PARENT VIRUSES AND THEIR DNA EXTRACTS

Strain designation	Sample	Strain characteristic			Average TCD_{50}/0·2 ml.*	Dilution of virus inoculated	No of mice inoculated*	No of mice developing tumours during months		Tumour development (%)
		CPE	HA titre	Plaque size				1–4	5–8	
C^+H^-	Parent virus	Strong	1·20	Small	$10^{6.5}$	10^0	42	3	5	19
						10^{-1}	30	—	5	17
	Virus after DNA extraction	Strong	1·20	Small	$10^{6.1}$	10^0	12	1	1	17
						10^{-1}	22	—	2	9
						10^{-2}	2	—	—	0
C^-H^+	Parent virus	Slight	1.960	Large	$10^{6.5}$	10^0	46	21	9	65
						10^{-1}	43	22	8	70
						10^{-2}	18	8	5	72
	Virus after DNA extraction	Slight	1:960	Large	$10^{6.1}$	10^0	7	4	1	71
						10^{-1}	17	9	—	53
						10^{-2}	2	1	—	50

* Accumulated data from six experiments.

Table 2 VIRUS ISOLATIONS AFTER NITROUS ACID TREATMENT AT *p*H 5·2 OF THE TWO POLYOMA VIRUS VARIANTS

Virus	Virus treatment	No of tubes inoculated	Virus isolations					Total No. of tubes positive
			Type of virus isolated	Original tubes	Subculture			
					1	2	3	
C^+H^-	Control untreated virus	300	C^+H^-	5	32	26	11	74
			C^-H^+	0	0	0	0	0
C^-H^+	Control untreated virus	287	C^-H^+	20	27	9	4	72
			C^+H^-	0	0	0	0	0
C^+H^-	Nitrous acid-treated virus	380	C^+H^-	*	26	30	21	77
			C^-H^+		0	0	0	0
C^-H^+	Nitrous acid-treated virus	299	C^-H^+	*	38	29	34	101
			C^+H^-		1	1	0	2
Mouse embryo cell monolayer control	—	84	—	0	0	0	0	0

* Unreadable as a result of cell damage.

Table 3. CHARACTERISTICS OF *SE* POLYOMA ISOLATED FROM DNA EXTRACTS OF VARIANTS TREATED WITH NITROUS ACID AT *p*H 5·2

Parent strain	Strain characteristic after nitrous acid treatment			Average TCD_{50}/0·2 ml.	Dilution of virus inoculated	No. of mice inoculated	No. of mice developing tumours during months		Tumour development (%)
	CPE	HA	Plaque size				1–4	5–8	
C^+H^-	Strong	1 : 20	Small	$10^{6\cdot1}$*	10^0	14*	—	1	7
					10^{-1}	32	1	3	12
					10^{-2}	8	—	—	0
C^-H^+	Strong	1 : 20	Small	$10^{6\cdot9}$*	10^0	48*	4	3	12
					10^{-1}	48	2	2	8
					10^{-2}	28	—	—	0

* Accumulated data from four experiments.

Table 4. CHARACTERISTICS OF VIRUS STRAIN DERIVED AFTER NITROUS ACID TREATMENT OF C^-H^+ VIRUS AT *p*H 5·2

Parent strain	Strain characteristic of mutant derived from nitrous acid-treated virus			Average TCD_{50}/0·2 ml.	Dilution of virus inoculated	No. of hamsters inoculated*	No. of hamster developing tumours during months		Tumour development (%)
	CPE	HA	Plaque size				1–3	4–8	
C^-H^+	Strong	1 : 20	Small	$10^{7\cdot1}$*	10^{-1}	8	4	—	50
					10^{-2}	21	—	2	10
					10^{-3}	20	1	2	15
					10^{-4}	22	—	—	0

* Accumulated data from four experiments.

tube inoculated. For elimination of the possibility of use of virus-contaminated tissue cultures as a result of latent viral infection, examination of mouse embryo cell monolayer control tubes was carried out in the same manner. Also the control virus titration of C^-H^+ virus type at limiting dilutions showed no appearance of C^+H^- variants. From the results of our experiments it seems that we are dealing with mutations induced by the nitrous acid treatment. However, no final conclusion on whether selection or mutation has taken place can be reached.

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A MOLECULAR MECHANISM FOR ENZYMATIC TRANSAMINATION

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IT is well known that many reactions catalysed by coenzyme-dependent enzymes can be reproduced in model systems containing only the coenzyme or an analogue of it. However, under these conditions the strict substrate specificity and reaction specificity that are characteristic features of the enzymatic process are, to a large extent, lost. The role of the protein moiety of the enzyme molecule in providing substrate specificity is evidently determined by the presence, in the active centre, of a 'binding site' that is complementary to the substrate. The questions that arise as to the means by which the action of an enzyme causes the selective cleavage of only some of the available covalent bonds in the substrate molecule are more complicated.

Elucidation of the absolute functions, in the catalytic process, of the specific protein of the enzyme (in particular, of an enzyme containing a bound coenzyme) is essential for any understanding of the principal features of enzymatic catalysis, namely, substrate specificity and specificity for a definite type of reaction. In fact, these are the features which, in the final reckoning, make possible the high velocities of enzymatic reactions.

In this article, which relates to the mode of action of pyridoxal phosphate-dependent enzymes (PALP-enzymes)*, we examine possible mechanisms determining the spatial interrelationships of coenzyme, apoenzyme and substrates during the course of the enzymatic reaction.

A general scheme for reactions catalysed by PALP-enzymes was proposed by A. E. Braunstein and M. M. Shemyakin[1] and by D. Metzler, M. Ikawa and E. E. Snell[2]. It was shown by Snell *et al.* that practically all the types of enzymatic reactions of amino-acids that are effected by this group of enzymes may be observed also in model systems containing pyridoxal or pyridoxal phosphate. In the case of such non-enzymatic catalysis, there is no necessity for the entire coenzyme molecule, and only its 4-formyl-3-hydroxypyridine part is needed.

Aspartate transaminase (AS-transaminase, aspartate : 2-oxoglutarate aminotransferase, *E.C.* 2.6.1.1) catalyses the reversible reaction:

$$\underset{\text{L-aspartic acid}}{HOOCCH_2CHNH_2COOH} + \underset{\alpha\text{-ketoglutaric acid}}{HOOC(CH_2)_2COCOOH} \rightleftharpoons$$

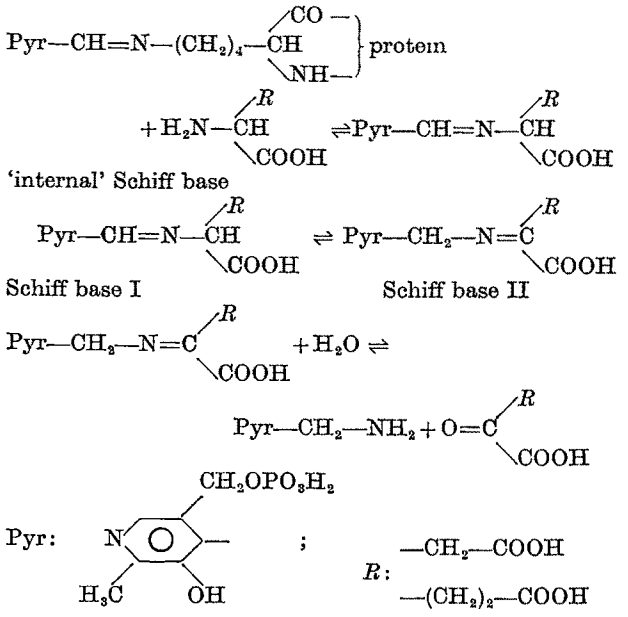
$$\underset{\text{oxaloacetic acid}}{HOOCCH_2CCOOH} + \underset{\text{L-glutamic acid}}{HOOC(CH_2)_2CHNH_2COOH}$$

It should be noted that other PALP-enzymes exist which specifically catalyse the α-and β-decarboxylation

* Abbreviations in the text: PALP, pyridoxal-5-phosphate; AS-transaminase, L-aspartate : 2-oxoglutarate aminotransferase (*E.C.* 2.6.1.1).

of aspartic acid and the α-decarboxylation of glutamic acid. Therefore, a detailed examination of the mechanism of the transamination reaction should contribute also to our understanding of the selectivity of enzyme catalysis.

Since pyridoxal phosphate exists in AS-transaminase in the form of an aldimine (Schiff base) with the ε-amino group of a lysine residue[3], the first step of the reaction appears to be a transaldimination, that is, a reaction of an amine with an imine, resulting in conversion of the 'internal' Schiff base of PALP with the lysine of the apoenzyme into a Schiff base with the substrate amino-acid. This is followed by tautomeric conversion of the so-called Schiff base I (aldimine) into the Schiff base II (ketimine); in the next stage the double bond of the latter intermediate is hydrolysed to yield a keto acid and protein-bound pyridoxamine phosphate:



Scheme 1

Extremely important to this sequence of reactions is the following circumstance. Each of the aforementioned steps has alternating requirements in regard to the ionization state of the 3-hydroxy group of the coenzyme.

The enzyme possesses maximum activity at *p*H 8–8·5; investigation of the spectral properties of the enzyme showed that in this range of hydrogen-ion concentrations, the phenolic group of the coenzyme exists in the ionized state[4].

In scheme 2*A* is shown the initial state of the protein-bound coenzyme, prior to interaction with the substrate. The existence of a non-covalent bond between the phosphate group of the coenzyme and the protein was established several years ago[5]. The presence of a bond between the apoenzyme and the nitrogen atom of the pyridine nucleus was postulated by Sizer and Jenkins[4] in order to account for the shift in the *pK* associated with the '*p*H-indicator' spectral change of the cofactor in AS-transaminase. This *pK* is shifted to the acid side (to 6·3) relative to the *pK* of the analogous transition, in the free imines of pyridoxal in aqueous solution (*pK* 10·8)[7]. Thus, protonation of the nitrogen atom in the pyridine nucleus by some proton-donating group of the protein leads to ionization of the 3-hydroxy group at lower values of *p*H. As a consequence, the dipolar ionic species of the PALP aldimine exists in the active centre of the enzyme at its *p*H-optimum, although this species is thermodynamically unstable in solution. Comparative studies of the fluorescence of the imine of pyridoxal phosphate with valine, and of AS-transaminase, confirmed the existence of such a state of ionization of the groups of the coenzyme[6]. Velick and Vavra[7] showed that the binding of aspartate to the enzyme is controlled by the dissociation of a proton with a *pK* of 6·3 which coincides with the *pK* of decolorization of AS-transaminase. Thus, the presence of an ionized phenolic group would seem to be an essential condition for the occurrence of the first step of the enzymatic reaction.

However, in this state the electron density in the pyridine ring of the coenzyme is significantly increased, and its properties as an electron-accepting group that provides for polarization of the carbonyl group in position '4' are, to a significant degree, lost. The subsequent reaction stages necessitate the presence of an un-ionized phenolic hydroxyl, in so far as the intramolecular hydrogen bond involving this group provides planarity in the conjugated system and returns to the pyridine nucleus the capacity to act as an electron acceptor.

While the aforementioned ionization state is also realized in the 'internal' Schiff base at *p*H < 6·3, this is not accompanied by transamination between the coenzyme and the ε-amino group of the lysine residue.

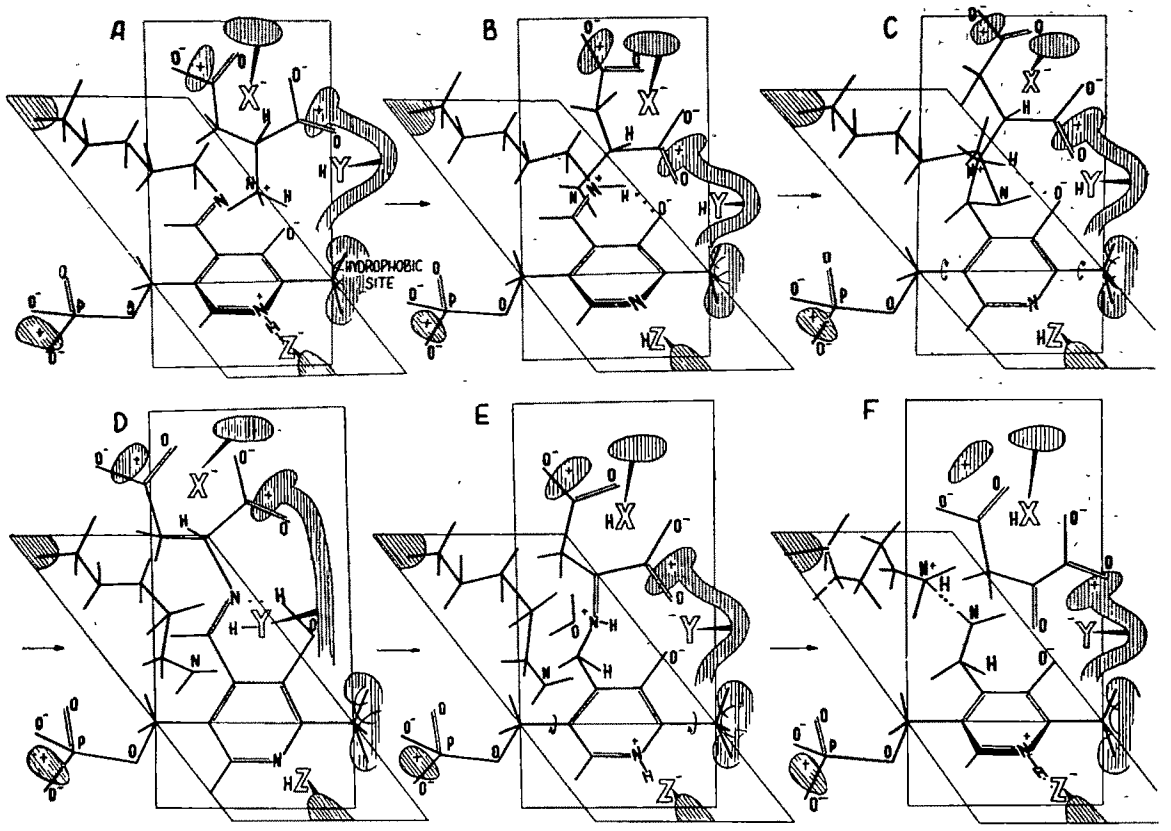
It is evident that any plausible mechanism describing enzymatic transamination must, on one hand, explain what is meant by the change in the state of ionization of the phenolic group which occurs in the course of this reaction, and, on the other hand, account for the absence of transamination in the 'internal' Schiff base over the whole range of *p*H from 5 (or less) to 10.

The results of our investigations of the mechanism of reaction of AS-transaminase with *cyclo*serine and its analogues provide evidence suggesting that the tautomeric interconversion of Schiff bases I and II in the transaminase-substrate complex is catalysed by functional groups of the active centre[8]. The recent investigations of Bruice and Topping[9] on non-enzymatic transamination reactions with the participation of pyridoxal also showed that the rate of the step involving the prototropic shift was substantially increased in the presence of imidazole, which apparently acts as an acid-base catalyst. Investigations of the parameters of luminescence and circular dichroism of the enzyme, and of its complexes with inhibitors, have provided evidence indicating that the coenzyme, under certain conditions, may acquire a definite degree of mobility. Thus, exposure of AS-transaminase at *p*H 8–9, where the enzyme exists in the active form, to light of wave-length 365 mμ (at this *p*H the absorption maximum of the protein-bound coenzyme is situated at 360–370 mμ) evokes a definite drop in the degree of polarization of fluorescence of AS-transaminase which is reversible in the dark. At the same time, the polarization of fluorescence in the absorption band of a dye, fluorescein isothiocyanate, with which the enzyme had been pre-treated, remained constant. It follows that the phenomenon that we observed was related to an increase in the degree of mobility of the enzyme-bound coenzyme, and not of the protein[10].

An examination of published data on AS-transaminase, in the light of our newly obtained results, enables us to propose a dynamic model for the functioning of the active centre of this enzyme (scheme 2).

A molecule of substrate amino-acid, the dissociable groups of which, at physiological values of *p*H, are ionized, is bound to a cationic site of the active centre above the plane of the pyridine ring of the coenzyme; under this condition, geometric interrelations are achieved that favour nucleophilic addition. Since maleic acid acts as a competitive inhibitor of AS-transaminase, but fumaric acid does not[12], the structure of this site must be such that it provides for binding of the aminodicarboxylic acid in a conformation with adjacent carboxylic groups.

Interaction between the negatively charged phenolic group of the coenzyme and the positively charged amino group of the substrate favours attainment of the correct



Scheme 2 Four-proton mechanism for enzymatic transamination

orientation of the reacting parts of the molecules. It is well known that nucleophilic addition to a C=N double bond depends on the degree of polarization of this bond and the nucleophilicity of the attacking reagent. However, the nitrogen atom of the substrate is positively charged, and in the unprotonated Schiff base the double bond is not significantly polarized (scheme 2*A* and 2*B*). In the model under consideration, the apparent contradiction is removed by the suggestion that the ionized hydroxyl group of the coenzyme accepts a proton from the amino group of the substrate, whereby this amino group acquires nucleophilic properties; at the same time, a positive charge appears on the formyl carbon atom, owing to formation of an intramolecular hydrogen bond. Protonation of the hydroxy group has a further important consequence, namely, a decrease in the basicity of the pyridine nucleus of the coenzyme, leading to breaking of the hydrogen bond between the pyridine nitrogen atom and the protein (scheme 2*B*).

The addition of the amino group of the substrate to the C=N bond of the enzyme results in the formation of a tetrahedral intermediate (*C*) that is further converted, with liberation of the ε-NH_2 group of lysine, into the enzyme-substrate Schiff base (scheme 2*C*→2*D*).

In so far as planarity of the coenzyme-substrate complex is a necessary condition for the subsequent tautomeric transition, formation of the 'Schiff base I' must inevitably be associated with a change in spatial position of the coenzyme.

In our opinion, this change is produced by way of rotation of the coenzyme around an axis passing through its 5-methylene and 2-methyl groups. The position of the axis remains fixed, owing to the fact that its end-points, the phosphate group on one hand, and the methyl group on the other, are bound to the protein. We have thus postulated, in this model mechanism, the existence of an additional site of attachment between the coenzyme and the protein, namely, a hydrophobic interaction between the methyl group of PALP and the side-chains of aliphatic amino-acid residues of the apoenzyme. The coenzyme turns around single C—C bonds which allow free rotation. Such rotation is made possible by cleavage of the covalent bond between the formyl group of the coenzyme and the ε-NH_2 group of lysine, and breaking of the hydrogen bond that links the pyridine nitrogen to a proton-donating group of the apoenzyme. The coenzyme, now fixed only at two points situated at the ends of the axis, rotates relative to the protein into a plane perpendicular to the original one, at the expense of transition of the tetrahedral carbon atom in position '4' into the trigonal form (scheme 2*D*). This results in the necessary orientation of the functional groups, *X* and *YH*, of the active centre, which catalyse the prototropic shift, in relation to the >C=N—CH-group of the enzyme-substrate complex. Translational displacement of the carboxyl groups of the substrate molecule, caused by rotation of the coenzyme, may contribute to bringing the catalytic groups of the protein, *X* and *YH*, into closer proximity with the coenzyme-substrate complex ('induced fit').

The tautomeric conversion of 'Schiff base I' into 'Schiff base II' probably proceeds by means of concerted general acid–general base catalysis[9]. Owing to this, the trigonal carbon atom in position '4' of the coenzyme-substrate complex is changed to a tetrahedral form, and the plane of the ring of the coenzyme reverts to its initial position with reconstitution of the hydrogen bond between the pyridine nitrogen and the apoenzyme (scheme 2*D*→2*E*). Formation of this bond leads to a lowering of the electron density on the oxygen atom of the phenolic group; the proton of the intramolecular hydrogen bond dissociates and catalyses hydrolysis of the C=N bond, to yield an α-keto acid and pyridoxamine phosphate. It is plausible that, in the amino-form of the enzyme, an additional hydrogen bond exists between the pyridoxamine phosphate and the protein, wherein the ε-NH_2 group of the lysine residue may act as the proton acceptor. This would

impart to the amino group of pyridoxamine phosphate the nucleophilic properties required for reaction with keto-substrates.

The foregoing dynamic model for the functioning of the active centre in AS-transaminase enables us to explain the role of each group of the coenzyme molecule in the process of enzymatic transamination and to define concrete functions of the apoenzyme. The protein moiety of the enzyme molecule not only catalyses the step of prototropic transition, but also provides for the appropriate mobile orientation of the coenzyme and substrate molecules that makes possible the realization of alternating electronic structures of the coenzyme, required at different stages of the transamination reaction.

From this dynamic model, it becomes clear that AS-transaminase cannot possess either α- or β-decarboxylating activity. In fact, for an α-decarboxylation reaction to occur, the necessary pre-conditions are complete protonation of 'Schiff base I'[13] and the absence of functional group catalysing prototropic transition. Less evident are the causes of the absence of β-decarboxylase activity in AS-transaminase, as β-decarboxylation involves the formation of 'Schiff base II'. Here the decisive factor is, apparently, the 'maleinoid' conformation of the substrate molecule, which hinders the formation of a cyclic transition complex that is characteristic for decarboxylation reactions of β-keto acids and their imines[13].

The proposed model is in good agreement with available experimental data concerning the mechanism of transamination; it gives a unified interpretation to a large number of seemingly contradictory facts, for example, the reactivity of the 'colourless' form of AS-transaminase, stability of the 'internal' Schiff base involving the ε-NH_2 group of lysine, the optical properties of complexes formed by the enzyme with quasi-substrates such as α-methyl-aspartate or β-hydroxyaspartate, etc.[14].

One of the essential features of the reaction scheme discussed here is the postulation of two spatially and functionally distinct sites in the active centre, between which a connexion is established by means of rotation of the coenzyme around an axis passing through the methylene and methyl groups of PALP; localized in one of these sites are the catalytic groups of the protein that are responsible, to a considerable extent, for the reaction type specificity of the enzyme; the other site serves to keep the coenzyme bound in the absence of substrate.

We believe that the conceptions expounded here may also provide a useful basis for the explanation of the molecular mechanisms of action of other types of PALP-enzymes.

We thank Prof. A. E. Braunstein and Prof. D. E. Metzler for stimulating discussions on this work.

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NUMBER OF MOLECULES OF ANTIBODY REQUIRED TO PROMOTE PHAGOCYTOSIS OF ONE BACTERIUM

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IT was established many years ago by Almroth Wright and Douglas that opsonic factors from serum were desirable components in a mammalian phagocytic system. In the absence of such factors the white cells of mammals were often unable to engulf foreign material such as bacteria, but the addition of specific antibody directed against the bacteria ensured efficient phagocytosis. The mechanism whereby the attachment of the protein antibody molecules to bacteria renders them amenable to the functions of the phagocytic cell is not really known, but it has been tacitly accepted that this must in part be due to a change in the surface charge on the bacteria consequent on the adsorption of a layer of antibody molecules. In recent years there has been a growing body of evidence that complement might be necessary for phagocytosis, and this adds complications to the foregoing easy explanation, involving surface charge alone. In order to begin to understand the process we have chosen to measure the minimum quantity of antibody which will permit the phagocytosis of one bacterial cell. The results in this article indicate that the number of molecules of antibody per bacterial cell seem far too small to alter the total charge on the bacterial surface and that other explanations must be sought.

Previous work has established that the normal mouse peritoneum contains several million phagocytic cells including a high percentage of macrophages which are capable in the presence of antibody of engulfing and killing Gram-negative bacteria with great rapidity. The essence of our method has been to inject 10^6 *Salmonella adelaide* into the peritonea of mice, together with varying amounts of antibody. In this way we have determined the minimum quantity of antibody which would permit the destruction of 99 per cent of the ingested bacteria within the space of 90 min. With no antibody all the bacteria remained viable over this period of time.

The *S. adelaide* antiserum was prepared by injecting rabbits with a boiled suspension of the organism grown for 24 h on nutrient agar plates. The inoculations were at 4-day intervals for 1 month.

S. adelaide lipopolysaccharide was prepared from organisms grown in the same manner by the phenol/water method of Westphal, Luderitz and Bister[1]. For haemagglutination tests we used sheep red blood cells coated with alkaline sensitized lipopolysaccharide as described by Crumpton, Davies and Hutchison[2]. Quantitative precipitin tests were carried out on the antisera following the method of Kabat and Mayer[3]. The precipitates were analysed for protein by the Folin–Ciocalteau method using bovine serum albumin as a standard protein.

The amount of antibody required for phagocytosis was determined by a number of steps as follows:

(1) *The opsonic activity of the whole antiserum.* For this purpose ten mice were injected intraperitoneally with 0·1 ml. containing approximately 10^6 *S. adelaide* with or without previous treatment with a dilution of the rabbit antiserum at 20° C for half an hour, At intervals during 90 min after injection one mouse was killed and the peritoneal cavity washed out with 1·5 ml. of Hanks's solution. Dilutions of the washout were made and plated out to enumerate the numbers of the injected bacteria which were still viable[4]. In this way it was shown that suspensions of the bacteria in as little as 0·1 ml. of a 1/500,000 dilution of antiserum gave 99 per cent kill (see Fig. 1).

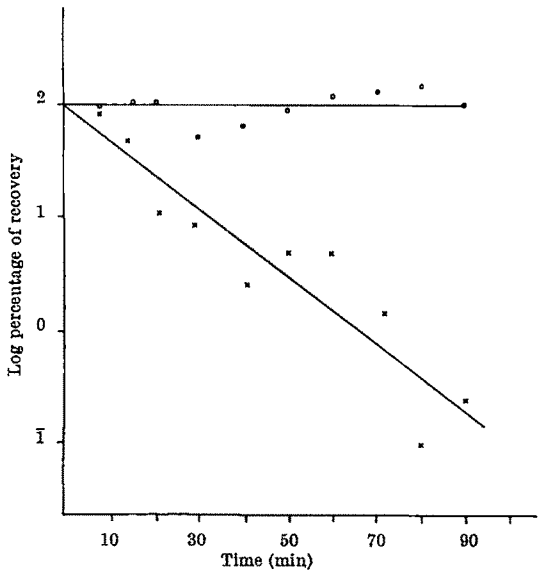


Fig. 1. Recovery of viable organisms after injecting *S. adelaide* intraperitoneally into mice together with dilutions of rabbit *S. adelaide* antiserum. O, Dilutions > 1/2,000,000; ×, dilutions < 1/500,000

(2) *The effect of 0·1 M mercaptoethanol on the opsonic activity.* The *S. adelaide* antiserum (1 ml.) was incubated for 1 h at 37° C with 0·1 M mercaptoethanol, neutralized with iodoacetamide and examined for its opsonic activity as above. The limit of activity was reached at a dilution of 1/50,000, indicating perhaps that a large percentage of the original activity was due to macroglobulin.

(3) *Determination of precipitating antibody against S. adelaide lipopolysaccharide.* When 2 ml. volumes of the antiserum were mixed with varying amounts of the purified lipopolysaccharide prepared from *S. adelaide*, protein analyses showed that 250 μg/ml. of antibody protein was the maximum amount precipitable. After removing the precipitates, the supernatant solutions, even from tubes containing considerable excess antigen, still produced intraperitoneal killing of *S. adelaide* up to a dilution of 1 in 2,000.

(4) *Estimation of γ G and γ M antibody content in the S. adelaide serum.* 2 ml. of the rabbit antiserum were equilibrated with 0·1 M phosphate buffer at *p*H 6·3 and passed through a 26 × 12 cm column of DEAE-cellulose at this molarity. This was exhaustively eluted with 0·1 M buffer before another batch elution was done using 1 M buffer. The two solutions were concentrated to the original serum volume and dialysed against 0·15 M sodium chloride. To each of these solutions was added 1 mg of *S. adelaide* lipopolysaccharide in a total volume of 5 ml. and the content of protein in the precipitate was determined by the Folin–Ciocalteau method. The 1 mg of lipopolysaccharide was known from the previous precipitin determinations to provide excess antigen, but the lipopolysaccharide–antibody systems do not form soluble complexes in antigen excess, so this is a valid method of finding the total antibody.

Total protein precipitated in the 0·1 M fraction = 205 μg/ml. original serum.

Total protein precipitated in the 1·0 M fraction = 66 μg/ml. original serum.

This totals 271 μg/ml. which is reasonably close to the figure of 250 μg/ml. for total antibody precipitated.

(4*a*) *Activity of γ G antibody.* Table 1 shows that after destruction of the γ M antibody by mercaptoethanol, the remaining opsonic activity of the serum is still effective at 1/50,000 dilution. The 0·1 M fraction above containing 205 μg γ G was active at 1/40,000 final dilution.

Table 1

	Final opsonic titre	Haemagglutinin
Original serum	1/500,000	1/500
Original serum + M.E.	1/50,000	
Purified γ M	1/150,000	
Purified γ G	1/40,000	
Original absorbed with *S. adelaide* or *S. manchui* etc.	1/5,000	1/2
Supernates from quantitative precipitin	1/2,000	1/4

(4*b*) *Activity of γ M antibody.* The original serum was active at 1/500,000 dilution and 90 per cent of this activity was lost after treatment with mercaptoethanol. This clearly inferred that the major part of the original serum activity was due to γ M antibody. To further establish this point, γ M antibody was isolated from antiserum by the following steps. 70 ml. of antiserum (2 × 35 ml.) were passed through 'Sephadex' *G*200 columns (62 × 45 cm) using 0·2 M *tris*-HCl buffers at *p*H 8·0 (ref. 5). The leading peaks were pooled, concentrated and centrifuged for 6 h at 80,000*g* to remove lipoproteins which floated to the top of the tubes and were discarded. The remainder was then dialysed against 0·1 M phosphate buffer *p*H 6·5 and applied to a DEAE-cellulose column (26 × 1·2 cm) previously equilibrated with this buffer. The eluate with 150 ml. of 0·1 M phosphate *p*H 6·5 was discarded and the macroglobulins eluted with 1·0 M phosphate buffer *p*H 6·5. The α_2 macroglobulins were then removed by block electrophoresis on polyvinyl chloride in veronal buffer 0·2 M *p*H 8·5 and the γ M globulins concentrated by pressure dialysis. This material gave a single sedimenting peak with uncorrected sedimentation coefficient of 18*S* on analysis in the Spinco model *E* ultracentrifuge and a single precipitin band on electrophoresis against sheep-anti-rabbit globulin antiserum.

The purified macroglobulin fraction was still active at approximately 1/150,000 dilution when returned to the original volume of antiserum fractionated. Bearing in mind the difficulties of purifying macroglobulins without aggregation or denaturation, these results strongly suggest that the major part of the original serum activity was due to γ M antibody.

(5) *Demonstration that the anti-lipopolysaccharide antibody is the active opsonin by elution from the lipopolysaccharide–antibody complex.* The antibody contained in 10 ml. of the rabbit antiserum was precipitated by the addition of 4 mg of the *S. adelaide* lipopolysaccharide representing the amount of antigen at the equivalence point. The precipitate was washed twice with saline, then dissolved in 2 M sodium chloride + 4·0 M urea in borate buffer *p*H 8·6. After 24 h at 4° C the suspension was centrifuged at 60,000*g* for 1 h and then dialysed overnight with stirring against 1 l. 0·1 M phosphate buffer plus 4 M urea at *p*H 6·5. The suspension was then passed through a DEAE-cellulose column (26 × 1·2 cm) previously equilibrated with the 0·1 M buffer containing urea. The column was eluted with 50 ml. of this buffer followed by 50 ml. of 1 M phosphate + 4 M urea at *p*H 6·5. The eluate in the latter buffer was concentrated to 2 ml. by pressure dialysis to give a final protein concentration of 150 μg per ml. This material agglutinated *S. adelaide* rapidly on a slide test and, when examined for its intraperitoneal opsonic activity, was found to be very active

up to dilutions of 1/40,000—the end-point was not reached in this experiment.

(6) *Confirmation that the antibody precipitable with lipopolysaccharide is the same as that responsible for the intraperitoneal killing.* That the antibody responsible for the intraperitoneal killing was the same as the 035 antibody measured by precipitation with lipopolysaccharide was made more certain by the finding that absorption with *S. manchui* or *S. alachua*, both 035 strains, reduced the opsonic activity to 1 in 5,000. Concurrently, the haemagglutinating titre against *S. adelaide*-sensitized red cells fell from 1 in 500 to 1 in 2 (Table 1).

We have demonstrated that in the rabbit antiserum used there were 66 μg of macroglobulin antibody directed against the 035 antigen of *S. adelaide*. This was effective in promoting intraperitoneal killing of the organism when diluted 1 in 500,000 and, on the assumption that all of this antibody was bound to the 10^6 bacteria injected, a simple calculation can be made of the numbers of antibody molecules per bacterial cell. For the calculations we used 10^6 as the molecular weight of γ M and 6×10^{23} as Avogadro's number. With the data given here this works out at eight molecules of γ M per bacterium. Similar calculations with the γ G data lead to a figure of 2,200 molecules of γ G per bacterium. Since it is rather unlikely that all the available antibody would be bound to bacteria at this high dilution, the actual numbers of bound antibody molecules per cell are probably less than those above.

A further simple calculation, using available data on the dimensions of immunoglobulin molecules, reveals that an organism like *S. adelaide* could accommodate approximately 10,000 γ M molecules in a close-packed monolayer on the surface of one bacterium. It seems, therefore, that the time-honoured idea that opsonization involves a change of surface charge due to coating bacteria with a layer of antibody is inaccurate at the least and probably invalid.

The striking difference in opsonic efficiency between γ G and γ M is reminiscent of the recent finding of a similar difference in haemolytic efficiency[6]. In the haemolytic system complement is involved and the relative efficiencies of the two types of antibody molecule can be explained by postulating that two closely adjacent molecules of γ G are needed to bind complement as a bridging molecule. On the other hand, the large γ M molecule may be able to fix complement in an intramolecular fashion. Could the same explanation apply to the opsonic system? Certainly there is ample evidence that complement, if present, will promote phagocytosis[7,8], but whether its participation is mandatory is uncertain. The most persuasive data are those of Nelson, who showed that erythrophagocytosis needs the formation of $EaC_{1,4,2,3c}$ before it occurs efficiently[9].

In our system the end-point measured was the loss of bacterial viability following phagocytosis. While this does not affect the calculations of the numbers of molecules necessary for the whole process to occur, it could be that complement is mainly involved after phagocytosis, in the intracellular events which led to death of the bacterium. Even allowing for this uncertainty, it seems that new explanations of the mechanisms of opsonization must be sought, encompassing the following points: (*a*) the small numbers of antibody molecules required; (*b*) the biological inertness of antibody itself, unless supplemented by other factors; (*c*) the enzymatic activities of the whole complement system after activation by antigen/antibody complexing. Nelson's suggestion that this opsonic mechanism involves some or all of the components of complement seems increasingly feasible[9].

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ESTABLISHMENT OF A STANDARD 'HOLLOMAN' CHIMPANZEE BLOOD GROUP PANEL AND ITS APPLICATION FOR IMMUNOGENETIC STUDIES

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THE rapid accumulation of new data concerning individual differences in chimpanzee blood has been reported in a series of articles[1-4]. The relative ease with which group-specific isoantisera have been produced in chimpanzees not only by isoimmunization but also by cross-immunization with human red cells is surprising when compared with the situation in man[5,6]. That a constitutional difference may be the explanation is suggested by the observation that many of the immunized chimpanzees become autosensitized[7,8]. If man were as readily sensitized as chimpanzees, the practice of blood transfusion would be seriously hampered.

At the time we published our previous report[1] as many as nine different standardized antisera were available, which defined more than 500 different blood group combinations in chimpanzees. In the meantime, antisera of additional specificities have been produced. Since for further progress standardized reagents are essential, this has created the need for a panel of chimpanzees of known blood groups to be used as reference standards. The establishment of such a panel became feasible when the large colony of chimpanzees maintained by the U.S. Air Force at the Holloman Air Force Base was made available for medical research purposes, specifically for transplanta-

Table 1. REACTIONS OF ANTISERA PRODUCED BY IMMUNIZED CHIMPANZEES (2 WEEKS AFTER THE THIRD INJECTION)

Red cells of	Human-type A-B-O group	Simian-type blood group				Reactions‡ with Billy's* serum			Reactions‡ with Hope's† serum			Reactions‡ with Lindsey's† serum			Reactions‡ with Mandy's† serum		
		V-A-B	C-E-F‖	G^c	H^c	Saline	Anti-globulin	Ficin	Saline	Anti-globulin	Ficin	Saline	Anti-globulin	Ficin	Saline	Anti-globulin	Ficin
1. Billy, No. 85	A	V.B	F	+	+	+	−	−	−	−	+ + ±	−	−	+ ±	−	−	−
2. Cindy, No 123	A	V.B	EF	+	+	+	−	+ + ±	−	±	+ + ±	−	−	+ ±	+ +	+ ±	+ +
3 Debbie No. 204	A	v.A	c	+	−	−	−	+ + ±	−	−	−	−	−	−	−	−	−
4. Dick, No. 110	A	v.B	EF	+	+	Tr.	−	+ + ±	−	−	+ +	−	−	+ +	+ +	+ +	+ ±
5. Guy, No. 197	A	V.B	c	+	+	+ +	+ + ±	+ + ±	−	−	−	−	−	−	−	−	−
6. Herbie, No. 194	A	v.AB	c	+	+	−	+ ±	+ + ±	−	−	±	−	−	+ +	−	−	Tr.
7. Hope, No. 136	A	v B	E	−	+	−	−	−	−	−	Tr.	−	−	+ +	−	−	+ + ±
8. Lindsey, No. 192	A	v.AB	F	+	−	Tr	+	+ +	−	−	+ +	−	−	−	−	−	−
9. Mandy, No. 208	A	V.B	EF	+	+	−	+	+ +	−	−	+ + ±	−	−	+ +	−	−	−
10. Possum, No. 169	A	v.B	EF	−	+	−	±	+ +	−	−	+ +	−	−	+ +	−	−	+ +
11. Rufe, No. 114	A	v.B	F	−	+	−	+ ±	+ + ±	−	−	+ +	−	−	+ +	−	−	+ +
12. Oscar, No. 211	A	V.A	E	+	+	+ +	+ +	+ + ±	−	−	−	−	−	+ +	−	−	−
Av. titre (units)§						4	20	6	0	0	12	0	0	5	4	20	3–12

* Cross-immunized with pooled human red cells from five individuals.
† Isoimmunized with red cells of individual chimpanzees other than those listed in this protocol.
‡ The strength of the reactions is indicated by the number of plus signs; + + + represents the strongest reaction possible, namely, a single large clump.
§ The number of units is the reciprocal of the highest serum dilution giving a one-plus reaction.
‖ See footnote to Table 4.

Table 2. REACTIONS OF THE ANTISERA OF THE FOUR IMMUNIZED CHIMPANZEES 6 WEEKS AFTER THE THIRD INJECTION

Red cells of	Titres with Billy's serum			Titres with Hope's serum			Titres with Lindsey's serum			Titres with Mandy's serum		
	Saline	Antiglobulin	Ficin	Saline	Antiglobulin	Ficin	Saline	Antiglobulin	Ficin	Saline	Antiglobulin	Ficin
Billy, No. 85	0	0	0	0	0	2	0	1	2	0	0	0
Hope, No. 136	0	0	0	0	0	3	0	0	0	0	0	2
Lindsey, No. 192	0	0	0	0	0	0	0	0	6	0	0	0
Mandy, No. 208	0	32	0	0	16	2	0	128	3	0	0	0

tion studies. From a total of ninety-seven chimpanzees tested, a permanent panel of twenty-seven animals has been selected for their blood types. Blood samples from this 'Holloman panel' are now available to other research workers, together with our antisera of standard specificities. This should foster the dissemination of information and encourage work elsewhere. At the same time some of the animals from the panel are being used for further immunization experiments. In this article, observations demonstrating the usefulness of the panel will be described.

In one experiment, fifteen chimpanzees from the panel were immunized, some with human blood and others with blood from chimpanzees selected for their blood types. The blood was injected by both the intravenous and intramuscular route, and in a few cases subcutaneously and/or intraperitoneally, in doses of 4–7 c.c. No adjuvant was used. Two weeks after the third injection, which was 3 months after the experiment was started, four of the twelve chimpanzees that were tested already showed the presence of isoantibodies (see Table 1). Of these four, one chimpanzee (Billy) had been immunized with human blood, and the other three with blood from individual chimpanzees.

The tests were undertaken using three different techniques, namely, the saline agglutination, the antiglobulin and the ficinated red cell methods. In addition to the direct tests, titrations were carried out as indicated on the last line of Table 1. As can be seen, the four chimpanzee antisera differed from one another in specificity. They also differed in specificity from all the antisera of our previous immunization experiments. It is of interest to note that when the four animals were re-tested a month later, without any intervening injections, their sera exhibited a change in specificity. As shown in Table 2, there was no longer convincing evidence of antibodies in Mandy's serum, while the sera of the other three chimpanzees now all gave parallel reactions. The experiments are being continued.

The tendency of antisera to change their specificity during the course of an immunization poses a serious problem for the production and maintenance of an adequate supply of standardized antisera. This situation also exists in man, though to a lesser degree, when volunteer donors are used for the production of anti-Rh sera, for example. In some cases it becomes necessary to absorb out interfering antibodies from the antisera. Such a problem recently encountered by us in the chimpanzee could be resolved in that way with the aid of the Holloman panel. We had noticed that the anti-**A**^{c} sera* obtained after the third immunization of the original anti-**A**^{c} donor (Malcolm) appear to be giving a higher percentage of positive reactions than anti-**A**^{c} sera from earlier bleedings. We then re-tested chimpanzees that had previously been found to be **A**^{c} negative, and found that some of them reacted positively with the new antiserum. Moreover, when chimpanzees on the Holloman Panel were tested in parallel against the new and the previous anti-**A**^{c} sera, discrepant results were obtained. It was then clear that the serum from the later bleeding contained antibodies in addition to anti-**A**^{c}.

Table 3. TITRATION OF SERUM OF CHIMPANZEE AFTER THIRD IMMUNIZATION

Tested against red cells of Name and No.	V-A-B type	Titres of unabsorbed serum by method of Saline agglutination	Anti-globulin method	Titres of serum after absorption with red cells of Val Saline agglutination	Anti-globulin method	Frans Saline agglutination	Anti-globulin method
Frans, 103	v.A	24	350	24	160	0	0
Went, 143	v.AB	16	350	24	240	0	0
Val, 169	V.B	12	96	0	0	12	56
Pix, 157	v.B	12	96	0	0	12	48
Randy, 170	V.O	0	0	0	0	0	0
Marc, 172	v.B	0	0	0	0	0	0

Absorption and titration experiments on this serum, as shown in Table 3, confirmed the presence of another antibody besides anti-**A**^{c}. The serum agglutinated in high titres not only red cells from chimpanzees known to be **A**^{c} positive (Frans and Went) but also some of those known to be **A**^{c} negative (Val and Pix). However, by absorbing the serum with the red cells of Val (**A**^{c} negative) the interfering antibody could be removed with scarcely any effect on the titre of the anti-**A**^{c} antibody. Conversely, absorption with Frans's red cells (**A**^{c} positive) removed the anti-**A**^{c} from the antiserum, leaving behind the newly formed antibody, which we now designate as anti-**I**^{c}. In this way, by suitable absorption of Malcolm's antiserum from recent bleedings, it was possible to produce two specific reagents, namely, anti-**I**^{c} as well as the old anti-**A**^{c}. The specificities of these two reagents were

* To avoid ambiguity, symbols for blood factors and their corresponding antibodies are printed in bold type, and symbols for agglutinogens, phenotypes and blood-group systems are printed in regular type.

Table 4. REACTIONS OF RED CELLS OF TWENTY-FIVE CHIMPANZEES OF KNOWN BLOOD TYPES WITH SERUM OF CHIMPANZEE MALCOLM

Serial No.	No. and name	Human-type A-B-O group	Simian-type blood groups V-A-B	C-E-F†	G^c	H^c	Reactions with Malcolm serum Unabsorbed	Absorbed with red cells of Val	Frans
1	157, Howard	O	v.B	F	−	+	+	−	+
2	190, Lavetta	A	v.B	c	+	+	−	−	−
3	232, Rose	A	v.B	EF	+	+	−	−	−
4	233, Bob	A	v.A	c	+	+	+	+	−
5	234, Gromek	A	V.B	c	+	−	−	−	−
6	235, Unnamed	A	v.AB	c	+	+	+	+	−
7	236, Mel	A	v.B	c	+	+	−	−	−
8	237, Glory	A	v.A	F	+	+	+	+	−
9	238, Shorty	A	V.O	EF	−	+	−	−	−
10	239, Floyd	A	v.A	c	−	+	+	+	−
11	243, Jerry	A	v.A	c	+	+	+	+	−
12	245, Chester	A	v.A(B)*	c	+	+	+	+	+
13	246, Clay	A	v.A	c	−	+	+	+	+
14	247, Unnamed	A	V.A	F	+	+	+	+	−
15	248, Unnamed	A	V.O	F	+	+	−	−	−
16	249, Unnamed	A	V.A	E	+	+	+	+	−
17	250, Unnamed	A	v A	c	−	−	+	+	−
18	251, Unnamed	A	V.B	E	+	+	−	−	−
19	252, Pepe	A	V.A	c	−	−	+	+	−
20	253, Tito	A	v.A	c	−	+	+	+	−
21	254, Fay	A	v.AB	c	+	−	+	+	−
22	255, Ike	O	v.A	c	−	−	+	+	+
23	256, Henry	A	V.O	c	−	+	+	−	+
24	257, May	A	V.O	c	−	−	−	−	−
25	258, Kay	A	V.O	F	+	+	+	−	+
	Controls								
A^c−I^c+:	Val, No. 169	A	V.B	E	+	+	+	−	+
A^c+I^c−:	Frans, No. 103	A	v.A	c	+	+	+	+	−

* Parentheses indicate weak agglutination with the respective reagent, or that clumping occurred only by the antiglobulin method and not by the saline agglutination method.
† The symbols used here have the following significance: c, cef; E, CEf; F, CeF; EF, CEF, and C, Cef.

established with blood samples from twenty-five chimpanzees of the Holloman Panel (Table 4).

The experiments just described demonstrate the value of the Holloman Panel for the continuation and further development of the immunogenetic studies on chimpanzees.

The experiments also underline the importance of obtaining large samples of sera at each stage of the immunization. For this purpose, plasmapheresis techniques have been adapted for use in chimpanzees and other non-human primates, in collaboration with Mr. J. Davis, Dr. P. Day and Miss Lynn Hiller of the National Institute of Arthritis and Metabolic Diseases. The methods of overcoming the problems of vasoconstriction and coagulation in plasmapheresis of non-human primates will be reported later.

The Holloman Panel is also being used in other immunogenetic investigations. Since the animals have all been under long-term observation, and have been investigated thoroughly to exclude the presence of communicable diseases, their blood is now being used for immunization experiments in man. Furthermore, the Panel is beirg used for investigations of platelet and leucocyte groups by Dr. N. R. Shulman[9], for studies on Gm and Inv serum groups in collaboration with Dr. P. Alepa and Dr. W. Terry[10], and for studies on cross-immune isoprecipitins in collaboration with Dr. E. Lichter of the National Institute of Allergy and Infectious Diseases.

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INHIBITION OF RADIATION-INDUCED LEUKAEMIA BY THE INJECTION OF HAEMATOPOIETIC TISSUE: A STUDY OF CHIMAERISM

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KAPLAN has shown that after four doses of sublethal X-irradiation spaced at weekly intervals *C57BL* mice develop lymphatic leukaemia[1]. It has been demonstrated that the thymus is essential for this leukaemia development[2], but that it need not be present during the irradiation. A mouse thymectomized before irradiation, which is given a syngeneic thymus from a new-born mouse as a subcutaneous graft soon after the final dose of irradiation, can, however, develop leukaemia[3].

For leukaemia development most of the haematopoietic tissue has to be irradiated; the shielding of one femur[4], the sternum[5] or the spleen[6] during irradiation greatly reduces the leukaemia incidence. The injection of syngeneic bone marrow cells soon after irradiation also inhibits the development of leukaemia[7], but allogeneic bone marrow is ineffective[8]. With reference to these results, Koller, Davies and Doak[9] wrote: "It seems likely (on the assumption that induced tumors arise directly from irradiated cells) that the ineffectiveness of allogenic bone marrow in altering the carcinogenic effect of the radiation is due to its failure to persist. Thus, when isogenic marrow, or shielding, was resorted to, the treated animals contained populations of irradiated and non-irradiated cells between which selective competition probably favored the non-irradiated cells and, consequently, fewer tumors arose. Animals treated with allogenic marrow, having eliminated the donor cells because of inadequate suppression of the immune responses, contained only irradiated cell populations and the incidence of tumors was therefore comparable to that in uninjected animals". This is supported by the fact that to get maximum inhibition of leukaemia development by syngeneic bone marrow cells a large number of cells must be injected intravenously soon after the last of the four doses of irradiation[10]. These would also be the conditions most likely to lead to the establishment of cellular chimaerism.

In order to test the hypothesis that leukaemia development is inhibited when the injected cells survive and proliferate in the irradiated host, it is necessary to be able to distinguish between the irradiated host cells and the unirradiated donor cells. By making use of a cytologically marked strain *CBA/T6T6* the two types of cell can be distinguished easily. (*C57BL* × *CBA/T6T6*)F_1 mice which have one small marker chromosome, were used as the irradiated hosts and were injected with *CBA/T6T6*

cells with two $T6$ markers or *BALB/c* cells which are unmarked. (*C57BL* × *CBA/T6T6*)F_1 mice were given four doses of 200 r. at weekly intervals starting when one month old. A 220-kV Westinghouse X-ray machine, half value layer 0·4 mm copper, focal distance 100 cm, dose-rate 60 r./min, was used with no added filtration. Within 1·5 h after the last dose of irradiation, the mice were injected intravenously with either 30×10^6 *CBA/T6T6* bone marrow cells, or 30×10^6 *CBA/T6T6* spleen cells, or 1×10^6 *CBA/T6T6* lymph node cells or 30×10^6 *BALB/c* bone marrow cells. In each case the cells were suspended in 0·4 ml. of medium '199'. Some mice were killed during the latent period between irradiation and the appearance of leukaemia and cytological preparations made, basically by the methods of Ford and Hamerton[11] and Rothfels and Siminovitch[12], to see whether dividing cells in the bone marrow, spleen and thymus were of host or donor origin. The rest of the mice in each group were left to find the leukaemia incidence. Mice were killed as they became recognizably leukaemic (from 112 days after the last irradiation).

To determine whether the leukaemia had arisen from the irradiated host cells or the unirradiated injected cells, either the spleen or thymus of the leukaemic mouse was transplanted into host and tissue-donor strain mice. Chromosome analysis was made of cells from the bone marrow, spleen and thymus of leukaemic mice.

Results

(1) *Leukaemia incidence.* The leukaemia incidence in the different groups of mice is shown in Figs. 1 and 2. Day 350 after the final irradiation was taken as an end-point for the experiments since the leukaemia incidence had nearly reached a plateau in all groups of mice by this time. The incidence was significantly lower in mice injected with *CBA/T6T6* bone marrow ($P < 0{\cdot}001$) or *CBA/T6T6* spleen cells ($P < 0{\cdot}001$) than in control mice. Neither injection of *CBA/T6T6* lymph node cells nor *BALB/c* bone marrow cells lowered the leukaemia incidence. In the mice injected wuth *BALB/c* bone marrow, the latent period between irradiation and the diagnosis of leukaemia was significantly longer than in control mice ($P < 0{\cdot}01$), and, at days 150, 200 and 250 after the final irradiation, there was a significant difference between leukaemia incidence in mice given allogeneic bone marrow and uninjected control mice. The final incidence in the two groups was not, however, significantly different.

(2) *Transplantation.* All leukaemias on being tested for transplantability proved only to grow in F_1 mice, suggesting that the leukaemias had arisen from the irradiated host cells and not from the injected unirradiated cells. Even in the few mice in which the injection of *CBA/T6T6* bone marrow or spleen cells had failed to inhibit leukaemia development, the leukaemias were nevertheless of F_1 type.

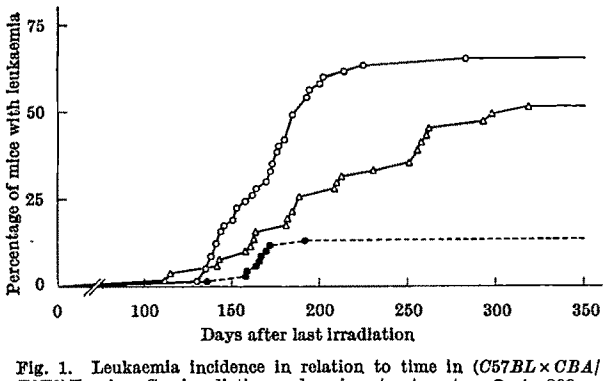


Fig. 1. Leukaemia incidence in relation to time in (*C57BL* × *CBA/T6T6*)F_1 mice after irradiation and various treatments. ○, 4 × 200 r.; △, 4 × 200 r. + *BALB/c* bone marrow cells; ●, 4 × 200 r. + *CBA/T6T6* bone marrow cells

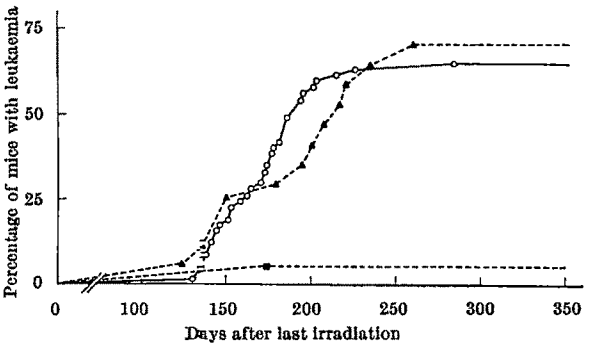


Fig. 2. Leukaemia incidence in relation to time in (*C57BL* × *CBA/T6T6*)F_1 mice after irradiation and various treatments. ○, 4 × 200 r.; ▲, 4 × 200 r. + *CBA/T6T6* lymph node cells; ■, 4 × 200 r. + *CBA/T6T6* spleen cells

(3) *Chromosome analysis of mice during the preleukaemic period.* The chromosome analysis of potentially leukaemic mice during the pre leukaemic period is shown in Table 1. It can be seen that mice injected with *BALB/c* bone marrow cells or *CBA/T6T6* lymph node cells had only host cells dividing, whereas after the injection of *CBA/T6T6* bone marrow or spleen cells, the donor cells predominated. The analysis of thymus and spleen was incomplete, but showed the same proportions of host and donor cells as the bone marrow, except in one mouse indicated in Table I.

Table 1. CHROMOSOME ANALYSIS OF MICE DURING PRE-LEUKAEMIC PERIOD

Treatment	Days after last irradiation	No. of mitotic cells identified in bone marrow	
		Host	Donor
4 × 200 r. + 30 × 10^6 *BALB/c* bone marrow cells	30	87	0
4 × 200 r. + 30 × 10^6 *CBA/T6T6* bone marrow cells*	40	0	119
	70	0	131
	125	1	64
4 × 200 r. + 30 × 10^6 *CBA/T6T6* spleen cells	30	1	66
	98	1	96
4 × 200 r. + 1 × 10^6 *CBA/T6T6* lymph node cells	77	56	0

* In one mouse 3/25 cells scored in the spleen were of host type.

(4) *Chromosome analysis of leukaemic mice.* Table 2 shows the results of chromosome analysis of leukaemic mice. Several of the leukaemias arising in the F_1 mice after injection of *CBA/T6T6* bone marrow, spleen or lymph node cells had appreciable numbers of cells with two $T6$ markers and more than forty chromosomes, although leukaemias transplanted only into F_1 mice. It should be noted that a few cells with two $T6$ markers were found in one leukaemic mouse which had been injected with *BALB/c* bone marrow cells, and that in two F_1 mice, which had been given *CBA/T6T6* cells, some cells with three $T6$ markers were seen.

(5) *Chromosome analysis of mice during post-leukaemic period.* Table 3 gives the results of the chromosome analysis of non-leukaemic mice which were killed more than 350 days after irradiation and injection of *CBA/T6T6* bone marrow or spleen cells. It can be seen that in most mice a large proportion of the dividing cells were of donor type, although there were also appreciable numbers of dividing host cells especially in the spleen. Three of the mice had some abnormal cells present, including a few with three $T6$ markers.

Discussion

(1) *Leukaemia incidence.* The leukaemia incidence after four sub-lethal doses of irradiation in the (*C57BL* × *CBA/T6T6*)F_1 mice used in these experiments was 65 per cent, which is significantly lower than that found in *C57BL* mice in this laboratory (90 per cent) and by Kaplan and Brown (95 per cent) (ref. 1). It is, however, very similar to that found by Ilbery[13] in (*C57BL* × *CBA*)F_1 mice, and by Law and Potter[14] in (*C57BL* × *A*) F_1 mice. Kaplan *et al.*[8], who used another strain combina-

Table 2. CHROMOSOME ANALYSIS OF LEUKAEMIC MICE

Treatment	No. of mitotic cells identified in: Bone marrow 1 *T6** Normal	Bone marrow 1 *T6** Abnormal	Bone marrow 0 *T6** Normal	Bone marrow 0 *T6** Abnormal	Thymus 1 *T6* Normal	Thymus 1 *T6* Abnormal	Thymus 0 *T6* Normal	Thymus 0 *T6* Abnormal	Spleen 1 *T6* Normal	Spleen 1 *T6* Abnormal	Spleen 0 *T6* Normal	Spleen 0 *T6* Abnormal	Lymph node 1 *T6* Normal	Lymph node 1 *T6* Abnormal	Lymph node 0 *T6* Normal	Lymph node 0 *T6* Abnormal
4×200 r. $+ 30 \times 10^6$ *BALB/c* bone marrow cells	—	—	—	—	6	44	0	0	0	20	0	0	—	—	—	—
	2	6	0	0	0	1	0	0	9	41	0	0	0	13	0	0
	50	0	0	0	34	0	0	0	—	—	—	—	—	—	—	—
	—	—	—	—	50	0	0	0	37	13	0	0	43	7	0	0
	0	8	0	0	10	40	0	0	18	32	0	0	0	50	0	0
(1)	23	67	0	10	0	64	0	0	12	28	0	2	38	7	0	4
	26	8	0	0	5	4	0	0	64	0	0	0	13	34	0	3
	1 *T6* Normal	1 *T6* Abnormal	2 *T6* Normal	2 *T6* Abnormal	1 *T6* Normal	1 *T6* Abnormal	2 *T6* Normal	2 *T6* Abnormal	1 *T6* Normal	1 *T6* Abnormal	2 *T6* Normal	2 *T6* Abnormal	1 *T6* Normal	1 *T6* Abnormal	2 *T6* Normal	2 *T6* Abnormal
4×200 r. $+ 30 \times 10^6$ *CBA/T6T6* bone marrow cells (2)	5	0	45	0	18	0	0	0	33	0	17	0	50	0	0	0
	—	—	—	—	0	49	1	0	8	3	39	0	—	—	—	—
	1	0	43	6	0	0	3	45	0	0	16	33	—	—	—	—
4×200 r. $+ 30 \times 10^6$ *CBA/T6T6* spleen cells (3)	0	0	24	26	0	2	0	35	0	0	0	70	—	—	—	—
4×200 r. $+ 1 \times 10^6$ *CBA/T6T6* lymph node cells	—	—	—	—	0	53	0	0	5	47	0	0	23	27	0	0
	50	0	0	0	49	0	0	1	50	0	0	0	58	0	0	0
	13	0	0	37	—	—	—	—	0	2	0	48	—	—	—	—
(4)	2	2	5	32	0	0	0	47	0	1	22	3	0	1	0	46

* Cells classified as 'abnormal' had more than 40 chromosomes or an abnormal chromosome present.

(1) Two cells in thymus, eight cells in spleen, and one cell in lymph node with three *T6* markers.

(2) One cell in spleen with three *T6* markers.

(3) Thirteen cells in thymus with three *T6* markers.

(4) Nine cells in bone marrow, two cells in thymus, three cells in spleen, one cell in lymph node with four *T6T6* markers (4*n*), one cell in thymus, one cell in spleen, two cells in lymph node with two *T6* markers (4*n*).

Table 3. CHROMOSOME ANALYSIS OF NON-LEUKAEMIC MICE

Days	Treatment	No. of mitotic cells identified in: Bone marrow Host	Bone marrow Donor	Thymus Host	Thymus Donor	Spleen Host	Spleen Donor
410	4×200 r. $+ 30 \times 10^6$	22	40	0	17	17	33
410	*CBA/T6T6*	0	50	1	9	6	14
412	bone marrow cells	3	47	0	33	2	4
417		2	41	0	50	3	21
417		4	46	0	17	29	21
420		0	50	—	—	36	14
477		5	45	4	3	26	24
477		1	49	0	21	5	45
477		0	50	0	2	5	45
501		4	45	0	2	14	36
504		33	17	—	—	33	17
518		1	49	—	—	7	43
548		21	29	0	8	15	35
548		0	50	0	30	6	44
590	*	1	49	—	—	4	28
600	†	2	48	1	0	5	5
731	‡	4	46	—	—	5	37
554	4×200 r. $+ 30 \times 10^6$	0	50	—	—	0	50
554	*CBA/T6T6*	0	50	—	—	0	50
571	spleen cells	0	50	—	—	0	37
624		0	50	—	—	0	38
641		4	46	—	—	18	15
648		2	48	—	—	26	24
719		0	50	—	—	0	50

* Five cells in bone marrow, five cells in spleen with three *T6* markers. Nineteen cells in spleen with one *T6* marker and more than forty chromosomes.

† Thirty-nine cells in spleen with two *T6* markers, one cell in spleen with three *T6* markers and more than forty chromosomes.

‡ Eight cells in spleen with one *T6* marker and more than forty chromosomes.

tion (*C57BL* × *BALB/c*)F_1, reported a much higher incidence (92 per cent). Although the overall incidence differed, the mean latent period between irradiation and the appearance of leukaemia was very similar in both parental *C57BL* and F_1 mice, which seems to indicate that the same leukaemia induction mechanism is operating in the F_1 mice as in the parental mice. This is one assumption which will be made in discussing the experiments reported here.

The leukaemia incidence in F_1 mice after the injection of *CBA/T6T6* bone marrow cells (14 per cent) or *BALB/c* bone marrow cells (51 per cent) confirms the results of Kaplan *et al.*[8,10] using *C57BL* mice, which showed that injection of syngeneic bone marrow results in a much decreased incidence, whereas allogeneic bone marrow is ineffective. The present experiments also showed that the injection of parental spleen cells was as effective as injection of parental bone marrow. This observation was, perhaps, foreshadowed by the findings of Lorenz *et al.*[6] that spleen-shielding during irradiation of *C57BL* mice resulted in a very low leukaemia incidence. It should be mentioned that many of the F_1 mice given parental spleen cells developed wasting disease, which can be attributed to a reaction of the injected, immunologically competent spleen cells against the other parental antigens of the F_1 recipient. The wasting disease was fatal in 40 per cent of the mice. This reaction was exaggerated when lymph node cells were injected and necessitated the reduction of cell dose; mice given 10×10^6 lymph node cells all died, while mice which received only 1×10^6 lymph node cells all survived.

In the present experiments the injection of parental lymph node cells was found to be ineffective in reducing leukaemia incidence below control levels. That this is not due solely to the small cell dose which could be injected is shown by unpublished experiments in this laboratory in which the injection of 30×10^6 syngeneic (*C57BL*) lymph node cells into *C57BL* mice failed to reduce leukaemia incidence.

The latent period was found to be not significantly different from that in the irradiated but uninjected F_1 mice, except in the mice which had been injected with allogeneic bone marrow, where there was a significant increase in the induction time. These were the only mice in which a host-versus-graft reaction could in theory occur. It is difficult to predict what the effect of this reaction might be, since the cell populations involved and their interaction *in vivo* are unknown. It is possible, however, that the reaction against the injected foreign (*BALB/c*) cells in some way retards the leukaemogenic process. One explanation of this could be that the (F_1) target cells for leukaemogenesis are involved in the anti-graft activity and that during this immunological reaction they are reduced in number. This could account for the increase in the induction period in these mice, if the time taken for leukaemia to appear depends on the build-up of a certain number of potentially leukaemic cells. It does, however, seem probable that a graft-versus-host reaction, which should have occurred in irradiated F_1 mice injected with parental spleen or lymph node cells, was ineffective in altering the time of onset of leukaemia and therefore did not affect as target cells the pre-leukaemic host cell population.

(2) *Transplantation.* All leukaemias arising in these experiments, including the few in mice injected with

CBA/T6T6 bone marrow or spleen cells, transplanted only into F_1 mice, strongly suggesting that they had arisen from irradiated host cells and not from the unirradiated donor cells. This test is widely accepted as being conclusive evidence that no parental leukaemic cells are present, but in some respects it may be unsatisfactory. F_1 mice will grow both parental and F_1 tumours. Parental mice, however, will reject F_1 cells and it is difficult to predict whether this rejection could affect the growth potentialities of a few (possibly leukaemic) parental cells mixed in with the F_1 cell populations. In the present experiments no artificial mixtures of known parental and F_1 tumours were used to test the validity of this point, and thus the transplantability test, showing as it did only host cell tumours, must be accepted with some, although slight, reserve.

(3) *Chromosome analysis of mice during the pre-leukaemic period.* The cytological analysis of pre-leukaemic mice showed that no abnormal cells were present. This is in contrast to the results reported in a recent paper by Joneja and Stich[17], who showed that 56 per cent of the mitotic cells in the thymus 7 weeks after irradiation were abnormal. However, in the experiments reported here the analysis of the thymus was inadequate. Joneja and Stich also found very few abnormal mitotic cells in the bone marrow.

The cytological analysis revealed that when either parental bone marrow or spleen cells were injected after the final dose of irradiation, chimaeras were obtained. Recently published results by Ilbery[13] indicated that by 60 days after irradiation none of the injected (*C57BL* × *CBA/T6T6*)F_1 cells could be found dividing in his irradiated (*C57BL* × *CBA*/H)F_1 hosts. This is possibly because he injected a far smaller number of bone marrow cells, 4×10^6 as compared with 30×10^6 in the present experiment. He found a leukaemia incidence of 30 per cent in bone marrow injected mice as compared with 15 per cent in similar mice in the present experiments. Despite this finding, the present results seem to indicate that there is a negative correlation between the incidence of leukaemia and the establishment of chimaerism (see Table 4); this bears out the postulate on which these experiments were designed. Unfortunately it is impossible to know whether a mouse analysed during the pre-leukaemic period would have become leukaemic or not, since cytological analysis necessitates killing the mouse. However, 6 per cent or 14 per cent respectively of the mice analysed after the injection of parental spleen or bone marrow cells should have developed leukaemia, but as so few were actually analysed in the pre-leukaemic period there remains the possibility either that animals can be ostensibly chimaeric during the pre-leukaemic period but still develop leukaemia of host-type (as shown by transplantability) or that some non-chimaeric mice were present but were not detected.

Table 4. RELATIONSHIP BETWEEN LEUKAEMIA INCIDENCE AND CHIMAERISM

Treatment	No. of mice	Leukaemia incidence (%)	Chimaerism
Control 4 × 200 r.	57	65	
4 × 200 r. + 1 × 10^6 *CBA/T6T6* lymph node cells	17	71	—
4 × 200 r + 30 × 10^6 *BALB/c* bone marrow cells	51	51	—
4 × 200 r + 30 × 10^6 *CBA/T6T6* bone marrow cells	66	14	+
4 × 200 r. + 30 × 10^6 *CBA/T6T6* spleen cells	18	6	+

(4) *Chromosome analysis of leukaemic mice.* Several analyses of leukaemias induced in *C57BL* mice by the appropriate irradiation which have been published, and unpublished observations in this laboratory show that chromosome abnormalities are a characteristic of these leukaemias. Thus, one might expect that leukaemias arising in mice injected with parental lymph node cells or allogeneic bone marrow, where there was no evidence for chimaerism during the pre-leukaemic period, would show abnormalities and this was the case. However, these abnormalities which relate solely to the leukaemogenic process complicate the present investigation, where it was hoped to identify both host and injected cells, in those groups which had been chimaeras during the pre-leukaemic period. Despite the fact that all leukaemias transplanted as host-type (F_1), there is a possibility that four populations of cells are present, namely normal and abnormal host cells and normal and abnormal donor cells. This supposes that the leukaemogenic agent(s) can induce abnormalities in the donor cells, without necessarily causing them to become leukaemic. Thus in leukaemias arising in mice injected with parental lymph node cells it is difficult to be sure whether the cells with two *T*6 chromosomes were donor cells or altered F_1 cells. However, no donor cells were found in the preleukaemic period, and all leukaemias transplanted as host type. It therefore seems more likely that the *T*6 chromosome tends to undergo non-disjunction in response to leukaemogenic stimuli. This supposition is supported by the finding of one leukaemic mouse in the group injected with allogeneic bone marrow, which had an appreciable number of cells with two *T*6 chromosomes. In these mice no cells of such a phenotype were introduced and therefore they must have derived either from the irradiated singly marked host, or, less likely, from the unmarked donor cell population. Overall there is, in mice injected with parental lymph node cells or allogeneic bone marrow cells, little evidence that the injected cells are involved in or affected by the leukaemogenic process.

It is of interest in this connexion that Kemp and his collaborators[15] and Hammouda *et al.*[16] have recently described cells among human leukaemic cell populations with two *Ph*¹ chromosomes. In a similar manner to that postulated here for the small *T*6 chromosomes, non-disjunction could have led to a duplication of the *Ph*¹ chromosome; such a mechanism for the development of aneuploid cells is perhaps particularly relevant to cells containing abnormally small chromosomes.

In the groups of mice injected with parental bone marrow or spleen cells there were very few leukaemias, but the cytological analysis of these was likely to have been complicated for the reasons detailed above. As before, cells with one and two *T*6 chromosomes were found as significant components of the leukaemic populations analysed. In one mouse a significant population of cells with three *T*6 chromosomes was observed. Despite the indication that donor cells were involved, it seems likely for the reasons already given, and the fact that all leukaemias transplanted only as host-type, that few if any donor cells are likely to have become leukaemic. It seems then that only if complete or nearly complete chimaerism is established is the leukaemia incidence greatly reduced. However, at the time that leukaemic mice were being killed for cytological analysis no comparable sample was taken from mice which were not at the time leukaemic. There is therefore no estimate of the incidence of chimaerism in non-leukaemic mice between 112 and 410 days after irradiation. To some extent this omission is remedied by the analysis of non-leukaemic mice in the post-leukaemic period.

(5) *Chromosome analysis of mice during post-leukaemic period.* The choice of day 350 after the final irradiation as the end of the leukaemic period was arbitrary. 350 days is about 70 days after the main leukaemic period, which is between 120 and 280 days after irradiation. A few leukaemias did, however, arise in most groups more than 350 days after irradiation. Chromosome analysis and transplantation of these showed similar results to those given previously. The results included in the analysis during the post-leukaemic period were in all cases of non-leukaemic mice. A few mice with abnormal chromosomes were found; similar findings are reported by Joneja and Stich[17]. It is possible that these mice were still in a pre-leukaemic condition. Some mice were still complete chimaeras, which shows that once chimaerism is established it can be long-lived. Many mice had appreciable numbers of host cells, but this was independent of the time after

irradiation and injection. This is of more interest to those working with radiation chimaeras, as it suggests that very late reversion can be a common phenomenon. These findings suggest that during the leukaemic period at least a few non-leukaemic mice with appreciable numbers of host cells would have been found. It seems that complete chimaerism is not a prerequisite for the non-leukaemic condition in these groups of mice.

The problem remains whether the inhibition of leukaemia development in mice injected with parental bone marrow or spleen cells is simply a matter of repopulation of the haematopoietic tissues (including the thymus) by the unirradiated donor cells, or whether the bone marrow liberates some humoral factor which is actively anti-leukaemogenic. Berenblum *et al.*[18] have shown that leukaemia development in *C57BL* mice can be inhibited by the injection of multiple doses of large amounts of sheep spleen homogenate, which suggests that there is a non-species-specific substance present in haematopoietic tissues which is capable of inhibiting leukaemia development. Further investigation is required to resolve this problem.

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CELL MULTIPLICATION RATES DURING DEVELOPMENT OF THE PRIMITIVE CARDIAC TUBE IN THE CHICK EMBRYO

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THE transformation of the primitive cardiac tube from a straight hollow blood-containing cylindrical structure into one with the form of an S-shaped loop during early embryogenesis of the heart has been described in detail from a morphological point of view[1–5]. There is as yet, however, little understanding of the mechanisms by which the change is effected. During this period of development, increases in the length and diameter of the tube must be accompanied by an increase in the total number of cells in the two layers which form the tube, namely, the myoepicardial layer (outer mantle) and the endocardial layer (inner mantle). Changes in configuration must be the result of rates of cell population expansion which differ at the same time or have different temporal sequences of change in circumscribed portions of the tube, or both. The present investigation was directed towards the elucidation of some of the patterns of cell multiplication during this period of development in the chick embryo heart by the use of autoradiography with tritiated thymidine. The 'radioactive index', as described by Messier and LeBlond[6] (that is, the percentage of cells in a specified tissue which incorporate tritiated thymidine after controlled exposure), was used as an indicator of relative rates of cell multiplication.

Thirteen chick embryos, from developmental stages 10–17, inclusive, as defined by Hamburger and Hamilton[7], were examined. These stages represent ages of approximately 35 h–3 days of incubation; they encompass the period of development from the onset of cardiac contractions in the straight tube to the fullest development of the S-shaped loop. 25 μc. tritiated thymidine, 6·7 c./mmole, contained in 0·1 ml. of normal saline, were injected through a hypodermic needle into the yolk of each embryo immediately below the area vasculosa. Incubation was continued thereafter for 1 h. The embryos were then fixed in 10 per cent neutral formalin, removed from their yolks, and their stages of development determined. Serial sections of the entire heart, 7μ thick, were cut in the transverse plane, and autoradiographs prepared by a slight modification of the dipping method of Messier and LeBlond[8]. Representative portions of both the myoepicardial and endocardial layers of each heart were then selected for determination of their radioactive indices in the following way: seven or eight levels, approximately equally spaced apart from the most caudal to the most cephalic ends of the heart tube, were selected. At each level four adjacent microscopic sections were examined. For the myoepicardial layer, in each of the sections at each level, between two and six identical areas, arbitrarily chosen but approximately equally spaced around the circumference of the layer, were marked off, each area being that portion of the layer enclosed by a microscope eyepiece grid which delineated an area of 1,000μ^2 under a magnification of × 970. The radioactive index was then calculated after counting, with a hand counter, the number of radioactively labelled cells and the total number of labelled and unlabelled cells in each area. Fig. 1 illustrates the method for the myoepicardial layer. The radioactive indices of the endocardial layer, which in all embryos was 1-cell-diameter in thickness, were determined by counting cells around the entire circumference of this layer in each section examined. Totals of between 6,000 and 23,000 cells in each embryo heart were counted.

After preliminary inspection of the data, the primitive heart tube of each embryo was divided anatomically along its cephalocaudad axis into three parts; the primordia of the atrial, ventricular and bulbar chambers. Because it is impossible to separate with certainty the atrial from the ventricular primordia before stage 12, the cardiac tube caudal to the bulbus at stages 10 and 11 was divided into cephalic and caudad halves, and the former classified for analysis as 'ventricle' and the latter as 'atrium'. Analysis was carried out to answer the following questions: (1) Do the mean radioactive indices of each layer of the three chamber primordia increase or decrease significantly with advancing development? (2) Is there a significant difference between the mean radioactive indices of the myoepicardial layer compared with those of the endocardial layer at any or all of the stages of development investigated? (3) Is there a significant difference between the mean radioactive indices of the myoepicardial layer of each of the three chamber primordia compared with each other at any or all of the stages of development? In addition, the radioactive

indices in the local areas of the myoepicardial layer were compared with each other to determine if there was any recognizable pattern to the anatomical distribution of areas with higher or lower indices along any linear axis or around the circumference of the tube.

Statistical analysis of the results consisted of a standard linear regression analysis[9] applied to the radioactive indices as functions of the stages of development. A time scale was sought which would make the radioactive indices increase (or decrease) linearly as a function of age (that is, time). Measurement of the stages of development (ranging from 10 to 17) in the variable log (stage 9) produced reasonably good straight lines. Thus, the regression equation used was:

$$RI = \alpha + \beta \log (S - 9) + e$$

where RI = radioactive index; S = stage; e = observational variability about the regression line; α = intercept of the regression line; β = slope of the regression line.

For each chamber primordium, data for both the myoepicardial and endocardial layers were analysed. Thus, six regression lines were fitted to the data, one for each layer of each chamber primordium. For each line the intercept and the slope were estimated by the method of least squares. These estimates and their standard errors are given in Table 3. The data from which the points were taken are presented in Tables 1 and 2. The best estimate of the radioactive index at any particular stage (S^*) was taken to be the value of the estimated regression line at that stage, that is:

$$a + b \log (S^* - 9)$$

where a = least squares estimate of α; b = least squares estimate of β. By usual techniques, a standard error was attached to this estimated regression value. This standard error involves the variability of the points about the line, the stages entering into the computation of the line, and the stage (S^*). These estimates and their standard errors were used to test for differences between the radioactive indices of the different chamber primordia and the two tissue layers of the tube by means of t-tests. The regression lines were computed using all the observations on all the embryos. Since some embryos had more observations made on them than others, the data were tested for variability due to embryos. None was found for the atrium or ventricle, but there was some evidence of embryo-to-embryo variability for the bulbus. An appropriate weighted regression was computed for the bulbus using the within-embryo and between-embryo variances. The difference between the weighted and unweighted regressions was so small that the latter is the one reported for the sake of consistency with the other two chamber primordia. In the figure in which the regression lines are plotted, the points represent averages for the embryos. Some averages are based on more observations than others, and this has been taken into account. The averages rather than the individual points are plotted to avoid cluttering the graphs with too many points.

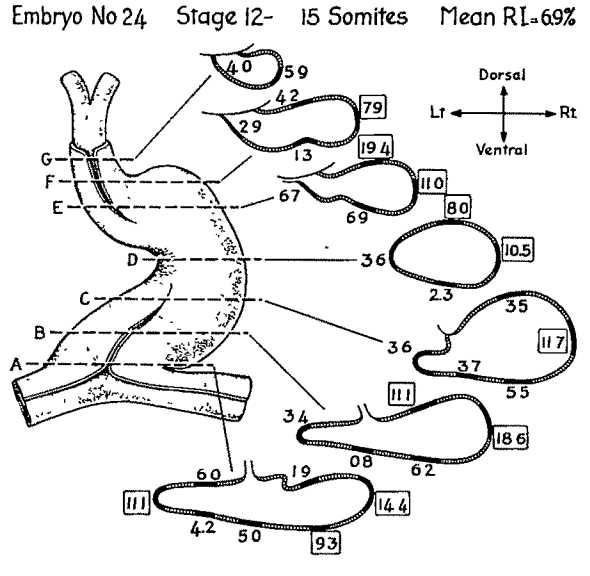


Fig. 1. Diagram of the method of selection of areas for determination of the radioactive index in the myoepicardial layer as exemplified by one of the embryos investigated. On the left is the primitive heart tube at stage 12−, as viewed from its dorsal aspect. The lettered hatched lines show the levels along the cephalo-caudad axis of the tube selected for analysis. Opposite each line is a semi-schematic drawing of the configuration of the myoepicardial layer in cross-section at that level. The blacked-in portions of the layer were those selected, at arbitrary points around the circumference of the layer, for counting. The figures opposite each blacked-in area are the radioactive indices (as percentages) for that area. The figures enclosed in line boxes are those which exceed the mean radioactive index for the entire heart tube

Table 1. RADIOACTIVE INDICES OF THE MYOEPICARDIAL LAYER OF EACH CHAMBER PRIMORDIUM

Embryo No.	Stage	Atrium mean N	Atrium mean R.I. (%)	Atrium mean S.E. (%)	Ventricle mean N	Ventricle mean R.I. (%)	Ventricle mean S.E. (%)	Bulbus mean N	Bulbus mean R.I. (%)	Bulbus mean S.E. (%)
38	10+	6	3·2	0·9	10	0 6	0·2	4	1·5	0·4
52	10+	8	1·2	0·4	8	1·0	0·4	6	1·3	0·5
51	11−	9	2 3	0·8	8	1·2	0·4	6	0·9	0·4
24	12−	17	7·1	1·2	11	7·5	1·5	3	4·2	0·9
48	12+	9	5·1	1 0	23	8 6	0·8	4	1·0	0 1
39	13+	10	9·7	1·0	21	10 4	0·6	7	3·9	1·0
64	14−	15	9 1	0·7	20	5·9	0·5	6	3·8	0·5
63	14	13	7·5	0·8	16	7·8	1·1	6	4·0	0·5
47	15	17	7·6	0·6	24	8·1	0·5	9	6·1	0·7
54	15	14	7·9	0·8	16	8·6	0 8	10	2·6	0 5
67	16	13	8·4	0·7	18	10·4	0 7	9	3·5	0·6
41	17	14	9·6	1·0	21	10·6	0·7	12	4·3	0·5
49	17	12	11·0	0 7	22	10·9	0·9	15	6·9	0 6

N is the number of determinations, each representing the mean of counts made at identical sites around the circumference of the myoepicardial layer from four adjacent histological sections. Sites were selected at two or more levels along the cephalocaudad axis of each chamber primordium.

Table 2. RADIOACTIVE INDICES OF THE ENDOCARDIAL LAYER OF EACH CHAMBER PRIMORDIUM

Embryo No.	Stage	Atrium mean N	Atrium mean R.I. (%)	Atrium mean S.E. (%)	Ventricle mean N	Ventricle mean R.I. (%)	Ventricle mean S.E. (%)	Bulbus mean N	Bulbus mean R.I. (%)	Bulbus mean S.E. (%)
38	10+	2	21·2	0 6	3	20 9	4·0	2	41·7	1·7
52	10+	3	22·7	0·9	2	23·9	4·9	3	26·0	4·0
51	11−	2	18·0	3 0	3	19·9	1·7	3	27·7	5·6
24	12−	3	26·7	7·4	3	14·2	4·2	1	25·0	—
48	12+	2	29 6	2·8	4	21·5	2·1	1	10·2	—
39	13+	2	24·0	0·2	5	25 9	2·6	2	22·6	5·9
64	14−	3	20·3	3·7	4	17·0	1·9	2	24·9	1·5
63	14	3	18 6	0·4	4	17·8	1 2	2	14·6	3·0
47	15	4	25·2	1·2	4	19 1	1·6	2	13·4	5·5
54	15	4	19·2	0·4	3	28·1	2·2	3	14·2	1·7
67	16	4	16·3	1 8	4	20·7	1 4	2	28 2	2·9
41	17	1	20 2	—	4	15·7	1·4	3	14·9	0 7
49	17	3	15·0	6·3	4	17·8	5·8	3	13·9	1·0

N is the number of determinations, each determination being the mean value of counts made around the entire circumference of the endocardial layer from four adjacent histological sections.

Table 3. DATA FROM WHICH THE REGRESSION LINES IN FIG. 1 WERE DRAWN AND THE SIGNIFICANCE OF THE SLOPES OF THESE LINES FOR THE MYOEPICARDIAL AND ENDOCARDIAL LAYERS OF EACH CHAMBER PRIMORDIUM

Tissue	Intercept	S.E.	Slope	S.E.	Scale	P-value of slope
Atrial myoepicardium	1·6	0·7	9·1	1·0	$\times 10^{-2}$	1%
Ventricular myoepicardium	0·7	0·7	11·0	1·0	$\times 10^{-2}$	1%
Bulbar myoepicardium	0·3	0·6	5·4	0·8	$\times 10^{-2}$	1%
Atrial endocardium	24 5	2·5	−5·7	3·8	$\times 10^{-2}$	10%
Ventricular endocardium	21·6	2·4	−2·4	3·5	$\times 10^{-2}$	N.S.
Bulbar endocardium	33·1	3·0	−20 0	4 5	$\times 10^{-2}$	1%

Fig. 2 shows the regression lines calculated to determine how the mean radioactive indices of the layers of each chamber change with advancing development, and Table 3 presents the data from which these lines were plotted. These show that there is a significant progressive increase in the mean radioactive indices of the myoepicardial layer in each of the chamber primordia with advancing development. There is no significant change in relation to age in the indices of the endocardial layer of the atrial or ventricular primordia, but the mean radioactive index

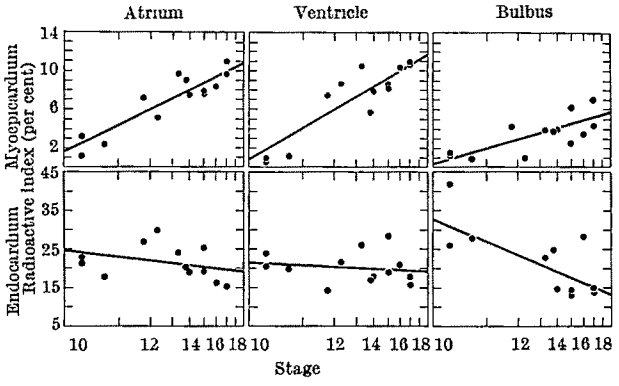


Fig. 2. Regression lines for the endocardial and myoepicardial layers of each chamber primordia plotted against stage of development. The stages of development are represented along the abscissa of the graphs on a semi-logarithmic scale. The data from which the points on the graphs are derived are recorded in Tables 1 and 2 and the values from which the regression lines were derived are in Table 3

of the bulbar endocardial layer decreases to a significant degree with advancing age.

Table 4 compares the mean radioactive indices of the endocardial layer with those of the myoepicardial layer at stages 10 and 17. In each of the chamber primordia at these two stages of development, the mean index of the endocardium is higher than that of the myoepicardium to a significant degree. Since the values for the myoepicardial layer increase with advancing development and those of the endocardium either do not change or decrease with age, the fact that there is equal significance to the difference between the two layers at the earliest and latest stages investigated indicates that the endocardial layer throughout the length of the tube has a significantly higher radioactive index during the entire period of development studied.

Table 4. COMPARISON OF THE RADIOACTIVE INDICES OF THE MYOEPICARDIAL AND ENDOCARDIAL LAYERS OF EACH CHAMBER PRIMORDIUM AT STAGE 10 AND STAGE 17

Stage	Tissue	Myoepicardial mean R.I.	*S.E.*	Endocardial mean R.I.	*S.E.*	*P*-value (difference)
10	Atrium	1·6	0 7	24 5	2·5	1%
	Ventricle	0 7	0 7	21 6	2 4	1%
	Bulbus	0 3	0·6	33·1	3·0	1%
17	Atrium	9·9	0·4	19·4	1·5	1%
	Ventricle	10·7	0 3	19·4	1 3	1%
	Bulbus	5 1	0 3	15 0	2 0	1%

Values are derived from the regression lines illustrated in Fig 1.

Table 5. COMPARISON OF THE RADIOACTIVE INDICES OF THE MYOEPICARDIAL LAYER OF EACH CHAMBER PRIMORDIUM AT STAGES 10 TO 17

Stage	Atrium	*S.E.*	Ventricle	*S E.*	Bulbus	*S.E.*	*P*-value of difference Atrium against bulbus (%)	*P*-value of difference Ventricle against bulbus (%)
10	1·6	0·7	0·7	0 7	0·3	0 6	N.S.	N.S.
11	4·4	0 4	4·1	0·4	1·9	0·4	1	1
12	6·0	0 3	6·0	0·3	2·8	0·3	1	1
13	7·1	0 3	7 4	0·2	3·5	0·2	1	1
14	8·0	0 3	8·5	0·2	4·0	0·2	1	1
15	8·7	0·3	9·3	0·3	4·5	0·2	1	1
16	9·3	0·3	10·1	0·3	4·8	0 3	1	1
17	9 9	0·4	10·7	0 3	5·1	0 3	1	1

Values are derived from the regression lines illustrated in Fig. 1.

Table 5 compares the mean radioactive indices of the myoepicardial layer of the individual chamber primordia with each other at each stage. Initial evaluation of the data showed clearly that there were no significant differences between the mean radioactive indices of this layer in either the atrial or ventricular primordia at any stage, and thus no more detailed analysis was carried out. Statistical evaluation of the differences between the bulbus and the other two chambers shows that the values for the bulbus are lower than those for the atrium and ventricle to a significant degree at all stages after stage 10.

No pattern of anatomical distribution of localized areas with higher or lower radioactive indices along any linear axis or around the circumference of the tube could be defined at any stage.

Messier and LeBlond[6] have demonstrated the validity of using the radioactive index determined with tritiated thymidine as an indicator of the rate of cell multiplication in expanding or renewing cell populations. The time of exposure of the tissue to the tritiated thymidine used in this study (1 h) was chosen arbitrarily. An older investigation of growth of embryonic tissues[10] concluded that the duration of the mitotic cycle in early stages of cardiac development in the chick probably is shorter than 1 h; thus, no conclusion can be reached as to whether the differences we have observed in the radioactive indices in different portions of the primitive cardiac tube are the result of variable percentages of cells being in the *S* phase of the cell division cycle at any one time, or whether they result from varying durations of the division cycle which allow more than one division during the hour of exposure, or both. There was not a sufficiently significant enough difference in the grain density of the label of the cells to use this criterion as a means of identifying different generations after incorporation of the label. Further investigations, using serial determinations of the radioactive indices at multiple short intervals of time after the injection of the tritiated thymidine, are necessary to define the duration of the *S* phase of cell division in these tissues. In any case, it is valid to accept differences in radioactive indices as indications of differing rates at which cell populations are increasing, whatever the mechanisms of the differences may be.

No studies similar to these on the primitive cardiac tube have been reported previously. Grohmann[11] has reviewed studies prior to 1961 on the mitotic index of various tissues in the chick embryo and used this method in evaluating the growth of the chick heart during embryonic and foetal development. He found an increase in the mitotic index of the ventricular myocardium from day 1·5 of incubation to day 4, after which the index fell gradually until hatching. This finding correlates with the increase in the mean radioactive index of the myoepicardial layer of the ventricular primordium found in this study, although our observations were made only until the third day of incubation. Grohmann also found considerable differences in the mitotic index in various portions of the heart. At day 1·5 of age the atrial and ventricular myocardial walls had similar mitotic indices; thereafter, until the thirteenth day, the ventricular indices were higher. The endocardial mitotic index exceeded that of the myocardium only on the second day, and then by less than 1 per cent. Differences in the technique of investigation, and the fact that Grohmann examined only three stages of development during the first 3 days of incubation without indicating the stages of development of his specimens in more detail by standards such as those of Hamburger and Hamilton, make his results non-comparable with those of this study.

The increase in the mean radioactive index of the myoepicardial layer of the entire tube during the period studied strongly suggests that there is a progressive increase in the growth increment of this structure. No data from serial determinations of heart size or weight are available to substantiate this interpretation. The reason for the lack of a similar increase in the indices of the endocardial layer with advancing development (and, in fact, the decrease of the values in the bulbar portion of the endocardium) is not apparent. An increase in the thickness of the myoepicardial wall may account for some of the rise in the index of the layer (especially compared with that of the endocardium which remains 1-cell-diameter in thickness), but this appears unlikely to be a major factor. The similarity between the radioactive indices of the atrial and ventricular primordia at all stages of development indicates that these two portions of the myoepicardial layer are growing at approximately equal rates; the bulbus is increasing its cell population

at a slower rate. Rough estimates of relative changes in the size of the chamber primordia, based on Patten's[12] drawings of the developing chick embryo heart, suggest that these correlate with ratio of the indices at various stages of development, but no firm conclusion regarding this can be reached because of doubt concerning the uniformity of the scale of the drawings. Because no significant patterns of growth in localized portions of the myoepicardial tube were found, this investigation is of no value in explaining how differential growth rates result in changes in configuration of the tube.

The high radioactive indices of the endocardial layer at all stages of development were an unexpected finding. One possible explanation is that cells of the endocardial layer are being lost continually from the layer either by sloughing into the adjacent blood stream or by migration through subendocardial tissue, or both; and that, therefore, there must be increased cell proliferation to replace the lost population as well as to increase with growth the total cell population of the layer. The role of direct haemodynamic pressure of the blood circulating through the tube on the cell multiplication rate of the endocardial layer is a specific factor which should be evaluated.

Other factors are probably of importance in any interpretation of both the positive and negative findings of this study and must be the subject of further investigation. Changes in multiplication rates in localized areas which occur more rapidly than at an interval of 1 h (the time of exposure to tritiated thymidine used in this investigation) could not be detected by this method and may be responsible for changes in configuration of the tube. Migration of cells through the myoepicardial and endocardial layers could not be traced. The presence or absence of multinucleated myocardial cells could not be evaluated, and a significant number of these would change the meaning of the radioactive indices. More complete understanding of the mechanisms which influence the morphological changes occurring during this period of development undoubtedly will require experimental interference with the normal sequences of these changes together with more detailed analysis of the cytodynamics of the cardiac tissue.

This investigation of cell multiplication rates of the primitive cardiac tube of the chick embryo from stages 10 to 17 of Hamburger and Hamilton, using the determination of the radioactive indices of various portions of the tube after the administration of tritiated thymidine, shows a progressive increase, with advancing development, in the growth increment of the myoepicardial layer, a lesser rate of cell proliferation in the bulbar primordium compared with that of the atrium and ventricle at all stages of development except the earliest, and a higher rate of cell multiplication in the endocardial layer compared with that of the myoepicardial layer at all stages of development studied. These cell population cytodynamic patterns form a basis on which further investigation of factors influencing the morphological development of the heart can be carried out.

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NEUROLOGICAL DEGENERATION AND REDUCED SERUM VITAMIN B_{12}-LEVELS IN CAPTIVE MONKEYS

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THE recent observation that many primates maintained in captivity on vegetarian diets quickly develop low levels of vitamin B_{12} in their serum[1–3] has led to further investigations to determine whether corroborative evidence of vitamin B_{12}-deficiency can be found. Thus the treatment of nine infant rhesus monkeys with the vitamin markedly increased their growth rates as compared with those of infants reared on non-supplemented vegetarian diets[4]. Atrophy of the mucous membranes of the tongue and cheek was found in nineteen monkeys with low serum levels and within a few weeks most of these lesions disappeared following injections of cyanocobalamin[5]. Preliminary evidence suggested that the low serum levels in rhesus monkeys might be related to their poor breeding performance, though so far it has not proved possible to improve fertility with cyanocobalamin alone[2,6].

The possibility that lesions of the nervous system might result from vitamin B_{12}-deficiency is also relevant as it is known that 'cage paralysis' occurs in captive monkeys and apes[7–10]. Certain resemblances to 'sub-acute combined degeneration' of the spinal cord in man—and in retrospect with vitamin B_{12}-deficiency—have been considered[11–13], although the conclusions were uncertain particularly because macrocytic anaemia was not demonstrated. In a review of degenerative demyelinating diseases in primates, Ruch[14] suggested that vitamin B_{12} may be implicated. It is known that demyelinating lesions in the nervous system occur in a number of other animal species (for example, chick, rat, calf) in vitamin B_{12}-deficiency states[15].

With these facts in mind a neurological study of three groups together comprising fifteen monkeys (*Macaca mulatta*) was undertaken.

Paralysed group (5 *monkeys*). These showed low serum vitamin B_{12}-levels and overt paralysis. Duration of captivity varied from 1·5 to 26 years. Neurological examination presented considerable difficulties. As well as the absence of subjective information and of signs elicited by commands, the recording of objective phenomena was problematical and interpreted cautiously. It is likely that only gross signs were recognized.

Paralysis usually followed a consistent pattern. The worst case presented as a flaccid paralysis of the hind limbs and tail. In the less-affected animals mobility was impaired so that either they did not climb, or they climbed clumsily and with difficulty, bearing the body-

weight mainly on the heels. Clumsiness in moving, though partly due to the weak grasp of the feet, seemed to be less evident if the animals watched their limbs. Sometimes there was intention tremor of the limbs. On examination, trophic changes on the heels and toes and loss of hair and skin on the tail were found. Though such lesions appeared to be painless, hyperaesthesia seemed to be caused in some animals by stroking or rubbing the digits or sole, and by pressure on the muscles and tendons of the leg. It proved almost impossible to test power or tone except in the most paralysed cases. Considerable flexion deformity was obvious in most of the affected animals. Knee, ankle and plantar reflexes, all easily obtainable in animals recently arrived in captivity, were usually absent. Sometimes reflexes in the arms could be obtained, but more frequently these were also absent.

Two animals showed severe, active sudanophilic, spongiform demyelination in the posterior and to a lesser extent the lateral columns of the spinal cord (Fig. 1) associated with axonal degeneration but not with significant gliosis: in one of these two cases there was active demyelination of anterior and posterior nerve roots and the anterior horns showed focal lymphocytic and microglial infiltration and neuronophagia. In the other three cases there was similar but less severe posterior and lateral column demyelination without significant axonal changes. Longitudinal sections showed that in all cases lesions in the white matter were more patchy than was apparent in transverse sections. In all five cases there was variable chromatolysis but no significant loss of the large anterior horn motor neurones.

One of the cases with less severe spinal degeneration also showed extensive, blotchy, more or less symmetrical, spongiform degeneration of the central white matter of the cerebral hemispheres (Fig. 2) accompanied by astrocytic hyperplasia and perivascular cuffing with lymphocytes, lipophages and eosinophil polymorphs. The only other animal with cerebral lesions had been captive for the longest period—26 years: throughout the cerebral cortex there was focal spongiform degeneration and gliosis, diffuse (sometimes laminar) neuronal loss, and atrophy of many residual neurones. Though there was no relevant vascular disease, these cortical changes were difficult to evaluate as the animal had been mildly diabetic for 4 years (on 20 units of insulin daily) and on one occasion 6 months before death was found in hypoglycaemic coma, which might have been a causative factor. It should be stressed that the spinal cord and peripheral nerve lesions in this animal were unlikely to have resulted from the diabetes as they were identical to the changes found in all other affected but non-diabetic animals.

Three cases showed severe, patchy, active sudanophilic demyelination of the peripheral nerves (Fig. 3), related proliferation of Schwann cells and mild lymphocytic and

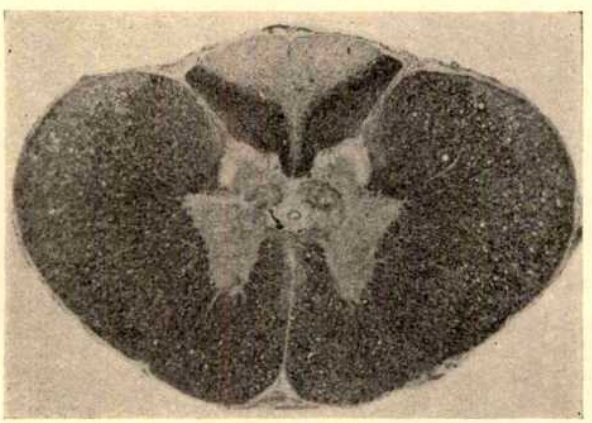


Fig. 1. Degeneration of posterior and lateral columns of spinal cord. Note spongiform appearance in lateral columns. Luxol fast blue-cresyl fast violet. (× *c.* 17)

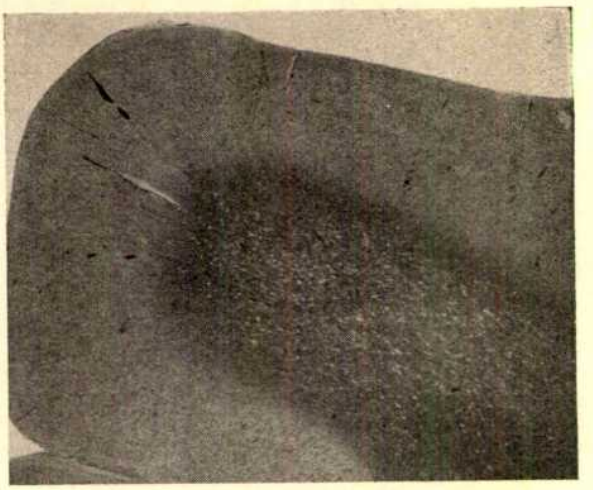


Fig. 2. Spongiform degeneration of the cerebral white matter. Luxol fast blue-cresyl fast violet. (× 8·5)

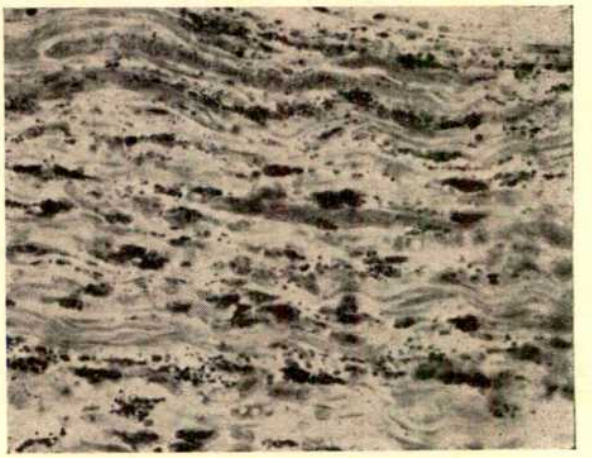


Fig. 3. Severe demyelination of a lateral popliteal nerve. Infiltration with sudanophilic lipophages. (Longitudinal frozen section.) Sudan 3 and 4-haematoxylin. (× 250)

lipophage infiltration; a fourth case showed similar but less-pronounced changes. Degeneration appeared to be most pronounced in the distal parts of the nerves. The peripheral nerves were not examined in the fifth case.

The optic nerves and tracts showed patchy demyelination in the single case in which they were examined.

Long-stay normal group (7 *monkeys*). These showed low serum vitamin B_{12}-levels but no paralysis. Duration of captivity varied from 3·5 to 16·5 years. There were neither trophic lesions on the extremities nor evidence of paralysis. Tendon reflexes in the legs and the plantar responses were usually absent.

Four animals showed mild but definite spongiform degeneration of the posterior and lateral columns of the spinal cord and patchy demyelination of the peripheral nerves, qualitatively similar to the lesions in the paralysed animals. Cerebral involvement was not found.

Short-stay normal group (3 *monkeys*). These showed reduced (and presumably still falling) serum vitamin B_{12}-levels but no paralysis. Duration of captivity was less than 2 years. The animals usually appeared normal with brisk reflexes and definite, repeatable plantar responses.

Two of these showed mild but unequivocal lesions of the spinal cord (Fig. 4) and peripheral nerves similar to those found in the previous 'long-stay normal group'. The brains were not affected.

General haematological findings. The serum vitamin B_{12}-levels were similar to those already reported by Oxnard[2]. Thus, in all the twelve 'long-stay' animals, the range was < 20–80 pg/ml. of serum (compare mean value

35, range <20–70 pg/ml. of serum in 34 'long-stay' animals found previously). The range in the 'short-stay' animals was 60–160 pg/ml., being between the limits previously found in 43 'recently captive' animals (mean value 233, range 110–680 pg/ml.) and those in animals captive for longer than 2 years. The 'short-stay' findings were complicated by the fact that the estimations were made in pregnancy which is known to be associated with reduced levels.

Anaemia was never found. Apart from 'very occasional larger red cells' in one paralysed animal, blood films and absolute values showed no evidence of macrocytosis. In the colony as a whole the routine haematological examination of ninety-one recently captive and seventy-six long-stay monkeys did not reveal anaemia or macrocytosis.

Folic acid assays and marrow smears have not yet been studied.

These preliminary findings indicate that captive monkeys with 'cage paralysis' showed reduced serum vitamin B_{12}-levels associated with spongiform degeneration of the spinal cord, patchy demyelination of peripheral nerves and occasional degeneration of cerebral white matter similar to the neurological lesions of human vitamin B_{12}-deficiency. There are minor differences: in the monkeys, the cord changes were less patchy and sometimes associated with degeneration of nerve roots and anterior horn motor neurones and with more pronounced reactive cellular infiltration.

Similar, but less severe, changes in the spinal cord and peripheral nerves were found in four out of seven clinically normal monkeys which had been captive for periods varying from 3·5 to 16·5 years; also in two out of three clinically normal animals captive for less than 2 years. The fall in serum vitamin B_{12}-level starts early in captivity[2] and therefore accompanies these sub-clinical lesions, which in one case were detected after only 8 months' captivity. It appears that such lesions eventually progress and result in overt paralysis, which affected one animal after only 14 months' captivity.

A cause-and-effect relationship between the reduced serum vitamin B_{12} and the neurological degeneration has not been established, although the similarity to human 'sub-acute combined degeneration' and to the degenerative demyelination in other species[15], together with the considerable objective improvement (at the time of writing) of four paralysed monkeys treated with parenteral cyanocobalamin, are very suggestive. The possibility that other nutritional elements are also deficient cannot be disregarded and it is noteworthy that similar pathological changes have been found in malnourished prisoners of war showing neuropathy[16], and in patients with neuropathy and adult coeliac disease[17,18]. Clinical evidence of chronic gastrointestinal disturbance was not observed in the monkeys. Furthermore, the absence of macrocytosis or other consistent haematological changes suggests that in this species erythrocytopoiesis is relatively resistant to vitamin B_{12}-deficiency.

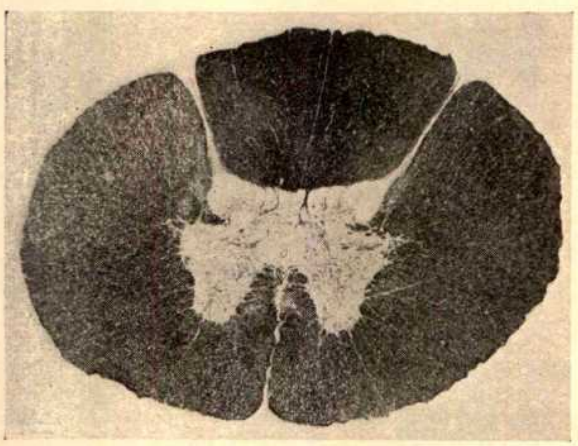


Fig. 4. Asymmetrical spongiform degeneration maximal in one lateral column of the spinal cord. Luxol fast blue-cresyl fast violet. (× *c.* 17)

It seems likely that dietary alterations consequent on captivity, rather than a decrease in absorption or an increase in requirements, account for the fall in serum vitamin B_{12}. Under natural conditions a limited intake of animal foodstuffs (such as insects or other small animals) or coprophagy could provide the small amount of vitamin B_{12} known to be necessary. The animals in the Birmingham colony now receive routine parenteral cyanocobalamin, but it is too early to assess its prophylactic effect either on long-stay or recently introduced inmates. Because of the relative infertility of captive primates with low serum levels[2] it is not yet known if this problem also applies to monkeys born in captivity or if myelination is defective in such circumstances, as has been shown in the chick, calf and rat[15].

Further pathological investigations are being undertaken on the foregoing fifteen and more recently killed animals. In particular, the possibility of faulty absorption of vitamin B_{12} is being considered. There was no evidence of parasitic infestation or of changes suggesting a primary bacterial or viral infection in any animal. The cellular infiltration occasionally noted appeared to be a secondary reaction to neural degeneration rather than the result of a primary infection. A single paralysed animal found to have gross cerebral cysticercosis at necropsy was not included in the series.

Samples of voluntary muscle were always examined but did not show noteworthy changes on routine histological examination, though specialized techniques for intramuscular nerves and nerve endings were not used. Such techniques will be necessary in future investigations, particularly as it appears possible that the peripheral nerves are affected by a distal atrophy or 'dying back'; this type of neural degeneration has aroused considerable interest in recent years[19,20].

Until the relationship of vitamin B_{12} (and other dietetic factors) to the neurological lesions is more precisely defined, the suitability of 'untreated' captive primates for certain types of neurobiological research is suspect. In particular, the association of mental symptoms with human vitamin B_{12}-deficiency sometimes without other neurological disturbance[21] must suggest caution in the use of such monkeys for behavioural and psychological studies. Meanwhile, there is much to be learnt from this experimental model of central and peripheral myelin degeneration.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Mass Ejection by Highly Evolved Stars

THE problem of the surprisingly high abundance of galactic helium which has been observed[1,2] was the stimulus for this work. This communication proposes a new mechanism for rapid mass ejection from highly evolved stars. The mechanism is effective only over a specific, relatively short period of time in the life of the star. The general problem of mass loss from stars is the subject of an excellent review by Weymann[3].

As Schwarzschild[4] shows (see his Fig. 28.3), the red giant phase of stellar evolution is characterized by a convective hydrogen envelope of very low density and a high-density helium core. Between these, in a very narrow transition zone, is the hydrogen-burning shell.

Let us examine closely the state of the star when the hydrogen-burning shell is far out and becoming quite thin. It is our basic contention that because of the high power of dependence of nuclear energy production on temperature, T (as T^{ν}, where $\nu = 13$–20 for the carbon cycle and $\nu = 3{\cdot}5$–6 for p-p burning), the shell will 'suddenly' stop burning. By 'suddenly' I mean in a shorter time than the Kelvin gravitational contraction-readjustment time, τ.

Iben[5] comments on the high sensitivity of the shell energy production rate to small changes in structure. One useful criterion might be when the linear dimension of significant fluctuations becomes comparable with the thickness of the shell.

The second basic contention is that, with the shell energy source stopped suddenly, the envelope will cool rapidly compared with τ. This is guaranteed by the fact that as the stellar envelope expands, highly efficient convective-energy transport supersedes radiative transport in more and more of the envelope. This fact can be appreciated from the calculations by Iben[5], Kippenhahn[6], Hayashi[7] and others.

Now, with the shell source having ended and the envelope relatively cool, the star must readjust gravitationally. The enormous, extended light hydrogen envelope is accelerated radially, that is, falls under gravity, into a heavy helium gas core across a relatively sharp boundary. This process is the classic Rayleigh-Taylor instability.

Whereas in the linear region, short wave-length perturbations grow fastest, in the non-linear range (perturbation amplitudes comparable with or larger than the wave-length, λ) the result is fundamentally different.

The first serious attack on the non-linear Taylor instability problem was made in a pioneering computer calculation ('Macbeth')[8] in 1952 by Pennington, Greyber, Wheeler *et al.*, whose results are not widely known. It is shown that the long wave-length perturbations eventually dominate in the non-linear range. Large spikes of heavy fluid penetrate deep into the light fluid region and bubbles of light fluid extend a small distance into the heavy fluid region. (It can be shown that the results carry over to problems of plasma-magnetic field interface instabilities. In 1957 I suggested a different application of the same process to astrophysics[9].)

Thus we conclude that shortly after the shell has stopped burning, large spikes of helium will surge out from the edge of the core, pushing hydrogen before it. Stellar rotation will merely modify the result. If small amounts of heavy elements or a strong magnetic field were to exist in the outer part of the dense core, they would also be brought to the surface of the star.

From a remarkable paper by Enrico Fermi and John von Neumann[10] we can obtain the result that in the late stages of the instability the heavy fluid should penetrate the light fluid with an acceleration

$$g\,\frac{\rho_1 - \rho_2}{\rho_1}$$

where ρ_1 is the density of the heavy fluid and ρ_2 is the density of the light fluid.

The fact that in our case ρ_2 is negligible compared with ρ_1, so that the acceleration of the spike is about g (the value of the acceleration of gravity at the shell), shows that very considerable mass loss can occur during the Kelvin contraction-readjustment time. Obviously it is to be expected that the Helmholtz instability would occur along the sides of the large spike because of the large velocity difference across the interface, and thus contribute to mixing, eventually breaking up the spike of heavy fluid.

Let us survey briefly some astronomical problems to which the proposed mechanism may be relevant:

(1) Clearly a similar ejection of helium, carbon and heavier elements would be caused by the very sudden end of a helium burning shell (energy production goes as T^{30}). This is relevant to the cosmic element abundance problem.

(2) It is interesting to note that a recycling calculation[2] over the age of the galaxy gave too little helium by a factor of 2—assuming that only at the final supernova phase was matter fed back into the interstellar medium. With the many generations of massive stars between the time of formation of the galaxy and the present, even a slightly earlier ejection of helium into the interstellar medium increases the predicted amount of helium significantly. This seems to explain the helium abundance paradox.

(3) Fowler, Burbidge, Burbidge and Hoyle[11], from a careful attempt to account for the chemical compositions of the peculiar A stars, deduce that a powerful mixing process has been at work so that the products of the interior nuclear synthesis have been brought to the surface. The mechanism outlined here appears to be the mixing process required for the A_p and A_m stars. Conceivably the symbiotic stars such as BF Cyg are in this phase now.

(4) The mechanism may explain the evolution of certain special types of stars which require very substantial mass loss of hydrogen reasonably to account for their chemical compositions. Examples are helium stars[12] such as HD 160641 and carbon stars such as R Cor Bor.

(5) The origin of planetary nebulae[13] and shell stars such as Zeta Tauri may be connected with the mechanism, by the formation of a gas shell from the ejected material.

(6) Conceivably a light element (deuterium, lithium, beryllium and boron) burning shell may form in a star contracting to the main sequence. The ejection of gas caused by the sudden end of such a shell could explain the nebulosity seen around many of these stars by Herbig.

(7) Kopal[14] remarks that many investigations are converging, on several grounds, to the conclusion that pulsations are limited to the outer layers of a star. I would propose that, in some stars, the mechanism triggers the stellar pulsations observed.

(8) If the Hoyle–Fowler massive objects can continue nuclear evolution (despite the relativistic instabilities calculated), perhaps by rotation, until shell burning proceeds to termination, the mechanism might explain

the luminosity variations[15] observed in quasi-stellar radio sources.

It is perhaps worth noting the obvious fact that the process discussed herein cannot show up in the present-day computing machine calculations[5–7] because all quantities are only radial-dependent in such calculations (spherical symmetry).

The mechanism suggests that mass loss should be greater for shells that end earlier (due to higher temperature dependence). Hence the CN cycle stars should have greater mass loss than stars on the *p-p* cycle. Whether the astronomical evidence supports such a prediction is not as yet known.

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A Possible Identification of the X-ray Source *Sco X-3*

THE purpose of this communication is to point out the positional coincidence of the X-ray source *Sco X-3* with an extended H II emission region which may be the remnant of a supernova explosion. Originally, a correlation between supernova remnants and X-ray sources had been predicted by theory and has been verified for the Crab Nebula[1] and for Kepler's supernova *SN* 1604 (ref. 1), although the latter identification has become very doubtful[2].

The position of *Sco X-3* is given as:

R.A. (1950)	Decl. (1950)
17h 23m	−44.3°

This position falls just within the upper opening of the U-shaped H II region No. 114 from Rodgers *et al.*[3,4]. This H II region is described by the authors as a faint, ring-like structure of 50 min width and extending over 5·5° × 5.5°.

Although the star density makes it impossible to classify the object in the Hα *Atlas*[4] according to van den Bergh's[5] criteria as belonging to class III, its general appearance fits remarkably well to that of known supernova remnants. Its size is comparable with the nebula around the radio source Vela *X*, to the Cygnus Loop, to *IC* 443, and to *S* 147, and the latter three objects share additionally its more or less ring-like structure.

No radio source has been reported yet in the direction of the H II region No. 114. However, Hayakawa *et al.*[6] have shown that the active time of radio emission of a supernova remnant may be as short as $\sim 10^3$ years, whereas the X-ray activity may last as long as $\sim 4 \times 10^4$ years. The lack of a detected radio source does not, therefore, bear much evidence against the proposed identification.

It may be noteworthy that the Muslim astrologer, Albumazar, observed in about A.D. 827 a reliably recorded supernova[7] in the tail of Scorpio. It became as bright as the Moon at quadrature ($\sim 10^m$) and was observed for 4 months. If this occurrence should be connected with the H II region No. 114, its present size would have to be explained by expansion during the past 1,140 years. If an absolute brightness of -16^m is assumed for the supernova at maximum, this places it at a distance of ~150 parsec; from this follows a very high expansion velocity of 13,000 km/sec. That rather unlikely value cannot be completely ruled out because of the uncertainties involved in our distance estimation and because expansion velocities of at least 7,000 km/sec are observed in supernovae of type II (ref. 8). An observational check could readily be made, because the annual apparent expansion rate of the H II region would have to be 17 sec/year.

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PHYSICS

Detection of Weak Microwave Modulation of Light

WE have devised a method of detecting very low levels of modulation on a laser beam, with the object of assessing the performance of microwave light modulators. The system may either be used simply to indicate the presence of modulation, or, in conjunction with a Fabry–Perot interferometer, to demonstrate the existence of optical sidebands as discrete frequencies.

Use is made of the well-known fact that, in general, the adjacent sidebands emerge from an optical modulator with a different plane of polarization from that of the carrier wave[1–4]. Thus, if crossed Nicol prisms are placed on either side of the modulator, the carrier will be suppressed and only the sidebands will emerge from the analyser prism. Kaminow[4] has in fact used such an arrangement to detect modulation from a high-power pulsed magnetron by receiving the emergent light on a photomultiplier tube and displaying the resulting output pulses on an oscilloscope.

The present modulator consists of a crystal of potassium dihydrogen phosphate in a rectangular *TE* 011 cavity fed from a 5-W continuous X-band source. The effective length of the crystal is about 1 cm, and a laser beam passing along the optical axis of the crystal is weakly modulated at the microwave frequency. The depth of modulation, estimated from published values of the electro-optic coefficients of potassium dihydrogen phosphate[5], is about 1 per cent, so that the intensity of the sidebands is 10^{-4} times that of the carrier. To display the optical modulation we impose an audio-frequency modulation on the

microwave source and insert a narrow-band amplifier[6], tuned to the audio-frequency, between the photomultiplier and the oscilloscope. The signal-to-noise ratio achieved with this system is of the order of 100 : 1.

Kaminow[7], using a 5-W X-band source and a more efficient modulator, has been able to photograph the splitting of the carrier and its adjacent sidebands, using a Fabry–Perot interferometer with dielectric mirrors designed for optimum performance at 6328 Å. The modulation index in this case was 0·03, so that the sideband intensity was about 10^{-3} times that of the carrier.

It is doubtful whether such a system would record sidebands as weak as those produced in our experiment, the resolution of the etalon being limited by light scattered from the principal set of rings. However, by incorporating a polarizer and analyser as already described, we may enhance the modulation index of the light entering the etalon, and so reduce the scattering problem. With such a system, used in conjunction with the narrow-band detector and a Fabry–Perot etalon with simple aluminized mirrors, the weak sidebands produced by our modulator are easily observed. The signal-to-noise ratio in this case is about 10 : 1, the reduction from the previous figure being due to attenuation of the light by absorption in the mirror surfaces. We would anticipate an improvement in signal-to-noise ratio of at least an order of magnitude if suitable dielectric mirrors were used in the Fabry–Perot etalon; a further improvement could be made by incorporating a lock-in detector of the type used in nuclear magnetic resonance systems.

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Nucleation of Thin Films

DURING an investigation into the electrical properties of evaporated thin films prepared under ultra-high vacuum conditions, it was observed that electrical charge on the surface of glass substrates can affect the nucleation density and initial growth structure of thin films of gold.

The substrates (Corning barium alumino silicate glass No. 7059) were cleaned by detergent wash, ultrasonic agitation and vapour de-greasing; after this process a small charge, generally positive, was found on the glass surface. This could be removed by baking the substrate in vacuum, or by condensing a small amount of gold on to the glass. The removal of positive surface charge appears to be a good indication that nucleation has occurred on the substrate. Negative charge disappears more slowly after nuclei have been formed. The vacuum plant used was diode getter-ion and titanium sublimation pumped, and it was observed that surface charge could be induced on the substrate during pump-down. The ion pump gave a positive charge and the titanium pump a negative charge. Both pumps were optically baffled from the evaporation chamber.

The effect of charge on nucleation was examined by polarizing the ends of a 3 in. × 1 in. substrate so that the surface facing the evaporation source had areas of both positive and negative charge. The surface charge on this electret was adjusted during pump-down to 10^{-8} torr so that the two ends were opposite in sign but about equal in magnitude. Gold was evaporated at a slow rate until the positive charge disappeared, which took about 6 sec after the shutter was opened. Evaporation was continued for a further 3 sec before the source shutter was closed, in order to allow further growth of the initial nuclei so that they were large enough to be observed in the electron microscope. Secondary nuclei formed during this evaporation would be so much smaller than the initial nuclei that they would not be observed.

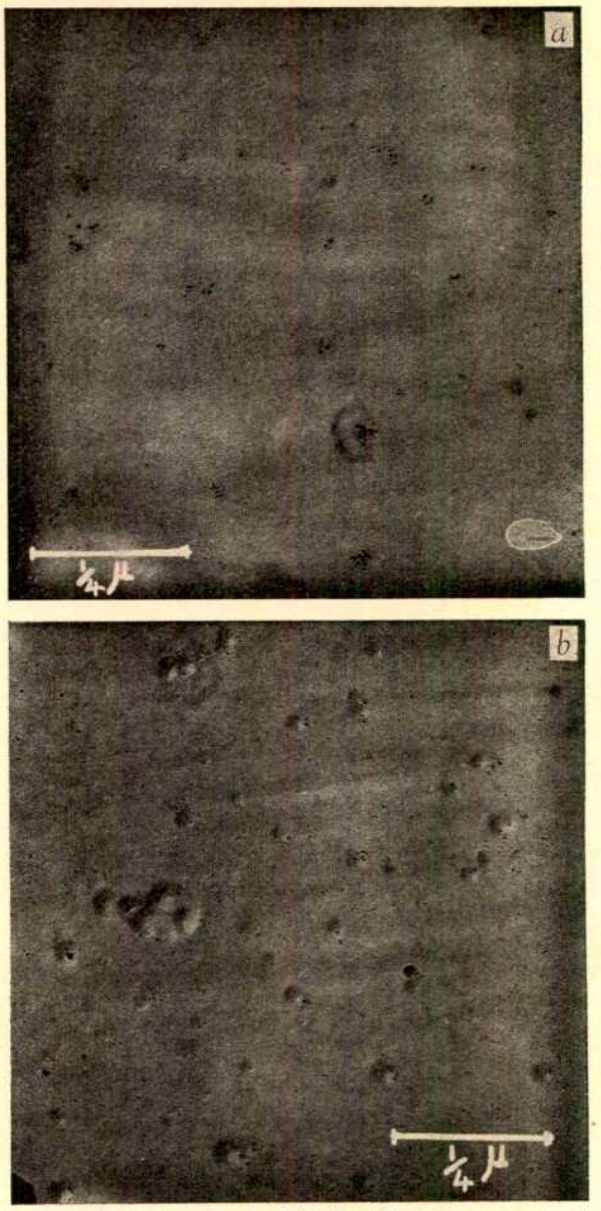


Fig. 1. *a*, Nucleation on a negatively charged surface: charge density $-1{\cdot}1 \times 10^{11}$/cm²; total nuclei density $4{\cdot}4 \times 10^{10}$/cm². *b*, Nucleation on a positively charged surface: charge density $+8 \times 10^{10}$/cm²; total nuclei density $7{\cdot}4 \times 10^{10}$/cm²

The specimens obtained were examined in an electron microscope by transferring the gold film to a thin carbon backing film, and the micrographs are reproduced in Fig. 1. Fig. 1*a* shows the negatively charged substrate, on which the nuclei are dispersed, and appear to be localized around surface imperfections in the glass.

Fig. 1*b* shows the positively charged surface which has a few nuclei present on surface imperfections, and a uniform background of small nuclei. In the positively charged case the total density of nuclei ($7{\cdot}4 \times 10^{10}$/cm²) is close to the charge density (8×10^{10}/cm²) while on the negatively charged surface the density of nuclei is three times less. If in the latter case we consider not the total number of nuclei but the number of cluster sites, the ratio of charge

to sites is 30 : 1. The average particle size of the uniform background in Fig. 1*b* is of the order of 30–40 Å.

The vapour was examined for charge and it was found that under the conditions of evaporation there were some positively charged gold ions, but these represented only 10^{-8} of the total. Free electrons were thermionically emitted from the hot source, but the total density of these during the complete evaporation was at least an order less than the charge density.

It is believed that this behaviour is consistent with nucleation at surface dipoles formed by a positively charged site and a vapour atom, the valence electron from the vapour atom being held by the positive site. On a negatively charged surface the valence electrons are not given up by the vapour atoms and they are not electrostatically bound to the site. The individual atoms will be free to travel some distance over the surface, and if in fact there is a small repulsion between the substrate and the atoms, nucleation will occur only in regions of field divergence on the surface, that is, at surface imperfections.

I thank Mr. J. Cleaver and Mr. J. D. Sinfield for preparing and examining the micrographs.

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Transmission Filters for Visible Light

SOLUTION filters have proved to be the most flexible and efficient type of filter. In order, however, to get a sharp cut, a suitable combination of solution filters and/or glass filters is necessary. Such a combination of filters suffers from very low transmission.

We report here the application of some dye solutions as transmission filters for the visible region of the spectrum. Such dye-solution filters have the advantages that (*a*) they have a fairly narrow range of transmission even when used singly, (*b*) the transmission region, as well as the peak, can be easily shifted in the desired direction by adjusting the *p*H of the solution, and (*c*) the maximum transmission of such filters is about 90 per cent on the average. Although a few workers[1–3] have made use of certain dye solutions as transmission filters, we have seen no reports of the shift of the transmission range of such filters with a change in *p*H. We have found that the same dye, at different values of *p*H, can be used in different parts of a filter combination for a sharp cut-off, and suitable mixture of

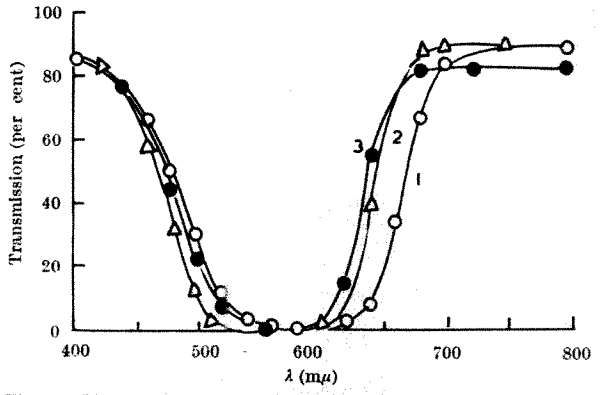


Fig. 1. Plot of percentage transmission against λ for methyl violet (40 mg/l.) in N/100 hydrochloric acid (curve 1), in water (curve 2) and in N/100 sodium carbonate (curve 3)

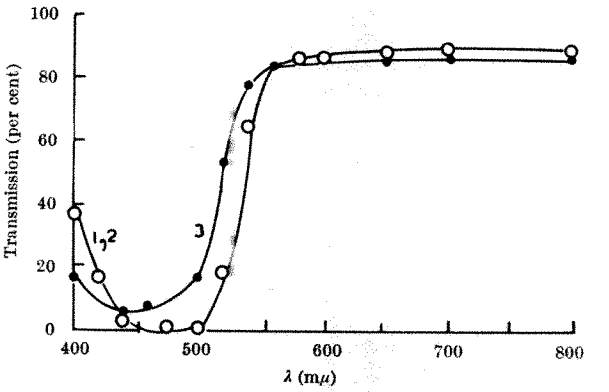


Fig. 2. Plot of percentage transmission against λ for acridine orange (84 mg/l.) in N/100 hydrochloric acid (curves 1, 2), in water (curves 1, 2) and in N/100 sodium carbonate (curve 3)

dye solutions are convenient for transmitting a fairly narrow range of visible light. Table 1 summarizes our observations with some dye solutions at different *p*H values, and plots of percentage transmissions against wavelengths are shown in Figs. 1–5.

The curves of Figs. 1–3 are analogous to those of sharp cut yellow and red glass colour filters (Corning Glass Works)[4]. By suitable combination with infra-red absorbing filters, these can be highly recommended as high-efficiency sharp cut-off filters. That the transmission

Table 1*

Dye	Concentration of dye (mg/l.)	in N/100 hydrochloric acid		in water		in N/100 sodium carbonate	
		Transmission ranges (mμ)	Wave-length at max. trans. (mμ)	Transmission ranges (mμ)	Wave-length at max. trans. (mμ)	Transmission ranges (mμ)	Wave-length at max. trans. (mμ)
Methyl violet	40	(i) < 400–500 (ii) > 600	~ 400 750	< 400–500 > 600	~ 400 700	< 400–500 > 600	~ 400 680
Acridine orange	84	(i) < 400–480 (ii) > 500	— 580	< 400–480 > 500	— 580	> 450	550
Methyl red	80	> 580	700	> 480	600	> 460	600
Disulphine blue *VN* 150	80	420–650	520	420–580	480	420–580	475
Mixed dye system		Methyl violet/disulphine blue = 1/50 v/v		Methyl violet/disulphine blue = 1/5 v/v			
		Transmission range (mμ)	Wave-length at max. trans. (mμ)	Transmission range (mμ)	Wave-length at max. trans. (mμ)		
Methyl violet (in water) Disulphine blue *VN* 150 (in N/180 hydrochloric acid)	80 80	420–620	520	420–580	480		

* All the dye-stuffs are either analytical reagent grade or microscopical stains quality. The spectral data are taken in a Hilger *H*700–307/57871 spectrophotometer in a 5-mm 'Pyrex' cell taking air as reference.

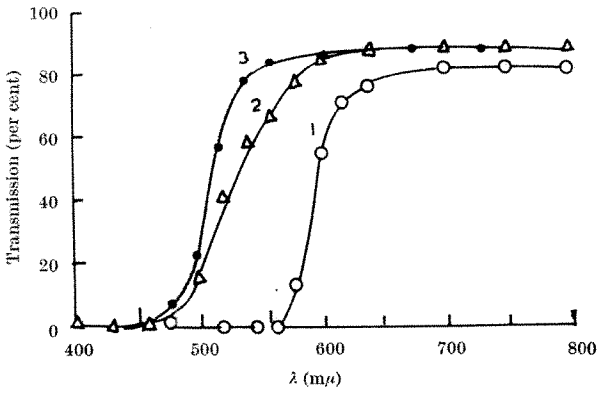


Fig. 3. Plot of percentage transmission against λ for methyl red (80 mg/l.) in N/100 hydrochloric acid (curve 1), in water (curve 2) and in N/100 sodium carbonate (curve 3)

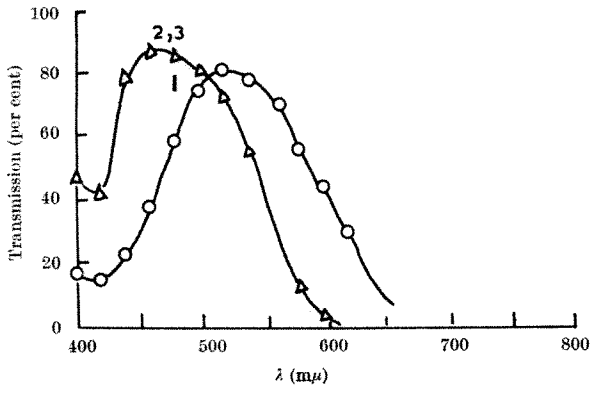


Fig. 4. Plot of percentage transmission against λ for disulphine blue *VN* 150 (80 mg/l.) in N/100 hydrochloric acid (curve 1), in water (curves 2, 3) and in N/100 sodium carbonate (curves 2, 3)

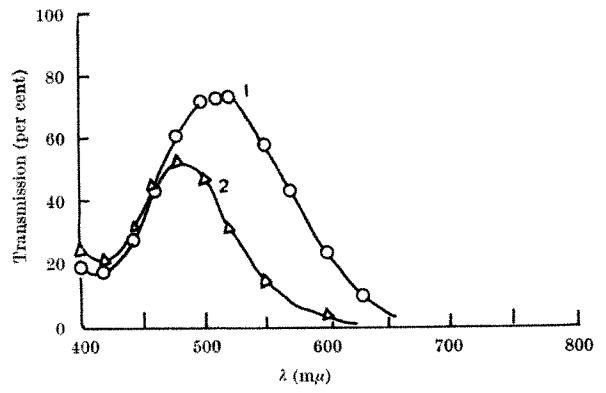


Fig. 5. Plot of percentage transmission against λ for mixed dye system: methyl violet (80 mg/l.) in water and disulphine blue *VN* 150 (80 mg/l.) in N/180 hydrochloric acid. Curve 1 refers to methyl violet/disulphine blue = 1/50 v/v. Curve 2 refers to methyl violet/disulphine blue = 1/5 v/v

region can be altered by changing the *p*H of the solution filters is evident from the curves in Fig. 4. The same effect can also be obtained in a mixed dye system by suitably adjusting the composition of the mixture. The curves obtained with a methyl violet/disulphine blue mixture have been found to bear a close resemblance to those of disulphine blue alone at different *p*H values (Figs. 4 and 5).

These solution filters have been compared with the Hilger and Watts 'Spekker' coloured glass filters. The latter suffer from the limitation that their percentage transmission value is very low (about 14–29 per cent), although the width of the transmission zone is about 60–70 mμ.

The dye solutions have been exposed to direct sunlight and also a 500-W tungsten filament lamp at a constant voltage of 230 V for about 24 h and have been found to be photo-stable.

We thank Prof. Santi R. Palit for his encouragement and the U.S. National Bureau of Standards and the C.S.I.R. (India) for financial assistance.

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Ionizing Radiation and Lightning Hazards

MR. P. J. GILLESPIE has recently questioned the claim that a small radioactive source attached to a lightning conductor can provide protection over a wide radius as a result of ionization of the surrounding air[1]. He points out that if this claim is justified, then users of large sources of ionizing radiations in hospitals and industry may be at considerable hazard from lightning.

A little further simple arithmetic illustrates well the improbability, to say the least, that a small radioactive source could be effective as a lightning conductor. According to Gillespie, a commercially available type has a source of 706 μc. radium 226 and is claimed to give protection over a radius of 100 m. At this distance the gamma-ray dose-rate is 6×10^{-5} mr./h or $1{\cdot}6 \times 10^{-11}$ r./sec. The average background of ionizing radiation (cosmic rays, Earth gamma-rays, etc.) in Britain gives a dose-rate of 100 mr./year or 3×10^{-9} r./sec. In terms of ion density, the natural background gives about 6 ion pairs/cm^3/sec, whereas at 100 m from the radioactive lightning conductor there is an additional 0·03 ion pairs/cm^3/sec. It is difficult to believe that this increase of 0·5 per cent in the ion density can significantly affect the path of a lightning stroke. Furthermore, the background ionization, the mean value of which is given above, varies considerably over the country, probably by a factor of at least two. For example, the granite city of Aberdeen and parts of Cornwall have backgrounds appreciably higher than most other places. Local variations in natural ionization must be considerably larger still. As Gillespie points out, a 3,000-c. cobalt-60 source can produce a dose-rate of 6×10^{-5} mr./h at 60 miles; alternatively, it can double the average background ionization at 3–5 miles. No evidence seems to have been produced that natural or man-made variations in the atmospheric ion density have any effect on the frequency or course of lightning strokes. The *reductio ad absurdum* of the claim that a small radioactive source can significantly increase the efficacy of a lightning conductor is almost complete.

One further important point should be mentioned. Gillespie's letter states that legislation governing the use of the radioactive lightning protection device has been passed under the Radioactive Substances Act, 1960. This surely must be a misunderstanding. To the best of my knowledge, the Radioactive Substances Act, 1960, is concerned entirely with registration, rules, facilities, etc., for the disposal of radioactive wastes and has no clause which could authorize the use of any particular radioactive device.

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[1] Gillespie, P. J., *Nature*, **208**, 577 (1965).

MR. GILLESPIE's recent communication[1] directs attention to the potential hazard created by the possibility of ionizing radiation attracting a lightning strike during a thunderstorm.

This hazard, if real, is a very grave one, and his letter is very timely, particularly in view of the increasing number of installations of kilocurie cobalt sources in radiotherapy departments. However, the figure he quotes of 0·00006 mr./h does not seem correct, because the natural background of radiation at ground-level, arising partly from cosmic radiation and partly from radioactivity mainly in building materials, gives rise to a radiation-level of about 0·012 mr./h.

It is obviously important for those who are responsible for the design of installations where large amounts of ionizing radiation are given out to know the correct figure.

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[1] Gillespie, P. J., *Nature*, **208**, 577 (1965).

PROF. ROBERTS's interesting comments on my communication regarding ionizing radiation and lightning substantiate my personal opinion that these devices are probably ineffective; this was one of the possibilities I mentioned originally.

However, it is of interest to note two statements made in sales literature issued by the manufacturers: (i) Their research work has shown that lightning will be effectively attracted if the atmospheric conductivity is doubled. (ii) The positive ions produced by the radiation will be attracted towards the negative cloud base, and in the strong electric field, such as exists immediately preceding or during a discharge, will initiate chain reactions which, by collision, increase the degree of ionization. This suggestion that an 'avalanche' ionization process occurs would, if well founded, answer the criticism that the very low dose-rates and therefore very low ionization would not be effective, as in these circumstances the ion density would be higher than the radiation alone would produce.

In view of the fact that it appears many thousands of these sources have been installed in nineteen different countries over the past eight years, and that many particularly vulnerable buildings, for example, chemical plants, explosive magazines, oil refineries, etc., have probably been fitted with them (at high cost—it may cost more than £2,000 to protect a large building), it seems very desirable to establish conclusively whether or not they are effective.

Regarding Prof. Roberts's statement that he is unaware of any legislation concerning these devices, the Order to which I referred was Statutory Instrument No. 1835, "The Radioactive Substances (Attachments to Lightning Conductors) Exemption Order, 1963", which came into force on December 1, 1963, under Section 2 (6) and (7) of the 1960 Radioactive Substances Act. There is a very similar statutory order applicable to Northern Ireland.

Mr. Ramsey raises an extremely interesting point; that even if a dose-rate of 6×10^{-5} mr./h has no effect on the path chosen by a lightning strike (and this point has still to be conclusively established), possibly some higher radiation dose-rate would.

Arguing by extremes, like Prof. Roberts, but in the other direction, it is generally accepted that intense beams of ionizing radiation can produce a highly conductive path through air, for example, many industries use antistatic devices consisting of radioactive sources which will effectively earth moving machine parts through the conductive path in air produced by the radiation beam. So, if one accepts the fact that ionizing radiation can be used to induce an electrostatic discharge, the pertinent question is, at what dose-rate and under what conditions could this be effective in relation to lightning?

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Velocity of Ultrasonic Waves in Single Crystals of Calcium Fluoride

THE elastic constants of crystals are commonly determined by measuring the velocities of propagation of acoustic waves in different principal crystal directions and applying well-known formulae[1]. For crystals of high perfection it has generally been reported that the measured velocity of sound of a given substance is substantially independent of impurity concentration and is a slowly varying function of temperature. There have been relatively few investigations of the dependence of acoustic velocity on impurity density due to the fact that, until recent years, the preparation of highly doped single crystals of high perfection was virtually impossible. We have investigated ultrasonic propagation in a number of calcium fluoride monocrystals grown in the <111> direction by the Czochralski technique. Some of the crystals were of high purity and others were doped with up to 2 per cent significant impurity for experiments on laser action. They were all of comparable perfection, with measured dislocation densities lying in the range 10^4–10^5 cm^{-2}. Samples for measurement were cylinders with opposite ends cleaved and finely polished to plane-parallelism.

The velocities of both longitudinal (v_L) and shear (v_S) ultrasonic waves in the crystals were determined for propagation in the <111> direction and care was taken to determine these quantities to an accuracy of better than 0·5 per cent by means of the pulse-echo technique. Quartz transducers were used and measurements were made by conventional methods. The time markers were calibrated by means of a nickel delay line. Some representative results of room-temperature measurements made with 10 Mc/s input pulses are given in Table 1.

Table 1

Crystal	Impurity	Colour	v_L (km sec^{-1})	v_S(km sec^{-1})
1	Pure	Colourless	6·50	3·98
2	Uranium	Dark pink	6·53	4·06
3	Uranium	Brown	6·67	4·09
4	Samarium	Green	6·44	4·02
5	Neodymium	Light green	6·45	4·03

The values of velocity are taken from a series of measurements on specimens of uniform colour cut from each crystal and the accuracy is believed to be better than 0·5 per cent so that the last figure in each case is significant. The values for pure material closely agree with the results quoted by Gerlich[2]. It seems clear that for crystals of calcium fluoride doped to about 1 per cent with the impurities mentioned in Table 1 the velocities of ultrasonic waves differ from the values for pure material, being greater in some cases and less in others, even though the samples are otherwise of comparable crystal perfection.

We thank the British Council for the award of a travelling scholarship to one of us (Yu. G. S.), G. W. Green of the Royal Radar Establishment for the supply of single crystals and Mr. J. Blitz for discussions about the measurements.

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[1] Mason, W. P., *Physical Acoustics and the Properties of Solids* (New York, Van Nostrand, 1958).
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GEOPHYSICS

Carrier of Remanent Magnetization in Certain Red Sandstones

MANY of the palaeomagnetic data which have accumulated during the past 10 years have resulted from extensive measurements on red sandstones, which often possess a stable and measurable component of remanent magnetization. At the present time, the process by which these rocks acquire their magnetization is not fully understood. Thermal demagnetization experiments show that haematite (α-Fe_2O_3) is generally responsible for the stable natural remanent magnetism, and this material occurs in two distinct forms in red sediments. One form is the red pigment, which gives the rocks their distinctive colour and is in the form of a coating on the quartz and feldspar grains and sometimes fills the interstices of the rock; the other form is black crystalline particles ('specularite'). Both these varieties of haematite are capable of carrying the natural remanent magnetism, through the acquisition of either depositional or chemical magnetization. The former process seems most likely to be associated with specularite[1], but the latter could be acquired by the pigment, if it were deposited from circulating iron-bearing solutions, or by the specularite if diagenetic alteration of magnetite to haematite occurred.

Except for field tests, there is no method of distinguishing between depositional and chemical magnetization, and I have attempted the alternative approach of determining whether the pigment or specularite (or both) carries the stable natural remanent magnetism of red rocks, using the technique of chemical demagnetization. This technique has been described elsewhere[2]; briefly, utilizing the fact that the pigment can be chemically dissolved out of the rock sample more rapidly than the specularite particles, the intensity of the natural remanent magnetism of a thin rock disk is measured at stages during treatment with acid; a record is kept of the amount of iron dissolved out during the intervals between measurements, and the point at which the pigment is completely dissolved is noted. A graph can then be drawn showing the decay of natural remanent magnetism and the decrease in iron content, and the proportion of the initial natural remanent magnetism remaining at the point where the pigment is dissolved is an estimate of the lower limit of the contribution of the specularite to the natural remanent magnetism of the rock.

Samples of red and purple sandstones from ten formations have been examined, ranging in age from Jurassic to Pre-Cambrian. With one exception (the Jurassic sample) there is some evidence that the natural remanent magnetism of the formations possesses a certain stability, either from thermal demagnetization, the existence of grouped directions of magnetization remote from the present field or the existence of reversals at a site. Disks of either 2·5 cm or 3·5 cm diameter and from 0·06 cm to 0·3 cm in thickness were used (according to porosity) and the solution carried out at 55° C in concentrated hydrochloric acid, intensity measurements being made with an astatic magnetometer. There is some uncertainty in the amount of haematite extracted at any stage, since the only possible iron measurement is of total iron (ferrous and ferric); it is probably not a serious source of error since the ferrous iron content of red beds is usually small compared with that of ferric iron. It does not affect the main object of the experiments, which is to measure the remaining natural remanent magnetism after the pigment has been dissolved.

Two types of results were obtained, examples of which are shown in Fig. 1. In Fig. 1*a* the pigment dissolves comparatively quickly and about 80 per cent of the original natural remanent magnetism remains at the point when the pigment has dissolved (shown by the vertical broken line); this represents the lower limit to

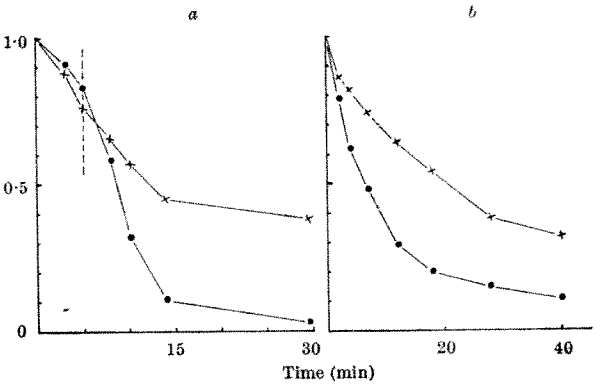


Fig. 1. Decrease of natural remanent magnetism (●) and iron content (×) during acid treatment of samples of red sandstone from (*a*) Moenkopi and (*b*) Lodore (normalized ordinates). The vertical broken line in diagram (*a*) marks the point at which the pigment has all dissolved

the percentage contribution of the specularite to the natural remanent magnetism. Before this point both specularite and pigment are being dissolved and it is uncertain whether the latter also contributes to the initial natural remanent magnetism. Additional information from the graph is that at least 75 per cent of the iron in the rock is in the form of the black particles and that at least 40 per cent of the total iron content is not contributing to the remanence. Results of this type were obtained from six of the ten formations, details of which are shown in Table 1. Except for the Chugwater samples, which came from different sites in Wyoming, the samples were from one site where two samples of the same formation were examined.

Table 1. SAMPLES OF RED SANDSTONES IN WHICH SPECULARITE CONTRIBUTES TO THE NATURAL REMANENT MAGNETISM

Formation and locality	Description of rock	No. of samples	Lower limit to percentage contribution of specularite to natural remanent magnetism
Kayenta, Arizona (Jurassic)	Coarse red-brown sandstone	1	85
Chinle, Utah (Upper Triassic)	Medium red-brown sandstone	1	90
Chugwater, Wyoming (Triassic)	Medium and fine red-brown sandstone	5	80
Moenkopi, Wyoming (Triassic)	,, ,,	2	70
Cutler, Arizona (Permian)	Fine red-brown sandstone	2	30
Supai, Colorado (Permian)	Medium red-brown sandstone	2	70

Where more than one sample of any formation was examined, the figure in the last column is an average value.

A different type of result is shown in Fig. 1*b*. The iron and natural remanent magnetism decay curves are more similar in shape, both decreasing smoothly, and there is usually more or less pigment remaining at the centre of the disk when the natural remanent magnetism has decreased to less than 10 per cent of its initial value. Under the microscope, these samples are seen to contain very little specularite compared with, for example, the Chugwater samples, but all contain varying amounts of pigment. Taken in conjunction with the similarity in the shape of the decay curves for iron and natural remanent magnetism, and the persistence of some of the pigment through to the end of the solution process, this strongly suggests that the pigment is contributing the majority, if not all, of the remanence observed in these rocks. The samples examined which show this type of behaviour are from the Taiguati formation, Bolivia (Permian), Deadwood formation, Wyoming (Mississippian), Lodore formation, Utah (Cambrian), and the Bass limestone (Pre-Cambrian) from two sites in Colorado. The Bass limestone samples are dark red and fine grained, and the samples from the other three formations are rather coarse purple sandstones. There is no significant change in the direction of magnetization of any of the samples

investigated, during the decay of the natural remanent magnetism.

It is concluded from these experiments that the pigment and specularite in red sandstones can both contribute to the natural remanent magnetism; it is not yet known whether they contribute to the natural remanent magnetism in rock in which specularite and pigment are both present, although there is one observation which may be relevant. In those cases where a curve of the type in Fig. 1*a* is obtained, a decrease in the rate of decay of natural remanent magnetism might be expected around the point where the pigment has just dissolved, if the pigment was contributing significantly to the initial natural remanent magnetism. However, in Fig. 1*a* the rate of decay is seen to be faster after the pigment has dissolved, and this is a general feature of this type of result. An explanation of this may be that the pigment is covering the specularite particles and the rate of attack of the acid on the latter will increase as the pigment is dissolved away. In view of these uncertainties, I believe that deductions from these curves should at present be confined to the minimum contribution of the specularite to the natural remanent magnetism, and to qualitative conclusions about the distribution of iron between specularite and pigment.

This work is supported by a grant from the Science Research Council, and I thank Miss E. Nubley for assistance with the measurements.

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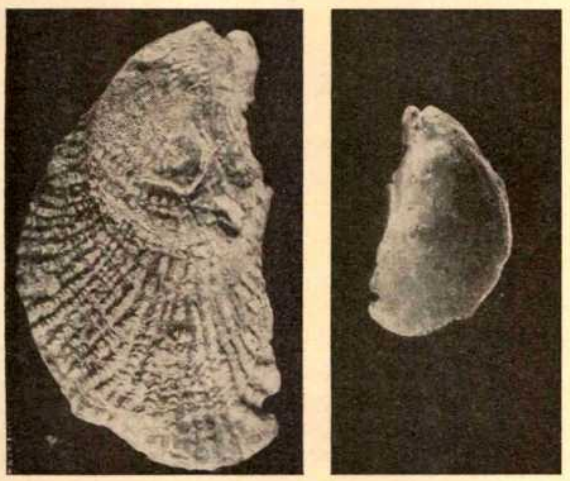
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GEOLOGY

Occurrence of *Ostrea eilatensis*: a New Oyster Species from the Cenomanian of Southern Israel and Egypt

THE Cenomanian sections in Central and Southern Israel are rich in ostreid fauna. The majority of fossil oysters represented are attributed to *Exogyra*; however, I have lately collected many specimens of morphologically variable species, unquestionably referred to *Ostrea*, from various localities in the Negev, Southern Israel.

The material on which the species is based consists of about twenty well-preserved specimens; most of them



a *b*

Fig. 1. *Ostrea eilatensis* sp. nov. Holotype, *HU*. 28427. (*a*) External view of young lower valve (×2). (*b*) Internal view of the same specimen (×1). Lower Cenomanian from the vicinity of Eilat



Fig. 2. *Ostrea eilatensis* sp. nov. External view of imperfect young lower valve, *HU*. 28429. Lower Cenomanian from the vicinity of Eilat. (×1)

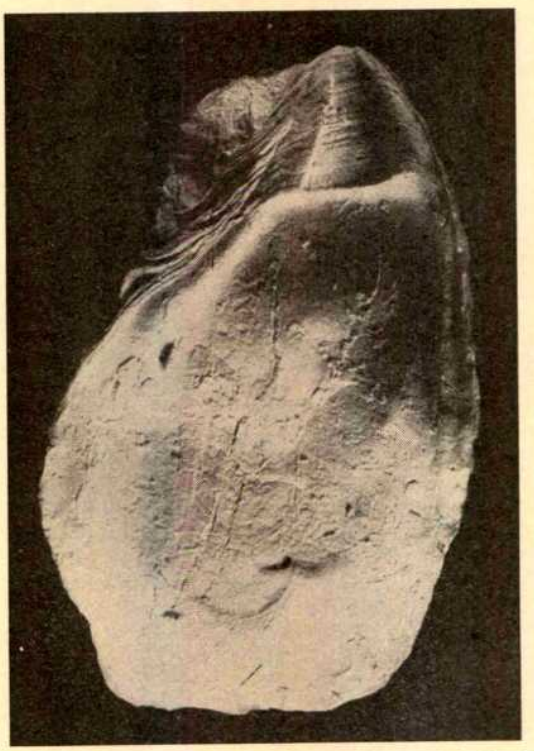


Fig. 3. *Ostrea eilatensis* sp. nov. Interior of adult lower valve, *HU*. 28430. Lower Cenomanian, Ramon. (×1)



Fig. 4. *Ostrea eilatensis* sp. nov. Holotype, *HU*. 28428. Interior of young specimen of upper valve. Lower Cenomanian from the vicinity of Eilat. (×1)

are of the lower valve. Representatives of the species are almost always embedded in soft marly strata.

The shell is subtriangular, inequivalve and inequilateral. The outlines of the valve are generally rounded, except those from the early stages which have a straight posterior border. The ligamental grooves are broad and shallow. The lower valve is convex and the umbo acutely angulated and prominent. Sculpture in the early stages of growth consists of longitudinal, densely crowded, numerous, somewhat sinuous rounded ribs, fanning out from the umbo; the number of the ribs is increased toward the

margin by branching and by the interpolation of new ribs. The surface is marked with concentric, somewhat imbricating growth lines that cross the ribs. Owing to the peculiar radial sculpture, the margin is marked by fine, rather obscure, crenulations. The early stages of growth possess a relatively small area of attachment, in contrast to the adult specimens, which are characteristically totally attached. An average-size specimen measures about 34 mm in length and 22 mm in greatest breadth. The corresponding dimensions of the largest collected specimen are 90 mm and 50 mm.

The upper valve is shorter than the other. It is flat or very slightly convex, and its surface is commonly marked only by prominent lines of growth. The interiors of both valves have deeply excavated sub-oval muscular impressions, situated on the posterior side, below the middle of their height, and the margins of the interior of the shell are elevated and slightly crenulated.

A great amount of variation is shown by this species, both in its form and in its sculpture; the fan-like fashion of radial ribbing on lower valve is transformed into coarse granulation in medium-sized specimens and the radial sculpture is totally eliminated in the adult specimens. When the shell is about half grown, the lower valve becomes less regular in form and attains its deepest excavation.

This well-marked species seems to have no close relatives in European and American Cenomanian faunas. It seems to me to be more nearly related to *Ostrea alicula* Hamlin, which has been reported from various Tethyal Aptian formations. The latter is distinguished chiefly by its smaller size, its more regular form, its more pointed apical region and the smaller number of radiating ribs on lower valve.

The specific name is derived from Eilat, where several of the best specimens studied were found.

This species seems to be confined to the Lower Cenomanian. It is common and widely distributed in Lower Cenomanian strata of the Ramon erosion cirque and the Eilat region, Southern Israel. Specimens collected from Cenomanian of the Gebel Minshera monoclinal structure of Northern Sinai, Egypt, are in good agreement with these from the type locality and Ramon.

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Salt Temperature: a Fundamental Factor in Salt Dome Intrusion

IN a previous article[1], I dealt with the emplacement of igneous masses (volcanic plugs, granite batholiths, diamond pipes, and serpentine bodies), salt domes, and such related bodies as mud volcanoes, ice piercements or pingos, and frost boils. In reviewing the subject for presentation at a recent symposium on salt domes in New Orleans, Louisiana[2], it dawned on me that heat is a very important factor and may actually initiate salt intrusion.

The prime motivating force for all intrusions is the weight of the overburden, or geostatic load, and in the case of salt, this is supplemented by buoyancy. In both, the force of gravity is the sole source of driving energy involved. Plastic flow of salt only occurs after deep burial—usually in excess of 15,000 ft. At shallower depths, it behaves as a competent rock and can withstand the geostatic load or may be folded together with the rock strata above and below without any evidence of plastic flow. Thus, salt domes are never found in areas where the evaporites have not been buried in excess of 15,000 ft. On the other hand, evaporites are unknown as metamorphic rocks[3], a fact which suggests that all traces

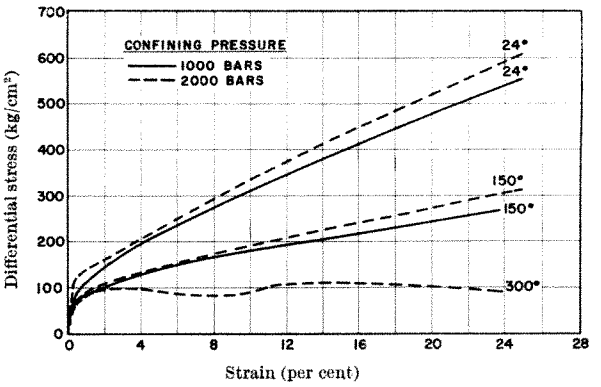


Fig. 1. Stress-strain curves for halite single crystals deformed dry in compression at 1,000 and 2,000 bars confining pressure and at different temperatures. Redrawn after J. Handin and R. V. Hager, jun., "Experimental deformation of sedimentary rocks under confining pressure: tests at high temperature"; *Amer. Assoc. Petrol. Geol. Bull.*, **42**, 2901 (1958)

of evaporites are expelled from sedimentary sequences by deeper burial.

A recent article by Kent[4] clearly demonstrates that salt has been extruded at the surface in several salt domes in Iran and has flowed downhill in the form of glaciers. It is physically impossible for salt to flow at the surface at atmospheric temperature and pressure. Salt is crystalline and has a hardness of 2·5, and as such would behave very much like limestone or any other competent rock. The only explanation for the glaciers would appear to be that the salt must have been hot when it was extruded.

The importance of temperature in salt dome tectonics has received very little consideration in the past. It is well known that temperature, as well as pressure, increases with depth of burial, but what sort of temperatures can be expected? A recent well[5] drilled by Amerada in the Gulf Coast salt dome area, Victoria County, Texas, has recorded a rock temperature in excess of 520° F at a depth of about 23,000 ft. On the evidence of palynology[6], the age of the Gulf Coast salt is Late Triassic to Early Jurassic and these strata lie at greater depth and at correspondingly higher temperature. It can reasonably be assumed that temperatures well above 500° F must have existed in the source salt beds from which the salt domes originated at the time of intrusion.

One of the earliest experiments on the effect of temperature on salt was by Cermak[7], who discovered that, by heating salt above 200° C (392° F), halite crystals can be bent quite easily. Above 200° C, salt flows without rupture. In 1958, Handin and Hager[8] published the results of laboratory experiments on the behaviour of salt at high pressures and temperatures (Fig. 1). Note that pressure has very little effect on yield stress, but that temperature is extremely important, and greatly reduces the ultimate strength and eliminates work hardening at 300° C (572° F). Both these conditions are operative at depths exceeding 25,000 ft., and with a pressure gradient of only about 100 kg/cm² (1,400 p.s.i.) salt behaves as a true plastic and flows indefinitely. It would appear that once salt has been heated above 400° F it becomes soft and plastic and is mobile. This plasticity can exist during the entire process of intrusion and even during extrusion at the surface. Thus, at the time of extrusion, salt can flow by simple gravity like a glacier—so long as it remains hot.

As pointed out by Orowan[9], fluids (liquids, glasses, and most non-crystalline materials) exhibit Newtonian viscosity, but crystalline materials behave as plastics and according to Andradian law. For an ideal plastic, the creep rate is zero until the stress approaches the creep limit. No deformation occurs until the yield point is reached, then any deformation at any velocity can result from the fixed stress. The salt does not flow: it moves

like a plug—very much like toothpaste being squeezed out of a tube.

In summary, the first essential for intrusion to result is that there must be a plastic medium (salt, mud or clay, ice, magma) and this must occur in the form of extensive sheets. In the case of salt, before buoyancy can begin to play any role, it must be buried at a depth of from 2,000 to 5,000 ft. Salt starts to become plastic below 12,000 ft. on account of pressure, and at about 25,000 ft. it becomes mobile on account of temperature. When it is mobile, it behaves hydrostatically and, as the surface load is never in exact balance, it will move laterally to places of less overburden pressure, where piercement or doming occurs. This state of balance is extremely delicate. Once initiated, flow would continue until the supply is exhausted.

The second requirement, then, is that there must be an imbalance in geostatic load distribution to initiate horizontal hydrodynamic flow. This lateral flow can only be due to geostatic load, but once piercement occurs, geostatic load plus the ever-increasing effect of buoyancy will cause the intrusive mass to rise rapidly through the overlying strata. Buoyancy only becomes a powerful force as the height of the intrusion increases to large proportions. At first, when no vertical relief exists on top of the evaporite layer, buoyancy is zero and piercement of the overlying strata cannot be caused by buoyancy alone. In the case of salt, buoyancy is known to be positive, but for magmas, it may be positive (up) or negative (down), depending on the density difference. Accordingly, buoyancy is not a requirement for intrusion, but is a modifying effect. Thus, intrusion of a very dense magma, such as kimberlite, can also occur.

As the salt moves into shallower overburden depths with their reduced geostatic load and lower near-surface compaction, the diameter of the stock generally increases. When the hydrostatic pressure of the salt (or magma) column balances the geostatic overburden pressure of the rock column on the source reservoir, or when the supply is exhausted, upward movement will cease. Thus, heavy basaltic magmas and even salt domes frequently do not reach the surface. It is postulated that the intrusive salt or magma should spurt into joints or fractures in the overlying rocks, speedily rising toward the surface, slowing down on reaching shallower depths (of less than 5,000 ft. in the case of salt), so that gentle flow may occur at the surface. Where the salt has reached the surface, it can flow out on the surface, or downhill like a glacier at the time of extrusion, but once it cools, it can only move downhill by very slow solid creep. This is in no way a measure of the rate of intrusion.

It should be pointed out that isopach thinning of a formation over a dome is not evidence of uplift during sedimentation unless erosional thinning can be demonstrated: thinning can also occur by stretching or by compaction of the strata above a dome at the time of intrusion.

In conclusion, heat is extremely critical for salt intrusion to result, and the energy driving the motion is gravitational potential energy derived primarily from the weight of the overburden, or geostatic load, and supplemented by the buoyancy effect caused by a density differential.

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Olivine Nodules in a Phonolite of the East Otago Alkaline Province, New Zealand

Numerous olivine nodules were recently reported by Sang Lyen[1] in a cossyrite phonolite flow from one of the lesser centres peripheral to the Dunedin Volcanic Complex, of Upper Miocene to Lower Pliocene age. The nodules are " . . . of varying size, widely scattered in the flow. Small nodules, a fraction of an inch across, may be found distributed widely, while larger types are mainly concentrated in the medial layers of the flow. . . ." Blocks up to 20 in. long were found.

The nodules are similar in appearance to those frequently found in basic lavas of alkaline affinities[2,3] and have similar mineralogy: olivine ($\beta = 1{\cdot}672 \pm 0{\cdot}002$), optically neutral, often with undulose extinction in wide subparallel bands; clinopyroxene ($\beta = 1{\cdot}685 \pm 0{\cdot}002$), $2V = 58°$ (ref. 1), bright green in hand specimen, with occasional exsolution lamellae of orthopyroxene; orthopyroxene ($\beta = 1{\cdot}672 \pm 0{\cdot}002$), very high negative $2V$; spinel ($n = 1{\cdot}80 \pm 0{\cdot}005$ (in O.U. 17738) and $1{\cdot}83 \pm 0{\cdot}01$ (in O.U. 17737)), brown interstitial grains, sometimes with narrow reaction rims against silicate.

The volume of exposed trachytes, phonolites, and salic pyroclastics accounts for about a quarter of the Dunedin Complex as a whole, while types intermediate between these and the basalts and basanites represent less than 5 per cent. On the other hand, trachytic lavas and pyroclastics made up almost all the initial eruptive phase of the main volcano, and over half the lavas of the third main eruptive phase were phonolite (estimates derived from incomplete computations by the late Prof. W. N. Benson).

In many continental alkaline provinces (such as East Africa) the overall volume of salic lavas and pyroclastics equals or exceeds that of associated basic eruptives, while flows of intermediate composition are but sparsely represented, as they are in the Dunedin Complex. Implicit in some recent work on the subject[4–7] is the idea that in such regions a mechanism other than fractional crystallization is required to account for the disproportionate amounts of basic, intermediate and salic lavas.

Olivine nodules in alkaline basalts are considered by many (see, for example, refs. 2 and 3) to represent subcrustal material. Assuming this to be a valid hypothesis, the occurrence of similar nodules in this phonolite would be evidence that it too was generated at sub-crustal levels.

Other evidence that some phonolite may originate at great depths is less directly provided by recent investigations of strontium isotope ratios in carbonatites and other rocks from alkaline igneous complexes[8,9]. The results are shown to be consistent with at least a sub-sialic source, both for the carbonatites and for the associated, comagmatic, igneous rocks. Phonolitic activity is not infrequently associated with carbonatite centres, as in East Africa, where field associations at least suggest that it may well be genetically related to carbonatite (see ref. 10), and could therefore have been generated from a similar deep-seated source.

I thank Prof. D. S. Coombs for helpful advice and discussion.

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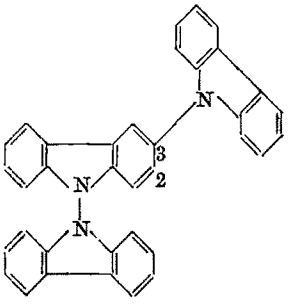
CHEMISTRY

Tricarbazyl

OXIDATION of carbazole with potassium permanganate in boiling acetone was reported in 1921 (ref. 1) to yield two crystalline products, (*A*) and (*B*), and an amorphous product. One of these crystalline products, (*A*), was later shown[2] to be 9,9′-dicarbazyl, and the other, (*B*), which was thought[1] to be a dicarbazyl on the basis of a cryoscopic molecular weight determination in benzene, was shown not to be identical with synthetic specimens of 1,1′- (ref. 3), 3,3′- (ref. 4) and 3,9′- (ref. 5) dicarbazyls. It was, however, proposed[6] that (*B*) might be either 1,3′- or 1,9′-dicarbazyl.

A recent paper[7] describing investigations of the oxidative dimerization of carbazole in various solvents prompts this report of structural investigations which have been carried out on the aforementioned compound (*B*), which has been prepared from carbazole by a method previously described[1].

The mass spectrum (mass spectra were recorded on an A.E.I. *MS* 9 spectrometer by Dr. J. M. Wilson) of compound (*B*) exhibits a molecular ion peak at $m/e = 497$, which, therefore, contrary to the previous proposal[1], shows it to be a tricarbazyl for which structure (I) is proposed on the basis of the following evidence.



(I)

(*a*) The mass spectrum of compound (*A*), 9,9′-dicarbazyl[2], which has a molecular ion peak at $m/e = 332$, shows the expected very easy fragmentation across the N—N bond to give a base-peak at $m/e = 166$. By comparison, the mass spectrum of (*B*) indicates, as well as a molecular ion peak at $m/e = 497$, the very easy loss of one carbazyl group (presumably by N—N bond scission as occurred in 9,9′-dicarbazyl) to give a base-peak at $m/e = 331$. Further fragmentation of the stronger C_3—N bond then affords much weaker peaks at $m/e = 167$ and 166.

(*b*) The infra-red spectrum (infra-red spectra were recorded in 'Nujol' on a Perkin Elmer model 237 spectrophotometer) of (*B*) shows no absorption in the N—H stretching region, indicating that the carbazole N-atoms must take part in the bonds between the three carbazole units. The complete spectrum is also very similar to that of 9,9′-dicarbazyl, but, unlike the latter spectrum, it exhibits a medium-strong band at $\nu_{max} = 812$ cm^{-1} and a weak band at $\nu_{max} = 879$ cm^{-1}, indicative of a 1,2,4-tri-substituted benzene nucleus[8], which therefore supports the attachment of the third carbazole unit to the 9,9′-dicarbazyl unit in (*B*) at either C_2 or C_3.

(*c*) That this linkage is between N—C_3 as shown in (I) and not between N—C_2 is supported by the observations[9] that C_3 in carbazole is more reactive than C_2.

Attempts to verify chemically structure (I) by selective reductive cleavage of the N—N bond to afford carbazole and 3,9′-dicarbazyl[5] have not been successful, probably due to the low solubility of (*B*) (ref. 1). Treatment with concentrated hydriodic acid at 150° C in a sealed tube for 15 h gave, contrary to previous observations[2], only carbazole.

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Crystal Structure of the Benzene *tris*(*o*-Phenylenedioxy) Phosphonitrile Trimer Complex

A DESCRIPTION of the inclusion compound *tris*(*o*-phenylenedioxy) phosphonitrile trimer has already been reported[1] which includes some preliminary crystallographic data and a short discussion of the possible arrangements of the molecular architecture. This communication describes a partial structure determination in two dimensions of the complex with benzene.

The crystals were hexagonal, unit cell $a = 11{\cdot}68$ Å, $c = 10{\cdot}077$ Å; space group either $P6_3$ or $P6_3/m$. The measured density of 1·395 g/c.c. is consistent with two molecules of the trimer and about one benzene molecule in the unit cell. The intensities of seventy-four independent $hk0$ reflexions were visually estimated, corrected for Lp factors and used to calculate the Patterson projection $P(u,v)$.

As reported in ref. 1, the two trimers must either be sited on the six-fold axis or one on each of the triad axes, but simple packing considerations show that only the sites on the three-fold axes lead to a structure containing suitable channels. The vector line of the *o*-phenylenedioxy group was obtained from the Patterson map, and a Fourier map based on a trial structure showed well-resolved atoms of the trimer arranged so that six trimers form one channel per unit cell around the benzene molecule which is sited at the origin. Initially the R value was high (> 40 per cent), but marked improvement was obtained (R fell to 21 per cent) when the trimer was moved through a small angle around the triad axis (the

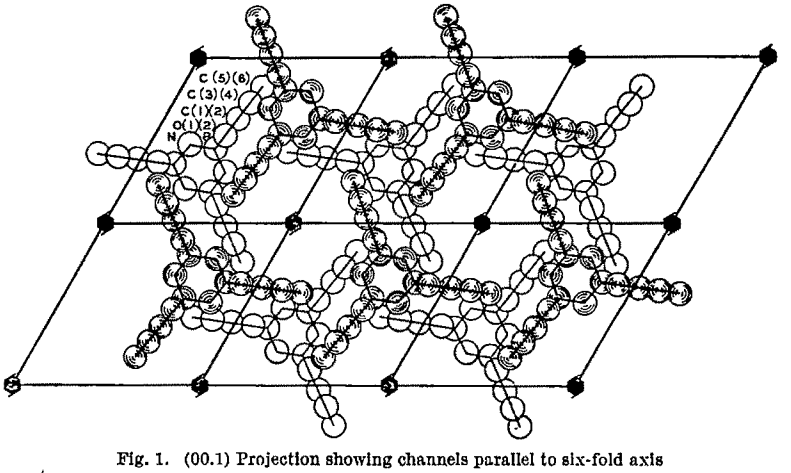


Fig. 1. (00.1) Projection showing channels parallel to six-fold axis

calculated structure factors are particularly sensitive to the azimuthal setting of the trimer) and further improvement to 18·6 per cent was obtained by small independent movements of the individual atoms of the trimer. It was assumed throughout that the planes of the phenylenedioxy groups were parallel to c, but only a least-squares refinement of three-dimensional data would prove this.

The effect on the diffraction transform was calculated for various orientations of the benzene molecule on the six-fold axis, but as the molecule exhibits rotational disorder its position is essentially indeterminate. The best position corresponded to the long axis of the benzene molecule parallel to or within 20° of the six-fold axis. Orientations where this axis was perpendicular to the plane of the molecule or to a C—C bond were eliminated because the long dimension of the molecule ~ 7 Å exceeded the diameter of the channel.

The atomic parameters of the trimer are:

	x	y	B
P	0·543	0·357	4·74
N	0·518	0·209	4·74
O (1) (2)	0·466	0·373	4·74
C (1) (2)	0·363	0·388	5·69
C (3) (4)	0·266	0·409	5·69
C (5) (6)	0·170	0·423	5·69

The packing of the trimers is such that channels, of internal diameters about 6 Å, are created as shown in Fig. 1 with the planes of the *o*-phenylenedioxy groups forming the channel walls. There is one channel per unit cell which is continuous throughout the structure and in this respect there is a close similarity to the channel structure of tri-*o*-thymotide.

Note. Dr. L. A. Siegel and Dr. J. H. van der Hende (American Cyanamid Co.) have recently completed a three-dimensional structure determination of this complex. The results, which were presented at the American Crystallographic Association, Gatlenburg, Tennessee, June 1965 (to be published in *J. Amer. Chem. Soc.*), are in agreement with ours.

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Fluorescence Spectrum of Phenol Vapour

INVESTIGATIONS of the fluorescence spectra of polyatomic molecules in the vapour phase suffer from the obvious difficulty that a suitable exciting source is generally not available. For this reason the fluorescence spectra of a very few polyatomic molecules have been reported so far. In this direction attempts have been made by many workers[1-3] to study the fluorescence spectra of monosubstituted benzenes using condensed sparks with different electrodes as suitable exciting sources.

Following the above technique, phenol vapour was excited by condensed iron spark rated 0·25 kW at 15,000 V with a condenser of capacity 0·005 mF kept in the circuit parallel to the spark gap. The complete experimental arrangement has been described elsewhere[4]. The spectrum was photographed on Kodak 'II 0' plates using a Hilger medium quartz spectrograph. An exposure of 80 h was used to gain sufficient intensity.

The fluorescence spectrum of phenol lies in the region 2750–3100 Å and consists of about fifty bands. The bands, except for a few, are sharp. A weak continuum has been found to overlap the entire spectrum. The wave-numbers (in vacuum) of these bands, with their approximate intensities and assignments, are collected in Table 1. The figures in parentheses are the difference in wave-numbers between the observed and the assigned values.

Table 1. FLUORESCENCE BANDS OF PHENOL

Wave-numbers (cm^{-1})	Intensity	Separation from 0–0 band	Assignment
36,389	*vw*	40	0+40 (0)
36,349	*vw*	0	0–0
36,289	*vw*	−60	0–61 (1)
36,123	*w*	−226	0–221 (−5)
36,108	*vw*	−241	0–241 (0)
36,039	*vw*	−310	
36,008	*vw*	−341	
35,823	*ms*	−526	0–526 (0)
35,788	*vw*	−561	0–526–35 (0)
35,762	*m*	−587	0–526–61 (0)
35,725	*vw*	−624	0–624 (0)
35,676	*vw*	−673	
35,653	*vw*	−696	
35,592	*w*	−757	0–526–221 (10)
35,572	*w*	−777	0–821+40 (4)
35,528	*s*	−821	0–821 (0)
35,496	*vw*	−853	0–821–35 (3)
35,466	*ms*	−883	0–821–61 (1)
35,390	*vw*	−959	0–1,001+40 (2)
35,348	*s*	−1,001	0–1,001 (0)
35,314	*vs*	−1,035	0–1,033 (−2)
35,280	*ms*	−1,069	0–1,001–61 (−7)
35,260	*s*	−1,089	0–1,033–61 (5)
35,167	*vw*	−1,182	
35,134	*w*	−1,215	0–1,001–221 (7) 0–1,256+40 (1)
35,093	*s*	−1,256	0–1,256 (0)
35,065	*vw*	−1,284	0–1,001–221–61 (−1)
34,923	*mw*	−1,326	0–1,367+40 (1)
34,982	*ms*	−1,367	0–1,367 (0)
34,946	*vw*	−1,393	
34,927	*vw*	−1,422	
34,851	*vw*	−1,498	
34,801	*vw*	−1,548	0–1,588+40 (0)
34,761	*w*	−1,588	0–1,588 (0)
34,711	*vw*	−1,638	0–2×821 (4)
34,676	*w*	−1,673	
34,629	*vw*	−1,720	
34,561	*vw, d*	−1,788	0–1,001–821+40 (−6)
34,519	*m, d*	−1,830	0–1,001–821 (−8)
34,461	*m, d*	−1,888	0–1,001–821–61 (−5)
34,347	*w, d*	−2,002	0–2×1,001 (0)
34,287	*m, d*	−2,062	0–2×1,033 (4)
34,225	*w, d*	−2,124	0–2×1,033–61 (3)
34,090	*w, d*	−2,259	0–1,256–1,001 (2)
34,024	*w, d*	−2,325	0–1,256–1,001–6 (−7)
33,253	*vw, d*	−3,096	0–3×1,033 (3)
33,196	*vw, d*	−3,153	0–3×1,033–6 (7)
33,060	*vw, d*	−3,289	0–1,001–1,256–1,033 (1)
32,995	*vw, d*	−3,354	0–1,001–1,256–1,033–61 (7)

v, very; *s*, strong; *m*, medium; *w*, weak; *d*, diffuse.

To explain the gross structure of the bands, phenol has been taken to belong to C_{2v} point group. The electronic transition involved is $^1B_1$–$^1A_1$, which is allowed from symmetry considerations. The 0–0 band and the bands involving totally symmetric vibrations, a_1, are expected to appear strongly. The vibrations b_1 and a_2 will give rise to weak bands. Transitions involving single quantum of b_2 vibrations are forbidden. In fluorescence spectra the intensity of the 0–0 band is generally reduced by self-absorption. The weak band at 36,349 cm^{-1} is taken as the 0–0 band. This is in agreement with the measured value in the emission spectrum[5]. The bands at 36,108, 35,823, 35,725, 35,528, 35,348, 35,314, 35,093, 34,982 and 34,761 cm^{-1} are separated from the 0–0 band by 241, 526, 624, 821, 1,001, 1,033, 1,256, 1,367 and 1,588 cm^{-1}. They are taken as the fundamental vibrations of the ground state.

All the vibrations, except 241 cm^{-1}, have been found to obey the symmetry properties of C_{2v} point group. The vibration 241 cm^{-1} which is involved in a very weak band at 36,108 cm^{-1} has also been reported in the emission[5] and absorption[6] spectra. This frequency has been assigned to b_2 symmetry type following the assignment of the corresponding frequency 242 cm^{-1} in the Raman spectrum[7]. This vibration is not expected to appear as a single quantum in the electronic spectrum. The occurrence of this vibration suggests that C_{2v} symmetry does not hold rigorously for this molecule, but is only approximately true, and the molecule probably belongs to some lower symmetry group.

A comparison of the fundamental ground state vibrations of phenol as observed in the Raman, infra-red[7], near ultra-violet absorption, emission and fluorescence spectra is given in Table 2.

Table 2. COMPARISON OF THE FUNDAMENTAL GROUND-STATE VIBRATIONS OF PHENOL

Raman	Infra-red	Near ultra-violet: Absorption	Near ultra-violet: Emission	Near ultra-violet: Fluorescence	Assignment
242		245	244	241	b_2 X-sens. bend. o.p.
531	531	527	526	526	a_1 X-sens. bend. i.p.
617	618	623	621	624	b_1 ring bend. i.p.
814	812	824	808	821	a_1 X-sens. stetch.
1,000		999	1,002	1,001	Ring
1,024	1,025		1,021	1,033	a_1 CH bend. i p.
1,253	1,253		1,261	1,256	a_1 X-sens. stetch.
				1,367	COH bend. i p.
1,603	1,604			1,588	a_1 ring stetch.

i.p., In plane. o p., Out of plane.

In addition to the main bands, some strong bands on the longer wave-length side, and weaker bands on the shorter wave-length side, have also been observed. They involve difference frequencies of −35, −61, −221 and 40 cm^{-1}.

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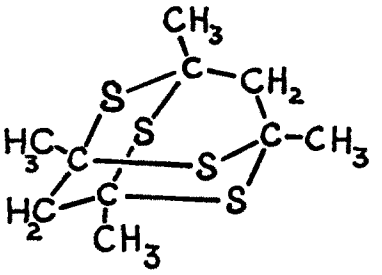
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Synthesis of Transition Metal Complexes of the Unknown Chelate, Dithioacetylacetone

ACETYLACETONE (acacH), from which the unknown chelate, dithioacetylacetone, can theoretically be obtained by substitution of two oxygen atoms with sulphur, is one of the most versatile bidentate chelates known[1]. In the enol form the hydroxylic proton can readily be replaced by a great many metal ions to give neutral complexes of the composition $M(acac)_n$. The co-ordination number of the metal is usually $2n$ and the resulting stereochemistry is tetrahedral, square planar or octahedral, although examples of square antiprismatic and square pyramidal co-ordination are known. The bonding to the metal is invariably through the two oxygen atoms, although it has been shown[2] recently that the α-carbon atom can bond to platinum in the compound trimethyl (acetylacetonyl)-2 : 2′ bipyridyl platinum (IV). Certain of the metal acetylacetonates undergo reactions characteristic of aromatic systems and these have been reviewed by Prof. Collman[3].

Recently, it has become apparent that bidentate sulphur chelates confer highly novel stereochemical, magnetic and spectral properties on transition metal ions which are due, in part, to the ease with which sulphur can participate in π-bonding with the central metal ion. We have been concerned, in this laboratory, with the chemical and physical properties of transition metal *N,N* disubstituted dithiocarbamates and, in particular, with the $^2T_2$–$^6A_1$ spin equilibrium exhibited by the iron (III) derivatives[4]. As a logical extension of these investigations we report here our attempts to combine the great versatility of acetylacetone with the remarkable properties so often possessed by transition metal complexes in which co-ordination is through sulphur atoms.

It should be noted that, although attempts have been reported for the synthesis of dithioacetylacetone by reacting a solution of acetylacetone in ethanolic hydrogen chloride with hydrogen sulphide, none were successful[5–7].



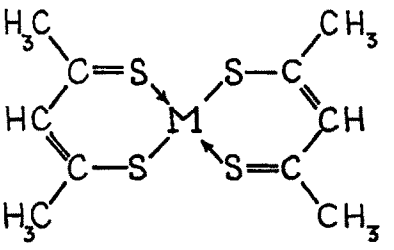
(I)

The main product of the reaction is a colourless, crystalline dimer which has been shown[8], by nuclear magnetic resonance spectroscopy and mass spectrometry, to have the structure (I). The dimer is characterized by exceptional resistance to chemical attack, and its conformation precludes chelating properties. Chaston *et al.*[9,10] have investigated a variation of the foregoing reaction, and have succeeded in isolating several new monothio-β-diketones, from which can be prepared brown, diamagnetic nickel complexes. In no case, however, were dithio-β-diketones prepared.

Isolation of the dimer (I) establishes that both oxygen atoms of the parent diketone are, in fact, substituted by sulphur atoms in the reaction medium. Accordingly, it seemed possible that, if the reaction were carried out in the presence of a suitable metal ion, dimer formation might be sufficiently inhibited for dithioacetylacetone to be isolated in the form of its metal complexes.

This experimental procedure has proved to be most effective and has led us to the successful synthesis of the compounds $Co^{II}(Sac\ Sac)_2$*, $Ni^{II}(Sac\ Sac)_2$, $Pd^{II}(Sac\ Sac)_2$ and $Pt^{II}(Sac\ Sac)_2$. These are crystalline, non-electrolytes in nitrobenzene, soluble in organic solvents but not in water, and are monomeric in the vapour state.

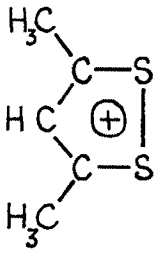
The solids are intensely coloured giving dark-red (Co^{II}, Ni^{II}), bright-red (Pd^{II}), or purple (Pt^{II}) solutions in benzene. $Co^{II}(Sac\ Sac)_2$ is paramagnetic with a magnetic moment, $\mu_{eff} = 2{\cdot}3$ Bohr magnetons, which is independent of temperature between 80° and 300° K. The nickel, palladium and platinum complexes are diamagnetic. A square planar co-ordination of the central ion by four sulphur atoms is therefore favoured as in structure (II).



(II)

Proof for the proposed formulation of these compounds is obtained from proton magnetic resonance spectroscopy and mass spectrometry. Thus Ni^{II} $(Sac\ Sac)_2$ exhibits just two proton resonance peaks in the expected ratio of 1 : 6. The mass spectra are characterized by strong parent ions at m/e = 321 [$^{59}Co^{II}(Sac\ Sac)_2$], m/e = 320 [$^{58}Ni^{II}(Sac\ Sac)_2$], m/e = 370 [$^{106}Pd(Sac\ Sac)_2$], and m/e = 457 [$^{195}Pt(Sac\ Sac)_2$] derived by electron loss from structure (II). The only other significant peaks in the spectra are due to ions at m/e = 131 which may have the

* The commonly accepted abbreviation for the acetylacetonate anion is 'acac'. We therefore suggest that 'Sac Sac' would be a suitable abbreviation for the dithioacetylacetonate ligand. On this basis the abbreviation for the monothioacetylacetonate ligand would be 'Sac ac'.



(III)

resonance stabilized (aromatic) dimethyl dithiolium cation structure as shown in III.

A preliminary examination of the chemical behaviour of the new complexes has indicated that they are not attacked by boiling alkali, slightly attacked by concentrated hydrochloric acid and decomposed by oxidizing acids. $Co^{II}(Sac\ Sac)_2$ is oxidized irreversibly by air when dissolved in pyridine, but in the absence of air a green solution is obtained in which pyridine molecules are probably co-ordinating to the central cobalt ion by way of the vacant axial positions.

Our attempts to isolate the parent ligand (Sac Sac H) from the metal complexes have, so far, proved unsuccessful.

All the compounds are characterized by electronic spectra rich in absorption bands (see Table 1). Those at lowest energies may be assigned to internal transitions within the *d* shell of the central ion. Those at higher energies arise from metal to ligand, ligand to metal and ligand to ligand electron transitions. We are, at present, carrying through molecular orbital calculations, in the Hückel approximation, by the procedures of Gray and Ballhausen[11], to define the transitions in greater detail.

Table 1. ELECTRONIC SPECTRUM OF $[Co^{II}(SAC\ SAC)_2]$

Wave-length (mμ)	Energy (cm^{-1})	Molar extinction coefficient	Solvent
1,480	6,750	20	Carbon disulphide
920	10,870	30	,, ,,
572	17,500	4,000	Benzene
462	21,600	3,200	,,
415 sh.	~24,000	~5,000	,,
360 sh.	~28,000	~15,000	,,
341	29,400	14,000	Carbon tetrachloride
273	36,600	28,000	,, ,,
249	40,100	~20,000	Methanol
224	44,600	~30,000	,,

The main absorption bands in the infra-red region are listed in Table 2. A detailed assignment of these bands is being secured by a normal co-ordinate analysis, based on a detailed knowledge of the molecular geometry. The preliminary results of a single crystal X-ray study[12] of $Co^{II}(Sac\ Sac)_2$ show that the crystals are orthorhombic with unit cell dimensions $a = 15{\cdot}6_4$ Å, $b = 14{\cdot}3_5$ Å, $c = 6{\cdot}0_5$ Å, and there are four units of composition Co(Sac Sac)$_2$ per unit cell. The space group is either *Cmca* or *C*2*cb*. For both space groups the cobalt atoms must be in special positions which are compatible with the square planar formulation. A tetrahedral cobalt environment is not consistent with the centrosymmetric space group nor, of course, the observed magnetism.

Table 2. SOME CHARACTERISTIC INFRA-RED BANDS OF DIVALENT METAL DITHIOACETYLACETONATES

Compound	Bands (cm^{-1})					
$[Co^{II}(Sac\ Sac)_2]$	1,490 s, br	1,157 s	1,005 m	840 s	740 s	550 m
$[Ni^{II}(Sac\ Sac)_2]$	1,485 s, br	1,159 s	1,012 m	833 s	750 s	557 m
$[Pd^{II}(Sac\ Sac)_2]$	1,485 s, br	1,150 s	1,010 m	830 s	750 s	550 m
$[Pt^{II}(Sac\ Sac)_2]$	1,485 s, br	1,155 s	1,010 m	835 s	750 s	550 m

The preparation of these compounds provides an excellent example of the important part which can be played by metal atoms in achieving the synthesis of normally inaccessible molecules. It seems possible that metal-ion-directed reactions, of a type similar to that described here, may well prove to be important in many biosynthetic processes involving sulphur.

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Reaction of Glutarimide with Phosphorus Pentachloride to form Chlorinated Pyridines

THE reaction of glutarimide with phosphorus pentachloride to give 2,3,6-trichloropyridine[1] yielded a material that was found to be quite impure. The infra-red spectrum of the crude product suggested that other chlorinated pyridines were also present.

Lochte *et al.*[2] described the preparation of 3- and 4-*cyclo*pentyl-2,5,6-trichloropyridines using the appropriately substituted *cyclo*pentyl-glutarimides and phosphorus pentachloride. They did not indicate whether other products were present. Lochte and Wheeler[3] later found that the pyridine derivative formed from 3-*cyclo*pentylglutarimide was impure, and they deduced from elemental analyses and molecular refraction data that the crude product was a mixture of 4-*cyclo*pentyl-2,6-dichloro- and 4-*cyclo*pentyl-2,3,6-trichloropyridines.

The crude product from our sealed-tube reaction was submitted to column chromatography on alumina using *n*-hexane as the developing solvent, and three pure compounds were isolated: 2,3,5,6-tetrachloropyridine, m.p. 89° (ref. 4); 2,3,6-trichloropyridine, m.p. 68° (ref. 1), and 2,6-dichloropyridine. The solution infra-red and ultra-violet spectra of these materials were identical with those of the known compounds. The pyridine derivatives were eluted from the alumina column with good separation and in the order given. 2,3,5,6-Tetrachloropyridine has not previously been reported as a product of this reaction.

The effects of time and temperature on the course of the reaction were studied and the data are shown in Tables 1 and 2.

Table 1 shows that a twelve-fold increase in time beyond 20 min has no effect on the total conversion to pyridine derivatives at the lower temperatures. In the case of the individual chlorinated pyridine derivatives, there seems to be a decrease in 2,6-dichloro- and an increase in 2,3,5,6-tetrachloropyridines at 100° C, but the changes are only slight. At 270° C, the increased time allowed for the reaction resulted in considerable alteration in the proportion of products.

When the temperature was increased, as shown in Table 2, the conversion to chlorinated pyridines increased slowly. The distribution of products also changed. Dichloropyridine showed a slow decline, trichloropyridine seemed to show a rise at 100° C followed by a steady decrease, and tetrachloropyridine showed a general increase. Pentachloropyridine did not appear until the reaction temperature reached 270° C, and then only a

Table 1. EFFECT OF TIME ON CONVERSION OF GLUTARIMIDE TO CHLORINATED PYRIDINES AND THE RELATIVE DISTRIBUTION OF PRODUCTS

Time of reaction	Per cent conversion to chlorinated pyridines at			Distribution of chlorinated pyridines*											
				Di-			Tri-			Tetra-			Penta-		
	55° C	100° C	270° C	55° C	100° C	270° C	55° C	100° C	270° C	55° C	100° C	270° C	55° C	100° C	270° C
20 min	55	45	—	37	9	—	55	65	—	8	26	—	0	0	—
40 min	47	48	—	39	15	—	53	60	—	8	25	—	0	0	—
90 min	41	47	—	38	7	—	53	62	—	10	31	—	0	0	—
120 min	57	—	—	37	—	—	52	—	—	12	—	—	0	—	—
240 min	41	49	—	38	6	—	53	68	—	9	36	—	0	0	—
7 h	—	—	71	—	—	11	—	—	46	—	—	41	—	—	1
24 h	—	—	—	—	—	0	—	—	14	—	—	61	—	—	25

* The values are in terms of per cent of total chlorinated pyridine. Di-, 2,6-dichloropyridine; Tri-, 2,3,6-trichloropyridine; Tetra-, 2,3,5,6-tetrachloropyridine; Penta-, pentachloropyridine.

Table 2. EFFECT OF TEMPERATURE ON CONVERSION OF GLUTARIMIDE TO CHLORINATED PYRIDINES AND THE RELATIVE DISTRIBUTION OF PRODUCTS

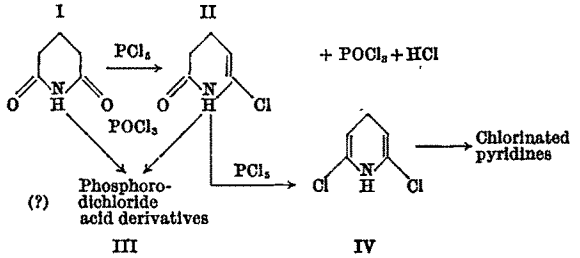
Temperature of reaction (°C)	Percentage conversion* to chlorinated pyridines	Distribution of chlorinated pyridines†			
		Di-	Tri-	Tetra-	Penta-
55	48 ± 8	38 ± 1	53 ± 2	9 ± 2	0
100	47 ± 3	9 ± 7	64 ± 6	30 ± 8	0
125	60	22	29	20	0
150	64	15	55	30	0
270	71	11	46	41	1
270	—	0	14	61	25

* The confidence limits are expressed at the 95 per cent level of significance. The values at 55° C and 100° C are the averages of all the determinations at these temperatures in Table 1; those at 125° C and 150° C are for a reaction time of 20 min. The values for 270° C represent 7- and 24-h reactions in that order.

† See Table 1.

very small amount was formed. However, as indicated in Table 1, the proportion of the penta- derivative increased markedly with a four-fold increase in time beyond 7 h.

These results are interpreted in the following way:



As the formation of II from I is taking place, the resulting phosphorus oxychloride also reacts with I and II. The net result of this simultaneous reaction involving phosphorus oxychloride is to effectively remove a portion of I and II from subsequent conversion to pyridine derivatives. When phosphorus oxychloride was used as a solvent for glutarimide and phosphorus pentachloride in this reaction, no chlorinated pyridines were formed. This tends to support the suggestion that the oxychloride does remove I and/or II from further reaction. When the temperature of the reaction is increased, the rate of chlorinated pyridine formation is increased relative to the rate of formation of by-products.

The nature of the by-products has not been investigated. However, when treated with water, the resulting compounds cannot be extracted by carbon disulphide. This suggests that the compounds represented by III are phosphorodichloridic acid derivatives. These are easily hydrolysed by water to give water-soluble phosphoric acid derivatives.

Compound IV, or a tautomer of IV, may be transformed to chlorinated pyridines by a successive series of chlorinations and dehydrochlorinations. The route to 2,6-dichloropyridine can be shown to involve the least number of steps, and consequently, is probably the first pyridine derivative formed.

Chlorination of the fully aromatic ring does not occur until the temperature of the reaction is increased. At 100° C and 20 min, it can be demonstrated that 2,6-dichloropyridine does not react with elemental chlorine. However, at 180° C and 50 h, chlorination of this compound with elemental chlorine does take place to give some 2,3,6-trichloro- and *sym*-tetrachloropyridines. At 285° C and 50 h, all of the chlorinated pyridines are almost quantitatively converted to pentachloropyridine.

At low temperatures, phosphorus pentachloride seems to be essential for the formation of higher chlorinated pyridines in this reaction. However, as the temperature of the reaction is increased, chlorination of the pyridine ring becomes an increasingly important factor in the final distribution of products.

The elemental chlorine required for these reactions is probably furnished by phosphorus pentachloride; at elevated temperatures, the pentachloride decomposes to form an equilibrium mixture with the trichloride and chlorine[5].

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Radiolysis of Monomethylhydrazine

IN an investigation of the effects of ionizing radiation on rocket fuels the radiolytic decomposition of monomethylhydrazine has been studied to determine whether the generation of radiolytic gases can be suppressed by the addition of free radical scavengers. The experiment consisted of preparing degassed specimens of monomethylhydrazine with and without free-radical scavengers, gamma-irradiating them with cobalt-60 to $8{\cdot}5 \times 10^6$ rads at 22°–25° C, and analysing the non-condensable gaseous products by gas chromatography.

The 'anhydrous' monomethylhydrazine used contained less than 2 per cent by weight of water and amines (Olin Mathieson Chemical Corporation). There is no reason

to believe that the water acts as a free-radical scavenger to affect the radiolytic decomposition. Water is actually combined with the solvent as CH_3NHNH_3OH, and this undergoes little basic dissociation. Furthermore, Baxendale and Mellows[1] have found that water at a concentration of 3 per cent in methanol does not scavenge radiolytic free radicals originating from the methanol.

The monomethylhydrazine and its solutions were protected from air-oxidation by a nitrogen atmosphere. Methyl methacrylate, pentadiene-1,3 and carbon tetrachloride were used as free-radical scavengers[2] at the large concentration of 5 per cent by volume. The scavenger 1,1-diphenyl-2-picrylhydrazyl could not be used in monomethylhydrazine since its solution slowly generated gas.

The radiolysis cell was a 50-ml. round-bottomed 'Pyrex' flask with a long neck and fitted with a horizontal break-seal on the lower neck, an upward slanted side arm of 10-mm diameter opposite, and a seal-off constriction on the upper neck. Above the seal-off constriction, the cell was sealed to the stopcock of a vacuum manifold having outlets to the air and to a high vacuum. The cell was flushed before use with nitrogen. The specimen (20 ml.) was introduced into the cell with a hypodermic syringe, frozen with liquid nitrogen and the side arm warmed with an air-heater while the frozen cell was flushed with nitrogen to remove any trace of the specimen that might be contaminating the side arm. The arm was sealed off at atmospheric pressure, the specimen degassed by freezing and pumping, and the frozen cell sealed off at 10^{-6} mm of mercury.

After irradiation, the specimen was frozen with liquid nitrogen, and the non-condensable products (hydrogen, nitrogen and methane) were transferred through the break-seal to a 1 l. calibrated gas-storage bulb by means of a Toepler pump. A 10 ml. portion was analysed quantitatively with a Perkin–Elmer gas chromatograph equipped with a thermistor detector, a 50-ft. Linde molecular sieve column at 30° C, and helium carrier gas.

Table 1. RADIATION YIELDS FOR MONOMETHYLHYDRAZINE WITHOUT AND WITH FREE-RADICAL SCAVENGERS

	Molecules/100 eV			
	$G(H_2)$	$G(N_2)$	$G(CH_4)$	Total
None*	4·7	4·0	3·4	12·1
Methyl methacrylate†	4·4	4·6	4·1	13·1
Pentadiene-1,3†	3·6	3·6	3·1	10·3
Carbon tetrachloride†	3·3	30·4	25·6	59·3

* Results are average of four experiments.
† Results are average of two experiments.

Table 1 shows radiolytic yields with methyl methacrylate and pentadiene-1,3 as scavengers. Unadulterated monomethylhydrazine produced a large quantity of radiolytic gas. A 20·0 ml. sample (17·5 g) generated 45·5 ml. of non-condensable gas, measured at 25° C and one atmosphere. The molecular composition is 38·7 per cent hydrogen, 33·5 per cent nitrogen and 27·8 per cent methane. The quantity of gas was not altered significantly by the addition of either methyl methacrylate or pentadiene-1,3 as a free-radical scavenger. This suggests that the gas results from a molecular or ionic reaction rather than a diffusion-controlled, free-radical reaction. Undoubtedly, condensable products are also formed, but the formation of the non-condensable products is plausibly accounted for by equation 1 because the gas composition is nearly equimolar, a single molecule of monomethylhydrazine contains the elements of one molecule of hydrogen, nitrogen and methane, and the decomposition is probably uni- or bi-molecular in nature:

$$[CH_3NHNH_2] \rightsquigarrow n\,H_2 + n\,N_2 + n\,CH_4 \quad n = 1 \text{ or } 2 \qquad (1)$$

The presence of carbon tetrachloride affects the radiolytic decomposition of monomethylhydrazine in a surprising way. Table 1 shows that the presence of carbon tetrachloride does not affect $G(H_2)$ significantly, but that it increases $G(N_2)$ and $G(CH_4)$ to values typical of chain reactions. But carbon tetrachloride is known to initiate free-radical chain reactions when irradiated in certain solvents[3,4]. This suggests that two major reactions occur simultaneously. One is the expected molecular (or ionic) reaction in unadulterated monomethylhydrazine which produces the expected equivalent yields of hydrogen, nitrogen and methane. This accounts for the hydrogen gas observed in the radiolysis, but does not account for all the nitrogen and methane gases. The other reaction is a chain reaction initiated by the carbon tetrachloride. It produces no hydrogen gas, but yields nitrogen and methane in amounts which are typical of chain reactions.

Radiolysis of the carbon tetrachloride is a prerequisite for the proposed chain reaction. This proceeds[5,6] according to the equation:

$$CCl_4 \rightsquigarrow Cl\cdot + \cdot CCl_3 \qquad (2)$$

Either of the reactive radicals from carbon tetrachloride will abstract a hydrogen atom from a suitable solvent and generate a new radical to propagate a chain[3,5,7] as suggested, for example, by Hannerz[3].

$$\cdot CCl_3 + R_1R_2CHOH \longrightarrow CHCl_3 + R_1R_2\dot{C}OH \qquad (3)$$

$$R_1R_2\dot{C}OH + CCl_4 \longrightarrow HCl + R_1R_2CO + \cdot CCl_3 \qquad (4)$$

Where the solvent is monomethylhydrazine, reactions (5) and (6) are proposed, taking into account (1) the hydrogen abstraction, (2) the probability of a bimolecular reaction, (3) the requirement (compare Table 1) of producing nitrogen and methane gases in equivalent amounts, (4) the requirement of not producing hydrogen gas, and (5) the necessity of generating a new radical for chain propagation.

$$\cdot Cl + CH_3NHNH_2 \longrightarrow HCl + N_2 + CH_4 + H\cdot \qquad (5)$$

$$\cdot CCl_3 + CH_3NHNH_2 \longrightarrow CHCl_3 + N_2 + CH_4 + H\cdot \qquad (6)$$

$$H\cdot + CCl_4 \longrightarrow HCl + \cdot CCl_3 \qquad (7)$$

These reactions produce two hydrogen radicals between them, and these propagate two identical chains. In the next chain step, a hydrogen radical is scavenged by the carbon tetrachloride according to reaction (7). Thus, the chain reaction in monomethylhydrazine would consist of the alternation of reactions (6) and (7).

The relative reactivity of carbon tetrachloride and monomethylhydrazine with hydrogen radicals is important. It appears that the carbon tetrachloride completely scavenges hydrogen radicals produced by means of reactions (5) and (6), in spite of the high chemical reactivity of monomethylhydrazine. If this were not true, the hydrogen radicals would almost certainly react with monomethylhydrazine molecules to produce hydrogen gas, and the value for $G(H_2)$ would be much greater than the observed value of 3·3 which is wholly accounted for by the molecular or ionic reaction. Reaction (7) is known to occur readily.

The apparent ability of carbon tetrachloride to scavenge hydrogen radicals successfully in monomethylhydrazine increases confidence that methyl methacrylate and pentadiene-1,3 would have scavenged free-radicals in this solvent had the solvent generated primary, radiolytic free-radicals. While these olefines can undergo radiolysis in monomethylhydrazine solution to produce hydrogen and other free-radicals, they act as self-scavengers and cannot, therefore, initiate a chain reaction leading to hydrogen, nitrogen or methane gas.

Approximately 210 molecules each of nitrogen and methane are produced before a chain is terminated. This may be calculated from the G values for monomethylhydrazine-carbon tetrachloride radiolysis in Table 1, a value of 2·6 for $G(\cdot Cl) + G(\cdot CCl_3)$ for pure carbon tetrachloride[5,8], and an estimation that the carbon tetrachloride (5 per cent by volume) absorbs 5 per cent of the total energy input. Chain termination may occur because

the propagating free-radicals combine, although other indeterminate reactions may be responsible.

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BIOPHYSICS

Electron Spin Resonance Study of Thermal Effects in Irradiated Deoxyribonucleic Acid

IT has recently been reported that the electron spin resonance spectrum of deoxyribonucleic acid (DNA) irradiated and measured at 77° K is different from that obtained after irradiation at room temperature[1,2]. We have observed that there are three distinct changes in the shape of the spectrum as the sample is warmed from 77° K to 300° K, the final spectrum being the same as that obtained by irradiating at 300° K.

A dry specimen of DNA, extracted from salmon sperm and given to us by the Department of Biophysics, King's College, London, was irradiated at 77° K to a dose of 1 Mrad with cobalt-60 γ-rays in an evacuated 'Spectrosil' sample tube. The derivative spectrum shown in Fig. 1 was recorded at 77° K on an X-band (9 Gc/s) electron spin resonance spectrometer. The sample was brought to various temperatures between 77° K and 300° K for about 5 min before recording the spectrum at 77° K. Changes in the spectrum were irreversible, and were dependent only on the annealing temperature but not on the annealing time at any given temperature. The spectrum after annealing at 100° K is given in Fig. 2 and shows slight differences from Fig. 1, the small amount of structure having disappeared completely. The largest change in the spectrum was observed after annealing at 210° K when a hyperfine spectrum of eight lines was observed. This spectrum is given in Fig. 3. Finally, the central line narrowed at about 260° K to give the spectrum shown in Fig. 4 which is also obtained after irradiation at room temperature. Thus energy transfer occurred at three different temperatures as the specimen was warmed from 77° K to 300° K.

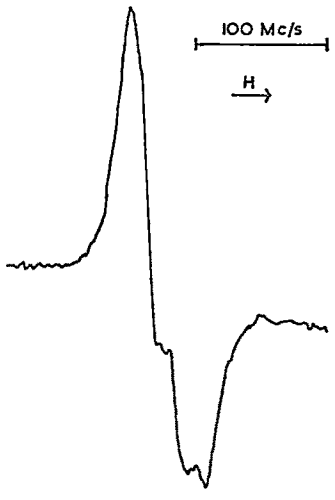


Fig. 1. Derivative spectrum of DNA irradiated and recorded at 77° K

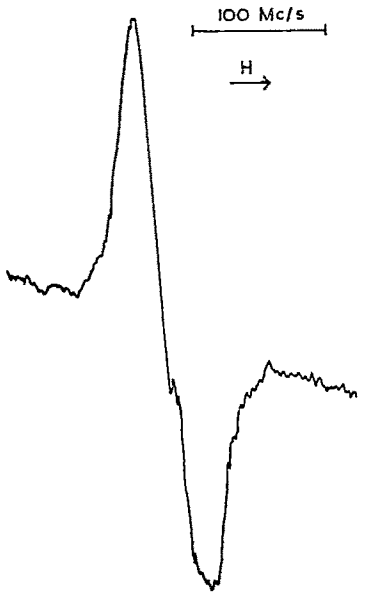


Fig. 2. Derivative spectrum of DNA irradiated at 77° K, annealed for 5 min at 100° K and recorded at 77° K

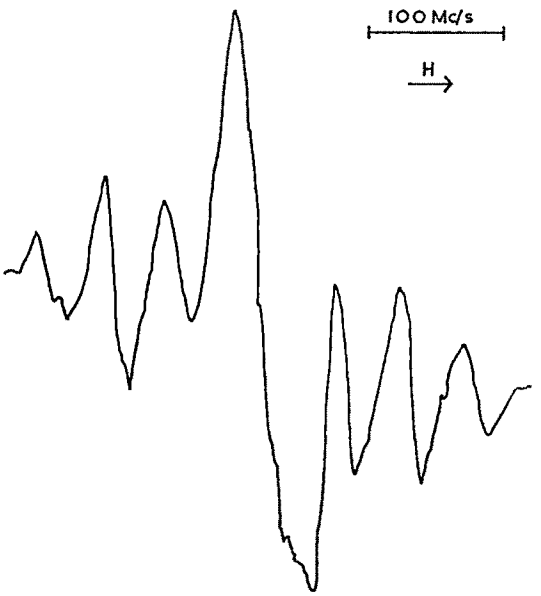


Fig. 3. Derivative spectrum of DNA irradiated at 77° K, annealed for 5 min at 210° K and recorded at 77° K

The single line observed at low temperatures had a peak-to-peak width of 50 Mc/s and the g-value at the centre, measured by comparing its position with that of the α,α-diphenyl β-picryl hydrazyl (DPPH) line, was found to be 2·0036 ± 0·0004. Salovey, Shulman and Walsh[3] have measured the corresponding g-value as 2·0030 ± 0·0005 and the peak-to-peak line width as 72 Mc/s. The total number of radicals decreased by about 15 per cent during the annealing. Alexander, Lett and Ormerod[1] observed a 30 per cent decrease in the number of radicals. However, when allowance is made for different radiation doses and also an estimated error of ± 25 per cent in our experimental measurements, it is doubtful if this difference is significant.

A similar annealing experiment was performed with polycrystalline thymine, thymidine and thymidine monophosphate. After irradiation and measurement at 77° K thymidine gave a spectrum similar to that of the eight-line hyperfine structure observed in DNA irradiated at room temperature. The spectra from thymine and

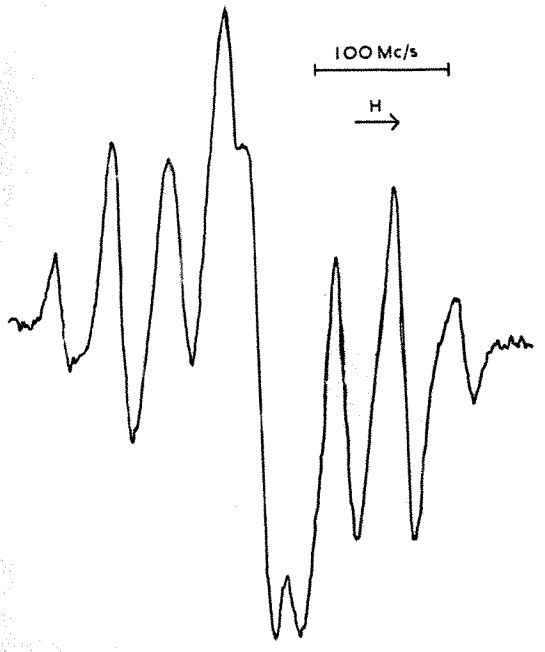


Fig. 4 Derivative spectrum of DNA irradiated at 77° K and recorded at 300° K. A similar spectrum is obtained by recording at 77° K after annealing for 5 min at 300° K

thymidine monophosphate were single lines with a small amount of hyperfine structure and the eight-line spectrum did not appear until the thymine had been annealed to 120° K and the thymidine monophosphate to 160° K. Assuming that the same radical is responsible for the eight-line spectrum in the four compounds, it is clear that the actual temperature of formation of this radical during the annealing process is very dependent on the detailed chemical structure. There is no simple explanation for these differences, such as an increase in temperature of radical formation as the molecular size increases.

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Compression of Human Red Cells during Centrifuging

THE question as to whether the mammalian red cell is or is not a 'perfect osmometer' in hypotonic media has been sufficiently discussed[1,2], the perfection of the osmometer being measured in terms of the value of a constant R. The red cells of heparinized blood are usually very good osmometers (R = 0·95–1·0) (refs. 3 and 4), and those of oxalated blood are poor osmometers (R = 0·5–0·8); the value of R, however, depends on the details of the methods used to measure it. Several explanations for the value of R being less than 0·95–1·0 have been suggested, for example, that some of the water in the red cell is bound, that electrolytes escape into hypotonic media, and that crenated or paracrystalline red cells have a real bulk modulus. It has also been suggested that red cells are compressed during high-speed centrifuging[5], the extent of the compression increasing as the quantity of water in the cell increases, and that compression of the cells accounts for values of R less than 0·9–1·0.

In the units used here, and supposing the red cells to be a uniformly packed mass, the equation which expresses the difference in osmotic pressure P between the cells, compressed with a force F, and the fluid surrounding them is:

$$P = 755 \left(\frac{g_1}{v_1 - d} - \frac{g_2}{v_2 + d} \right)$$

where g_1 and g_2 are the electrolytes (in g) in the cells and in the surrounding fluid respectively, where v_1 and v_2 are the volumes of the cells and of the surrounding fluid, and where d is the volume of water transferred at equilibrium from the cells to the surrounding fluid by the force F, which is about 10^6 dynes/mm^2.

First taking normal red cells with a fractional water content of 0·7 and compressing them with the force F, one can obtain a series of values of d_1 and from these a series of values of P_1; then, taking swollen red cells with a fractional water content of 1·4 in a tonicity of 0·5, one can obtain another series of values of d_2 and from these another series of values of P_2. The values d_1 and d_2 for which $P_1 = P_2$ are compared in terms of the volumes of the normal and swollen cells respectively; this gives the compression of the normal and of the swollen cells. The latter is the greater, and from these values R can be calculated.

If $v_1 = v_2$ initially, and if the compression of the normal cells is 0·14, that of the swollen cells is 0·18 and an R-value of 1·0 is reduced to 0·93. If $v_1 = 0{\cdot}5\, v_2$ initially, the compression of the normal cells is 0·1 and that of the swollen cells is 0·19 and an R-value of 1·0 is reduced to 0·89. These reduced values of R, however, are minimum values, because even when $v_1 = v_2$ or $0{\cdot}5\, v_2$ the cells and the supernatant fluid are unlikely to remain homogeneous when the compressing force is applied.

The compressing forces and the compression of the cells at high centrifugal force accordingly exist, but are too small to account for R-values less than about 0·9. Many R-values less than 0·9, however, have been obtained for human red cells of heparinized or defibrinated blood.

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BIOCHEMISTRY

Pinguinain : a Simple Method for its Crystallization

RECENTLY we reported[1] the separation of pinguinain, the enzyme present in the fruit of the tropical plant *Bromelia pinguin* L. Using the technique of gel-filtration, we isolated the enzyme from the fruit juice and from crude acetone precipitates which also contained carbohydrates and low molecular weight peptides. Further work on this enzyme led to its crystallization, the procedure for which is the object of this communication.

The starting material used was the juice of the fruits. One hundred millilitres of the juice, which had a *p*H of 3·5–4·0, was neutralized by the addition of 1·0 N sodium hydroxide. At *p*H 7·0–7·2, a dark precipitate was formed and allowed to stand for about 15 min. The

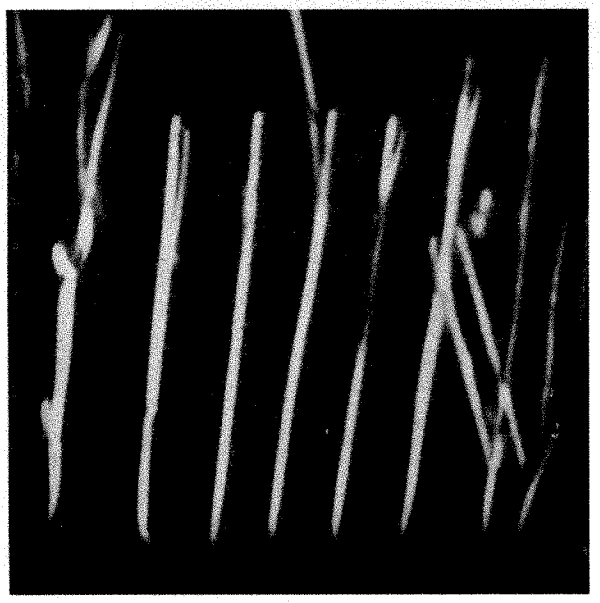


Fig. 1. Pinguinain crystals (average length 150μ) obtained after lyophilization in the presence of 10^{-4} thioglycollic acid. (×400)

suspension was centrifuged for 30 min at 25,000 r.p.m. in a Spinco model *L* preparative ultracentrifuge using an *SW* 25·1 swinging bucket rotor. The clear supernatant was removed and the precipitate, which contained large molecular weight impurities, was discarded. The *p*H of the supernatant was restored to the original. This procedure did not have any deleterious effect on the enzymatic activity of the juice.

The enzyme was isolated from any remaining large molecular weight impurities and peptide material by gel-filtration, using the conditions previously described[1]. Since the isolation of relatively large amounts of enzyme was intended, we prepared cylindrical columns having dimensions of 40 × 5 cm, a gel bed of 600 ml., a void volume of 200 ml. and a rate of flow of 160 ml. per h. Under these conditions 100 ml. of juice was fractionated in 5–6 h. The eluted fractions were collected with the aid of an automatic fraction collector operating on a volume basis (3·0 ml./fraction). Protein contents of the fractions were estimated qualitatively by precipitation with 10 per cent trichloroacetic acid and spectrophotometrically by absorption at 280 mμ. Enzymatic activity was determined as previously described[2]. The fractions containing the protein were pooled. Usually the bulk of the enzyme was found in fractions totalling a volume of 250 ml. The amount of enzyme recovered was 1·5 ± 0·1 g/100 ml. juice, as estimated spectrophotometrically. This was the average yield for ten different preparations made. An $E^{1\%}_{1cm}$ of 22·0 was used for the estimation of protein concentration.

The enzyme solution was exhaustively dialysed against 4 l. of 10^{-4} M thioglycollic acid (Difco Laboratories, Detroit, Michigan). Dialysis was performed for 3 days with three changes of the dialysing solution daily. A portion of the solution was removed for gel-filtration and the remainder was frozen, kept frozen for 2 h and lyophilized. The crystalline material obtained was needle-like (Fig. 1). Crystals had an average length of 150μ, were insoluble in cold 95 per cent ethanol, but dissolved readily in slightly acid or alkaline solutions. They showed least solubility at *p*H 5·5. Crystals could be made to grow by suspending them in 95 per cent methanol and allowing them to stand in the cold (−10° C) for 1 week or longer. Using this procedure, crystals longer than 500μ could be obtained. Kjeldahl digestion indicated that the crystalline material had a nitrogen content of 15·0 per cent. 5 per cent of the total nitrogen appeared to be amide nitrogen. The sulphur content, estimated from methionine and cystine determinations, was 1·1 per cent. Crystals showed a largely diminished enzymatic activity, but this was easily restored with reducing agents containing sulphydryl groups (Table 1). The presence of an SH group compound seems to be necessary for crystallization to occur since lyophilization in the absence of free sulphydryl groups yielded amorphous material.

Table 1. ACTIVATION OF CRYSTALLIZED PINGUINAIN*

Sample No.	Activator	% ^{131}I albumin converted to TCA-soluble radioactivity
1	None	1·0
2	1·0 ml. 0·05 M thioglycollate	43·3
3	2·0 ml. 0·05 M thioglycollate	38·9
4	1·0 ml. 0·05 M L-cysteine, HCl	28·7
5	2·0 ml. 0·05 M L-cysteine, HCl	34·9
6	1·0 ml. 0·05 M sodium cyanide	0·9
7	2·0 ml. 0·05 M sodium cyanide	0·9
8	1·0 ml. 0·05 M ascorbate	0·6
9	1·0 ml. 0·10 M ascorbate	0·6

* Incubation performed in 0·1 M acetate buffer, *p*H 4·0 at 37° C for 20 min. Incubating mixture contained 3·0 mg crystallized pinguinain: 80 mg human serum albumin with 0·4 μc. iodine-131 labelled albumin as tracer; activators as indicated and a total volume of 10 ml.

We consistently noticed that the lyophilization procedure caused a change in the elution pattern of the protein material. The dialysed solution, prior to freezing and lyophilization, appeared homogeneous on gel-filtration in 'Sephadex *G*-100' and '*G*-200', using eluting buffers with *p*H ranging from 4·0 to 7·4. The crystalline material, however, showed two different absorption peaks at 280 mμ (Fig. 2). On 'Sephadex *G*-100' gel-filtration of the crystalline lyophilized material and collection of fractions of the effluent volume (2 ml. each), the faster eluting peak was collected in fractions in which the original prelyophilized enzyme solution appeared when subjected to gel-filtration. This peak contained enzymatic activity proportional to protein concentration and appeared to be homogeneous when further subjected to gel-filtration ('Sephadex *G*-100' or '*G*-200', 0·1 M acetate *p*H 4·6; phosphate saline buffer *p*H 7·3). On treatment of this component with fluorodinitrobenzene and acid hydrolysis of the dinitrophenyl (DNP) protein (5·7 N hydrochloric acid, 18 h), a single ether-insoluble-dinitrophenyl derivative was formed as shown by two-dimensional paper chromatography (butanol–acetic acid–water, 4 : 1 : 1; isobutanol–*n*-butanol–formic acid–water, 2 : 1 : 1 : 3). The second peak appeared in later fractions. The material in the second peak could not be precipitated with trichloroacetic acid, did not show

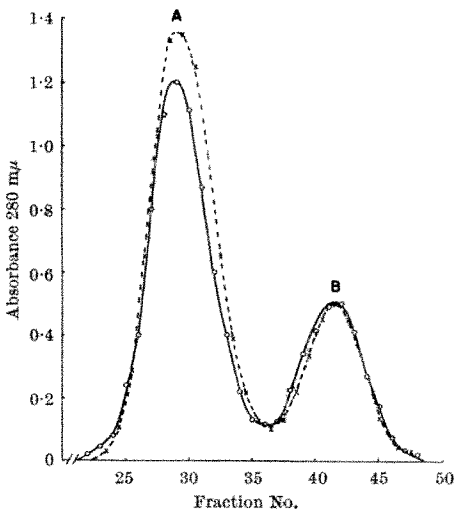


Fig. 2. 'Sephadex *G*-100' (0·01 M acetate, *p*H 4·6) filtration pattern of pinguinain. ——, Lyophilized crystalline material. Note peaks *A* and *B*. The original enzyme solution (prior to crystallization) had an elution pattern identical to peak *A*, but no peak *B*. - - - - -, Pattern for peak *A* of the crystalline preparation after re-dialysis and lyophilization. Notice the reappearance of a peak *B* identical to peak *B* of the original preparation

enzymatic activity and appeared heterogeneous when subjected to paper chromatography.

In our attempt to rule out the possibility that the slower eluting peak might have resulted from concentration of impurities in the original enzyme solution, we performed the following experiment: A solution of crystallized pinguinain was prepared and subjected to gel-filtration in 'Sephadex *G*-100', in a column having dimensions of 40 × 4 cm, using 0·1 M acetate buffer *p*H 4·6 as eluting buffer. The two absorption peaks at 280 mμ appeared. The fractions containing the faster eluting peak were pooled, dialysed as already explained, and lyophilized. Solutions of the crystallized enzyme preparation and of the re-filtered and re-lyophilized material from the faster eluting peak were again subjected to gel-filtration. The two solutions yielded very similar elution patterns (Fig. 2). The slower eluting component reappeared in the crystallized material obtained from the faster eluting peak, thus verifying that the appearance of this component is due to a partial breakdown of some of the protein molecules caused by the crystallization and lyophilization process. Whenever crystallization under these experimental conditions occurs, the optical density ratio between the two components is about 2 : 1.

These results indicate that crystalline pinguinain can be obtained in very high yields using a rather simple method but that its lyophilization causes a partial breakdown of a certain proportion of the molecules. The loss of activity occurring after crystallization is reversible. Crystallization can be accomplished only in the presence of compounds containing free sulphydryl groups. Pinguinain seems to be composed of a single peptide chain, as shown by the production of a single DNP derivative.

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Occurrence of 5-Hydroxy-L-tryptophan as a Free Plant Amino-acid

USING labelled amino-acids and chromatographic techniques, it has been shown that 5-hydroxytryptophan is an intermediate in the conversion of tryptophan to the physiologically important base 5-hydroxytryptamine (serotonin) in higher animals[1,2]. The hydroxylation of tryptophan in the 5 position has also been demonstrated in *Chromobacterium violaceum*[3] and in tissue slices of *Citrullus vulgaris* (watermelon)[4].

In these animal, bacterial and plant cells, however, the concentration of 5-hydroxytryptophan is low and confirmation of its presence has rested on indirect evidence rather than on the isolation of the compound itself.

As part of a programme designed to investigate the distribution of 'non-protein' amino-acids in leguminous plants and investigate possible toxic or other physiological effects, which they may produce when eaten by man and higher animals, we have examined the seed of *Griffonia simplicifolia*, a plant of reputed medicinal value, the leaves of which are fed to sheep and goats in West Africa to stimulate reproduction[5]. Ethanolic (50 per cent) extracts of this seed were found to contain high concentrations (estimated at > 1 per cent dry seed weight) of a compound which reacted with ninhydrin, gave a blue-purple colour with Ehrlich's reagent and occupied an unusual position for a plant amino-acid on two-dimensional chromatograms. Tests with α-nitroso-β-naphthol[6] indicated that the compound was a 5-hydroxyindole, and the ultra-violet absorption maxima shown by partially purified aqueous solutions, at acid and alkaline values of *p*H, corresponded with those given in the literature for 5-hydroxytryptophan.

Extracts of the 'unknown' were chromatographed in seven different solvent systems and subjected to high-voltage ionophoresis on paper at four different values of *p*H using authentic samples of 5- and 6-hydroxy-D,L-tryptophan (kindly given by Dr. S. F. Contractor) as 'markers'. In all systems the 'unknown' moved with 5-hydroxytryptophan. In the solvent system previously used to resolve 5-hydroxy-D,L-tryptophan[7], and also in methanol : pyridine : water (20 : 5 : 1 by volume) which was found to separate the optical isomers equally well, the compound from *Griffonia simplicifolia* ran with the slower-moving 5-hydroxy-L-tryptophan. The colour given by the 'unknown' with Ehrlich's reagent was identical both in shade and in rate of development with that given by authentic 5-hydroxytryptophan, and a characteristic change of colour, from purple through green to brown, was observed when 'spots' of the plant amino-acid and of synthetic 5-hydroxytryptophan on paper chromatograms were developed successively with ninhydrin and Ehrlich's reagent.

After isolation and recrystallization the infra-red spectrum of the purified compound was found to be identical with that of synthetic 5-hydroxy-L-tryptophan (supplied by Calbiochem).

The quantitative recovery of this amino-acid from the seed, its distribution, biosynthesis, fate in the plant and the possibility of it being directly or indirectly responsible for the physiological effects attributed to *Griffonia simplicifolia* are at present being investigated.

We thank Miss J. P. O'Donovan for technical assistance, Mr. R. C. Hider for determining the infra-red spectra and Mr. M. A. Apau for help in obtaining plant material.

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Acetate and Other Carboxylic Acids as Precursors of Ethylene

THE fungus *Penicillium digitatum*, which is the common green mould of citrus fruit, produces a small amount of ethylene which is of physiological significance to its host. The mechanism of ethylene synthesis by the fungus and other plant tissues has been explored in several laboratories by supplying postulated precursors specifically labelled with carbon-14 or tritium. Burg[1] reviewed this work in 1962 and concluded that ethylene is "synthesized fairly directly from sugar, but the steps between glucose and the final product have completely evaded detection". Wang *et al.*[2] have fed specifically labelled sugars and acids to *Penicillium digitatum* and concluded that an "intimate

relationship between ethylene and the tricarboxylic acid cycle is indicated". Burg and Burg[3] provided apple slices with a number of specifically labelled compounds and stated that it is "presumptuous to conclude [that] the relationship [between Krebs cycle acids and ethylene] is very direct".

This communication reports the production of ethylene of high specific activity in relation to the carbon dioxide evolved by *Penicillium digitatum* when acetate-2-^{14}C was fed and re-affirms by an improved method that pyruvate-3-^{14}C yields ethylene of higher specific activity than does pyruvate labelled in the 1 or 2 positions[4]. An improved method for the collection and counting of the carbon dioxide and ethylene is described which is less tedious and does not require shaking or handling of the fungal mats. Disturbing the mats lowers the ethylene production.

Cultures of *Penicillium digitatum NRRL* 1202 isolate were grown in stationary Fernbach flasks at 20° C on citrate medium as described by Gibson[4]. When high ethylene production was observed ($\geqslant$40 μl./h/culture), the medium on which the culture was growing was sucked off and 100 ml. of fresh citrate medium was added. About 25 μc. of ^{14}C-labelled compound was added and ethylene-free air was passed over the culture at a rate of 60 ml./min. The effluent of the culture was dried and passed through a Beckman infra-red gas analyser to determine the total carbon dioxide and then to the 250-ml. ionization chamber of a Cary model 31 electrometer to determine the total radioactivity in the gas stream. Carbon dioxide in the effluent of the electrometer chamber was removed by two 'Ascarite' tubes in series, and the last traces of water and most of the volatile organic compounds by a glass trap held in a dry ice bath. Ethylene was then adsorbed on 0·25 g of silica gel (Davidson 40–60-mesh refrigeration grade *PA*-100) contained in a copper U-tube of 3/16 in. diameter held in a dry ice bath as suggested by Pratt[5]. After adsorbing ethylene for 15–60 min, silica gel in the copper U-tube was connected in the helium input line of a thermal conductivity gas chromatograph. When the tube was transferred to a 40° C bath, ethylene was released immediately from the silica gel and was resolved by a 0·125 × 18-in. column of alumina (Alcoa *H*-151, 60–80 mesh). The ethylene peak was trapped in a 250-ml. Cary flow-type ionization chamber for determination of radioactivity. That the radioactivity was in ethylene was checked periodically by absorption and release from mercuric perchlorate[6] and recounting.

Table 1. SPECIFIC ACTIVITY OF CARBON DIOXIDE AND ETHYLENE AT HOURLY INTERVALS AFTER ADDING SPECIFICALLY LABELLED COMPOUNDS TO CULTURES OF *Penicillium digitatum*

Compound	Gas	Disintegrations per min/μ mole: Hours 1	2	3	4	5	6
Acetate 1-^{14}C	C_2H_4	nil	nil	nil	15		
	CO_2	4,290	4,080	2,950	1,950		
Acetate 2-^{14}C	C_2H_4	3,520	8,880	7,120	6,180	4,150	
	CO_2	1,470	2,410	2,200	1,830	1,650	
Pyruvate 2-^{14}C	C_2H_4	30	40	150	140	300	125
	CO_2	630	1,000	1,100	1,060	1,070	990
Pyruvate 3-^{14}C	C_2H_4	1,110	4,440			4,130	3,840
	CO_2	100	460			740	700
Malate 3-^{14}C	C_2H_4	880	1,950	1,900	2,830	3,110	
	CO_2	1,310	1,560	1,670	1,600	1,600	

The purity and activity of the isotopes used were checked by paper chromatography and scintillation counting.

The specific activity of ethylene and respiratory carbon dioxide at various times after the addition of specifically labelled acids is shown in Table 1. When acetate-1-^{14}C was used, the specific activity of carbon dioxide was very high during the first hour and decreased rapidly thereafter. There was no activity in ethylene during the first 3 h and only a trace during the fourth hour. On the other hand, when acetate-2-^{14}C was supplied, the specific activity in ethylene was twice that of carbon dioxide for the first 5 h and the pools leading to both carbon dioxide and ethylene were maximally labelled during the second hour.

When pyruvate-2-^{14}C was fed, the specific activity in ethylene was at first less than one-tenth that of carbon dioxide, while pyruvate-3-^{14}C yielded ethylene with a specific activity of about ten times that of carbon dioxide for the first 2 h. Carbon dioxide from pyruvate-3-^{14}C became labelled more slowly than the 2 position. This was to be expected if pyruvate was oxidized through the TCA cycle.

Carbon dioxide from malate-3-^{14}C was labelled at about the same rate as was carbon dioxide from pyruvate-2-^{14}C. This finding is consistent with oxidation through the TCA cycle. Ethylene was labelled more slowly, the ratio of specific activity in ethylene to that in carbon dioxide increasing from 0·6 to 1·9 over 5 h. This suggests that some malate may be converted to pyruvate and then to ethylene by a path leading away from the TCA cycle.

If our interpretation of the data on malate is correct, only one carbon, C2 of acetate or C3 of pyruvate, is incorporated extensively in the ethylene molecule. The rates at which ethylene becomes labelled suggest that the path to ethylene synthesis is not through steps of the TCA cycle, but rather that TCA cycle intermediates go to pyruvate or glycolate and acetate where C2 or C3 combines with another molecule which in turn leads to ethylene synthesis.

These observations of ethylene production by *Penicillium digitatum* are in marked contrast to those of Burg and Burg[3], who showed that ethylene initially synthesized by apple tissue became labelled equally well from acetate labelled in either 1 or 2 positions and pyruvate in the 2 or 3 positions. The specific activity in ethylene was much less than that of carbon dioxide. It is probable that ethylene is formed by a different mechanism in fruit.

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Induction of Tyrosine Transaminase in Rat Liver by Actidione

ACTIDIONE (*cyclo*heximide) inhibits protein synthesis in yeasts, certain protozoa and mammalian cell lines[1–3]. It is a toxic compound for some mammalian species, for example, rats; however, in these cases, hydrocortisone acts as an antidote against such poisoning[4]. We recently reported[5] that actidione in rats stimulates the adrenal cortex by way of the anterior pituitary. The stress reaction protected rats against actidione while hypophysectomy or adrenalectomy made them more sensitive to actidione poisoning[5]. Furthermore, mice (which are normally highly resistant to actidione poisoning[4,5]) became sensitive to actidione after adrenalectomy[5].

The effect of actidione may thus be profoundly modified by hormonal factors. In the rat, repeated injection of actidione (0·2 mg for five consecutive days) brings about a stress-induced increase of adrenal protein and RNA; but the DNA increase, which occurs after repeated administrations of ACTH and accompanies a large increase of total RNA[6], is blocked by actidione[5]. An accumulation of RNA in the microsomal fraction of rat liver has also been observed[5].

In unicellular organisms, inhibition of protein synthesis can be produced by a smaller dose of actidione than is needed for the inhibition of DNA synthesis[7]. The synthesis of an inducible enzyme, such as β-galactosidase, was reported to be inhibited by actidione even in bacteria where actidione does not inhibit the growth[8]. It was of interest, therefore, to investigate whether or not actidione inhibits the induction of enzymes such as tyrosine-α-ketoglutarate transaminase (TT) and tryptophan pyrrolase (TPO) in the rat liver: this was especially so because both these enzymes are known to be inducible by hydrocortisone[9–11].

Male Sprague–Dawley albino rats (average body-weight 200 g) were used for these experiments. TT and TPO were assayed by Knox's methods[12,13]. Actidione injected intraperitoneally to the extent of 1 mg per kg body-weight inhibited the induction of TPO, assayed 4 h after administration of substrate (200 mg) or by hydrocortisone (10 mg). The addition of microsomes or of haematin to assayed mixtures did not affect these results. In contrast, actidione did not abolish the induction of TT by hydrocortisone. Furthermore, in the absence of hydrocortisone, actidione increased the level after 4 h. A similar, although smaller, effect also occurred in hypophysectomized rats. The effect was, therefore, not caused by the release of corticosteroids by way of the anterior pituitary, but suggested a possible direct induction of TT by actidione. This conclusion was warranted by further experiments with adrenalectomized rats. An increase in the level of liver TT of some 250–300 per cent was observed 4 h after administration of actidione. The same results were obtained using a modified Briggs reaction[14] for assay of tyrosine transaminase. The induction of TT by actidione increased linearly with time for the period of 2·5 h and reached a maximum after 4 h. Puromycin hydrochloride (Nutritional Biochemicals) completely prevented the induction of TT. These results are summarized in Table 1.

Table 1. EFFECT OF ACTIDIONE (*Cyclo*HEXIMIDE) ON TYROSINE-α-KETOGLUTARATE TRANSAMINASE ACTIVITY IN RAT LIVER

Exp. No.	Animal No.	Treatment	Enzymatic activity after 4 h	Change (%)
(1) Intact	1	Saline	54	—
	2	Hydrocortisone	204	+277
	3	Hydrocortisone + actidione	388	+618
	4	Actidione	358	+562
	5	Actidione	418	+674
(2) Hypophysectomized	6	Saline	83	—
	7	Hydrocortisone	277	+233
	8	Actidione	275	+231
	9	Actidione	286	+244
	10	Actidione	280	+237
(3) Adrenalectomized	11	Saline	50	—
	12	Actidione	238	+370
	13	Actidione	212	+320
	14	Actidione	220	+340
	15	Actidione + puromycin	47	—
	16	Actidione + puromycin	57	—

Enzymatic activity is expressed in μmoles of *p*-hydroxyphenylpyruvate formed/g liver/h at 37° C, 4 h after treatment. 10 mg hydrocortisone sodium succinate (*o*) injected intraperitoneally. Actidione (Upjohn, Kalamazoo, Michigan) 1 mg/kg body-weight. 15 mg puromycin hydrochloride (Nutritional Biochemicals) was given at −1 and 0 h, and 10 mg in 1 h from the administration of actidione.

Assay of TT performed with 0·3 ml. supernatant (15 min at 30,000*g*) of 10 per cent (in saline) homogenate of liver. The reaction mixture with all supplements (see ref. 13) incubated for 15 min at 37° C. The reaction stopped with 1 c.c. 30 per cent TCA. Spectrophotometric readings made at 310 mμ.

These findings demonstrate that an inhibitor of protein synthesis, such as actidione, may also act as an inducer for the synthesis of the enzyme tyrosine transaminase, perhaps by stimulating the action of hydrocortisone. This 'pseudohormonal' action of actidione may explain the toxicity of actidione in certain mammalian species and also the fact that hydrocortisone may act as an antidote in actidione poisoning. It does not explain why a similar effect of 'pseudohormonal' induction is not observed in the case of TPO, but only the inhibition of enzyme induction. One possible explanation may be that the targets for induction of TPO and TT have a great affinity for both hydrocortisone and actidione, but a different degree of specificity required to bring about the synthesis of each of these enzymes; in the case of TT, hydrocortisone may be replaced by actidione for the induction of the enzyme, whereas for TPO the enzyme can be induced only by hydrocortisone.

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2-Acetamidofluorene and 3-Methylcholanthrene: Differences in Binding to Rat Liver Deoxyribonucleic Acid *in vivo*

THE relationship between mutagenesis and carcinogenesis is still a matter of controversy[1]. The problem is particularly unresolved with respect to liver cancer induced by chemicals. Although hepato-carcinogenic nitrosamines are known to alkylate liver DNA *in vivo*[2], similar binding to liver DNA *in vivo* has not been reported for the two other most intensively studied groups of liver carcinogens, namely, the fluorenamines and the aminoazo dyes. Although, at present, one cannot make definitive interpretations of the functional significance (with respect either to DNA replication or to DNA transcription) of the binding of a carcinogen to the DNA in a liver cell, studies are needed which correlate carcinogenicity of various compounds with their binding *in vivo* to gene material. This report describes the binding of the strongly hepato-carcinogenic agent 2-acetamidofluorene[3] (*N*-2-fluorenylacetamide, AAF) to rat liver DNA after intraperitoneal injection. In a parallel *in vivo* experiment the aromatic hydrocarbon, 3 (or 20) -methylcholanthrene (MC), which is not carcinogonic for rat liver[4] (although it does cause significant alterations in both RNA[5] and protein[6] synthesis in this organ), was found not to bind to rat liver DNA.

Fischer male rats, 8–10 weeks old, weighing 140–180 g, were injected intraperitoneally with either 6 mg of AAF-9-^{14}C (synthesized by Drs. E. K. and J. H. Weisburger[3], radiopurity greater than 98 per cent by paper chromatography in *cyclo*hexane–*t*-butanol–acetic acid–water, 16 : 4 : 2 : 1) or 3 mg of MC-^{3}H, generally labelled (Nuclear Chicago, Des Plaines, Ill., radiopurity greater than 95 per cent by paper chromatography in methanol–ether–water, 4 : 4 : 1). The rats were killed at the times

indicated in Table 1. In each experiment 3 g of liver from each of two identically treated rats were pooled and highly purified nuclei were isolated as described previously[7]. The nuclei were suspended evenly in approximately 8 ml. of 0·32 M sucrose, 0·002 M magnesium chloride, 0·001 M potassium phosphate, *p*H 6·8, and lysed by the addition of 42 ml. of 6·5 M caesium chloride, containing 0·005 M trisodium EDTA. Gentle homogenization with the loose pestle of a Dounce homogenizer was necessary to assure complete lysis of nuclei. The final density of the lysate was 1·66. The DNA was separated from the other nuclear constituents by centrifuging 9-ml. portions of the nuclear lysate for 64 h at 45,000 r.p.m. and 20° C in an angle rotor[8] (International rotor No. 965, *T*-60 ultracentrifuge). Under these conditions, the DNA forms a sharp band just below the middle of the tube at its equilibrium density of 1·70 (ref. 9); RNA is sedimented to the bottom of the tube and protein migrates to the top. Fractions of 0·5 ml. were collected by punching a hole in the bottom of the tube with a 27 gauge needle, and absorbancy at 260 mμ was measured on 0·1 ml. aliquots after dilution to 3·0 ml. (Fig. 1). Radioactivity assays were made on 0·2 ml. aliquots from all fractions (Fig. 1) by adding 200 μg carrier DNA, precipitating with ice-cold 10 per cent trichloroacetic acid (TCA), washing with ice-cold methanol, and dissolving the precipitate in 1 ml. of hyamine hydroxide before counting in a liquid scintillation counter. Caesium chloride densities were determined by refractive index measurements[10], after correcting for the presence of sucrose in the nuclear lysates.

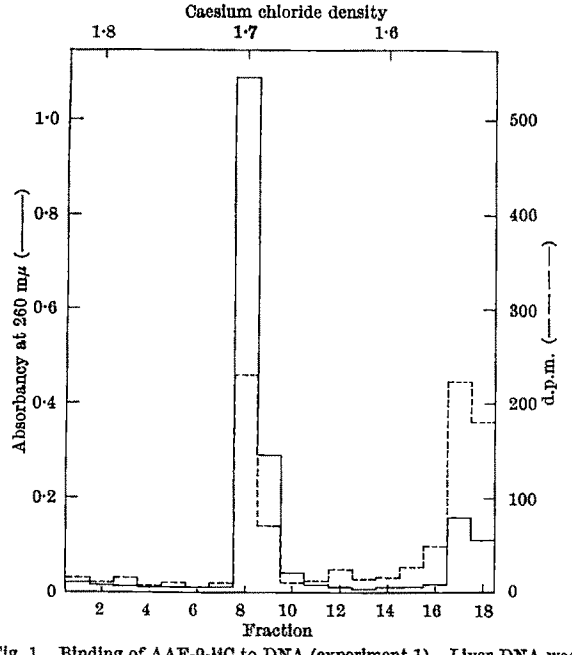


Fig. 1 Binding of AAF-9-^{14}C to DNA (experiment 1). Liver DNA was banded by density equilibrium centrifugation in caesium chloride. Fractions of 0 5 ml. were collected Absorbancy figures are those obtained after 1 : 30 dilution of aliquots (1 cm light path). The plotted radioactivity figures are those obtained on separate 0·2-ml. aliquots

The results in Table 1 and Fig. 1 show that AAF or a metabolite thereof binds to rat liver DNA, while no binding of MC to DNA was detectable. It is of particular interest that binding of AAF to DNA occurs within 2 h of administration of the compound (experiment 1). Table 1 also shows the total radioactivity in the entire nuclear lysates after precipitation with ice-cold 10 per cent TCA and washing the precipitate with cold methanol. In experiments 1, 2 and 3, about 50 per cent of this radioactivity was resistant to two extractions with hot 10 per cent TCA (90°, 15 min each time) and two subsequent extractions with methanol at room temperature. In experiment 4, in contrast, essentially none of the MC radioactivity in the nuclear lysate (precipitated by cold TCA) was extracted by treatment with hot TCA and methanol.

Table 1. BINDING OF 2-ACETAMIDOFLUORENE AND 3-METHYLCHOLANTHRENE TO RAT LIVER DNA AFTER SINGLE INTRAPERITONEAL INJECTION

Experiment	Labelled compound	Duration of isotope treatment	Total d.p.m. in entire initial homogenate	Total d.p.m. in entire nuclear lysate precipitated by cold TCA	μMoles of compound bound to DNA per mole DNA-phosphorus
(1)	AAF, 220 μc /rat	2 h	$36·6 \times 10^6$	$7·1 \times 10^4$	14
(2)	AAF, 220 μc./rat	23 h	$7·8 \times 10^6$	$12·5 \times 10^4$	17
(3)*	AAF, 220 μc./rat	23 h	$2·7 \times 10^6$	$1·8 \times 10^4$	4·8
(4)	MC, 450 μc./rat	23 h	$10·2 \times 10^6$	$2·3 \times 10^4$	<0·08 (Lowest limit of detection)

AAF-9-^{14}C, specific activity 8 mc /mmole, was administered in 0·2 ml. of dimethyl sulphoxide. MC-^{3}H, specific activity 40 mc./mmole, was administered in 0·5 ml of dimethyl sulphoxide. For each experiment 2 rats were used and their livers pooled at the time of killing.

* In experiment 3 the rats were pre-fed for 3 weeks with 0·03 per cent AAF in a semi-synthetic diet before injection and then placed on standard ration after injection; in experiments 1, 2 and 4 the rats were fed *ad lib.* on standard commercial laboratory ration.

In all experiments the banding density of the DNA indicated that the protein remaining bound to the DNA during density equilibrium centrifugation was less than 3 per cent. Furthermore, the low buoyant density of proteins would not allow AAF, bound only to protein, to form a radioactivity peak overlapping the DNA band. At this point the nature of the binding of AAF to DNA is unknown. According to Marroquin and Farber[11], who have previously reported binding of AAF to liver RNA *in vivo*, the possibility that nucleotides might become labelled from carbon dioxide derived from AAF-9-^{14}C is quite unlikely. The level of binding of AAF to DNA is approximately of the same order of magnitude as has been found for binding of single doses of carcinogenic aromatic hydrocarbons to mouse skin DNA[12] or for binding of physiologically significant doses of actinomycin *D* to rat liver DNA[13].

It is of interest that MC, which is not carcinogenic for rat liver, did not bind to rat liver DNA *in vivo*. This is in direct contrast to its behaviour in mouse skin, for which MC has been found to be both a potent carcinogen[4] and a firm binding agent to DNA *in vivo*[12]. It is conceivable that species and organ differences in the carcinogenicity of MC may be related to the extent of DNA binding, which in turn may be related to differential metabolism of this hydrocarbon. Further investigations on the binding of liver carcinogens and their non-carcinogenic analogues to DNA are in progress and will be published elsewhere in greater detail.

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Stimulation of Fermentation in Yeasts by Acetoin and Oxygen

In Warburg experiments with 'resting' cells, most yeasts show a Pasteur effect, that is, fermentation of glucose is inhibited under aerobic conditions. This effect is generally regarded as an inhibition of fermentation by respiration, for example, as a result of a lack of inorganic phosphate (P_i) or adenosine diphosphate (ADP)[1,2].

However, in yeasts belonging to the genus *Brettanomyces*, fermentation of glucose is inhibited under anaerobic conditions[3,4]. This 'negative Pasteur effect', as it was named by Custers[3], is attributed to an initial shortage of nicotinamide-adenine dinucleotide (NAD)[5], brought about by the activity of redox systems in the cell after the addition of glucose[6]. It can be abolished by various carbonyl compounds, reoxidizing $NADH_2$ enzymatically, and by oxygen, reoxidizing $NADH_2$ by way of the respiratory chain to NAD, which then takes part in the fermentation[5]. Hence the term 'negative Pasteur effect' is less appropriate and may even be confusing, since it suggests a close relationship with the Pasteur effect. For this reason, the term 'Custers effect' is here proposed for this inhibition of alcoholic fermentation under anaerobic conditions.

The question arose whether an inhibition under aerobic conditions and an inhibition under anaerobic conditions may occur in one and the same yeast; in other words, whether a Pasteur effect may be masked by a Custers effect, or vice versa.

In order to elucidate this point, further experiments were performed on the effects of a carbonyl compound (acetoin) and oxygen in low concentrations on fermentation in yeasts, using the method given in detail by Wikén *et al.*[4].

With *Brettanomyces* spp., addition of acetoin (10^{-3} M) or of small amounts of oxygen (such as 0·1 per cent of the gas phase) to the anaerobic yeast suspensions stimulates fermentation of glucose to rates even larger than those in air (Fig. 1, Table 1). Apparently, at certain low oxygen tensions, the anaerobic inhibition of fermentation is overcome by oxidation of $NADH_2$ by way of the respiratory chain, whereas the depressing effect of respiration on fermentation, known as the Pasteur effect, does not yet manifest itself to the full extent. It is concluded that in *Brettanomyces* spp. the Pasteur effect is obscured by the Custers effect, but may become evident after the anaerobic inhibition of fermentation is abolished by addition of acetoin or oxygen in low concentrations.

Furthermore, acetoin enhances anaerobic fermentation in many yeasts showing a Pasteur effect; low concentrations of oxygen may have a similar effect (Fig. 2, Table 2). It is concluded that in these yeasts a partial

Table 1. FERMENTATION IN *Brettanomyces* SPECIES

Species	Strain *CBS*	Culture medium	Culture age (h)	Suspension liquid	Fermentation (μl. carbon dioxide in 90 min per 20 mg wet cell weight)					
					Aerobic (in air)	Aerobic $+10^{-3}$ M acetoin	Anaerobic (in nitrogen)	Anaerobic $+10^{-3}$ M acetoin	In presence of low concentration of oxygen	Oxygen admitted (%)
Brettanomyces anomalus	3026	I	22	*B*	277	294	≤2	467	337	0·43
		III	15	*B*	182	192	≤4	368	224	0·43
Brettanomyces bruxellensis	96	I	20	*B*	346	349	≤4	407	394	0·45
Brettanomyces custersianus	4805	III	22	*B*	6	17	≤8	155	—	—
Brettanomyces custersii	5512	III	25	*B*	406	401	66	492	426	0·27
Brettanomyces intermedius	1943	I	21	*A*	456	450	≤8	674	579	0·12
Brettanomyces lambicus	75	I	21	*A*	156	169	≤1	351	160	0·09
Brettanomyces nanus (ref. 7)	1945	III	37	*A*	100	150	≤6	290	—	—
Brettanomyces schanderlii	2796	I	44	*A*	364	429	2–6	653	561	0·09

The yeasts were grown in shake cultures at 30° C in a synthetic liquid medium (I) or in yeast water with 2 per cent glucose (III). After being collected and washed, the cells were suspended in distilled water (*A*) or in 0·05 M potassium dihydrogen orthophosphate, pH 4·5 (*B*). Acetoin was added together with glucose from the side arm of the Warburg flasks at $t=0$. The glucose concentration in the Warburg vessels was 0·1 M. To obtain the desired oxygen concentrations, measured volumes of air were admitted shortly before $t=0$ to anaerobic Warburg vessels. The manometric experiments were performed at 30° C. The values for the aerobic fermentation were calculated without correction for respiration (ref. 4).

Table 2. FERMENTATION IN SPECIES BELONGING TO OTHER GENERA

Species	Strain *CBS*	Culture medium	Culture age (h)	Suspension liquid	Fermentation (μl. carbon dioxide in 90 min per 20 mg wet cell weight)					
					Aerobic (in air)	Aerobic $+10^{-3}$ M acetoin	Anaerobic (in nitrogen)	Anaerobic $+10^{-3}$ M acetoin	In presence of low concentration of oxygen	Oxygen admitted (%)
Endomycopsis bispora	1890	III	24	*A*	8	21	1	79	103	0·14
Endomycopsis fasciculata	5514	I	23	*B*	14	14	5	209	68	0·25
Endomycopsis monospora	2554	III	23	*A*	3	7	2	52	26	0·09
Endomycopsis scolyti	4802	III	15	*B*	47	47	36	57	95	0·22
Endomycopsis wickerhamii	4106	III	16	*B*	20	18	2	222	216	0·37
Saccharomyces lactis	683	I	20	*B*	99	111	338	449	421	0·10
Saccharomyces lodderi	2757	I	21	*B*	603	590	1,430	1,615	1,541	0·10
Saccharomyces marxianus	712	I	22	*B*	12	27	138	276	185	0·10
Saccharomyces rhodanensis	4840	I	16	*B*	25	31	42	155	154	0·44
		III	19	*B*	9	4	35	409	443	0·44
Saccharomyces rouxii	732	III	22	*B*	502	489	1,138	1,090	1,272	0·06
Pichia bovis	2616	III	18	*A*	37	6	33	220	52	0·12
Pichia saitoi	4910	I	21	*B*	−13	−7	592	638	634	0·05
Pichia terricola	2617	III	28	*B*	9	7	37	131	41	0·06
Hansenula anomala	110	III	22	*B*	−3	−15	112	254	114	0·07
Hansenula capsulata	1993	III	17	*A*	11	—	119	214	136	0·11
Hansenula holstii	4140	III	17	*A*	72	—	198	394	254	0·32
Hansenula jadinii	1600	I	16	*A*	181	198	336	419	—	—
Hansenula schneggii	113	I	18	*A*	6	−9	395	475	—	—
Hansenula silvicola	1705	III	16	*A*	−20	—	21	—	168	0·12
Schwanniomyces occidentalis	1153	III	22	*B*	86	97	855	915	873	0·06
Schwanniomyces persoonii	2169	III	16	*B*	58	61	711	767	796	0·05
Torulopsis apicola	2868	I	63	*A*	17	36	33	18	92	0·09
Torulopsis ernobii	1737	III	22	*B*	7	5	21	33	111	0·28
Torulopsis vartiovaarai	4289	I	23	*B*	35	44	482	534	539	0·37
		III	23	*B*	278	278	685	732	657	0·15
Candida melinii	601	III	22	*B*	−4	−14	8	14	118	0·49
Candida pelliculosa	605	III	19	*B*	22	13	132	293	167	0·20
Candida tropicalis	94	III	24	*A*	37	37	786	880	742	0·14
Candida utilis	621	III	24	*B*	20	−9	689	760	632	0·09
Trichosporon behrendii	2452	III	22	*B*	24	26	49	67	64	0·26
Trichosporon fermentans	2529	III	24	*B*	10	13	369	421	348	0·09

See legend to Table 1.

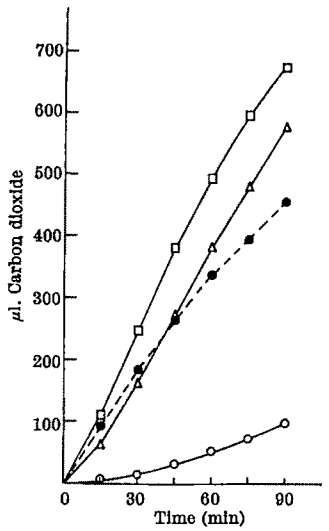


Fig. 1. Glucose fermentation by *Brettanomyces intermedius*, *CBS* 1943. ●, Aerobic fermentation with or without 10^{-3} M acetoin; ○, anaerobic fermentation; □, the same with 10^{-3} M acetoin; △, fermentation with 0·12 per cent oxygen

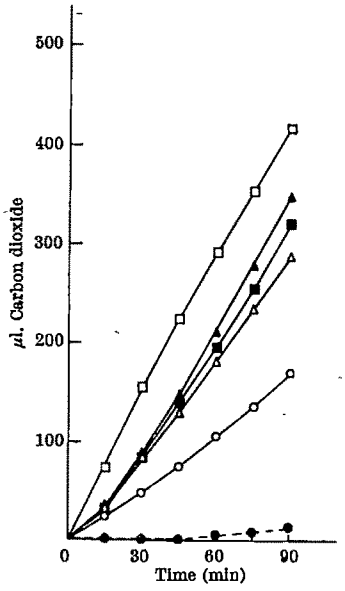


Fig. 2. Glucose fermentation by *Trichosporon fermentans*, *CBS* 2529. ●, Aerobic fermentation with or without 10^{-3} M acetoin; ○, anaerobic fermentation; □, the same with 10^{-3} M acetoin; △, fermentation with 0·06 per cent oxygen; ▲, the same with 0·09 per cent oxygen; ■, the same with 0·18 per cent oxygen

inhibition of fermentation under anaerobic conditions is masked by the Pasteur effect.

As may be seen from Tables 1 and 2, the occurrence of a Pasteur effect and a Custers effect, respectively, is not restricted to yeasts grown in a particular medium or suspended in a particular system. As might be expected, acetoin has little effect on aerobic fermentation. As regards the effect of acetoin on anaerobic fermentation, it must be emphasized that not all yeasts will necessarily have a dehydrogenase system for the oxidation of $NADH_2$ by acetoin. The oxygen concentrations, chosen arbitrarily, will not necessarily be the optimal ones for obtaining a maximum fermentation rate, and this is shown in Fig. 2.

In summary, it is concluded that in one and the same yeast, fermentation may be inhibited: (1) by higher oxygen tensions leading to a shortage of, for example, P_i or ADP as a consequence of respiratory chain phosphorylation; (2) by anaerobic conditions leading to a shortage of NAD.

Depending on the ratio between these two inhibitions, a Pasteur effect or a Custers effect is observed as a net result. An optimal oxygen concentration may be found at which the rate of fermentation is maximal.

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Induction of Enzyme Activity in Pea Tissues by Animal Hormones and by Puromycin

IT was shown previously[1] that the ability of pea stem segments to synthesize benzoylaspartate is greatly increased by pretreatment with indolyl-3-acetic acid (IAA) and that this inducing effect is suppressed by actinomycin *D* and by puromycin, inhibitors of ribonucleic acid[2] (RNA) and protein synthesis[3], respectively. In extending this work it has been found that certain animal hormones, and more surprisingly puromycin, also act as inducers of the aspartate conjugation system.

Segments of pea epicotyls excised from the apex of the third internode were treated for 16 h in the appropriate buffered medium and then transferred for 2 h to 10 mg/l. of benzoic acid (carboxyl-^{14}C). The segments were extracted in 80 per cent ethanol containing sodium carbonate and the amount of benzoylaspartate formed was estimated as described previously[1].

The data of Table 1 show that thyroxine, hydrocortisone and testosterone elicit a three- to five-fold increase in benzoylaspartate production and that their inducing activity, like that of IAA, is completely suppressed by the presence of 5 mg/l. of actinomycin *D* during pretreatment. It has already been established[1] that this level of actinomycin strongly inhibits RNA synthesis in pea stem segments. In another experiment, with varying concentrations of thyroxine (Fig. 1), a maximum response was obtained at 100 mg/l. (seven-fold induction); inclusion of actinomycin abolished induction at all thyroxine concentrations tested. The extent of induced conjugate formation in response to the animal hormones is somewhat variable, and in particular a clear optimal concentration as in Fig. 1 is often not obtained. For thyroxine and testosterone at least, this is probably because they are present in fine suspension, being initially dissolved in a small quantity of ethanol (insufficient to affect growth or conjugation), which is then dispersed in the aqueous incubation medium. Thus the effective external concentration can be expected to vary with the degree of dispersion from sample to sample.

Table 1. EFFECT OF ACTINOMYCIN *D* DURING PRETREATMENT ON THE INDUCTION OF BENZOYLASPARTATE FORMATION BY VARIOUS HORMONES

Pretreatment	Benzoylaspartate formed μg	Benzoylaspartate formed Per cent conversion
Buffer	1·2	5·7
AM	0·0	0 0
L-Thyroxine 200 mg/l.	6·8	25·9
L-Thyroxine 200 mg/l. + AM	0·0	0·0
Hydrocortisone 200 mg/l.	3·3	15·9
Hydrocortisone 200 mg/l. + AM	0·0	0·0
Testosterone propionate 200 mg/l.	3·9	21·4
Testosterone propionate 200 mg/l. + AM	0·0	0·0
IAA 50 mg/l.	6·7	51·5
IAA 50 mg/l. + AM	0 0	0 0

Incubation conditions were as described in the text. Actinomycin *D* (AM) was used at 5 mg/l.

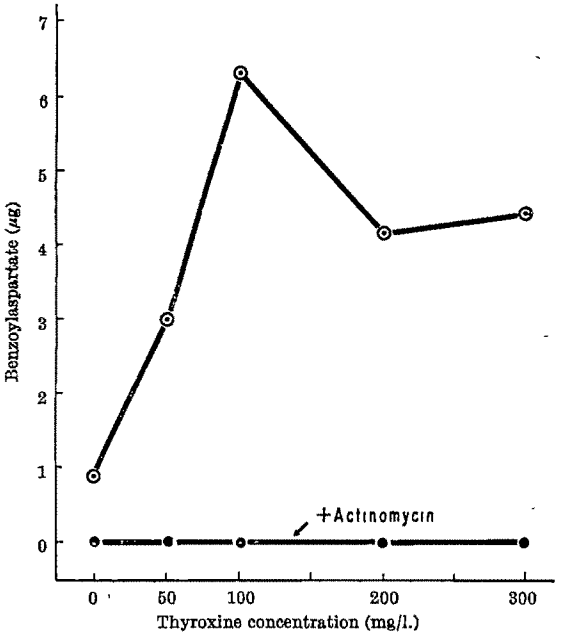


Fig. 1. The effect of pretreating pea stem segments for 16 h in various concentrations of thyroxine with (●) or without (○) 5 mg/l. of actinomycin *D* on the amount of benzoylaspartate formed after transference for 2 h to labelled benzoic acid (10 mg/l.)

When the action of puromycin on the hormonal induction is investigated, certain apparently anomalous results appear. Puromycin effectively represses benzoylaspartate synthesis induced by testosterone, hydrocortisone and by IAA (Table 2), but fails to reverse the effect of thyroxine. Moreover, with puromycin alone during pretreatment, in the absence of any hormone, conjugate formation is increased three-fold. Data on the fresh weight increase of the tissues during pretreatment (Table 2) show that in the cases where puromycin is effective in suppressing hormonal induction, growth is severely retarded, whereas the treatments of puromycin alone or in combination with thyroxine are relatively non-inhibitory. It would appear as if applications of hydrocortisone, testosterone and indolyl-3-acetic acid in some way increase tissue susceptibility to puromycin, one manifestation being that synthesis of the conjugation enzyme becomes puromycin-sensitive. Such a difference is not, however, reflected in the overall rates of protein synthesis (Table 3): puromycin, whether in combination with thyroxine or with testosterone, inhibits leucine incorporation to about the same extent (85 per cent and 80 per cent, respectively). The hormones themselves reduce leucine incorporation, an effect previously noted for IAA under similar conditions[1]. This action is not incompatible with induction of an enzyme representing a small proportion of the total protein synthesized.

Table 2. Effect of Puromycin during Pretreatment on the Induction of Benzoylaspartate Formation by Various Hormones

Pretreatment	Benzoylaspartate formed (μg)	Gain in fresh wt. (%)
Buffer	1·4	24·2
PM	4·3	14·6
PM + actinomycin 5 mg/l.	0·0	9·5
L-Thyroxine 100 mg/l.	4·9	24·1
L-Thyroxine 100 mg/l. + PM	5·6	16·9
Testosterone propionate 100 mg/l.	3·8	21·8
Testosterone propionate 100 mg/l. + PM	1·1	2·4
Hydrocortisone 100 mg/l.	7·6	6·7
Hydrocortisone 100 mg/l. + PM	0·4	−3·6
IAA 50 mg/l.	9·2	49·2
IAA 50 mg/l. + PM	0·8	−0·6

Incubation conditions were as described in the text. Fresh-weight gains were measured after the 16-h pretreatment period. Puromycin (PM) was used at 10^{-3} M.

Table 3. Effects of Thyroxine, Hydrocortisone and Puromycin on the Incorporation of Leucine-^{14}C into Protein

Treatment (2 h+3 h)	Specific activity of protein (c.p.m./mg)
Buffer	867
Puromycin	290
L-Thyroxine 100 mg/l.	472
L-Thyroxine 100 mg/l. + PM	131
Hydrocortisone 100 mg/l.	512
Hydrocortisone 100 mg/l. + PM	178

Pea stem segments were treated for 2 h in the appropriate medium prior to the addition of DL-leucine-1-^{14}C ($2·6 \times 10^{-5}$ M) for a further 3 h. All media contained 15 mg/l. of benzylpenicillin. Incorporation was determined in a manner similar to that of Osborne (ref. 4). Puromycin was used at 10^{-3} M.

The anomalous puromycin effect may be compared with similar reports of actinomycin-induced increases in enzyme activity[5] and in ferritin protein synthesis[6]. Increased alkaline phosphatase activity in response to both actinomycin and puromycin has been noted[7]. At this stage it is scarcely worth speculating on the interpretation of the puromycin-induced increase in benzoylaspartate synthesis, except to note that this action of puromycin is actinomycin-sensitive (Table 2) and that actinomycin alone has no such effect (Table 1 and Fig. 1). Thus activation of pre-existing enzyme by puromycin is not a likely explanation.

Carlisle *et al.*[8] reported that locust extracts containing the moulting hormone ecdysone showed activity in a dwarf pea gibberellin bioassay, while gibberellic acid injected into locust larvae mimicked the action of ecdysone on moulting. The present report serves as a further indication that some animal and plant hormones may, superficially at least, have certain properties in common.

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Proposed Catalytic Role for Histidine in Pyridine Nucleotide-linked Alcohol Dehydrogenases

The appearance of histidine close to essential thiol groups in a number of alcohol dehydrogenases[1], coupled with various inactivation[2] and inhibition[3] experiments, suggests the possibility of histidine involvement in the catalytic step of certain pyridine nucleotide-linked enzymatic oxidation-reduction processes. The purpose of this communication is to propose a simple concerted mechanism which allows histidine to fulfil such a role by both substrate and coenzyme activation.

Assume that in the ternary complex of enzyme–coenzyme-substrate, the alcohol or carbonyl group of the substrate is located in sandwich fashion between the imidazole ring of histidine and the nicotinamide ring of the pyridine nucleotide, as shown in Fig. 1 (I) and (II). The two rings are positioned so that the α-nitrogen of imidazole and the pyridine nitrogen are close to each other and may be slightly tilted to bring the two nitrogens closer together. Oxidation and reduction are viewed as proceeding by a concerted and virtually cyclic mechanism (I⇄II) which would allow a low-energy common transition state, intermediate in structure between (I) and (II). The direction of electron movement is indicated by the arrows.

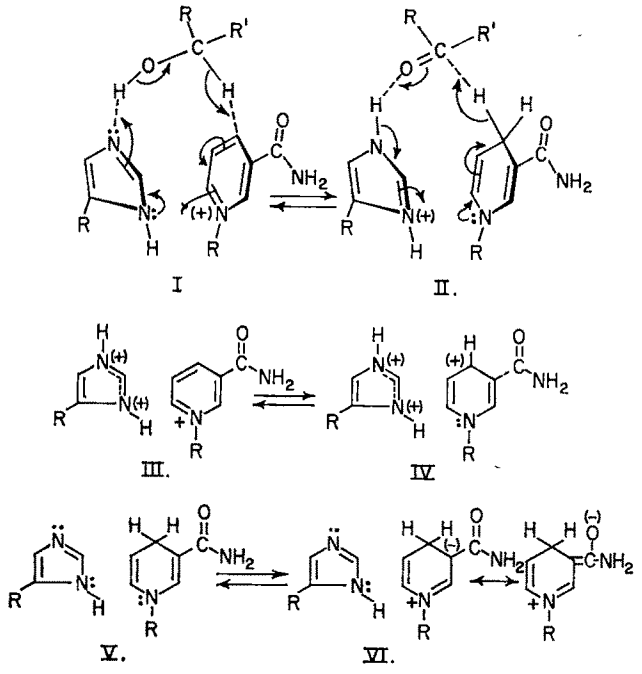


Fig. 1. Proposed participation of enzyme histidine in enzymatic oxidation-reduction processes

Catalytic aid with respect to the substrate, which has been previously suggested by Winer and Schwert[3] for lactic dehydrogenase, may be understood by visualizing the reaction in discrete steps even though the process is considered as concerted and the relative timing of proton and hydride transfer cannot be defined. In the oxidative direction proton transfer from the alcohol to the imidazole nitrogen would generate partial negative charge on oxygen and facilitate the transfer of hydride ion to the pyridinium ring. In the reductive direction, protonation of the carbonyl group by imidazolium would generate partial carbonium ion character on the carbonyl carbon, thereby facilitating the transfer of hydride ion from the reduced pyridine nucleotide. Alternately, assuming hydride transfer as the ultimate step in the oxidative direction and microscopic reversibility, reaction in the reductive direction would require initiation by hydride transfer with stabilization of the incipient negative charge on oxygen by the imidazolium proton.

A second catalytic function based on charge localization in the two nitrogen-containing rings may lead to coenzyme activation. (I) and (II) represent the mutually stabilized charge situation with respect to the two opposing nitrogens —positively charged nicotinamide nitrogen faced by the odd pair of electrons on imidazole or vice versa. (The relative position of the two nitrogen atoms in question is in accordance with the suggestion of Kosower[4] that the characteristic spectral shift of enzyme-bound NADH is due to the proximity of a quaternary ammonium group.) Again, by representing for convenience a concerted process in discrete stages, it may be seen that a movement of the hydroxyl proton closer to the imidazole nitrogen results in positive charge being placed on both imidazole nitrogens and creates the energetically unfavourable situation of opposing positively charged imidazolium and pyridinium nitrogen (III). In (II), a movement of the imidazolium proton towards the carbonyl leads to the energetically unfavourable situation (V) of opposing odd electron pairs on the two nitrogen atoms in question. Both unfavourable situations may be relieved by charge redistribution as shown in the canonical resonance representations (IV) and (VI) with important consequences concerning the ease of hydride transfer. In (IV), the delocalization of positive charge to the 4-position of the nicotinamide and the creation of partial carbonium ion character should facilitate hydride transfer from the alcohol substrate to that position. In (VI), placement of negative charge at the 3-position, where it may in turn be delocalized by way of the carboxamide carbonyl function, would facilitate reduction of the substrate carbonyl by promoting the expulsion of hydride ion from the 4-position of the dihydronicotinamide. Thus, the aforementioned scheme provides a mechanistic pathway whereby the imidazole ring of histidine can promote rapid hydride transfer in oxidation reduction reactions by 'activating' both the substrate and the coenzyme.

In theory, with the nucleotide fixed in the type of folded conformation necessary for adenine–nicotinamide energy transfer, the imidazole ring of the pyridine nucleotide itself could serve the role postulated for a histidine residue. While the concept of the pyridine nucleotide carrying the potential for its own activation as well as that of the substrate is attractive, intuitively this would appear to assign too passive a role to the enzyme.

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Succinylation of Gamma Globulin

THE chemical modification of proteins with succinic anhydride was shown by Habeeb *et al.*[1] to result in an unfolding of the compact conformations of several proteins in neutral aqueous buffer. This was attributed to the large increase in net negative charge produced by the replacement of the positively charged ε-NH_3^+ groups of lysine residues by negatively charged-$NHCOCH_2CH_2COO^-$ groups. An intensive study of the specificity of this reaction and of its conformational effects was made by Cherry[2] with the protein bovine serum albumin; the results have been briefly reported[3]. Utilizing these findings, Klotz and Keresztes-Nagy[4] made the interesting observation that the electrostatic repulsions introduced by succinylation could result in the complete dissociation of non-covalently linked sub-units of proteins, in particular, those of haemerythrin, at neutral *p*H without the addition of any other dissociating agents such as urea or detergent. We have now shown that rabbit γ-globulin, succinylated either before or after partial reduction of interchain disulphide bonds[5], can be separated into its constituent heavy and light peptide chains[5,6] by gel-filtration in aqueous buffers at *p*H near neutrality without the use of detergents[7]. Furthermore, the isolated succinylated heavy and light chains are quite soluble in such buffers, whereas isolated but unmodified heavy chains are insoluble[5].

An experiment in which succinylation was performed after partial reduction of the γ-globulin is described. 500 mg of rabbit γ-globulins (Fraction II, Pentex, Inc., Kankakee, Illinois) was reduced by the method of Fleischman *et al.*[5]. The protein was dissolved in 25 ml. of 0·55 M *tris* buffer, *p*H 8·2, and 0·325 ml. of 2-mercaptoethanol was added. After standing at room temperature for 1 h, the solution was cooled in an ice bath, and to it was added 1 g of iodoacetamide, dissolved in 25 ml. of *tris* buffer and also ice-cooled. After 1 h, the solution was dialysed against 0·2 M potassium chloride in the cold room.

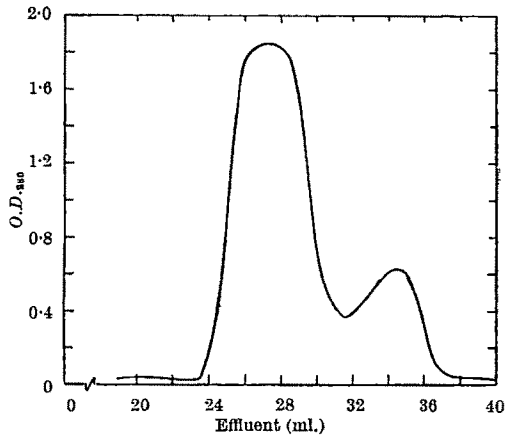


Fig. 1. Gel-filtration of rabbit γ-globulin after mild reduction followed by complete succinylation (see text for details). Column was 'Sephadex *G*-100', 1 × 57 cm column, equilibrated at room temperature with 0·01 M *tris* buffer, *p*H 8·2

Succinylation of this protein solution was carried out by the procedure of Cherry[2]. To the solution, cooled in an ice bath, was added 125 mg of solid succinic anhydride. The stirred solution was maintained at *p*H 8·0 by the careful addition of 5 M potassium hydroxide. The succinic anhydride was completely dissolved after about 40 min, and the mixture was allowed to react at *p*H 8·0 for 1 h thereafter. The solution was then dialysed against 0·01 M *tris* buffer, *p*H 8·2.

Analysis for the completeness of the succinylation of the ε-NH_3^+ groups of lysine was performed by the method of Cherry[2]. This procedure consists of exhaustive dinitrophenylation of the residual ε-NH_3^+ groups of the succinylated protein with dinitrofluorobenzene, hydrolysis in 4 N hydrochloric acid at 105° for 22 h, and determination on a Beckmann model 120 amino-acid analyser of the amount of ε-*N*-(2,4-dinitrophenyl)-lysine present. This compound appears on the short column chromatograms after the arginine peak. The succinylation of the γ-globulin was in this manner shown to be 96–97 per cent complete under the conditions used.

Separation of the succinylated chains was carried out by gel-filtration on a column of 'Sephadex *G*-100' equilibrated with 0·01 M *tris* buffer, *p*H 8·2 at room temperature. A typical elution pattern is shown in Fig. 1. The ratio of areas under the two peaks is 80 ± 2 : 20 ± 2. The heavy chain fraction is completely excluded from the interior of the gel, and the light chain fraction emerges quite close to it. The lower retention of the succinylated chains by the gel as compared with the unmodified chains in 1 M propionic acid may be due to an unfolding of the chains with a consequent enlargement of their molecular domains, or to aggregation. The tendency of the succinylated chains to aggregate is demonstrated by the failure of the chains to separate if gel filtration is carried out at higher salt concentrations; attempts to separate the chains by gel filtration in 0·1 M sodium chloride, 0·1 M *tris* buffer, *p*H 8·0, were not successful.

The two fractions were identified as follows. Each fraction was further purified by a second gel filtration similar to the first and then subjected to amino-acid analysis. The values obtained for each fraction were in satisfactory agreement both with published values[8] and with values obtained from a control which had been withdrawn prior to succinylation and separated into authentic heavy and light chains by gel filtration in 1 M propionic acid[5].

Identical chain separation is achieved if the protein is first succinylated, and then reduced and alkylated, under the same conditions used here. However, the succinylated protein is more susceptible to reduction of its disulphide bonds than is the native molecule. As measured by carboxymethylcysteine content, about nine disulphide bonds are reduced in the former case as compared with 4–5 in the latter[8]. This observation is similar to that of Habeeb[9], who found that succinylation of bovine serum albumin increased the number of disulphide bonds susceptible to mild reduction, presumably as a result of the unfolding of the molecule produced by succinylation.

Succinylation of γ-globulin thus provides a means of obtaining modified heavy and light chains which are readily separable and soluble in a neutral aqueous buffer. Modification is complete and specific for the ε-NH_3^+ groups of lysine[2]. Tryptic digestion of the succinylated chains should produce characteristic fragments terminating in arginyl residues which might prove useful in structural studies of the immunoglobulins.

It has recently been reported by Fuchs and Sela[10] that the reaction of rabbit γ-globulin with *N*-carboxy-D,L-alanine anhydride resulted in polyalanylation of more than 75 per cent of the lysine residues in the molecule. The heavy-chain fraction prepared from polyalanylated γ-globulin was found to be much more soluble at *p*H's near neutrality than an unmodified heavy-chain fraction[10].

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PHYSIOLOGY

Denervation and the Electrogenesis of the Sodium Pump in Frog Skeletal Muscle

THE sodium pump in frog skeletal muscle has been found[1] to be electrogenic in experiments in which sodium-rich muscles were immersed in recovery fluid containing 10 mM potassium and the mean membrane potential measured by means of the microelectrode technique[2] during active sodium excretion. It was found that the measured potential, E_m, was greater than the potassium-equilibrium potential, E_K, by about 11 mV, suggesting that uptake of potassium ions may not be tightly coupled to excretion of sodium ions in a 1 : 1 manner[3], but that potassium ions may be drawn into the muscle fibres under the influence of the potential generated by the sodium pump. The electrogenic nature of the excretion of sodium ions in muscle has also been confirmed in other laboratories[4,5].

Experiments have now been carried out with a preparation consisting of companion sartorii connected to the spinal cord of decapitated frog by way of the sciatic nerves. The sartorii were made sodium-rich[6] by overnight immersion in cold potassium-free Ringer–Conway fluid containing 120 mM sodium. The rest of the preparation was in a cold moist chamber similar to that already described for rat muscle[7]. In this fluid, muscle ionic sodium increased from about 25 to 86 m.equiv./kg while ionic potassium decreased from 88 to 36 m.equiv./kg wet weight of muscle. Muscles were then made to excrete

sodium by re-immersion for 2 h at room temperature in Ringer–Conway fluid containing 104 mM sodium and 10 mM potassium. As already described for rat[7], one muscle of each pair was cut free of the nerve before re-immersion in recovery fluid and the sodium ion excretion and potassium ion uptake by innervated and denervated muscles were compared. The results are shown in Table 1.

Table 1. FINAL CONCENTRATIONS OF SODIUM IONS AND POTASSIUM IONS IN MUSCLES AFTER RECOVERY AND E_m MEASURED 10 MIN AFTER RE-IMMERSION OF SODIUM-RICH MUSCLES AND 5 MIN AFTER DENERVATION

Muscles with nerve			Denervated muscles			
Na^+ (m.equiv./kg)	K^+	E_m mV	Na^+ (m.equiv./kg)	K^+	E_m mV	E_K
33·1±2·9	87·9±2·5	63·6±0·9	45·9±2·3	78·9±2·4	72·0±0·9	59·2±3·2

In another series of experiments the mean membrane potential of innervated sodium-rich sartorii was measured about 10 min after re-immersion in recovery fluid. The muscle was then cut free of the nerve and the potential measurements resumed after an interval of about 5 min. One of the innervated companion muscles was analysed for sodium ions and potassium ions immediately after enrichment with sodium ions, while the other in each case was analysed after the potential measurements described. The mean value of E_K for the set of muscles was then calculated and compared with the measured potentials. From the results (Table 1) it appears that the membrane potential of innervated muscles is much closer to E_K, while sodium ion excretion is stimulated at the same time. The sodium pump clearly becomes more electrogenic following denervation of the muscle. The increase in potential observed on denervation is associated with reduction of sodium ion excretion, which might be explained on the basis of reduced potassium ion uptake or permeability. This view is in keeping with the poor accumulation of potassium ions observed in denervated rat muscles during sodium ion excretion as compared with innervated ones[7]. The lower E_m of innervated muscles might be expected to facilitate excretion of sodium ions by reducing the energy barrier to this process[8]. A similar stimulation of excretion of sodium ions has been observed by Horowicz and Gerber[9] when E_m of muscles was reduced by treatment with azide.

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Transaminase Deficiency in Alcoholics and Cases of Peptic Ulcer

DURING vitamin treatment of ten male and five female alcoholics it was found that, in the majority of cases, internal rate of conversion of vasodilating nicotinic acid to non-vasodilating nicotinamide was much slower than with controls, as manifested by the incidence of flushing with low doses (50–75 mg nicotinic acid). Very similar results were obtained with five non-alcoholic peptic ulcer cases, duration of flushing being of the order of 30 min and extending over face, neck, arms and sometimes as far as the legs.

Six controls who had not suffered any chronic gastric abnormalities or from alcoholism required doses of 150–200 mg of nicotinic acid to produce similar flushing, but it was further noted that, with both experimental and control subjects, ingestion of food a short time after administration of nicotinic acid induced flushing with lower doses than when fasting. For example, a control subject who required 200 mg to produce a marked flush when fasting needed only 150 mg if food was taken 10–15 min after administration.

The findings suggest that the same enzymes are implicated in the metabolism of foodstuffs and nicotinic acid, food taking the priority of use. In view of present theories of enzyme defects in alcoholics[1] and the frequent occurrence of peptic ulcers in alcoholics, a common enzyme defect is possible. Whatever intermediate steps may be involved, transamination is necessary for conversion of nicotinic acid to nicotinamide, and involvement of a transaminase such as pyridoxal-5-phosphate appears probable.

Preliminary findings from an investigation in progress suggest that in many alcoholics and peptic ulcer cases there is a defective utilization of vitamin B_{12}, lack of which can lead to deficiency of transmethylase[2,3], ribonuclease[4], cytochrome-oxidase[5] and various dehydrogenases[6]. Defective transmethylation could result in deficiency of synthesized pyridoxal-5-phosphate and consequently impaired transamination.

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Actinomycin D and the Regulation of Apoferritin Synthesis in Rat Liver

IRON administration increases the net synthesis of apoferritin in rat liver *in vivo*[1]. Correspondingly, liver slices prepared from rats dosed with iron incorporate ^{14}C-leucine into apoferritin *in vitro* more readily than do slices from untreated controls[2]. The mechanism by which the iron acts is unknown, although it has been postulated that intracellular iron in erythroblasts may influence genetic loci[3]. In the following experiments actinomycin *D*, an inhibitor of DNA-dependent RNA synthesis[4], was administered to rats prior to a dose of iron, and liver slices were later tested *in vitro* for incorporation of ^{14}C-leucine into apoferritin. Actinomycin *D* inhibited the stimulatory effect of iron, indicating that derepression of genes and the synthesis of messenger RNA are involved in the regulatory response to the metal.

Male albino rats of the Sherman strain weighing 150–250 g were fasted for 18 h in metabolism cages, lightly anaesthetized with ether, and given 7 mg iron intravenously as iron dextran ('Imferon', Lakeside Lab., Milwaukee). At various intervals thereafter livers were removed from groups of four rats, sliced with a Stadie–Riggs microtome, and 2·0 g of pooled slices incubated in 15·0 ml. medium at 37° C with continuous shaking and gassing with 95 per cent oxygen–5 per cent carbon dioxide. The incubation medium described by Matioli and Eylar[3] was used except that 3·3 μc. of ^{14}C-L-leucine (222 mc./mmole, New England Nuclear Corp., Boston) and 1 μmole carrier L-leucine were added to 15 ml. medium along with 3·0 ml. normal rat serum in place of serum albumin and transferrin. After 3 h of incubation, 1·5 g slices were homogenized in 10 volumes of 0·01 M sodium chloride, the homogenate heated to 72° C for 10 min, and precipitated

proteins removed by centrifugation at 15,000*g* for 20 min. Triplicate aliquots of the supernatant solutions were mixed with 40 μg of carrier crystalline rat liver ferritin and then treated with a rabbit antihorse spleen ferritin antiserum which had been shown to cross-react with crystalline rat liver ferritin[5]. (In later experiments crystalline horse spleen ferritin was used as carrier and found to yield results comparable with the rat liver ferritin.) The immune precipitates were centrifuged after 18 h, washed three times with 10 ml. 0·9 per cent saline, dissolved in 0·5 ml. of 0·25 N sodium hydroxide at 100° C, and aliquots taken for estimation of protein[5] and of radioactivity in Bray's solution[6] in a liquid scintillation spectrometer. Fig. 1 illustrates representative results from two of four experiments. Increased incorporation of ^{14}C-leucine *in vitro* was evident 2 h after iron administration, with a maximal effect, almost ten-fold that of the control value, at approximately 16 h.

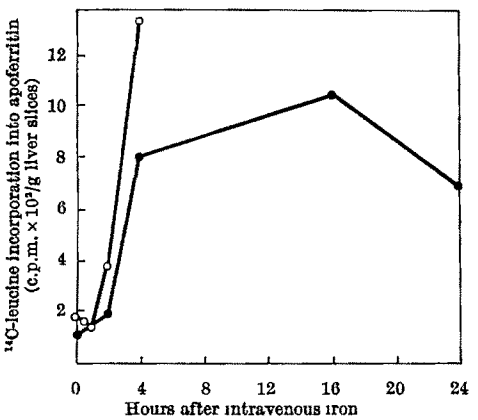


Fig. 1. Incorporation of ^{14}C-leucine into apoferritin by rat liver slices *in vitro* at various times after intravenous iron dextran. Incorporation of ^{14}C-leucine is expressed per gram liver slices. Two experiments are shown, and four rats (weighing 200–300 g) were tested at each time interval

The effect of actinomycin *D* on the iron-stimulated incorporation of ^{14}C-leucine into apoferritin and on ^{14}C-uridine incorporation into ribonucleic acid was then studied. The following three groups of four rats were tested in each experiment: untreated, treated with iron dextran as here, or given 1–4 μg/g body-weight of actinomycin *D* intraperitoneally or intravenously 30 min before iron dextran. 4 h after iron the livers were removed and pooled slices tested with ^{14}C-leucine as already outlined and, separately, under identical conditions except that uridine-2-^{14}C (25 mc./mmole, New England Nuclear Corp.) replaced radioactive leucine. Slices incubated with ^{14}C uridine were later homogenized in ten volumes of isotonic saline, and the method of Schneider[7] used to precipitate, wash, and extract nucleic acids with hot trichloroacetic acid. The extracts were washed three times with equal volumes of ether to remove trichloroacetic acid (which quenches in the liquid scintillation solution used) and aliquots taken for estimation of ribose[7] and of radioactivity in Bray's solution. Table 1 summarizes the results of four experiments. In each instance actinomycin *D* clearly inhibited incorporation of uridine into RNA and of leucine into apoferritin, and the effects were comparable quantitatively. Thus the greatest inhibition of incorporation into apoferritin, 91·2 per cent in experiment 4, corresponded to the greatest decrease in incorporation into RNA, 80·3 per cent.

The results indicate that DNA-dependent synthesis of messenger RNA is a necessary step in the events which are initiated by iron and lead to increased synthesis of apoferritin. The initial site of action of the iron remains unknown, although one possibility is that it directly affects a regulatory gene[8]. Further analysis of the control of apoferritin synthesis *in vitro* may clarify the nature of the genetic regulation, a type of control which appears to underly the action of certain hormones and other substances[9].

Table 1. EFFECT OF ACTINOMYCIN *D* ON IRON-STIMULATED INCORPORATION OF ^{14}C-LEUCINE INTO APOFERRITIN AND ON ^{14}C-URIDINE INCORPORATION INTO RNA BY RAT LIVER SLICES *in vitro*

	Exp. group	Incorporation ^{14}C-leucine into apoferritin (c.p.m. × 10^3 per g liver slices)		Incorporation ^{14}C-uridine into RNA (c.p.m. × 10^3 per μmole ribose)	
(1)	No iron	2·32		2·60	
	Iron	8·62		3·40	
	Actinomycin + iron*	3·93		1·44	
	Percentage inhibition		54·4		57·7
(2)	No iron	2·18		6·52	
	Iron	15·92		5·48	
	Actinomycin + iron*	7·41		1·71	
	Percentage inhibition		53·4		68 7
(3)	No iron	1·28		4·79	
	Iron	4·88		5·69	
	Actinomycin + iron	2·74		2·52	
	Percentage inhibition		43·8		55·7
(4)	No iron	0·73		6·79	
	Iron	5·06		5·06	
	Actinomycin + iron	0·44		0 99	
	Percentage inhibition		91·2		80·3

* Doses (μg/g body-weight) of actinomycin *D* were 1 μg (intraperitoneal) in experiment 1, 2 μg (intraperitoneal) in experiment 2, and 4 μg (intravenous) in experiments 3 and 4.

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Demonstration of Endogenous Pyrogen in Afebrile Rabbits

A VARIETY of environmental factors, including position, are known to influence temperature regulation in experimental animals. Rectal temperature will fall 1°–2° C over a 2-h period in rabbits fastened to a board[1]. In addition, securely restrained rabbits in a normal, standing position will develop hypothermia of as much as 2·7° C during a 30–90 min period and exhibit reduced febrile responses to pyrogens when compared to 'free' animals[2]. Fekety recently reported a fall in baseline rectal temperatures when rabbits were shorn, restrained supine, or chilled; furthermore, large doses of endotoxin, adrenaline, or endogenous pyrogen failed to elicit fever[3]. It was postulated that the hypothermia resulted from increased heat loss, since covering the shorn and supine rabbits with blankets restored normal pyrogenic responsiveness[3]. As some of these differences in febrile reactivity might be due to alterations in the production of endogenous pyrogen, this possibility was investigated.

New Zealand albino rabbits of either sex weighing approximately 2 kg from the same colony (National Institutes of Health) were used. Animals were housed in air-conditioned rooms and the experiments were performed in the same environment. Upright animals were restrained with loosely fitting collars in wooden stocks which permitted freedom of movement of their torsos; prone animals were securely restrained with their legs

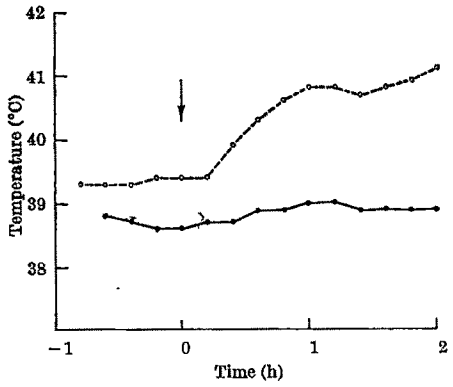


Fig. 1. Mean temperature responses of 6 upright (○ - - - ○) and 5 prone (●——●) rabbits to 5 μg of endotoxin given at zero hour (arrow). Animals were exsanguinated at 2 h

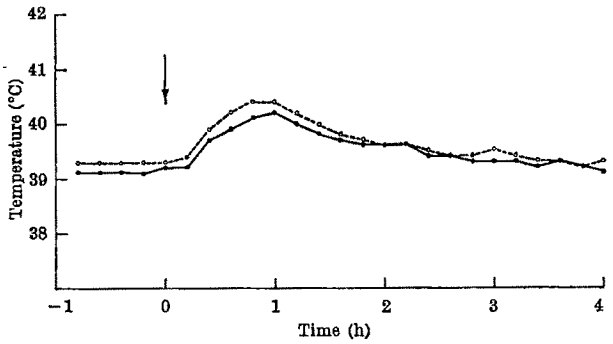


Fig. 2. Mean temperature responses of normal rabbits given sera obtained at 2 h from animals in Fig. 1; 10 c.c. of pooled sera from upright (○ - - - ○) and prone (●——●) rabbits administered at zero hour (arrow). Curves represent mean responses of 8 animals (upright donors) and 5 animals (prone donors)

extended. Both groups were trained in their respective positions for 3–5 h on the day before the experiments. Temperatures (°C) were monitored with thermistors connected to a recording telethermometer and inserted 4–6 in. into the rectum. *E. coli* endotoxin (Difco) was dissolved in sterile, pyrogen-free saline at a concentration of 5 μg/ml. prior to intravenous injection into a marginal ear vein. All equipment was sterile and pyrogen-free. Endogenous pyrogen was prepared as described by Atkins and Wood[4]: animals were injected with 5 μg of endotoxin and bled by cardiac puncture 2 h later. After clotting, serum was separated by centrifugation, pooled (2 animals per pool), stored at 4° C and used within 3 days. Following incubation for 1 h (37° C), 10 c.c. portions were injected into a marginal ear vein of normal recipient animals. Fever index for each animal was determined by plotting temperature versus time on 1 × 1 in. standard graph paper (1 h and 1 deg. each equal 1 in.), and the area in cm^2 under the 5-h fever curve was measured with a compensating polar planimeter.

Following the administration of 5 μg of endotoxin, upright rabbits developed fever, in contrast to prone rabbits which remained essentially afebrile (Fig. 1). Despite these differences, sera obtained 2 h after endotoxin injection from these two groups contained similar amounts of endogenous pyrogen (Fig. 2). Mean fever indices of the animals that received the endogenous pyrogen were not different: mean fever index ± standard error of 8 normal rabbits receiving sera from the upright (febrile) animals was 10·1 ± 0·82 cm^2, and that of 5 rabbits receiving sera from the prone (afebrile) rabbits was 11·5 ± 0·79 cm^2.

In addition, 2 prone rabbits bled through femoral arterial catheters experienced a fall in white blood cell count to 25 per cent of control values within 10 min after endotoxin injection, which persisted throughout the 2-h period. These changes in leucocytes are similar to those noted in upright animals following challenge with endotoxin. These findings would support the concept that endogenous pyrogen is derived from leucocytes during the leucopenic response to endotoxin and that this mechanism is unaffected by position or lack of febrile response in restrained rabbits.

These data demonstrate the production of endogenous pyrogen in the absence of fever and suggest that the lack of fever is due to either a decreased responsiveness of the thermoregulatory centres or an inability to conserve heat normally. Increased heat dissipation could be due solely to positional factors through the exposure of relatively hairless areas, as suggested by others[3], or to the increase in body surface area added by stretching the animals out; these explanations are supported by the fact that merely covering the animals will restore pyrogenic responsiveness[3]. It is noteworthy, however, that since a wide variety of positions will affect temperature, a more complex mechanism than simply heat loss may be responsible for the observed changes. All the experiments described, including our own, which demonstrated altered febrile reactivity, used animals which were securely restrained; thus, immobilization, regardless of position, may have a profound influence. Since the administration of cortisone will block the febrile response to endotoxin in normal animals[5], it is possible that the magnitude of the endogenous adrenocortical response in restrained animals is sufficient to suppress fever.

We thank Mr. J. Edelin for expert assistance.

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Growth Hormone Secretion in Response to Stress in Man

A WIDE variety of emotional and physical stimuli result in increased adrenocortical activity. Thus plasma cortisol-levels rise in response to trauma, surgery, fever and hypoglycaemia[1–3]. This has been attributed to a stress control mechanism which depends on the integrity of a cerebro-hypothalamo-pituitary-adrenal pathway and overrides the normal 'negative-feedback' control mechanism[4]. An increased secretion of growth hormone has been shown during insulin and tolbutamide induced hypoglycaemia, during exercise and during acute or chronic starvation[5–8]. This has been attributed to an increased demand for energy substrates and to be mediated via the hypothalamus. The possible role of stress in relation to growth hormone release does not appear to have been considered.

Emotional stress. Our interest in this possibility arose from the findings in a medical student, who was told that he had received a large dose of insulin and would experience severe hypoglycaemic symptoms. In fact, saline and not insulin had been injected. His plasma sugar remained unaltered, but there was a demonstrable increase in his plasma cortisol measured fluorimetrically[9] and growth hormone determined by radioimmunoassay[10] (Table 1*A*). Saline injection in four other control subjects, without prior suggestion, produced no changes in plasma sugar, cortisol or growth hormone.

Pyrogen. In two male and two female subjects we have shown an increase in growth hormone secretion to pyrogen

Table 1. RESPONSE OF PLASMA GROWTH HORMONE TO STRESS

	Control period	Min after injection 30	60	90	120
A Emotional stress; male subject*	1·1	5·2	10·5	—	8·7
B Pyrogen; 2 males and 2 females	3·0 ±2·8†	3·1 ±2·8	3·1 ±2·8	18·9 ±4·5	18·4 ±11·9
C Pyrogen + glucose; female subject	<1·0	<1·0	3·7	10·7	7·0
D Lysine 8-vasopressin; 3 male subjects	<1·0†	<1·0	<1·0	1·8	2·0
E Lysine 8-vasopressin; 2 female subjects	2·0 1·1	6·3 11·7	32·0 21·4	21·7 21·8	6·6 25·2

Results for plasma growth hormone expressed as μmg/ml.
* Injected with saline.
† Mean level and where possible the standard deviation.

(0·005 μg/kg body-weight, Organon) as evidenced by elevated circulatory growth hormone levels 90 min after injection (Table 1*B*). The plasma cortisol increased at the same time, but the plasma sugar did not change significantly. The responses in the male and female subjects were not different. The results suggest that a pyrogen-induced stress stimulates both pituitary growth hormone and ACTH secretion which is not due to a measurable hypoglycaemia. Similar rises in circulating growth hormone with the maintenance of normal blood sugar levels have been found after exercise in the fasting state. This has been attributed to the increased metabolic demands for lipid substrates stimulating secretion since the elevated plasma growth hormone levels are suppressed by glucose[7,11].

Pyrogen and glucose. The growth hormone response to pyrogen would appear to involve a different mechanism not previously demonstrated since it was not suppressed by a continuous intravenous infusion of glucose (Table 1*C*). Thus a rise in plasma sugar levels from 90 to 135 mg/100 ml. was observed during the 60–120 min period after the injection of pyrogen when growth hormone secretion was stimulated.

Lysine 8-vasopressin (LVP). The adrenocortical response to stress requires the secretion of corticotrophin-releasing factor (CRF) from the hypothalamus and thence pituitary ACTH release and adrenal cortisol secretion. Studies were carried out using lysine 8-vasopressin, a compound having corticotrophin-releasing activity in man[12], to determine whether it was possible in normal subjects to stimulate cortisol secretion without a concomitant stimulation of growth hormone secretion. In five normal subjects, infusion of synthetic LVP (Sandoz) at the rate of 5 P.U./h for 2 h produced the expected elevation in plasma cortisol-levels, the individual maximum cortisol increments ranging from 8·9 to 25·9 μg/100 ml. The infusion had no significant effect on growth hormone levels in the three male subjects (Table 1*D*), but a marked increase was observed in the two female subjects studied (Table 1*E*). There was no association between the presence or absence of a response in growth hormone to LVP and the rises in plasma cortisol or the levels of plasma sugar which were essentially unchanged throughout the test. Further work is required to confirm and elucidate the apparent sex difference in growth hormone response to LVP. It may be noted, however, that oxytocin and lysine vasopressin increase plasma free fatty acids in puerperal women and female dogs but cause decreases in male dogs[13,04].

Summarizing, stimulation of growth hormone secretion associated with adrenocortical activity occurred in response to emotional stress and to the injection of pyrogen. This was not associated with hypoglycaemia. The plasma growth hormone response to pyrogen secretion was not suppressed by an induced hyperglycaemia. Stimulation of cortisol secretion by lysine 8-vasopressin was accompanied by a rise in plasma growth hormone levels in women but not in men. Plasma sugar-levels were unchanged in both men and women.

We thank Dr. G. F. Marrian and Dr. V. Wynn for their support of this work, and Mrs. J. Gray and Miss J. Riccardo for skilled technical assistance. We thank Dr. V. H. T. James for the analyses of plasma cortisol used in this investigation.

Note added in proof. Since this letter was submitted, Glick, Roth, Yalow and Berson (*Rec. Prog. Horm. Res.*, **21**, 241; 1965) have reported an elevation of plasma growth hormone after major surgery, not suppressed by glucose administration

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PHARMACOLOGY

Inhibition of Ribonucleic Acid Synthesis in Mouse Skin by Actinomycin *D* and 7,12-Dimethylbenz[*a*]anthracene

IT has recently been demonstrated that the initiation of skin tumorigenesis by 7,12-dimethylbenz[α]anthracene (DMBA) is dramatically inhibited by topical applications of actinomycin *D*[1,2]. In view of the well-known inhibitory action of actinomycin *D* on DNA-dependent RNA synthesis[3], it was proposed that this observation helps support the hypothesis that the early events of chemical tumorigenesis involve alterations in the expression of specific genetic information. In the present study we describe the effect of topical applications of actinomycin *D*, of DMBA, and of the two combined on the synthesis of RNA in mouse skin. The experimental conditions employed exactly simulate those used to inhibit tumour formation[1].

As pointed out by Sinclair and McCarter[4], it is not possible to incorporate sufficient radioactivity into the RNA of skin by administering radioactive ribonucleosides intravenously, intraperitoneally, or subcutaneously to constitute a reliable assay of RNA synthesis. We were able, however, to obtain a highly reproducible measurement of RNA synthesis using a slight modification of the method described by Sinclair and McCarter[4]. This involved incubating narrow strips of full-thickness skin (5 mm × 3 cm), excised from treated areas of the back of the mouse, in 5 ml. of Eagle's medium containing 50 μc. of ^{3}H-uridine (7·1 c./mM). The incubation was carried out at 37° C in a shaking water bath for varying lengths of time. At the end of such time, the reactions were terminated by removing the excised pieces of skin from the incubation medium and either freezing at −20° C for biochemical analysis or fixing firmly mounted pieces in alcohol–acetic acid (3 : 1) for eventual sectioning and autoradiography.

The incorporation of ^{3}H-uridine into skin RNA was determined both by autoradiography of 3-μ-thick sections of skin and by a previously described biochemical procedure[5]. That incorporation of ^{3}H-uridine occurs exclusively into RNA is indicated by the data in Fig. 1, in which it is shown that ribonuclease action or treatment with

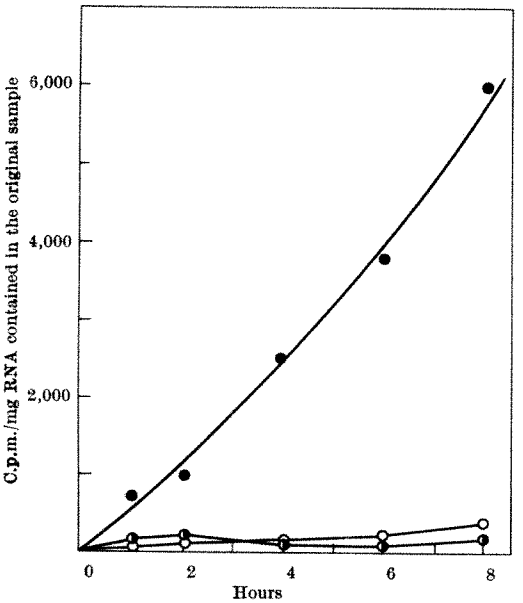


Fig. 1. Kinetics of the incorporation of ^{3}H-uridine into RNA of mouse skin. Narrow strips of full-thickness skin were removed from the backs of previously shaved, random bred Swiss mice and incubated as described in the text. After the appropriate incubation time, the strips were removed, frozen, and stored for two to three days, then thawed and freed of the panniculus carnosus and adipose tissue. The resulting skin was minced with scissors and exhaustively ground in 5 ml. of 0·01 M phosphate buffer, *p*H 7·0, containing 0·14 M sodium chloride using a power-driven glass-teflon tissue grinder. An equal volume of 20 per cent trichloroacetic acid was added and each sample washed as described previously (ref. 5). Closed circles represent the acid precipitable radioactivity of washed samples following an incubation at 37° C in 2 ml. of phosphate-saline, *p*H 7·0, for 20 min. Open circles represent acid-precipitable radioactivity in the same samples incubated as above except that 200 μg of ribonuclease was added at the start of each incubation. Half closed circles represent acid-precipitable radioactivity from those samples which were suspended in 0·3 M potassium hydroxide and incubated at 37° C for 18 h. Radioactivity and RNA determinations were conducted as previously described (ref. 5). Each point represents the average value obtained from four independent determinations

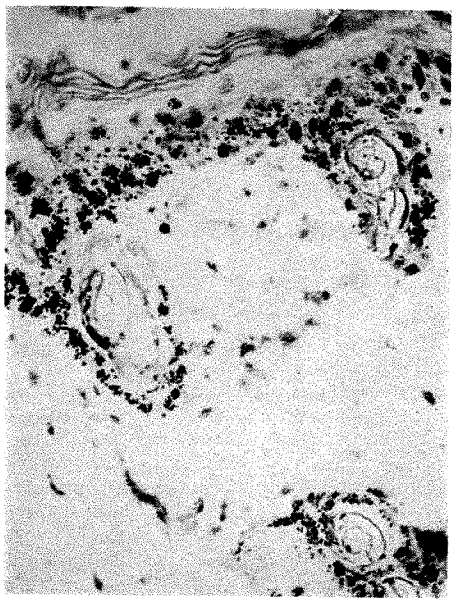


Fig. 2. Autoradiograph of a section of mouse skin. Narrow strips of skin were incubated for 4 h *in vitro* with Eagle's medium containing ^{3}H-uridine. Deparaffinized sections (3μ) of skin, washed in 5 per cent trichloroacetic acid at 0° C for 5 min, were covered with Kodak 'AR10' stripping film. After 3 weeks of exposure, slides were processed and stained through the emulsion with toluidine blue. Note that the heavy labelling is restricted to the cells of epidermis and hair follicles

0·3 M potassium hydroxide under conditions known to hydrolyse RNA preferentially[6] releases essentially all the radioactivity into an acid soluble form.

The autoradiograph shown in Fig. 2 clearly illustrates that only the interfollicular and follicular epidermal cells are significantly labelled. The cells of the dermis are virtually unlabelled and consequently do not contribute appreciably to the radioactivity found in the RNA fractions of skin such as those shown in Fig. 1 and Table 1. RNA synthesis, therefore, is principally associated with those cells known to constitute the actual tumour or papilloma rather than with dermal cells which are not an integral part of the tumour at early stages of development[7].

As shown by the data in Table 1, a single small dose of DMBA, equivalent to that used to initiate the tumorigenic process[1], partially inhibits the synthesis of RNA at early time. By the third day following the application of DMBA, however, full recovery of RNA synthesis has occurred. In direct contrast to the relatively rapid onset and short duration of the inhibitory activity of DMBA is the observation that the maximal inhibitory effect of actinomycin *D* on the synthesis of RNA is not realized until the third day and that recovery of RNA synthesis does not begin until 11 days after its initial application. Furthermore, the data in Table 1 indicate that, at the dosage used, DMBA does not significantly influence the duration or extent of inhibition of RNA synthesis as produced by six topical applications of actinomycin *D*.

Table 1. INHIBITION OF THE INCORPORATION OF ^{3}H-URIDINE INTO THE RNA OF MOUSE SKIN

Time after first application	Percentage inhibition DMBA	Act *D*	DMBA + Act *D*
8 h*	—	0 ± 6	—
16 h	18 ± 5	20 ± 5	15 ± 5
24 h	22 ± 5	30 ± 2	25 ± 7
2 days	0 ± 5	—	—
3 days	6 ± 5	84 ± 5	72 ± 5
4 days	0 ± 5	81 ± 4	78 ± 3
5 days	—	90 ± 3	—
8 days	0 ± 6	86 ± 7	86 ± 2
11 days	0 ± 5	81 ± 5	79 ± 2
14 days	0 ± 5	0 ± 3	15 ± 3
21 days	0 ± 5	—	5 ± 5

* In this experiment only the first three actinomycin *D* treatments were given.

To the shaved back of general-purpose Swiss mice, either a single dose of 15 μg of DMBA in 0·2 ml. of acetone was applied to the entire back, or 15 μg of actinomycin *D* in 0·2 ml. of acetone was applied 2, 5, and 8 h before and 2, 5, and 8 h after the application of DMBA. When actinomycin *D* alone was applied, the same dose and time sequence was used except that acetone was substituted for DMBA. The controls, on which the percentages shown above are based, were treated similarly with acetone. Before excising a narrow strip of skin for the incubation, the backs were swabbed with 95 per cent ethanol to remove any excess actinomycin *D* or DMBA. Biochemical analysis of the incorporation of ^{3}H-uridine into skin RNA was carried out as described in Fig. 1. Each value listed above represents the average inhibition found in from 3 to 8 determinations.

It is clear from these observations that the synthesis of RNA has not been inhibited by actinomycin *D* at the time of DMBA application. In fact, significant inhibition by actinomycin *D* is delayed 24 h, and then the degree of inhibition is equivalent only to that produced by DMBA alone. This suggests that 'initiation', a term which designates the early irreversible events of skin tumorigenesis, is not complete until at least 24 h following the application of the hydrocarbon, since actinomycin *D* treatment at that time successfully blocked tumour formation[1]. Furthermore, it has been shown by Bates *et al.*[2] that actinomycin *D* application 1 day after DMBA treatment is at least as effective in inhibiting tumour formation as the simultaneous application of both agents. It therefore seems probable that the initiation process is not complete until at least 48 h after DMBA application. In contrast to the apparent delay in the complete initiation of tumorigenesis, DMBA functions rapidly to inhibit the synthesis of RNA in mouse skin. The inhibitory action is transitory, however, and the completion of the initiation process appears to occur after the diminution of this effect. Therefore, it seems highly probable that, although DMBA inhibits RNA synthesis, as was recently shown by Sinclair and McCarter[3], this action by itself does not constitute the irreversible event of chemical tumorigenesis.

Brookes and Lawley have observed that the binding of DMBA to DNA is more persistent than is its binding to protein which is maximal during the first 48 h (ref. 8). Since our findings suggest that the first irreversible event of tumorigenesis occurs 48 h after the application of DMBA, the binding of DMBA to DNA sites may be related

to this event. Their observation that a good correlation exists between the carcinogenic potency of various hydrocarbons and the extent to which these hydrocarbons bind DNA is consistent with the above suggestion.

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IMMUNOLOGY

Appearance of Natural Antibodies in Young Rabbits

NATURALLY occurring antibodies to antigens of erythrocytes, tissues such as liver, and microbes are invariably detectable in the serum of fully grown animals but are not found in the serum of the new-born. Whether these antibodies arise as a result of normal physiological maturation or as a result of inapparent immunization has puzzled immunologists for many years. There is evidence that the blood group and microbial antibodies may be formed as a response to ingested antigens, since their production has been prevented in some cases by rearing the animals in a germ-free environment. Germ-free chicks, for example, promptly developed antibodies to blood group B antigen after the oral administration of *Escherichia coli* 086, an organism with blood group B antigenic determinants[1]. The phenomenon of naturally occurring complement-fixing serum substances reactive with extracts of mammalian tissue, including an animal's own tissue, is not readily explained[2,3]. It is possible that these substances, which are serum globulins, are not antibodies formed in response to antigenic stimulation. When combined with tissue for which they have an affinity, they may conceivably be aggregated and thereby fix complement[4].

There are certain difficulties in accepting the thesis that all naturally occurring antibodies arise as a result of inapparent immunization. Obviously, immunization may account for some, but not all, of these antibodies. The production of antibody to blood group B substance in germ-free animals by immunization with *E. coli* 086 does not exclude the possibility that antibodies may arise by other mechanisms in addition to immunization. The low levels or absence of bactericidal antibodies in the germ-free animal may be explained also as a result of physiological abnormalities[5] related to their development in an unnatural environment. In addition, germ-free animals with minimal levels of gamma-globulin do not always have lower levels of bactericidal or phage-neutralizing antibodies than their litter-mates reared in a conventional environment[6,7]. These observations are not conclusive in that germ-free animals are subjected to a variety of antigenic substances in sterile foods and viruses. Nonetheless, pelagic whales, the bacterial flora of which lack intestinal coliform organisms, possess bactericidal antibodies against *E. coli* in titres equal to those of human subjects[8]. Finally, it has been pointed out that the naturally occurring blood group antibodies in man such as anti-P_1 and anti-Le^a react more strongly at lower temperatures, whereas antibodies arising after an identifiable stimulus such as anti-Rh react more strongly at 37° C (ref. 9) and normal rabbit bactericidal antibodies may be heat labile (56° C for 30 min) in contrast to the heat stability of immune bactericidal antibody[10].

The present communication attempts to investigate this problem further by determining the time of appearance of four antibodies directed against three markedly different antigens. Naturally occurring bactericidal, bacteriophage neutralizing and complement-fixing antibodies against normal rabbit and rat tissue were titrated in the serum of healthy, non-immunized rabbits from a few weeks after birth until the rabbits had reached maturity. The methods used have previously been described[3,11,12].

Results of representative titrations of serum collected at approximately 10-day intervals over a 217-day period from an individual rabbit are given in Table 1. In no case was antibody detectable before rabbits were 28 days of age. Bactericidal and phage neutralizing titres steadily increased as the animal aged. Complement-fixation titres became detectable at ≤9 days of age and reached what appeared to be a maximum at 59 days of age. Bleedings obtained beyond 217 days, up to 270 days, revealed no further increase in titre and these results were not included in the table. Several other rabbits the sera of which were similarly tested gave essentially the same results.

Table 1. APPEARANCE OF NATURAL ANTIBODIES IN RABBIT

Age of rabbit (days)	Bactericidal titre* against *Shigella dysenteriae*	Neutralization titre† against coliphage *T2*	CF antibody titre‡ against: Rabbit liver	CF antibody titre‡ against: Rat liver
18	<5	<5	<3	<3
28	<5	<5	<3	<3
38	<5	17	<3	<3
49	5	27	3	3
59	69	149	9	9
69	40	61	3	3
80	28	38	9	9
91	23	70	3	3
111	52	54	3	9
122	151	394	9	9
132	211	510	9	9
154	289	510	9	9
164	222	294	9	9
175	211	345	9	9
217	250	222	9	9

* Reciprocal of serum amount required to kill 50 per cent of an inoculum of about 2×10^7 cells of the organism (ref. 11).
† Reciprocal of serum amount required to neutralize 50 per cent of 10^3 *T2* coliphage particles (ref. 12).
‡ Antigens were saline extracts of the tissue in a complement-fixation procedure previously described (ref. 3).

Many mammals synthesize relatively little gamma globulin at birth and the ability to produce these globulins develops early in post-natal life[13,14]. From the data of Table 1, it can be seen that antibodies to the various antigens all appeared at about the same time. The fact that antibody was first detected by phage neutralization is probably due to the greater sensitivity of this system. It does not seem likely that the growing rabbit would simultaneously encounter antigens that induce antibody formation toward *Shigella*, *T2* coliphage, rabbit or rat liver. If natural antibodies are merely globulin molecules with affinities toward any antigen to which the animal can respond[15], the production of natural antibodies might reasonably be expected to parallel the appearance of immunoglobulins in the serum. It is possible that rabbits aged 40 days encounter a wide spectrum of antigenic determinants found in bacteriophage protein, cell walls of *Shigella*, and the cytoplasmic constituents of mammalian tissue. More likely, however, is the postulate that certain globulins arise at this time as a result of normal physiological maturation and that globulins react with certain antigens because of chance chemical correspondence with certain specific combining groups. The appearance of tissue complement-fixing 'auto-antibodies' at the same time as the bacterial and viral antibodies also suggests that these substances may be formed by related mechanisms.

The results of this investigation and other considerations indicate the possibility that natural occurring antibodies may not always arise as a result of immuniza-

tion. It may not be possible to obtain an experimental answer to the question of the origin of the naturally occurring antibodies due to the fact that animals live in a microbial world with highly antigenic substances that are necessary for their development.

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Antibody Production by Single Spleen Cells: a Comparative Study of the Cluster and Agar-plaque Formation

DURING the past few years, two new methods have been described for use in investigating *in vitro* production of antibodies against sheep erythrocytes by isolated cells. In the first method[1,2] a suspension of spleen cells is mixed with sheep erythrocytes, embedded in agar or carboxymethylcellulose and incubated with guinea-pig complement. Under these conditions, plaque formation occurs as a consequence of the production of haemolysins by certain spleen cells. With the second method[3,4] a suspension of spleen cells is incubated with sheep red blood cells, but the cells are not embedded in a semi-solid medium. In this environment clusters of agglutinated sheep erythrocytes are formed around a number of spleen cells. In the present investigation the results obtained by these two methods are compared and the observed differences are discussed.

The agar-plaque technique was performed as described by Jerne[5]. For the investigation of the cluster formation our previously described technique[3] was slightly modified: the L_1 medium was replaced by Eagle's medium with 5 per cent calf serum (absorbed with mouse erythrocytes) and during incubation the tubes were not agitated but the cells were allowed to settle on the bottom of the slanting culture tube. These modifications resulted in a significant increase in the number of clusters formed.

First the possibility was investigated that cluster formation in this system was due to the absorption of cytophylic antibodies[6] on certain spleen cells. A pooled suspension of spleen cells from non-immunized mice was divided in four aliquots containing nine million cells each. Two samples suspended in 1 ml. medium containing 0·3 ml. normal mouse serum were kept in melting ice for 1 h[6]; the other two samples were kept in the same way in medium containing mouse-anti-sheep serum. The haemagglutinating titre of the antiserum was in two successive experiments 1 : 3,200 and 1 : 25,600 respectively. After incubation the cells were washed in the cold until no or only slight agglutinating activity was found in the washing fluid. The washed suspensions were incubated in the usual way with sheep erythrocytes. In both experiments, between 0·04 and 0·3 per cent of the spleen cells kept in control serum and between 0·1 and 0·3 per cent of the cells kept in antiserum formed clusters. An identical treatment of spleen cells from immunized mice neither increased nor decreased the number of cluster-forming cells. Injection of non-immunized mice with strong haemagglutinating antisera, causing a titre in the blood of these animals of 1 : 640, resulted in no increase of the number of cluster-forming cells in the spleen, when these animals were killed 24 h after the last injection. All these figures were very close to the values found in non-immunized control mice ($0{\cdot}15 \pm 0{\cdot}07$ per cent). More evidence against the assumption that cluster formation is only due to antibodies adhering to the surface of some spleen cells was provided by the observation that the cluster of sheep erythrocytes around the spleen cells often consisted of several cell layers. It seems reasonable to assume that antibodies absorbed on the cellular surface could cause only the agglutination of one layer of erythrocytes. The number of layers could be determined by adding a drop of acetic acid to the suspension in the haemocytometer during microscopical observation. The gradual lysis of the erythrocytes revealed that the number of erythrocyte layers surrounding the nucleated cell sometimes amounted to four. Moreover, the nucleated cell could sometimes be loosened from the clustered erythrocytes by mild shaking, while the cluster of erythrocytes remained intact.

The question arose whether plaque and cluster formation are two manifestations of the same phenomenon or belong to different processes in antibody formation. If the first supposition holds true it should be possible to convert a cluster into a plaque by the addition of complement. This possibility was investigated by two different methods:

(*a*) Guinea-pig complement was added to a cluster containing suspension of spleen cells and incubated at 37° C. During microscopical observation of at least 2 h no perceptible lysis of clustered sheep erythrocytes was observed nor was a significant decrease in the number of clusters found. When, however, a small amount of antiserum was added to the suspension, both free and clustered sheep erythrocytes were lysed.

(*b*) Spleen cells from immunized mice were first incubated for 30 min in a suspension with sheep erythrocytes and thereafter this cluster containing suspension was mixed with agar and poured on a Petri dish. With a suitable magnification the already formed clusters embedded in the agar were clearly visible. In a certain area of the plate all clusters were marked by a small circle, scratched in the bottom of the plastic Petri dish. A second incubation and the addition of complement resulted in a clear-cut plaque formation on the agar plate (Fig. 1). However, of a total number of 913 clusters so marked, none was found to form a plaque after incubation with complement. From this observation it can be concluded that the antibody producing cells produce either a plaque or a cluster. Production of lysis and agglutination by the same cell, if it occurs, must be a very rare phenomenon.

In order to investigate the relation between cluster and plaque formation, the haemolysin and haemagglutinin titres and the numbers of cluster- and plaque-forming cells in the spleen of $(DBA/2 \times C57BL)F_1$ and $ND2$ mice were determined at different time intervals following a single intravenous or intraperitoneal injection of sheep erythrocytes. (The $ND2$ mice were obtained from the Radiobiological Institute, Organization for Health Research TNO, Rijswijk (Z.H.).) So far as plaque formation is concerned, the data are in reasonable agreement with the results of Jerne[5] and Friedman[7]. The cluster formation and the titre of the haemagglutinating antibodies follow a somewhat different course (Table 1). The numbers of plaque-forming cells and the haemolysin

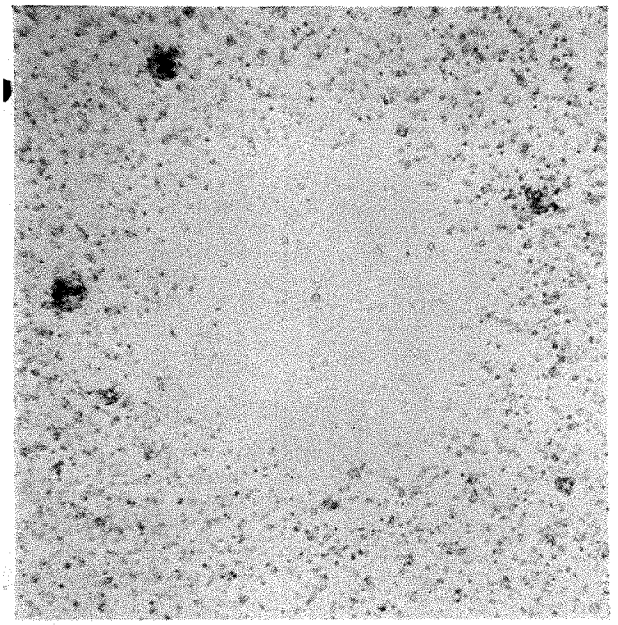


Fig. 1. Microphotograph of an agar plate containing both clusters and plaques formed by spleen cells from mice immunized with sheep red blood cells

titres show a clear-cut tendency to decrease after a sharp rise at about the fourth day, whereas the numbers of cluster-forming cells and the haemagglutination titres level off at about the sixth day. The *ND2* mice which received an intraperitoneal injection of sheep cells show this difference even more pronounced than the $(DBA/2 \times C57BL)F_1$ mice which received an intravenous injection (Table 2).

In smears made of cluster containing spleen cell suspensions and stained with either May–Grünwald and Giemsa or with methyl green–pyronin, cluster-forming cells could be identified by a rim of adherent erythrocytes around the cell. It was found that nearly all observed cluster-forming cells had the morphology of lymphoid cells with a preference for the younger forms. All these cells showed a positive pyroninophilic cytoplasm. Cells which had ingested carbon particles after intraperitoneal injection of indian ink were in a few instances found to form small clusters. Polymorphs, on the other hand, were never surrounded by agglutinated erythrocytes.

Table 1. COURSE OF THE CLUSTER AND PLAQUE FORMATION BY SINGLE SPLEEN CELLS AND OF THE ANTIBODY TITRE IN $(DBA/2 \times C57BL)F_1$ MICE IMMUNIZED WITH SHEEP RED BLOOD CELLS

Days after antigen injection*	Number of plaques per 10^6 spleen cells	Number of clusters per 10^6 spleen cells	Antibody titres Haemolysins†	Haemagglutinins
3	159	2,600	1 : 45	1 : 32
4	782	11,000	1 : 324	1 : 128
5	613	24,000	1 : 912	1 : 256
6	330	26,000	1 : 630	1 : 256
7	166	9,800	1 : 575	1 : 128
8	81	11,300	1 : 426	1 : 256
11	44	13,100	1 : 316	1 : 512
12	26	11,400	1 : 380	1 : 256
13	22	14,000	1 : 426	1 : 256

* The mice were intravenously injected with 0·5 ml. of a 10 per cent suspension.
† Titre of 50 per cent haemolysis.

Table 2. COURSE OF THE CLUSTER AND PLAQUE FORMATION BY SINGLE SPLEEN CELLS AND OF THE ANTIBODY TITRE IN *ND2* MICE IMMUNIZED WITH SHEEP RED BLOOD CELLS

Days after antigen injection*	Number of plaques per 10^6 spleen cells	Number of clusters per 10^6 spleen cells	Antibody titres Haemolysins†	Haemagglutinins
3	107	1,000	1 : 14	1 : 4
4	938	9,000	1 : 602	1 : 28
5	552	15,000	1 : 398	1 : 256
6	661	33,000	1 : 346	1 : 256
7	155	19,000	1 : 223	1 : 512
10	97	24,000	1 : 186	1 : 1,024
11	72	23,000	1 : 141	1 : 1,024

* The mice were intraperitoneally injected with 0·5 ml. of a 20 per cent suspension.
† Titre of 50 per cent haemolysis.

The results indicate that cluster formation is caused by an antibody producing cell. Since plaque formation also is caused by antibody production the results suggest that two different classes of cells are present, one class producing haemagglutinating, the other haemolysing, antibodies. These findings are supported by the results of Talmage and others[8–10], which indicate that antisera against sheep erythrocytes may contain components which differ in haemolytic and combining activity. The relatively high number of cluster-forming cells in the spleen with the number of plaque-forming cells in the spleen may reflect either a higher sensitivity of the cluster method or a real difference in the mechanism of these two systems.

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PATHOLOGY

Effect of Alimentary Lipaemia on the Glycoproteins and the Congo Red-binding Capacity of Albumin in Healthy and Atherosclerotic Individuals

BENNHOLD's investigations[1] demonstrated that Congo red, which is known to be bound by albumin, injected intravenously in patients suffering from analbuminaemia, is eliminated from the circulating blood at a considerable rate and that, following the injection of albumin preparations, it reappears in the blood stream. This phenomenon illustrates the significance of albumin as a carrier protein. In the mechanism regulating the release of intravenously injected dyes from the blood stream the processes of adsorption between the dyes and the plasma proteins and the vascular or extravascular proteins, respectively, play a decisive part. On the basis of the foregoing it can be stated that the physico-chemical interactions occurring between the serum proteins and the transported substances make it possible for the latter: (1) to be eluted from the tissues and to become adsorbed by the carrier protein; (2) to be transported in bound form; and (3) to be discharged from the carrier protein at a site which possesses a greater affinity for the transported substance (the site of utilization, storage or excretion and elimination).

Considering that, on one hand, the transport capacity of proteins is limited, while on the other there is competition between the various substances for the available transport surfaces, both the reduction of the transport capacity of the plasma proteins, as well as any excessive increase in the amount of one of the substances transported, may lead to the appearance of certain pathological

Table 1

		Hours after ingestion of 50 g butter									
		0		4			6			7		
		A.M.	*S.D.*	*A.M.*	*S.D.*	'*P*' <	*A.M.*	*S.D.*	'*P*' <	*A.M.*	*S.D.*	'*P*' <
Congo red-binding capacity. Ext. units	A	0·23	0·08	0·19	0·06	0·20	0·18	0·07	0·05	0·17	0·06	0·01
	H	0·27	0·04	0·28	0·05	0·20	0·26	0·04	0·90	0·22	0·03	0·30
'*P*' <		0·30		0·01			0·01			0·01		
Total lipids (mg/per cent)	A	696	23	973	135		880	56		823	91	
	H	661	17	909	34		745	38		664	10	
Lipoprotein fractions (per cent)	A	23	4	8	6		10	5		11	6	
α	H	33	3	23	3		27	4		33	3	
β	A	75	4	75	4		76	4		76	3	
	H	67	3	70	3		70	3		67	3	
chylomicrons	A	2		17	7		14	7		13	6	
	H	0		7	2		3	1		0		
Total neutral glycoproteins (mg per cent)	A	450	30	415	43	0·01	398	26	0·001	410	40	0·01
	H	380	11	401	36	0·20	393	28	0·20	389	23	0·30
'*P*' <		0·001		0·40			0·50			0·20		
Glycoprotein fractions per cent albumin	A	13·0	2·9	7·2	3·3	0·001	6·6	3·1	0·001	8·3	3·6	0·01
	H	20·3	1·6	13·8	2·3	0·001	12·1	2·9	0·001	18·6	2·6	0·02
'*P*' <		0·001		0·001			0·001			0·001		
α-1 globulins	A	18·1	1·2	19·7	3·0	0·20	19·9	2·0	0·01	19·5	1·7	0·01
	H	15·4	1·1	18·7	1·1	0·001	17·0	1·1	0·001	15·3	1·4	0·60
'*P*' <		0·001		0·70			0·001			0·001		
α-globulins	A	34·0	3·2	37·9	2·0	0·001	38·7	1·8	0·001	38·0	2·1	0·001
	H	27·3	1·0	32·6	1·9	0·001	33·5	1·6	0·001	29·2	0·9	0·001
'*P*' <		0·001		0·001			0·001			0·001		
β-globulins	A	23·2	1·4	21·8	1·6	0·05	21	1·6	0·001	20·8	1·9	0·001
	H	22·8	1·2	20·1	1·7	0·001	21·4	1·8	0·001	22·7	1·9	0·90
'*P*' <		0·60		0·05			0·60			0·05		
γ-globulins	A	11·7	1·3	13·4	2·0	0·001	13·9	2·1	0·001	13·5	1·9	0·01
	H	14·2	1·6	14·7	1·8	0·02	15·9	1·9	0·001	14·2	2·1	0·90
'*P*' <		0·01		0·20			0·05			0·40		

A.M., Arithmetical mean; *S.D.*, standard deviation; A, atherosclerotic; H, healthy. '*P*'s' were calculated using Student's '*t*' test, between the initial values and the values obtained 4, 6 and 7 h after the ingestion of the butter, as well as between the result of atherosclerotic and healthy individuals.

phenomena similar to thesaurismosis. As an example illustrating this, we may mention the following pathological processes. The discharge of the carrier protein loaded with non-metabolized bilirubin (which has not been excreted by the liver), due to the lack of conjugase in kernicterus of the newborn, may be facilitated by the administration of sulphamides. These are adsorbed even more intensively by the albumin than is the bilirubin and take the place of the latter on the carrier protein. Here the bilirubin is bound to the secondary extravascular surface in basal ganglions, as well as on other parts of the organism. Similar mechanisms are also apparent in the pathogenesis of Wilson's disease, of siderosis and so on.

Due to the action of lipoprotein lipases the absorbed lipids follow a definite pathway through the various plasma lipoprotein fractions—the fatty acids released being bound by albumin[2].

According to Leszler's investigations[3], in which he followed the intravenous administration of phenolphthalein, indigo carmine and also tripaflavin, up to their elimination, a considerable lowering of the serum cholesterol-level may be observed. This phenomenon can be explained by the competition which exists between the dye and the cholesterol for the transport surface of the albumin which is available. Frederickson[4] demonstrated that consumption of carbohydrates leads to a lowering of the free fatty acid-content of the plasma, as well as a lowering of the turnover of chylomicrons. The effect on the plasma glycoproteins brought about by changes in lipoprotein-level has yet to be elucidated. Similarly, few data are available regarding the pathological changes due to the correlations existing between the glyco- and lipo-proteins.

In the course of investigating alimentary lipaemia, we studied the simultaneous changes in the capacity of albumins to bind Congo red, as well as those occurring in the lipo- and glyco-proteins, both in healthy and in atherosclerotic individuals.

Ten patients suffering from atherosclerosis were examined, as well as ten healthy students, who in the morning of the test had ingested 50 g of butter. Beforehand, and 4, 6 and 7 h following the consumption of the fat, we determined: (1) the Congo red-binding capacity of albumin (the Congo red was precipitated through a saturated sodium chloride solution, while the albumin-bound dye remained in the solution. After standing for 24 h, the solution was filtered and the extinction of the filtrate was determined)[5]; (2) the total lipids present using Swahn's method; (3) the paper electrophoretic patterns of the lipoprotein fractions; (4) the total neutral glycoproteins according to our own method[6]; and (5) the paper electrophoretic patterns of the glycoprotein fractions. Our results are shown in Table 1.

In atherosclerotics the alimentary hyperlipaemia is associated with a marked decrease in total neutral glycoproteins—a phenomenon which does not occur in healthy individuals. The neutral glucide content of albumin, as well as that of β globulin, is decreased, while that of α-1, α-2 and γ globulins is increased. These modifications indicate a tendency towards a slower return to normal in the case of atherosclerotics than in healthy individuals. Measurements of the capacity of albumins to bind Congo red in healthy individuals failed to show any significant modifications; in atherosclerotics this capacity decreased 6 and 7 h after the ingestion of the fat. We believe that we are here confronted with a decrease in the transport capacity of albumin and β-globulins. A fact in favour of this mechanism is that the decreased Congo red-binding capacity, arising as a result of alimentary lipaemia, is similar to that brought about *in vitro* by treatment with sulphamide and barbital sodium, as well as that in the serum of patients suffering from epidemic hepatitis.

On the basis of the foregoing, the following working hypothesis can be formulated. In atherosclerosis we may note the appearance of various causes capable of modifying the transport medium. Apart from a decrease of the lipoprotein lipase and a lowering of the transport capacity of proteins caused by a disproteinaemia, the depolymerization of the fundamental substance may lead to the introduction into the blood stream of a large amount of glucidic fragments. This may lead to competition for the transport surfaces, thus facilitating the de-adsorption and elimination from the circulating blood of the less strongly bound lipids. Our investigations confirm the validity of the foregoing suppositions regarding the nature of this pathogenetic mechanism. Undoubtedly, we have to deal with a decrease of the transport capacity of albumin and β-globulins, as compared with the available lipids and glucides. In the latter case—irrespective of the complex and multilateral origin of these modifications—

it can be stated that the decrease in the transport capacity facilitates the de-adsorption of lipids, thus facilitating their elimination from the blood. As a result of the continuous increase in the lipids available to the tissues, after a certain period, a shift in the site of fixation from the carrier protein towards the secondary surfaces of the endothelium into the vascular walls can be noted. If the pathogenetic picture of atherosclerosis with the multiple role of the modifications affecting the transport medium could be completed, it would undoubtedly contribute to a better understanding of this disease and open new vistas in its therapy. This could be achieved by increasing the blood transport capacity by way of drugs and through the prevention of the excessive loading of the transport capacity of plasma proteins.

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Testicular Calcinosis in Domestic Ruminants

CALCIFICATION in the testes of bulls has been the subject of a few reports over several decades[1–3]. Accounts of the condition have been mainly concerned with its incidence in surveys and theories on its aetiology. It is established now that the condition is usually bilateral in its occurrence, varies in degree, and is by no means rare. There is general support for a hypothesis that semen statis is a fundamental factor in the development of the condition[4,5]. Doubt has been cast on the reproductive significance of such calcification with the statement that, *per se*, it does not necessarily indicate infertility[3]. The association between the condition and fertility has not otherwise been closely considered.

We have investigated calcinosis (using Selye's terminology[6]) in the testes of domestic ruminants by assessment of gross calcinosis on cut surfaces of affected testes from sheep, goats and bulls with known breeding histories and by occasional histo-pathological examination of affected testes. Our aim has been to obtain clarification on (*a*) the degree of association between testicular calcinosis, sperm abnormalities, and infertility; (*b*) the sequence of events in the tubular tissue leading to calcium deposition; (*c*) the consistent and several characteristics of the condition in cattle, goats and sheep. The broad results of these observations to date are summarized as follows.

Testicular calcinosis occurred in association with lesions in the epididymis or adjacent testicular parenchyma. Masses of dead spermatozoa gathered in related collecting tubules and occluded them; such tubules ruptured with spermatocoele and granuloma formation as a common result. Seminiferous tubules in affected testes showed atrophic change and testicular shrinkage was sometimes apparent. The calcification process appears to become established by way of saponification, with granule formation in sperm masses.

Generalized testicular calcinosis was associated with reduction in semen qualities and hence with varying degrees of infertility. Advancing degrees of gross calcification progressed through oligospermia, of the order of 4,000 sperms per ml., to azoospermia.

Intermediate degrees of bilateral calcification were associated with some fluctuations in the quality of ejaculates. Fair to poor motility of spermatozoa was a fairly constant feature on microscopic examination of warmed samples of whole semen. Abnormal morphology varied, but consisted chiefly of defects of the neck and tail, for example, separated heads and tails, and bent tails.

Subjects with unilateral calcinosis of an intermediate degree gave semen samples which varied considerably, particularly with regard to the percentage of abnormal sperm forms. Motility was usually poor, seldom good. Advanced cases were sterile whereas intermediate cases showed varying degrees of fertility about or below the lower level of common fertilizing rates (for example, 60 per cent lambing rate to a Suffolk ram with unilateral calcinosis to an intermediate degree). Some very mildly calcified testes have been found in subjects with histories of average fertility, but no reputedly highly fertile sire has been found with gross testicular calcinosis.

No true age incidence is apparent; advanced cases have been found in young subjects.

Compared with the widely confirmed incidence of up to 30 per cent in bulls, the approximate incidence of significant calcification appears to be relatively low in sheep (3 per cent) but high in goats (up to 40 per cent), in which latter species the condition, in its advanced form, obviously contributes to the common sterility of males.

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RADIOBIOLOGY

Stable Bromine and Activation Analysis in Protein Tracer Studies

ALTHOUGH the judicious use of radioisotopes such as iodine-131 and iodine-125 for tracing proteins in metabolic studies in man may result in very little iatrogenic exposure of the subject to radioactivity, a general method for labelling such proteins which would not expose the patient to even this small amount of radiation would be preferable, especially in infants and pregnant women. In the present investigation, the feasibility of using neutron activation in the study of the metabolism of proteins labelled with stable bromine, bromine-79 and bromine-81 was explored the latter isotopes have suitable cross-sections for thermal neutron capture, bromine-79 being activated to bromine-80 (half-life of 4·4 h) and bromine-81 being activated to bromine-82 (half-life of 36 h) (ref. 1).

Human serum albumin and human serum IgG were labelled with stable bromide which contained equal parts of bromine-79 and bromine-81. 1·0 ml. of 0·1 N hydrochloric acid was added to 4 μmoles of potassium bromide in 0·05 ml. water; the bromide was oxidized to bromine with 0·05 ml. of 12·5 per cent sodium hypochlorite solu-

tion, and this was added promptly to 1 μmole of the protein dissolved in 2 ml. of 0·1 M sodium bicarbonate buffer, *p*H 9·4, and kept in an ice-bath. After 5 min, the brominated proteins were passed through a column of Dowex 1 and then dialysed for 24–48 h against several changes of 0·1 N sodium chloride. In an identical manner, additional aliquots of albumin and γ-globulin were radio-brominated using bromine-82 labelled sodium bromide with stable potassium bromide as a carrier; more than 98 per cent of the radioactivity in the latter protein preparations was precipitable with 10 per cent trichloro-acetic acid (TCA) and there was less than 1 atom of bromine per molecule of protein. Human serum albumin and IgG were also labelled with iodine-125 using a nitrous acid method[2].

Adult albino female mice were injected intravenously with 0·025 μmoles of the stable brominated proteins in 0·1 ml. of 0·15 M sodium chloride, and the disappearance of the proteins was followed by obtaining serum samples at regular intervals beginning 5 min after the injection. The serum samples obtained from a group of mice at a given time after injection were pooled, and the serum pools were dialysed against several changes of 1 N ammonium hydroxide for 3–4 days to remove as much Na^+, K^+, Cl^-, Br^- and brominated protein degradation products as possible to avoid radio-activation of these elements. 0·5-ml. portions of dialysed sera were placed in the centre of 3 cm lengths of polyethylene tubing, 5 mm in internal diameter, and the samples were dried at 70° C for 4 h (ref. 3). Both ends of the tubing were sealed with heat, the tubes were placed in a nylon container and then exposed for 24 h in the Brookhaven graphite-moderated research reactor in a flux of approximately 9×10^{12} thermal neutrons/cm²/sec. After irradiation, the tubes were left behind lead shielding for 24–48 h to permit decay of short-lived activation products after this period; both ends of the tubes were cut off, and the irradiated samples were recovered by boiling the tubing in 5 N sodium hydroxide for several minutes followed by two washings in distilled water. The samples were then assayed in a 400 channel scintillation spectrometer with a 3 in. sodium iodide crystal: the energy spectrum of bromine 82 was apparent, but sodium-24 was found to be present in variable concentrations in most of the samples. The sodium-24 spectrum was subtracted from the energy spectrum of the sample and the amount of bromine-82 present was estimated from the remaining gamma emission having an energy peak at 0·787 MeV. The amounts of bromine-82 in the sera were plotted against time after injection, the amount found in the sera obtained 5 min after injection being taken as 100 per cent. Using this method, the half-life of stable brominated human albumin in mice was found to be 18·5 h (Fig. 1) and that of stable brominated human IgG was 2·5 days (Fig. 2).

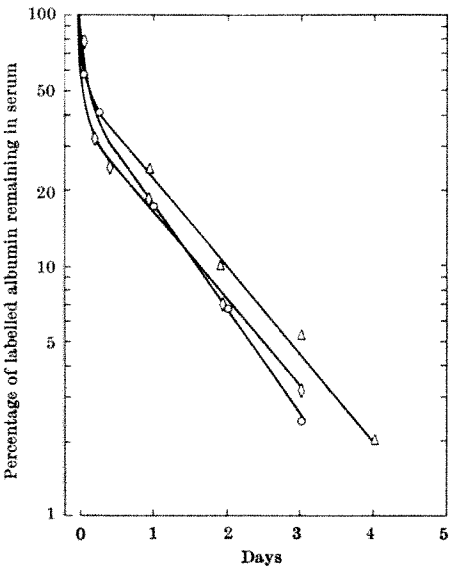


Fig. 1. Disappearance of intravenously injected human albumin-^{125}I (△), albumin-^{82}Br (◇), and stable brominated albumin (○) from the serum of the mouse. Stable brominated albumin was detected by neutron activation analysis

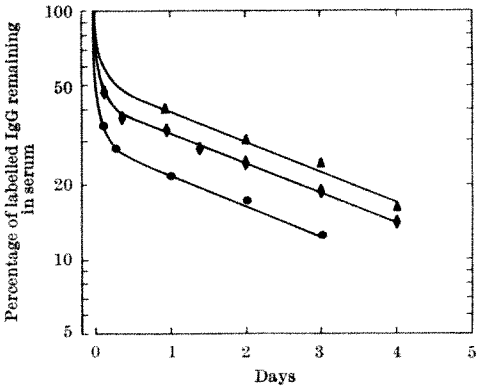


Fig. 2. Disappearance of intravenously injected human IgG-^{125}I (▲), IgG-^{82}Br (◆), and stable brominated IgG (●) from the serum of the mouse. Stable brominated IgG was detected by neutron activation analysis

To compare the turnover of these stable brominated proteins with that of radio-isotopically labelled proteins, albumin labelled with bromine-82, albumin labelled with iodine-125, IgG labelled with bromine-82, and IgG labelled with iodine-125 were injected intravenously into separate groups of mice. Sera were obtained from the mice at regular intervals and aliquots of the sera were dialysed against 0·1 N sodium chloride; after dialysis, 99 per cent of the radioactivity in each serum sample could be precipitated with TCA. The half-life of albumin-^{82}Br was 18 h and that of albumin-^{125}I was 19 h (Fig. 1); IgG-^{82}Br had a half-life of 2·4 days and that for IgG-^{125}I was 2·5 days (Fig. 2).

Thus, the turnover of the stable brominated plasma proteins in mice as assayed by neutron activation appeared to be similar to the turnover of their radio-brominated and their radio-iodinated analogues. The theoretical limit of detection under the conditions of irradiation and detection used in this study was calculated to be 1×10^{-11} moles of bromine, but the actual limit of detection under these conditions was found to be 1×10^{-9} moles of bromine. Simply by lengthening the time of irradiation and utilizing samples of greater size, the smallest amount of bromine detectable by this method is readily decreased more than tenfold. This investigation supports the feasibility of utilizing stable brominated proteins and neutron activation analysis for the study of plasma protein metabolism where exposure of the subject to even small amounts of radiation is undesirable.

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BIOLOGY

Concentration of Entries in Binary Arrays

NUMERICAL methods for the classification of elements into sets commonly begin with an assortment of individuals defined by the possession or non-possession of a number of attributes, and seek to provide final sets of highly similar individuals[1,2]. The attribute-groups which form the basis of such a classification may also be of interest, in which case the system may be transposed and the attributes classified into groups by reference to the individuals[3]. Finally, attempts have been made[4] to extract 'noda', simultaneously defined by a final set of individuals and by a final set of attributes. Existing methods for the extraction of such noda sub-divide individuals and attributes independently and collate the results by reference to a 'two-way table'; but such methods do not provide the most efficient concentration of positive entries. It should in principle be possible to obtain each block substantially independently of the others, concentrating its entries by reference to such individuals and attributes as are involved in it; but, except in the trivial case of the 'black-and-white' table, it is not to be expected that the blocks will then dispose themselves conveniently within the cells of a conventional two-way table. Some may be expected to do so, but some will almost certainly transgress cell-boundaries and/or occupy only part of the fixed cells.

The situation can be improved if, at any stage, the incipient divisions in the two directions can be made comparable, so that division can be effected of either individuals or attributes, whichever gives the greater concentration. This would be possible with existing systems if a probabilistic parameter were in use, so that the two probabilities could be directly compared; but the computation would be extremely heavy, and the purpose of this communication is to suggest an altogether simpler strategy which will serve a similar purpose. Suppose a data-matrix to be divided into two groups of A and α individuals specified by two groups of B and β attributes; the table is now divided into four cells with possible maximum entries of AB, αB, $A\beta$ and $\alpha\beta$; let the actual positive entries be a, b, c and d respectively; and write $N=(A+\alpha)(B+\beta)$ and $n=(a+b+c+d)$. Although we now have the form of a 2×2 table, the usual null hypothesis based on marginal totals is inapplicable, since it may require a cell to contain more unities than there are spaces available (for example, that $a>AB$). We therefore state a new null hypothesis. The proportion of unities to total spaces is n/N: call this k. The hypothesis states that this proportion occurs in each of the four cells, that is, that the unities are disposed in proportion to the spaces available. For the first cell the expected number of unities is ABk, and of zeros $AB(1-k)$; the corresponding observed numbers are a and $(AB-a)$. It is easily shown that the contribution of this cell to a χ^2 is

$$\frac{(a-ABk)^2}{ABk(1-k)}$$

and the remaining three contributions can be written down by symmetry. Since A, B, α and β are given, only one parameter—n—is used in calculating the expectations, and the resulting χ^2 has three degrees of freedom. Mather[5] has shown how such a χ^2 can be partitioned into three additive components, each with one degree of freedom; using his methods we obtain the partition:

For the A/α division $\dfrac{\{(a+c)\alpha-(b+d)A\}^2}{k(1-k)A\alpha N}$

For the B/β division $\dfrac{\{(a+b)\beta-(c+d)B\}^2}{k(1-k)B\beta N}$

For the interaction $\dfrac{(a\alpha\beta-bA\beta-c\alpha B+dAB)^2}{k(1-k)A\alpha B\beta N}$

We note five properties of interest: (i) the maximum value of the total χ^2 is N, achieved by any division of the table into completely full and completely empty cells; (ii) empirical tests suggest that the direct comparison of the A/α and B/β divisions is more profitable than consideration of either the total χ^2 or the interaction; (iii) k must be calculated anew at each successive division, or else the χ^2 will have four degrees of freedom and the rationale of the partition will fail; (iv) χ^2 components for successive divisions are not monotonic, and do not define a stopping rule, which must be based on density and overall content of cells; (v) the system can be immediately generalized to arrays of more than two dimensions.

In any divisive system, since consideration of all dichotomous choices is normally impracticable, a restricted set of divisions must be designated. We have adopted the usual divisive strategy of considering only monothetic splits; but as a test we have enumerated all 961 divisions of a 6×6 table, and have been reassured to find that the highest contributions in both directions were from monothetic divisions. The method has been applied to a 15×27 set of ecological data of 20 per cent overall density, and produced five cells, which had a mean density of 81 per cent and accounted for 70 per cent of the positive entries; it is noteworthy that these cells cannot be contained within a conventional two-way table. This very satisfactory degree of concentration suggests to us that the method will find particular value in the analysis of tables which, by conventional methods, concentrate at an unacceptably low level. It is being tested on larger-scale ecological data, and the results will be submitted for publication to an ecological journal in due course.

We thank Mr. P. Macnaughton-Smith, of the Home Office Research Unit, for his advice.

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The Nature of Pseudovacuoles in Cyanophyceae

THE nature of the pseudovacuoles, or gas vacuoles, which occur in bacteria and Cyanophyceae, is still enigmatic, although a number of reviews have been devoted to them.

The opinion which still prevails is that put forward by Fritsch[1]. After discussing the various observations and opinions with respect to Cyanophyceae he concluded that "the vacuoles develop at the bottom under anaerobic conditions (by a kind of gas-producing fermentation), and that as a result of the buoyancy thus acquired, the forms in question float to the surface". He doubted "whether material possessed of pseudovacuoles is actually in a healthy condition and whether cell-division continues".

Observations on a strain of *Oscillatoria agardhii*—a typical plankton—in axenic culture[2] (the first in which pseudovacuoles were isolated bacteria-free) showed that this view did not hold true for all cases. *O. agardhii* is strictly photo-autotrophic and damaged by organic nutrients—even by glucose at concentrations as low as 0·01 per cent. This in itself does not exclude the possibility of the gas being produced by a kind of fermentation, but at least it renders it impossible to prove.

It was possible to show that in this alga, pseudovacuoles are not simply gas-bubbles in a colloidal protoplasmic matrix but are instead cell-organelles of definite shape. They are elongated and multiply by fission. When centrifuged they decrease considerably in number, but after a few hours they gradually reappear in similar shape and arrangement as before.

These structures can be found during healthy propagation in all trichomes of the algae provided they remain floating, in raw and in pure cultures, and also in *Aphanizomenon gracile*. They are also present when cultures are kept under continuous illumination, and thus deficiency in oxygen is out of the question.

The possibility has to be considered that the pseudovacuoles, which no doubt keep the trichomes afloat, are caused by true inner exudation of gas.

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Histochemical Studies on the Rediae and Cercariae of *Echinoparyphium recurvatum* Linstow

ALTHOUGH information is available on the effects of trematode larvae on the gonads and digestive diverticulum of their molluscan hosts[1–3], surprisingly little is known regarding the feeding and enzyme systems of larval trematodes.

The preliminary results of this investigation deal with the acid and alkaline phosphatases and non-specific esterases of the redia of *Echinoparyphium recurvatum* occurring within the host snail *Lymnaea pereger*. Histochemical tests for alkaline and acid phosphatase were carried out on both fixed (10 per cent neutral formalin) and unfixed cryostat sections of rediae removed from the host tissues and supported in mammalian liver, according to the methods of Gomori as outlined by Pearse[4]. Incubation times: alkaline phosphatase 24 h at 37° C, and acid phosphatase 5·5 h at 37° C. Inhibitors used as controls were 10^{-2} M potassium cyanide and 10^{-2} M sodium fluoride and, made up in water, these were found to be effective. Non-specific esterases were demonstrated by the indoxyl-acetate method after Pearse[4].

A weak acid-phosphatase activity was seen in the cuticle of the redia and a stronger reaction in the cuticle and suckers of developing cercariae. The caecal walls and contents together with the oral lips of the pharynx were strongly acid phosphatase positive. Alkaline phosphatase did not appear to be present in the cuticle wall of the redia but was seen in the suckers and cuticle of developing cercariae which is probably associated with cellular differentiation. The caecum, caecal contents and the oral lips of the pharynx (Figs. 1 and 2) were strongly positive. The walls of the lateral excretory ducts (Fig. 3) and bladder were also strongly active.

Erasmus[5] has shown alkaline phosphatase activity in the cuticle of the sporocyst of *Cercaria X* Baylis. Dusanic[6] also demonstrated alkaline phosphatase in the cuticle of the sporocyst of *Schistosoma mansoni*. Cheng[7] investigated the distribution of phosphatases in the redia of *Echinoparyphium* sp. where it was seen to differ somewhat from that in redia of our specimens. Acid phosphatase was not demonstrated by Cheng except in the caecal contents, while alkaline phosphatase was seen in the cuticle. A similar distribution of alkaline phosphatase was seen elsewhere although not in the pharynx.

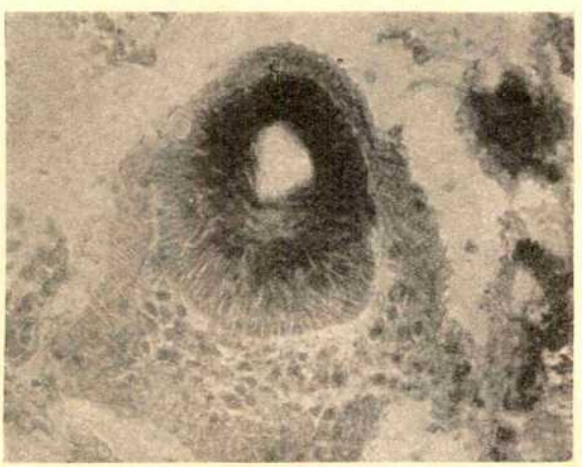


Fig. 1. Longitudinal section of pharynx of redia showing alkaline phosphatase activity in the walls lining its lumen. (× *c.* 600)

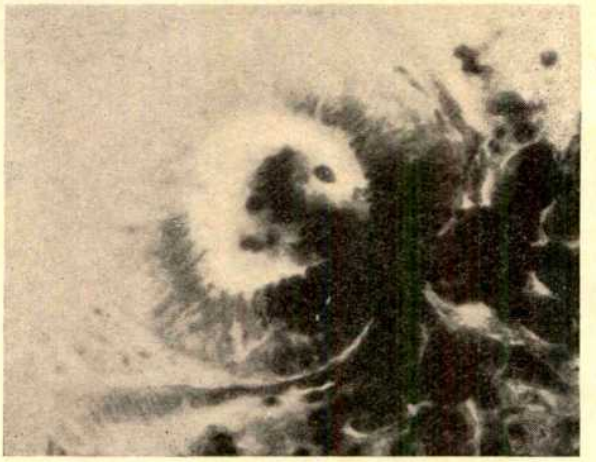


Fig. 2. Longitudinal section of pharynx of redia ingesting liver cells showing alkaline phosphatase activity (dark regions) in the oral lips and surrounding tissues. (× *c.* 600)

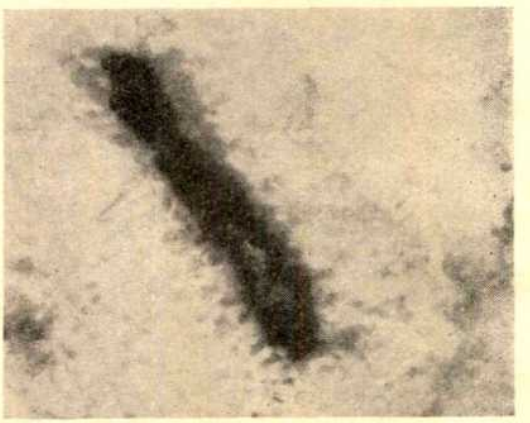


Fig. 3. Longitudinal section of primary excretory duct of a cercaria showing alkaline phosphatase activity in its walls. (× *c.* 1,200)

The biological role of the non-specific phosphatases is still not understood and the position is reviewed by Nimmo-Smith and Standen[8]. They suggest that the cuticular phosphatases in the adult worms of *Schistosoma mansoni* may be functioning in the uptake of nutrients. Cheng suggests that the alkaline phosphatase in the redial wall of *Echinoparyphium* sp. may be functioning either for the phosphorylation of substances for passage through the body wall[9–13] or in the hydrolytic catabolism of glycogen in the surrounding host tissues[1–3,14]. The pharynx in *E. recurvatum* may be involved in the secretion of phosphatases (Erasmus[12,13] demonstrated that cuticular phosphatases in *Moniezia expansa* can hydrolyse substances outside or in the immediate environment). There certainly appears to be a zone of high phosphatase activity at the oral lips (Fig. 1) and in the immediate vicinity of the pharynx during ingestion of supporting liver tissue (Fig. 2). It seems likely that the presence of phosphatases in the pharynx caecal walls and cuticle is correlated with absorption of nutrients, if this is in fact their function. Cheng[14] suggests that indigestible debris along with host enzymes may be regurgitated from the redial caecum, and that these enzymes account for any local histolysis.

Coil[15] demonstrated alkaline phosphatase in the excretory system of trematode cercariae, particularly in the tubules leading from the flame cells. Alkaline phosphatase activity has been shown in the lateral excretory ducts of the cercariae of *Echinoparyphium recurvatum* which corresponds with the distribution found by Cheng, and also in the walls of the bladder. Roche[16] points out that the proximal renal tubules of vertebrate kidney are also highly active for alkaline phosphatase. It seems possible that a role of resorption of nutrients such as glucose may be the function of alkaline phosphatase in the excretory system of trematodes.

A strong activity of non-specific esterase was found in the oral lips of the pharynx, and to a lesser degree in the cuticle of the redia. Activity in unidentified tissues was also seen in developing cercariae. The significance of this enzyme activity is not as yet clear. The presence of fats in sporocyst and redial stages has been described by Ginetzinskaya[17], and it is possible that the esterase activity described here may be associated with some aspect of fat metabolism within the redia.

This work was carried out in the Department of Zoology, University College of South Wales and Monmouthshire, Cardiff.

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MICROBIOLOGY

Role of Carbon Dioxide and Acetate in Biosynthesis by Sulphate-reducing Bacteria

SULPHATE reduction by bacteria is one of the most important biogeochemical processes for which microorganisms are responsible. Sulphate-reducing bacteria obtain energy for biosynthesis from the very small amount released in the reduction of SO_4-ions with the participation of easily activated hydrogen of certain organic compounds and with hydrogen gas. Previous work[1,2] and my recent experiments have shown that the principal sulphate reducer, *Desulphovibrio desulphuricans*, in pure culture, can only use for this purpose the hydrogen of formate, isobutanol, lactate, pyruvate and ethanol, which are oxidized during the reduction of sulphate to carbon dioxide, butyrate and acetate as the case may be. Among these substances, only the last-mentioned three suffice as sole sources of both energy and carbon for growth. The others are used merely as sources of energy; carbon for biosynthesis must be supplied in this case by the addition of yeast extract[3,4].

Such a limited number of possible energy and carbon sources for bacterial sulphate reduction does not correspond with the very wide distribution of this process in Nature. The experiments described here provide some explanation for this apparent contradiction. The strain of *D. desulphuricans* used was isolated from the underground water of an oilfield. During attempts to grow this strain with formate or hydrogen gas as sole energy source I found that normal growth is dependent on the presence of a small amount of acetate in the medium. Stimulation of growth begins with the addition of 10 mg/l. of sodium acetate and 40 mg/l. gives optimum growth and sulphide production. As the acetate is not oxidized by this bacterium it could only serve as a source of carbon for biosynthesis. In Table 1 are presented data demonstrating almost complete removal of added acetate from the medium in the culture of *D. desulphuricans* grown in the presence of formate.

Table 1. REMOVAL OF LABELLED ACETATE FROM THE MEDIUM BY CELLS OF *D. desulphuricans* DEVELOPING IN FORMATE MEDIUM*

Radioactivity of sodium acetate added (15 mg/l.) (c.p.m./l.)	Radioactivity of bacteria (c.p.m./l.)	
	Count of bacteria sedimented on 'Millipore' filter	Count of bacterial carbon recovered as barium carbonate
$4{\cdot}25 \times 10^5$	$3{\cdot}88 \times 10^5$	$3{\cdot}43 \times 10^5$

* Medium: 3 g potassium monohydrogen phosphate, 1 g sodium dihydrogen phosphate, 0·2 g ammonium chloride, 0·1 g magnesium sulphate, 1·5 g sodium sulphate, 6 g sodium formate and 1 l. of water.

Another source of carbon for *D. desulphuricans* which may provide about 30 per cent of the carbon needed for biosynthesis is exogenous carbon dioxide. This was shown by my previous experiments with *D. desulphuricans* isolated from reservoir mud[5] and grown in a lactate medium. To show the part played by carbon dioxide and acetate as carbon sources experiments were carried out using labelled compounds: $H^{14}COONa$, $H^{14}CO_3$, $^{14}CH_3-{}^{14}COONa$.

The cultures were grown in mineral medium with the addition of one of the labelled compounds. The specific activities were measured of carbon of the supplied compounds at the beginning and end of the experiment and of carbon of the bacterial cell material. In order to prevent any decrease of the specific activity of the labelled carbon dioxide or acetate with unlabelled molecules formed by bacterial metabolism, a high concentration of the former (6–8 g/l.) was supplied in the medium. The proportion of the cell carbon originating from the labelled compound was calculated as a percentage[6] from the values of specific activity.

Table 2. ACETATE AND CARBON DIOXIDE IN THE BIOSYNTHESIS OF *D. desulphuricans*

Source of energy		Additional sources of carbon for cell synthesis				Percentage of cell carbon derived from the labelled substance
		^{14}C-labelled		Unlabelled		
Substance	Conc. g/l.	Substance	Conc. (mg/l.)	Substance	Conc. (mg/l.)	
Hydrogen gas	Gas phase	$NaH^{14}CO_3$	1,000	Na-acetate	5	48·0
,,	Gas-phase	,,	1,000	,,	30	31·7
Na-formate	6	,,	5,000	,,	20	31·5
,,	6	,,	5,000	Na-acetate + peptone	20 + 200	30·6
Ethanol	3	,,	1,000	—	—	31·4
Na-lactate	4	,,	5,000	—	—	29·9
Hydrogen gas	Gas phase	$^{14}CH_3$-$^{14}COONa$	20	$NaHCO_3$	1,000	66·8
Na-formate	6	,,	40	,,	1 000	70·1
,,	6	,,	100	$NaHCO_3$ + yeast extract	1,000 + 200	30·2
,,	6	,,	20	Glucose	100	31·6
,,	6	,,	40	Isobutanol	200	32·3
Na-formate-^{14}C	6	—		Na-acetate	40	2·4
Ethanol	3	$^{14}CH_3$—$^{14}COONa$	8,000	—	—	68·5

The results show (Table 2) that *D. desulphuricans* is not capable of true autotrophy—as was previously pointed out by Postgate[3] and Mechalas and Rittenberg[4]. But, according to these authors, only 5–10 per cent of the cell carbon originates from carbon dioxide and the rest from the yeast extract added. The strain that I used assimilated 30–50 per cent of the carbon of the exogenous carbon dioxide when grown in an atmosphere of hydrogen with trace amounts of acetate. Without the latter the growth of cultures inoculated with washed cells was marginal. If bacteria were grown in the presence of formate or in more complex lactate or ethanol medium, carbon dioxide assimilation was consistently equal to 30 per cent. Even the addition of yeast extract and peptone did not lower this value. This phenomenon definitely distinguishes *D. desulphuricans* from the various heterotrophs and facultative autotrophs which show decreased carbon dioxide utilization in the presence of complex organic matter[6].

Experiments with labelled acetate have demonstrated the possibility of *D. desulphuricans* synthesizing all its cell material from carbon dioxide and acetate carbon, assimilated in the proportion 1:1, resulting in a ratio of C_1 to C_2 of 1:2. In the presence of C_3-, C_4- or C_6- compounds the proportion of cell organic matter originating from acetate is lowered from 68–70 per cent to 32–40 per cent. These data show the possibility of partial assimilation of other carbon sources including glucose, isobutanol, yeast extract (but not peptone) for biosynthesis, together with acetate.

When *D. desulphuricans* is grown in media rich in hydrogen gas, formate or ethanol its energy-yielding metabolism appears to be spatially separated from cell synthesis: connexion occurs only through the bound energy and hydrogen transfer. The carbon dioxide and acetate formed inside the cells during the oxidation of formate or ethanol are not directly involved in the synthesis of cell material (Table 2). Utilization of these compounds proceeds only from the medium.

The cells grown in ^{14}C-formate medium are practically unlabelled. Cells grown on unlabelled ethanol in the presence of a large amount of ^{14}C-acetate become labelled at a level corresponding to the utilization of 70 per cent of the external labelled acetate (the remaining 30 per cent of cell carbon is derived from carbon dioxide).

The results given here show that the sulphate reducers can normally grow using the simple basic products of the anaerobic decay of organic material: hydrogen gas formate and acetate, without any lactate and yeast extract. This perhaps explains the wide distribution and great intensity of the process of carbon dioxide reduction in natural conditions.

The capacity of *D. desulphuricans* to use the energy of hydrogen and formate oxidation for the biosynthesis of cell material by C_1+C_2 reaction is similar to that of some formate-oxidizing and purple bacteria[7].

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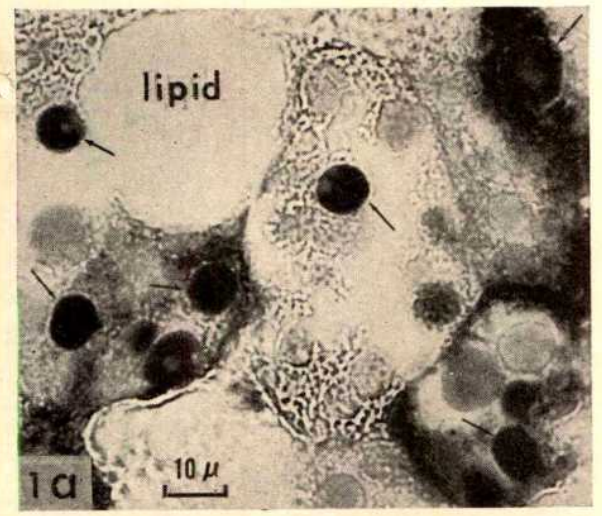
ENTOMOLOGY

Sequestration of Protein by the Fat Body of an Insect

THE fat body in an insect commonly contains a single cell type which is functionally diverse. At various times it stores and mobilizes fat, protein and glycogen which it may have synthesized or sequestered. In general the fat body becomes filled with reserves as metamorphosis approaches[1]. Larvae of *Aëdes* fed on casein build up reserves of protein granules in the fat body[2], and in *Drosophila* larvae, protein granules are induced to form in the middle of the third stadium by a hormone from the ring gland[3]. In *Calpodes* larvae the protein granules are probably translocated endocuticle. They arise suddenly within the Golgi vesicles 30–35 h before pupation, at a time when the thick larval endocuticle is being resorbed by the moulting fluid. At about 10 h before pupation other granules containing both protein and ribosomes arise from the isolation of endoplasmic reticulum in cytolysomes[4]. Although the formation and occurrence of protein granules in the fat body are well documented, there is little information on the origin of the protein.

In silkworm (*Bombyx*) larvae the fat body synthesizes protein[5] which appears in the blood. It has generally been assumed that the protein granules are also synthesized by the fat body. For example, Butterworth *et al.*[3] directed attention to the enlarged vesiculate nucleoli, typical of cells synthesizing protein, which occur at the time the protein granules begin to form in the fat body of *Drosophila*. Also, mitochondria are reported to synthesize protein[6], and according to Gaudecker[7] the protein granules arise from mitochondria in *Drosophila*. The question arises whether or not the protein granules are synthesized from amino-acids *in situ* or are synthesized elsewhere, merely to be sequestered by the fat body.

The following experiments show that, in *Calpodes*, the fat body sequesters blood proteins to form the granules and synthesizes rather little of them. If the fat body synthesizes the protein, then if we inject tritiated amino-acids into the haemocoel we should expect: (1) that the incorporation would be high in the fat body at the time of granule formation relative to other times, and (2) that the incorporation would be higher in at least some of the granules than in the rest of the fat body cell. To test this, a series of twenty-eight larvae were injected with ^3H-tyrosine (dose 20 μc./g) for a 2-h period of incorporation at various stages in the fifth and pupal stadia. Autoradiographs were prepared and grain counts made. Incorporation into the fat body was no higher at the time of protein granule formation than at other times in the stadium. In contrast, there was considerable incorporation into the epidermis when the protein granules were forming and endocuticle was being resorbed. The moulting fluid contains proteolytic enzymes[8] and presumably the cuticular protein is taken up by the epidermis as amino-acids. If the epidermis synthesizes protein from the products of digestion of the larval cuticle, we should expect a high rate of incorporation at this time (Table 1). This would not be true if it were merely passing the amino-acids from the digested cuticle into the blood. A comparison was also made between the incorporation into the granules and into the rest of the fat body cell (excluding the large area occupied by lipid from the count). The granules do not have any higher incorporation than the rest of the cell and no granules seem strongly labelled (Table 1). The incorporation into the protein granules themselves was remarkably low at all stages of their formation. Under comparable conditions, incorporation into the pupal cuticle is four to six times as high (Table 1). Most of the granules appear during a period of about 12 h, and the rate of protein deposition is probably no lower than the rate of deposition in the cuticle. Similar results were obtained after the incorporation of ^3H-leucine. The low incorporation in the granules is not due to too short a period for uptake and synthesis *in situ*. The incorporation only increases slightly as the period is lengthened to 16 h, spanning the greater part of the time during which the granules are forming (Table 2). The results make it unlikely that the fat body synthesizes much of the protein in the granules which form there. We there-



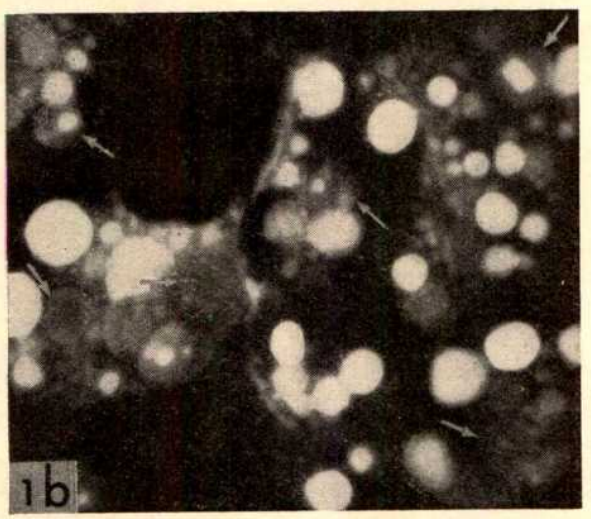


Fig. 1. The fat body of *Calpodes ethlius* during the formation of the protein granules 4 h after the injection of peroxidase (1 mg/g) into the haemocoel. Frozen sections were treated with benzidine to show the location of peroxidase. *a*, Viewed by transmitted light the peroxidase containing granules appear dark; *b*, the same field viewed by fluorescence shows many other protein granules or parts of granules not containing peroxidase which fluoresce brightly. Arrows mark some of the peroxidase containing granules (×720)

fore explored the alternative hypothesis that the fat body sequesters protein which it does not synthesize.

If the fat body sequesters proteins from the blood, it should be possible to trace the passage of a foreign, recognizable protein into the granules. Horse radish peroxidase was chosen for this purpose[9]. The enzyme (1 mg in 0·1 ml. Ringer's solution) was injected into larvae when the protein granules were forming. Four hours later the larvae were fixed in cold glutaraldehyde and frozen sections prepared. The sites of peroxidase activity were visualized by the blue or brown colour developed after treatment with alcoholic benzidine hydrochloride. When viewed by transmitted light some of the granules were entirely coloured, others were colourless and some may have resulted from the fusion of smaller granules of both sorts (Fig. 1*a*). The granules are easily distinguished by fluorescence microscopy. The natural granules fluoresce yellow when excited by ultra-violet light, but this is masked in granules containing peroxidase after the benzidine reaction (Fig. 1*b*). We may conclude that the fat body sequesters proteins from the blood to form the protein storage granules.

Table 1. NUMBER OF GRAINS PER 100μ^2 AFTER A 2-H INCORPORATION PERIOD OF ^{3}H-TYROSINE (20 μc./g) AT THE TIME OF PROTEIN GRANULE FORMATION IN THE FAT BODY OF *Calpodes*

Fat body	Epidermis	Fat body protein granules	Pupal cuticle
14±1	20±3	14±1	About 50*

* The grains over the cuticle are so dense that counts are difficult.

Table 2. NUMBER OF GRAINS PER 100μ^2 OVER THE PROTEIN GRANULES IN THE FAT BODY AFTER VARIOUS INCORPORATION PERIODS OF ^{3}H-TYROSINE (20 μc./g) AT THE TIME OF THEIR FORMATION

4 h	8 h	16 h
15±2	12±1	10±1

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VIROLOGY

Change in Chang Liver Cells from Virus Susceptibility to Resistance mediated by Persistent Infection with Echovirus Type 9

CERTAIN mutants of echovirus type 9 are able to destroy a variety of stable epithelial cell lines—Chang liver, *KB* and *FL*. This genetic character, designated by us *cha*$^+$, has been used to differentiate the various mutants of echovirus type 9, some occurring in the *cha*$^+$ and others in the *cha*$^-$ forms[1,2]. A persistent infection may be established in vigorously growing Chang liver cells when a low virus inoculum of a *cha*$^+$ mutant is used. This infection is characterized by virus multiplication to high titre in the absence of microscopically visible cellular damage. Such a virus carrier state can be maintained for long periods of time by regular changes of growth medium and passage of the cells, and may finally result in spontaneous cure and total loss of susceptibility of the cells to homologous virus challenge. This communication deals with certain aspects of this virus-cell system.

From the Quigley strain[3] of echovirus type 9, a mutant was obtained through the action of nitrous acid[4]. An important characteristic of this mutant is its ability to produce plaques on both Chang liver and primary monkey kidney cells, which was useful in the assay of the virus. This mutant has been used to initiate a persistent infection in Chang liver cells using a low virus inoculum, of approximately 10^3 P.F.U. per 3×10^6 cells, and maintaining the cultures in a cell growth medium. The growth medium consisted of Earle's balanced salt solution, lactalbumin hydrolysate 0·5 per cent, 0·01 per cent yeast extract and 15 per cent calf serum. The virus-carrier state was initiated and maintained in Chang liver cells grown in this medium in milk dilution bottles. The number of cells per bottle varied between 2×10^6 and 5×10^6. Virus titrations were carried out in primary monkey kidney cell monolayers, in tubes or plates.

After inoculation and an adsorption period of 1 h, virus could not be detected in the supernatant fluid of the cell cultures when tested on three occasions during the first 5 days (Fig. 1). At the beginning of the second week, the virus titre in the supernatant fluid was $10^{4.5}$ P.F.U./ml. Virus titres were afterwards maintained at the level of $\geqslant10^{5.5}$ P.F.U./ml. for a number of weeks. Medium was

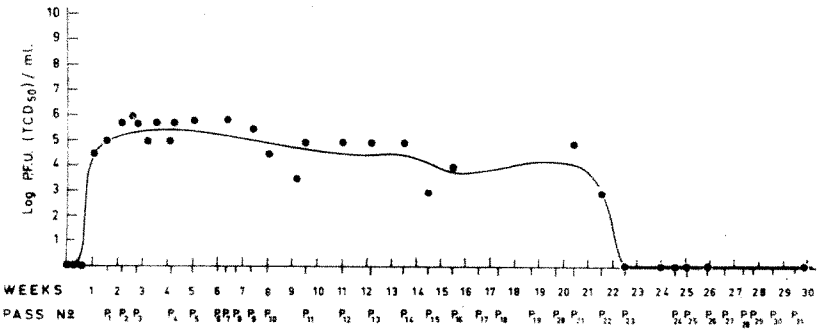


Fig. 1. Titres in supernatant fluids of Chang liver cell cultures persistently infected with echovirus type 9

changed and cell passages were carried out every fourth and seventh day respectively, the cells multiplying at a rate similar to that of uninfected cells. The virus concentration in the supernatant fluid remained more or less constant up to the fourteenth week, when virus titres started to fall abruptly. No virus could be detected starting from the twenty-second week. Repeated attempts to isolate virus from the cells on monkey kidney and Chang liver cell monolayers, either by plating out up to 10^7 intact cells, or by the seeding of cells disrupted by ultra-sound or by freezing and thawing, were unsuccessful. The cell cultures thus obtained have been free from infectious virus now for twenty-five consecutive passages.

Several experiments were carried out in order to elucidate the mechanism of this persistent infection and the nature of the 'spontaneous cure' which terminated the virus carrier state. The number of infected cells was determined at the fourth, eleventh and fifteenth passage levels. This was done by plating out varying numbers of cells on monkey kidney indicator cells, after treatment of the versene-dispersed cell suspensions with echovirus type 9 antiserum. The percentage of cells yielding 1 P.F.U. was calculated and found to be 5·0 per cent, 2·8 per cent and 1·1 per cent respectively. These results would indicate that (*a*) only a small proportion of the cells yields infective virus, which varied at these passages between 1 in 20 and 1 in 95, and (*b*) with the passage of the persistently infected cultures, the number of cells yielding virus decreases. In addition, at the eleventh and fifteenth passage levels, the ratio of P.F.U. from whole and disrupted cells was determined and found to be close to unity, indicating that each yielding virus cell releases only 1 P.F.U. at a time. These results are in accord with those reported by Deinhardt *et al.*[5] for MCN_{NDV} carrier cultures, and suggest that the mechanism of the chronic cell infection may be mediated by interferon[6]. In preliminary experiments, small amounts of interferon were detected in the supernatant fluids of the carrier cultures at the third, eleventh and fifteenth passage levels but not in the spontaneously cured cells.

The most intriguing finding was the spontaneous disappearance of the virus from the $Chang_{Echo\text{-}9}$ carrier cultures. The spontaneously cured $Chang_{Echo\text{-}9}$ cells were tested for susceptibility to homologous as well as to heterologous viruses. The strain of echovirus type 9 which had initiated the virus carrier state was used for homologous challenge, and poliovirus type 1 and coxsackievirus type B_1 as heterologous viruses. All three viruses are cytopathogenic for parent Chang liver cells. Uninfected parent Chang liver cells, kept and passaged in an identical growth medium, served as controls. Preliminary experiments carried out in tube cultures have indicated that the cured $Chang_{Echo\text{-}9}$ cells are resistant to a challenge dose of $10^{7\cdot5}$ P.F.U. of echovirus type 9 which constituted a multiplicity of infection of 320. Table 1 shows the mean values of three challenge experiments. The spontaneously cured $Chang_{Echo\text{-}9}$ cells are completely resistant to challenge with homologous virus. On the other hand, these cells are highly susceptible to both poliovirus type 1 or coxsackie virus type B_1, the efficiency of plating of these viruses on spontaneously cured $Chang_{Echo\text{-}9}$ cells being almost equal to that on control Chang liver cells. All the challenge tests were carried out in the absence of serum. Once resistance had been established, serum was not necessary for its maintenance.

Table 1. HOMOLOGOUS AND HETEROLOGOUS CHALLENGE OF SPONTANEOUSLY CURED $CHANG_{Echo\text{-}9}$ CARRIER CELLS

Cells used for plating	No. of P.F.U. after challenge with* Echovirus type 9	Poliovirus type 1	Coxsackie virus type B_1
Spontaneously cured $Chang_{Echo\text{-}9}$	0·0	8×10^6	$5{\cdot}5 \times 10^6$
Control—Chang liver	9×10^6	$1{\cdot}2 \times 10^7$	2×10^7

* Numbers represent mean values of three experiments.

The nature of this cell resistance is as yet unknown. Adsorption experiments indicate that it is not due to lack of specific virus receptors, since 95 per cent of echovirus type 9 are adsorbed to the cells within 1 h at 37° C, which is similar to its rate of adsorption to parent Chang liver cells. Walker and Hinze[7] have shown in *C-M* carrier cultures that these are specifically resistant to homologous but not to heterologous challenge, and that 80–95 per cent of the cells contain viral antigen. It is possible that in our system resistance may also be due to the presence of viral antigen. However, the *C-M* cultures, unlike the spontaneously cured $Chang_{Echo\text{-}9}$ cells, have been shown regularly to release virus. In most virus carrier cultures studied (see refs. 8 and 9), resistance is due to the presence of virus in the cells; when the cultures are cured with antiserum, their susceptibility to homologous virus is restored. In a number of virus carrier systems[10–12], increased cell resistance to homologous challenge is observed in the absence of virus release.

Vogt and Dulbecco[12] interpreted this increased resistance on the basis of selection of resistant cell mutants preexistent in the cell population. Such a mechanism may also explain the cell resistance obtained in the $Chang_{Echo\text{-}9}$ system, in which the selective pressure resulted in repopulation of the cultures with resistant cells. One may speculate on the possibility that similar mechanisms operate in the development of cell resistance under natural conditions. As an example may serve the ill-understood 'intestinal resistance' to poliovirus—either wild or induced by attenuated strains—obtained by repeated infections of the intestinal tract. This intestinal resistance is also type-specific in analogy with the $Chang_{Echo\text{-}9}$ spontaneously cured cells.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, May 2

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 2.30 p.m. and 5 p.m.—Colloquium on "Current Topics in Tropospheric Propagation".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 5 p.m.—Annual General Meeting.

UNIVERSITY OF LONDON (at the Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, London, W.2), at 5 p.m.—Dr. D. C. Phillips: "The Structure and Function of Lysozyme" (first of a series of four Almroth Wright Lectures).*

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Annual General Meeting, followed by Dr. R. J. Wicker: "Some Thoughts on Sweetening Agents, Old and New" (chairman's address).

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Annual General Meeting, followed by Dr. W. T. Norris: "M.H.D. Generation of Electricity".

Tuesday, May 3

UNIVERSITY OF LONDON (at the Royal Free Hospital School of Medicine, 8 Hunter Street, Brunswick Square, London, W.C.1), at 5 p.m.—Prof. M. J. Blunt (University of New South Wales): "The Structure and Function of Macroglia".*

UNIVERSITY OF LONDON (at Westminster Medical School, Horseferry Road, London, S.W.1), at 5.15 p.m.—Dr. J. A. Waddell: "Flow in Collapsible Tubes or the 'Waterfall' Phenomenon".*

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Mr. F. Walley: "Some Special Applications of Prestressed Concrete to Civil Engineering Structures".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Prof. R. J. Harrison: "Marine Mammals and the Sea". (Afternoon lecture for Fourth Form and Sixth Form Boys and Girls from Schools in London and the Home Counties. To be repeated on May 4, 10 and 11.)

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Prof. J. W. Dungey: "The Magnetosphere" (Inaugural Lecture)*.

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, Royal School of Mines, London, S.W.7), at 5.30 p.m.—Prof. D. W. Fuerstenau: "Mineral-Water Interfaces. I. Principles and Problems of Interfacial Behaviour".*

UNIVERSITY OF LONDON (at the Institute of Archaeology, 31–34 Gordon Square, London, W.C.1), at 5.45 p.m.—Prof. J. D. Evans and Mr. A. C. Renfrew: "Excavation in the Neolithic Settlement at Saliagos, Antiparos, 1965".*

PLASTICS INSTITUTE, LONDON SECTION (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Annual General Meeting.

Wednesday, May 4

COLOUR GROUP (Great Britain) (at the National Gallery, Trafalgar Square, London, W.C.2), at 2.30 p.m.—Fifth Annual General Meeting. 3 p.m.—Mr. H. Rohemann: "Art and Technique in the Restoration of Pictures".

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1), at 5 p.m.—Prof. J. F. C. Kingman. "An Approach to the Study of Markov Processes".

ROYAL SOCIETY OF MEDICINE, HISTORY OF MEDICINE SECTION (at 1 Wimpole Street, London, W.1), at 5.15 p.m.—Annual General Meeting, followed by Dr. Charles Newman: "Medical Education in Our Time".

INSTITUTION OF CHEMICAL ENGINEERS (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Dr.-Ing. Walter Ludewig: "Highlights in the History of BASF".

ROYAL MICROSCOPICAL SOCIETY (at the Institute of Ophthalmology, Judd Street, London, W.C.1), at 5.30 p.m.—Conversazione.

UNIVERSITY OF LONDON (in the Gustave Tuck Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. Alfred Tarski: "On Undecidable Theories" (further lectures on May 11 and 12)*.

PHARMACEUTICAL SOCIETY OF GREAT BRITAIN (at 17 Bloomsbury Square, London, W.C.1), at 6 p.m.—Prof. G. B. Marini Bettòlo: "The Evolution of Pharmacopoeias in Europe".

Thursday, May 5

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Dr. F. H. C. Crick, F.R.S.: "The Genetic Code" (Croonian Lecture).

UNIVERSITY OF LONDON (at Bedford College, Regent's Park, London, N.W.1), at 5.15 p.m.—Prof. L. S. Hearnshaw: "The Comparative Study of Mental Development".*

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Informal Discussion—"Report on the Washington Conference on Desalination" introduced by Prof. Silver.

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. P. J. Lambeth, Mr. J. S. T. Looms, Mr. A. Stalewski and Mr W. G. Todd: "Surface Coatings for H.V. Insulators in Polluted Areas"; Dr. F. H. Last, Mr. T. H. Pegg, Mr. N. Sellers, Mr. A. Stalewski and Mr E. B. Whittaker: "Live Washing of H.V. Insulators in Polluted Areas".

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, Royal School of Mines, London, S.W.7), at 5.30 p.m.—Prof. D. W. Fuerstenau. "Mineral-Water Interfaces. II. The Adsorption of Surfactants".*

CHEMICAL SOCIETY (at the Royal Institution, 21 Albemarle Street, London, W.1), at 6 p.m.—Prof. J. D. Roberts (California Institute of Technology): "Studies of Conformational Equilibration by Nuclear Resonance Spectroscopy" (Centenary Lecture).

INSTITUTE OF REFRIGERATION (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, S.E.1), at 6 p.m.—Meeting on "Medical Applications of Refrigeration". Dr. D. K. Brooks: "Total Body Cooling in Man"; Dr. D. E. Pegg: "The Preservation of Living Cells and Tissues at Very Low Temperatures".

SOCIETY OF CHEMICAL INDUSTRY, MICROBIOLOGY GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Annual General Meeting. Prof. G. F. Gause (Institute of New Antibiotics, Moscow): "Some Aspects of Antibiotic Research".

Friday, May 6

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. F. Kingsley Sanders: "The Beginnings of Cancer".

Monday, May 9

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Prof. L. Berry: "The Nile in the Sudan".

UNIVERSITY OF LONDON (at the Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, London, W.2), at 5 p.m.—Dr. R. C. Valentine: "The Construction of a Virus" (second of a series of four Almroth Wright Lectures).*

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "An Engineer's Ideas on Some of the Electromagnetic Fundamentals" opened by Mr. G. F. Shotter.

SOCIETY FOR VISITING SCIENTISTS (at the English Speaking Union, Dartmouth House, 37 Charles Street, London, W.1), at 7.30 p.m.—Discussion Meeting on "Teaching Methods in Biology and Medicine". Chairman: Dr. J. R. Ellis. Speakers: Prof. Marc Klein: "The Training of the Doctor in France"; Dr. J. Badenoch: "The Tutorial System at Oxford".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

SENIOR LECTURER and a LECTURER (with good academic qualifications and specialized in one of the following fields: circuit engineering or communications or computer engineering) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING—The Principal, Lanchester College of Technology, Priory Street, Coventry (May 3).

DEMONSTRATOR/SENIOR DEMONSTRATOR IN THE DEPARTMENT OF GEOGRAPHY—The Registrar, The University, Newcastle upon Tyne, 2 (May 4).

HISTOLOGICAL TECHNICIAN IN THE DEPARTMENT OF PHYSIOLOGY, to run a histological laboratory in a new research group being set up to study the formation and regeneration of nerve connections—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (May 4).

JUNIOR FELLOW (preferably with experience of research in mycology) IN BOTANY—The Registrar, University Senate House, Tyndall Avenue, Bristol, 2 (May 4).

JUNIOR FELLOW IN THE DEPARTMENT OF PHYSICAL CHEMISTRY to partake in a programme of research on the synthesis and crystallization of polymers, and the influence of polymer morphology on the kinetics of polymer degradation, and the structure of polymer carbons—The Registrar, University Senate House, Bristol, 2 (May 6).

LECTURER (Grade II) IN THE DEPARTMENT OF PHYSICAL CHEMISTRY—The Registrar, University Senate House, Tyndall Avenue, Bristol, 2 (May 6).

ASSISTANT LECTURER (with a thorough training and research experience in X-ray crystallography and an interest in its applications of mineralogical problems) IN CRYSTALLOGRAPHY IN THE DEPARTMENT OF GEOLOGY IN THE FACULTY OF SCIENCE—The Registrar, The University, Manchester, 13, quoting Ref. 102/66 (May 7).

ASSISTANT LECTURER (with a wide interest in cytology so as to be able to lecture to students of both departments) IN CYTOLOGY IN THE DEPARTMENTS OF BOTANY AND ZOOLOGY IN THE FACULTY OF SCIENCE—The Registrar, The University, Manchester, 13, quoting Ref. 84/66 (May 7).

ASSISTANT LECTURER (with an honours degree and experience in research, industry or teaching) IN ELECTRONICS IN THE DEPARTMENT OF APPLIED PHYSICS—The Registrar, Welsh College of Advanced Technology, Cathays Park, Cardiff, quoting Ref. No. DN741 (May 7).

LECTURER IN THE DEPARTMENT OF SOCIOLOGY—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (May 7).

LECTURER or ASSISTANT LECTURER (preferably with a particular interest in recoil chemistry or radiation effects in solids) IN CHEMISTRY to specialize in radiochemistry—The Registrar, The University, Manchester, 13, quoting Ref. 103/66 (May 7).

LECTURER or ASSISTANT LECTURER (with qualifications and experience in either Greek philosophy or logical theory or, preferably, both) IN PHILOSOPHY—The Registrar, University College of Swansea, Singleton Park, Swansea (May 7).

LECTURERS or ASSISTANT LECTURERS (2) (with research experience in any of the branches of electrical engineering) IN ELECTRICAL ENGINEERING IN THE FACULTY OF SCIENCE—The Registrar, The University, Manchester, 13, quoting Ref. 99/66 (May 7).

LECTURER or ASSISTANT LECTURER IN STATISTICS IN THE DEPARTMENT OF MATHEMATICS—The Registrar, The University, Leeds, 2 (May 9).

RESEARCH ASSISTANT (suitably qualified graduate with interest/experience in the field of either physical chemistry or physical biochemistry) IN THE DEPARTMENT OF CHEMISTRY AND BIOLOGY—The Principal (Dept. N), Harris College, Corporation Street, Preston (May 9).

ASSISTANT KEEPER (with some training and an interest in invertebrate zoology) IN ZOOLOGY at the Manchester Museum—The Registrar, The University, Manchester, 13, quoting Ref 105/66 (May 14).

LECTURER (with special interests in high polymer chemistry) IN PHYSICAL CHEMISTRY—The Secretary, The University, Aberdeen (May 14).

RESEARCH ASSISTANT (graduate with a special interest in electron microscopy) IN BOTANY—The Professor of Botany, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (May 14).

RESEARCH ASSISTANTS (with a university degree, or equivalent, in chemistry or physics, and preferably experience in electronics) IN THE DEPARTMENT OF CHEMISTRY to participate in a research programme, financed by S.R.C., on the application of electron spin resonance technique to the study of chemical reactions—The Secretary, The University, Aberdeen (May 14).

RESEARCH DEMONSTRATORS (with research interests either in organic or physical chemistry) IN CHEMISTRY—The Registrar, University College of Swansea, Singleton Park, Swansea (May 14).

LECTURER or ASSISTANT (registered medical practitioner) IN THE DEPARTMENT OF FORENSIC MEDICINE—The Registrar, The University, Leeds, 2 (May 16).

LECTURER (with a distinguished academic record in mathematics, preferably with interests in a branch of applied mathematics and some experience in computing) IN MATHEMATICS at Wollongong University College, University of New South Wales—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, May 16).

THIRD FOUNDATION CHAIR IN BIOLOGY, La Trobe University, Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, May 16).

LECTURER (with experience of modern cartography, preferably in association with a large national mapping organization) IN CARTOGRAPHY at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, May 18).

LECTURER (non-medical, preferably with experience in immunology) IN CHEMICAL MICROBIOLOGY IN THE DEPARTMENT OF BACTERIOLOGY—The Registrar, The University, Liverpool, quoting Ref. CV/16/N (May 20).

LECTURER (with special interests in physical organic chemistry) IN ORGANIC CHEMISTRY—The Secretary of the University Court, The University, Glasgow (May 20).

LECTURERS or ASSISTANT LECTURERS (3) IN THE DEPARTMENT OF SOCIOLOGY AND SOCIAL ANTHROPOLOGY (one appointment will be in social anthropology and two in sociology)—The Registrar, The University, Hull (May 20).

ASSISTANT LECTURER/LECTURER IN PSYCHOLOGY—The Registrar, The University, Keele, Staffs (May 21).

ASSISTANT LECTURER (preferably with an interest in some aspect of animal physiology) IN ZOOLOGY—The Registrar, The University, Manchester, 13, quoting Ref. 109/66 (May 21).

SENIOR LECTURER (medically qualified) IN PHYSIOLOGY—The Registrar, The University, Sheffield (May 21).

LECTURERS/ASSISTANT LECTURERS IN MATHEMATICS, CHEMISTRY, PHYSICS AND BIOLOGY at the Royal University of Malta—The Inter-University Council, 33 Bedford Place, London, W.C.1 (May 30).

PROFESSOR OF EDUCATION at the University of Waikato, Hamilton, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (May 30).

SENIOR LECTURER IN THE DEPARTMENT OF PATHOLOGY—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (May 30).

LECTURER IN PHYSICS (Theoretical) to join Dr. S. Doniach in a research group working on applications of quantum field theory to problems in solid state physics—Prof. M. Blackman, F.R.S., Physics Department, Imperial College of Science and Technology, London, S.W.7 (June 3).

CHAIR OF ELECTRONIC COMPUTATION IN THE DEPARTMENT OF APPLIED MATHEMATICS—The Registrar, The University, Sheffield (June 4).

ASSISTANT EDITOR (with experience of research and publication in medical or veterinary entomology, good critical faculty and ability to write lucid and concise English, but preferably a reading knowledge of other European languages) AT THE COMMONWEALTH INSTITUTE OF ENTOMOLOGY, LONDON, S.E.7, for duties which will include producing and editing the Institute's publications—The Secretary, Commonwealth Agricultural Bureau, Farnham Royal, Slough, Bucks (June 15).

BIOCHEMICAL GRADUATE (preferably postdoctoral) for the PROFESSORIAL SURGICAL UNIT LABORATORY to take part in research and supervise the biochemical work—The Secretary, St. Mary's Hospital Medical School, Paddington, London, W.2.

BIOCHEMIST (well qualified with suitable research experience) to lead a small group investigating the biochemistry of the mammary gland and related topics—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref. 66/N/15.

CHIEF TECHNICIAN IN THE DEPARTMENT OF ZOOLOGY to be in charge of the Technical Staff (25) of the laboratories—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh.

LECTURER or ASSISTANT LECTURER IN MATHEMATICS (Pure or Applied)—The Secretary, Royal Holloway College (University of London), Englefield Green, Surrey.

LECTURERS/ASSISTANT LECTURERS (4) (preferably with interests in one or more of the following subjects: vertebrate zoology, embryology, physiology, histology or marine ecology) IN THE ZOOLOGY SECTION OF THE DEPARTMENT OF BIOLOGICAL SCIENCES—The Registrar, Portsmouth College of Technology, Hampshire Terrace, Portsmouth.

PHYSIOLOGIST to join a small team of scientists studying the human environment—D. C. Page, Head of Personnel Services, The Electricity Council Research Centre, Capenhurst, Cheshire, quoting Ref. N/19.

RESEARCH ASSISTANT (suitably qualified chemist) for isotopic tracer studies of combustion mechanisms—Dr. C. F. Cullis, Department of Chemical Engineering and Chemical Technology, Imperial College of Science and Technology, London, S.W.7.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

A Fuel Policy for Britain. (A PEP Report.) Pp. iii+236. (London: Political and Economic Planning, 1966. Orders to PEP, 98 Kingston Road, S.W.19.) 50s. [23

Agricultural Research Council: Radiobiological Laboratory. ARCRL Report No. 15: Interim Report—Radioactivity in Milk, 1965. Pp. vi+10. (London: Agricultural Research Council, 1966. Obtainable from H.M. Stationery Office.) 2s. 6d. net. [23

Commonwealth Mycological Institute. Mycological Papers. No. 101: Microfungi. I: African Species of *Unicinula*; Some Species of *Fusicladiella*; Various Hyphomycetes, Mainly Tropical. By F. C. Deighton and K. A. Pirozynski. Pp. 43. 15s. No. 102: Rust Fungi III: On Alangiaceae, Amaranthaceae and Amaryllidaceae. By Geoffrey F. Laundon. Pp. 52+9 plates. 20s. No. 103: Dematiaceous Hyphomycetes, VI. By M. B. Ellis. Pp. 46. 15s. C.M.I. Descriptions of Pathogenic Fungi and Bacteria. Set 8, Nos. 71–80. Pp. 20. 5s. (Kew: Commonwealth Mycological Institute, 1965.) [23

The Council of Engineering Institutions. Annual Report 1965. Pp. 15. (London: The Council of Engineering Institutions, 1966.) [23

The Carnegie United Kingdom Trust. Fifty-second Annual Report 1965. Pp. viii+70. (Dunfermline, Fife: The Carnegie United Kingdom Trust, 1966.) [23

Manchester Public Libraries. The 113th Annual Report of the Libraries Committee, 1964–1965. Pp. 12. (Manchester: Manchester Public Libraries, 1966.) [23

National Research Development Corporation. Sixteenth Annual Report, 1964–65. Pp. 53+8 photographs. (London: H.M. Stationery Office, 1965.) 5s. net. [23

Proceedings of the British Ceramic Society. No. 5 (December, 1965): The Physics and Chemistry of Ceramic Surfaces. (Papers presented at a Meeting of the Basic Science Section held at the Royal Aeronautical Society, London, on December 15–17, 1964.) Pp. iv+205. (Stoke-on-Trent: British Ceramic Society, 1965.) 60s. [23

Aslib Cranfield Research Project. Factors Determining the Performance of Indexing Systems. By Cyril Cleverdon, Jack Mills and Michael Keen. Vol. 1: Design. Part 1: Text. Pp. 1–120. Part 2: Appendices. Pp. 121–377. (London: Aslib, 1966.) 45s. [23

Proceedings of the Symposium on Mixing—Theory Related to Practice. (A.I.Ch.E.—I.Chem.E. Symposium Series No. 10.) Pp. 38. (London: The Institution of Chemical Engineers, 1965.) 60s.; 9 dollars. [23

English Electric Valve Co. Ltd. Abridged Valve Data: Power Valves, Microwave Tubes, Electron Optical Devices, Cold Cathode Tubes, Vacuum Capacitors. Pp. 38. (Chelmsford: English Electric Valve Co. Ltd., 1966.) [23

Journal of Materials Science, Vol. 1, No. 1 (February 1966). Pp. iv+1–116. Subscription rates (inc. surface mail): 160s. (23 dollars) for an annual volume of 4 issues. Single copies 50s. (7 dollars). (London: Chapman and Hall, Ltd., 1966.) 23

The Zoological Record, Vol. 100, Section 16 (1963): Amphibia. Compiled by Pauline Curds, Sarah E. Bunney and Olga M. Arnold. Pp. 108. (London: The Zoological Society of London, 1965.) 20s.; 2.82 dollars. [23

National Vegetable Research Station—Notes on the Station and Its Work. Pp. 8. (Wellesbourne, Warwick: National Vegetable Research Station, 1966.) [23

The College of Advanced Technology, Birmingham. The Report of the Academic Advisory Committee on The University of Aston in Birmingham Designate. Pp. 32. (Birmingham: The College of Advanced Technology, 1966.) [23

Multi-Lingual Glossary of Industrial Diamond Terms: English/German/French/Dutch/Italian/Spanish/Swedish/Japanese. Pp. 180. (London: Industrial Diamond Information Bureau, 1966.) 20s. [23

The Macaulay Institute for Soil Research. Annual Report 1964–1965, (No. 35). Pp. 59. (Craigiebuckler, Aberdeen: The Macaulay Institute for Soil Research, 1966.) [23

Other Countries

Jahresbericht der Hamburger Sternwarte in Bergedorf fur das Jahr 1964. Pp. 13. Astronomische Abhandlungen der Hamburger Sternwarte. Band 7, Nr. 4: Die δ Cephei- und RR Lyrae Veränderlichen im Erweiterten Kapteyn—Feld SA 98 (Monoceros). Von Arthur Arno Wachmann. Pp. 205–244. Band 7, Nr. 5: Analytische und Numerische Untersuchung der Singularitäten Homogener, Rotierender Weltmodelle Von Christoph G. Behr. Pp. 245–318. Band 7, Nr. 6: Das Spektrum des BO V-Sterns τ Scorpii. Von Michael T. Scholz. Pp. 319–338. (Hamburg–Bergedorf: Hamburger Sternwarte, 1965.) [12

Mitteilungen aus der Biologischen Bundesanstalt fur Land- und Forstwirtschaft, Berlin-Dahlem. Heft 116, (November 1965): Nomenklator, Geographische Verbreitung und Wirtsbereich des Gelbrostes, *Puccinia striiformis* West. Von Prof. K. Hassebrauk. Pp. 75 (Berlin-Dahlem: Biologischen Bundesanstalt für Land- und Forstwirtschaft, 1965.) 17.50 D.M. [12

Nuclear Energy Information Center, Warsaw. Review Report No. 19: Hydrogen—The Carrier of Life. By M. Taube. Pp. iv+138. (Warsaw: Nuclear Energy Information Center, 1965.) [12

The Carlsberg Foundation's Oceanographical Expedition Round the World 1928–30, and previous "Dana" Expeditions. "Dana" Report No. 64: The Fishes of the Family Bregmacerotidae. By Umberto d'Ancona and Geminiano Cavinato. Pp. 91. 27 D.kr. "Dana" Report No. 67: Larvae of Decapod Crustacea. The Oceanic Penaeida: *Solenocera—Certaaspis—Ceratаspides* By P. Heegaard. Pp. 147. 55 D. kr. (Copenhagen: Andr. Fred. Høst and Son, 1965 and 1966.) [12

United States Department of the Interior: Geological Survey. Professional Paper 349-D: Geology of the Western Great Smoky Mountains, Tennessee. By Robert B. Neuman and Willis H. Nelson. Pp. iv+81+plates 1–6. Professional Paper 441: Water Resources of the Upper Colorado River Basin—Technical Report. By W. V. Iorns, C. H. Hembree and G. L. Oakland. Pp. iv+370+plates 1–3. Professional Paper 479: Geology of the Sierra Blanca Area, Hudspeth County, Texas. By Claude C. Albritton, Jr. and J. Fred Smith, Jr. Pp. vi+131+plates 1–9. Professional Paper 480: Geology of the Sierra Diablo Region, Texas. By Philip B. King. Pp. vi+185+plates 1–16. Professional Paper 487: Geology and Structural Control of Ore Deposition in the Creede District, San Juan Mountains, Colorado. By Thomas A. Steven and James C. Ratté. Pp. iv+90+plates 1–7. (Washington, D.C.: Government Printing Office, 1965.) [12

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PROPER PLACE FOR TRANSPORT

WHAT is to happen about transport? Evidently there are huge and important changes in prospect. A decade or so from now, there will have been yet another transformation in the way in which people and their goods are moved from place to place. Old techniques are being faced with attenuation or even extinction, sometimes because better methods of travelling have come along but sometimes simply because the old methods have become intolerable.

The development of recent decades most obviously likely to be continued is the tendency for alternative methods of travel to co-exist, and so to offer potential travellers a choice. Within large cities, underground transport is usually an alternative to several ways of travelling on the surface. Roads, railways and airlines are in competition, and there are still people who cross the North Atlantic by sea. (Most freight goes that way, of course.) Choices between co-existing alternatives are usually made on rational grounds, although this does not imply that cheapness is all that matters.

In circumstances like these, even minor technical developments can trigger off marked changes in the pattern of transport. In Britain, electric traction promises to increase the distance over which railways can win passengers from airlines. Quite modest improvements of public transport in cities could do much to diminish congestion from motor cars. Oil tankers displacing 300,000 tons like that ordered from a Japanese yard by Gulf Oil could decisively affect the pattern of petroleum distribution from the major oilfields and—at the same time—encourage the pipeline operators, who offer the simplest and often the cheapest means of bulk transport. Then, there is the Boeing 747 aircraft, which is likely to do for people what the huge tankers will do for petroleum—trunk route transport will flourish, but getting off the beaten track will be increasingly troublesome. All these changes, promised or merely possible in the pattern of transport, have in common what is, in the broadest sense, an economic stimulus.

From this point of view, the benefits of new technical developments may be different from what their supporters intend. Thus, ironically, it could be that the first—and perhaps even the only—beneficial consequence of the Anglo-French project to build the *Concord* supersonic airliner will be to ensure that the operating costs of slower aircraft are steadily reduced. More soberly, there could well be a time, in the early 'seventies, when huge subsonic aircraft ply across the North Atlantic and similar routes, and smaller and faster aircraft travel less busy but longer routes. (It does not, of course, follow that the British and French Governments will recover their expenditure on the *Concord*.) Yet again, diversity seems to promise that the pattern of transport will be helped to find its most economic form. But what kind of diversity would be best?

Fast transport between cities separated by a few hundred miles is becoming urgently necessary in densely populated areas, particularly in Europe, North America and Japan. The United States Government is financing a number of exploratory investigations bearing on specific problems such as linking the major cities on the Atlantic seaboard. However, it remains to be seen whether the result will really reach beyond schemes for patching up the existing railway network to some of the more ambitious schemes which are sometimes heard of—monorails, pneumatic tubes with trains inside, and deep-bored tunnels intended to enable trains to oscillate from one city to another with no expenditure of energy except for overcoming friction and air resistance. One difficulty is that these transport studies, although well supported, are not being given the kind of attention lavished, for example, on getting to the Moon. In Britain, the somewhat comparable development of hovercraft, also likely to be important over distances of a few hundred miles (by sea or dry land), is not moving forward as vigorously as it might because of a tendency to expect that this device should show a profit from the beginning. Then intra-city transport systems of radically new design are being explored chiefly on the backs of envelopes.

There may eventually be even greater benefits to be won by planning cities, and indeed whole countries, in such a way that the advantages of novel kinds of transport networks can be exploited to the full. Within existing cities, for example, populations tend to be uniformly distributed on the ground, although with a density decreasing outwards from some central zone. The interactions between the distribution of population and an existing transport network tend to be limited to the proclivity for population to distribute itself, over the course of time, in such a way that all transport links are equally congested. It is, however, entirely conceivable that some quite different pattern of population would lend itself more easily to the use of fast transport links. If, within cities, populations were to be gathered into a number of more or less separate concentrations, it might be possible to win great advantages from potentially fast means of travel—monorails for example—which are not likely to be economic as simple replacements for existing underground railways. In other words, there is a strong case for asking that the fabric of a city and the means of transport used within it be designed as a delicately integrated whole. Similarly, cities should be designed or encouraged to develop in such a way as to cater more efficiently for the need to move people and goods easily from one to the next. This, after all, is how the great oil companies organize their affairs (although even they find it difficult to regulate the disposition of the eventual users of petroleum products).

How are ambitious plans of this kind to be formulated? Engineers by themselves are able to handle only part of the problem—the design and development of better ways of travelling. Architects and city planners are equally limited in what they can accomplish on their own, and are habitually but understandably accustomed to regard existing means of transport as permanent parts of the fabric of society.

A more active and vigorous interchange between city planners and transport engineers is a first necessity. Happily, there are signs of such a development in several countries—in the United States and Britain for example—and the use of computers for solving problems of planning is likely to foster the development. But there is also a need to attempt more deliberately to define the potential benefits of the co-ordinated planning of buildings

and transport. Obviously the money will have to come from governments, but responsibility for seeing that these developments begin rests principally with the technical community.

ASPHALT ECOLOGY

NATIONAL Nature Week, concerned with drawing attention to the place of non-human living things in Britain, has passed off without incident and without excessive sentimentality. The floods of argument which might have come from those who hold that all human interference with the environment is either dangerous or iniquitous did not materialize. Nobody seems to have been urging that fertilizers—not to mention insecticides —are poisonous. Animals of all kinds have had a fair showing, and the protective instincts of the British people have been lavished not merely on those animals commonly to be found in nursery rhymes, but also on stoats, starlings and snakes. The proceedings of the past week or so have been informed by a hard-headed appreciation that living things of all kinds give such great pleasure to all kinds of people that they are indispensable on that account alone But there is also growing up a keen awareness of how ecological considerations can have profound economic and social consequences even in localities where the density of human population is so great that most things are artificial. Evidently the propaganda of recent years has had a beneficial effect, which does great credit to bodies such as the Nature Conservancy, the Council for Nature and the host of smaller societies and associations, some local and some specialized, which have been drumming up support for constructive conservation.

It remains to be seen what will be the consequences of this activity. In Britain, growing concern for the problems of conservation has been accompanied by a resurgence of interest in the acquisition and the management of natural countryside. Buying up the remaining tracts of unspoiled coastline is an obvious but worthy goal. Extending the system of national parks is sensible, although the new arrangements for their administration which have recently been spelled out by the British Government are defective in many ways (see p. 574 of this issue of *Nature*). These, however, are the more passive components of a policy on conservation. There is also a growing need for machinery that will more actively ensure that the human environment is sensitively adapted to human needs. Ridding rivers of pollution is high on the list of priorities, and there is bound to be great interest in the proposals in the United States for cleaning major rivers which have been heavily polluted by industrial and other effluents. If, as some proclaim, it will be possible to turn the Hudson into a pleasant flowing stream again, there will be grounds for hoping that salmon may yet be fished from the Thames at London Bridge. But there are also the coal-tips and other spoil heaps to be carted away. Most people breathe heavily polluted air, and deserve better. And given the difficulty inescapable in crowded countries of finding somewhere to swim on warm days, might not schemes be seriously considered for making enough inland water space to satisfy the growing need? In other words, there is no sharp dividing line between conservation in the traditional sense and environmental engineering on the grand scale.

THE STRUCTURE OF POLYMORPHISM

Genetic Polymorphism

By Prof. E. B. Ford. (All Souls Studies, No. 5.) Pp. 101. (London: Faber and Faber, Ltd., 1965.) 21*s*. net.

AT the beginning of the century those sharp discontinuities of variation in wild populations, which we know as polymorphisms, were a corner-stone of the case against Darwinism. Now the evidence which their study has afforded is one of the chief pillars of the Neo-Darwinian structure which genetics has been putting together for the past forty years. For, as Prof. Ford points out, polymorphism provides one of the relatively few situations in which evolutionary change proceeds in such a way and at such a speed as to be detected and analysed by observation and experiment. And no one has contributed more than Prof. Ford and his school to the analysis and understanding of polymorphism.

Much of the material discussed in *Genetic Polymorphism* has been used earlier by Prof. Ford in his *Ecological Genetics*; but now it is analysed specifically for the information it can give about the structure and evolution of polymorphic systems. Evidence is drawn from a wide range, from heterostyly in plants, from mimicry, especially in the butterfly *Papilio*, from the moth *Panaxia*, from snails, from the chromosome studies of *Drosophila* species, and from man; and, indeed, in a number of these cases the monograph provides a valuable summary of literature spread over many years and many journals. What emerges is that polymorphisms may serve a variety of functions and may operate in a variety of ways, but that they have a common genetical structure arising from a common response to the impact of natural selection.

Genetically a polymorphic system comprises two elements, the switching gene, or super-gene, on the segregation of which depends the determination of the characteristic differences in the population; and the background genotype, on the balance of which depends the adjustment of the different phenotypes to the function they discharge. Not all the evidence is presented for this bipartite structure, especially in relation to the determination of incompatibility in plants; but the evidence given is ample for the conclusions reached. It would, however, have been of interest to have had included an account of Thoday's production of polymorphism by artificial selection under experimental conditions in *Drosophila*, for this brings out especially well the point that Prof. Ford makes about the variety of the switching systems that the same form of selection can build up.

The genetic structure of polymorphism is treated not just statically as something that has evolved, but dynamically as something that continues along its path of evolutionary progression, even at times outlasting the forces of selection to which it owes its origin. We are shown the switching system as progressing from gene to increasingly complex super-gene, and the background genotype as the manifestation of a co-adaptation that arises and persists as the impact of natural selection determines. Like all systems, too, polymorphism has its inertia. The system may come into being as a result of forces other than heterozygous advantage, indeed in many cases it obviously has done so, but once the system is in being heterozygous advantage may arise merely as a by-product of the growth of the switching super-gene. And once it has arisen it can hold the polymorphism in the population even after its initial cause has vanished.

Prof. Ford's monograph is a short one, but it is packed with interest, not least in the chapter on man. We are indebted to him for it. KENNETH MATHER

SILICON TECHNOLOGY FOR THE SEMICONDUCTOR INDUSTRY

Silicon Semiconductor Technology

By W. R. Runyan. (Texas Instruments Electronics Series.) Pp. ix+277. (New York: McGraw-Hill Book Company, Inc.; Maidenhead: McGraw-Hill Publishing Company, Ltd., 1965.) 132*s*.

GERMANIUM and silicon have dominated semiconductor technology for the past twenty years and the progress made with their preparation has maintained for them leading places in semiconductor physics. The spur for the technological developments was not, however, primarily one of expectations of discoveries of new physical effects but rather the prospects of the large-scale manufacture of junction transistors. Germanium received most attention at first; that rare element, previously little investigated, was soon to be made available more nearly completely purified and with greater crystalline perfection than any other substance. Its purification owed much to the development of zone refining, while the production of the large monocrystals with the controlled levels of impurity content required by the makers of devices was made possible only by the greatly improved control of the Czochralski method of pulling crystals from the melt and the adaptation of zone refining to zone levelling. No book has integrated these developments, other than by collecting together many of the original papers.

Similar studies with silicon started a little later; here again the spur was the prospect of industrial exploitation. Compared with germanium, silicon is a very abundant element and its supply does not depend on residues from other industries. Its conductivity, too, can be made *n*- or *p*-type, but its energy gap is one and a half times as great. For a given donor or acceptor concentration, it therefore retains its extrinsic properties to higher temperatures, and junction devices made in it can be used at temperatures up to 150° C or higher. The oxide which can easily be grown on it is not hygroscopic and might be expected to protect junctions from the detrimental effects of water vapour which were so noticeable with early germanium devices.

Progress with silicon benefited from that with germanium, but was not spectacular at first; the higher melting point and greater chemical reactivity presented problems. None proved insuperable, however, and the advantages predicted began to be realized. The silicon devices now available include high-voltage rectifiers, thyristors handling currents of 100 amp or more, transistors with gain-bandwidth products of 1,000 Mc/s, varactor diodes, solar cells and fully integrated electronic circuits; they testify to the success of the basic technology of this element. But, once again, no author had devoted a book to silicon technology until this recent addition to a newly launched series.

Silicon Semiconductor Technology is an educational book as well as one of reference. The order of presentation might be questioned, but frequent users will have little complaint to make on that score. After a short historical note, the second and third chapters describe briefly the chemical purification of silicon and methods for casting it into several shapes. The fourth chapter is more detailed in its account of crystal growing. It concentrates on pulling from the melt and growth from the vapour phase on to a substrate. The segregation of impurities during pulling or zone melting is well looked after, with data for several important elements. The next chapter surveys crystal systems before examining the diamond lattice in particular and telling how to determine the orientation of silicon crystals and the presence of twinning and of grain boundaries; but it fails to link up with the defects of the crystals grown by the methods of the previous chapter. The sixth chapter returns to doping procedures, listing the behaviour of the elements in common use as donors and acceptors, without adding much quantitative information to that already given. Diffusion of dopants is the subject of the next chapter; here the author keeps device technology to the fore with his choice of geometrical conditions and detailed attention to boron and phosphorus, the two elements of which the diffusion must be so accurately controlled in the planar technology. The next three chapters describe very competently the electronic, optical and other physical properties of monocrystalline silicon, relating them to composition. They would not be out of place in a book on semiconductor physics, but are just as needed here, for so many physical properties have to be taken account of in device technology. The final chapter switches to metallurgy, in the form of diagrams of binary phase systems, nearly forty in all, with silicon as one of the two elements throughout.

The author pleads that any description of surface behaviour would demand far more space than he could spare; but some account of the technology of thermally grown layers of silicon dioxide, and their role, was surely justified. Room for a chapter could have been found by omitting some absorption spectra and the phase diagrams of silicon and many metals with which it is only rarely associated, and by condensing some other figures.

The book is excellently produced, and almost all the basic technological data vital to the silicon semiconductor industry are presented clearly and in sufficient detail. Although the writing offends occasionally, particularly by its frequent and sometimes indiscriminate use of 'This' and 'this', the book is commended to everyone with a serious interest in the subject. J. R. TILLMAN

SOLAR RADIO ASTRONOMY

Solar Radio Astronomy

By Mukul R. Kundu. Pp. xi+660. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1965.) 150*s*.

RADIO astronomy has taken a vigorous place in modern research since the Second World War. For ten years it was a compact subject, not too large to be covered by one reasonably comprehensive text. By 1956 Shklovsky recognized that this could not continue, and his book of that year dealt only with radio objects beyond the solar system. Now Dr. Kundu has shown that it requires a substantial book to cover the solar part of radio astronomy. It is very satisfying that, at the present time, the solar part is clearly defined and there is very little ambiguity concerning the material that should be included in a book entitled *Solar Radio Astronomy*.

The introductory chapters, 1–4, cover the relevant optical observations, radio propagation and emission, and observing techniques. The radio propagation theory is not very complete and reference to other texts may be necessary. Three radio emission processes are discussed under the titles "Bremsstrahlung", "Gyro-synchrotron Radiation", and "Cerenkov Radiation". These sections give the essentials of thermal, synchrotron and plasma emissions, all of which are required for explaining solar phenomena. There is a useful discussion on the Ginzburg and Zheleznyakov theory of coupling between plasma and electromagnetic waves. The developments of interferometry for solar radio purposes are described.

The central theme of the book is contained in Chapters 5–12, which deal systematically with the emission described as: quiet sun; slowly varying emission; cm bursts; dm bursts; meter bursts of types II, III, *U* and V; type IV radiation; noise storms and type I bursts. Each type of radiation is treated in a separate chapter and this makes it somewhat difficult to interrelate the burst phenomena until the threads are gathered together in Chapter 11. The great significance of the type IV synchrotron emission and its various manifestations is well portrayed.

Chapters 13–18 are more of a miscellany. Radio emission variations are so widely used as quantitative measures of some forms of solar activity that it is necessary to discuss the factors with which they correlate. This has led to a chapter on solar X-ray emission which points out the X-ray relations but rather neglects the rest of the ionizing ultra-violet spectrum. The radio phenomena of individual sunspot groups are shown to affect their relations with geomagnetic storms, polar cap events and cosmic ray bursts observed on Earth. These lively modern problems are well discussed. It has also been necessary to discuss the observations of those discrete cosmic sources which can be seen through the extended outer regions of the corona. Such observations make it possible to trace the characteristics of the solar wind most of the way towards the Earth. Finally, reference is made to the direct radar reflexion obtainable from the Sun.

The book is well set up and well illustrated. It keeps very close to the research problems of the past few years and it does not minimize the difficulties of interpreting the highly varied phenomena. It will be essential for all who are studying either radio astronomy or solar phenomena, and should have a wide use for general reference.

C. W. ALLEN

WHAT IS A ROBOT?

Human Robots in Myth and Science

By Prof. John Cohen. Pp. 156+16 plates. (London: George Allen and Unwin, Ltd., 1966.) 35*s*. net.

PROF. COHEN uses the word 'robot' in the broadest possible sense as any self-regulating artefact which operates without human aid. The first five chapters of *Human Robots in Myth and Science* are really concerned with mythology and literature which are of interest to the psychologist and the historian rather than to the applied physicist. The applied physicist is concerned with much more precise definitions of terms since he has the task of carrying the industrial revolution into its second stage. The first industrial revolution was the replacement of human muscle power by heat engines. The second is concerned with the replacement of human attention by automatic devices.

In my opinion the word 'robot' should only be applied to one particular group of automatic devices. Thus we can distinguish between the computer, automatic control devices, the telearchic machine, specialized machines and the true robot. Basically, the computer can only carry out operations with numbers, although these can be highly complex and very fast and include remembering and taking definite action according to the result of an earlier stage of the calculation. Except when a deliberate random element is built in, the computer is, however, mainly carrying out as accurately as possible and very fast the instructions given to it by men. Automatic control devices can take in, from a small number of instruments, numerical observations of physical factors and operate a number of controls on a piece of equipment in a way which has again been built into them by a man. The telearchic machine is simply a device for enabling a man, seated at a control panel in a convenient and comfortable position, to receive sense impressions from a dangerous region and to instruct a machine movement by movement in carrying out complex operations. Specialized machines save labour by enabling a man to do very much faster and more efficiently something that he could do crudely with his hands. This category also includes machines for providing a substitute for a missing part of a man.

The true robot is like a man to the extent that it can do a whole variety of different tasks and in that it receives a certain number of sense impressions so that it can adapt its operations to a limited range of external circumstances. However, it can only (like the computer or the automatic control device) act in precisely the way in which it has previously been instructed by a man. It can also only carry out movements according to its previously given instructions. This definition of a robot surely gives a clear answer to the question posed in the title of the final chapter "Is Man a Robot?". Man differs basically and essentially from a robot in the fact that he can use free will to work out where he wants to go and what he wants to attain and then make his own plan of operations. In Prof. Cohen's book the ascetic is quoted as an example of the most robot-like of humans. I should take exactly the opposite view. The ascetic is the man whose thoughts about his purpose in life have led him to do the actions which are most uncomfortable from the point of view of his physical body.

Neither robots nor computers will ever be able to make an original invention, a really creative work of art, to think of a new scientific hypothesis or to write a good book either on philosophy or on fiction. This is quite simply because not only do they not have free will but they also lack what the author refers to as "the seat of emotion". No creative work of any kind can be done by purely logical processes of juggling concepts or numbers about. It requires the working together of logical understanding and the emotional process of creativeness. It follows that if humans have a capability for having useful emotions and a possibility of free will which can never be built into any machine, then we have a perfect moral right to treat machines as slaves to serve our purposes. Prof. Cohen discusses the possibility of using animals as slaves, but there is little doubt that in the next 20 years machines can be developed to serve the needs of mankind far more efficiently than animals. There is already in the United States a machine with one hand which can carry out two hundred successive handling operations exactly as it has been instructed. These operations are stored on magnetic tape.

The big research problem for the applied physicist is to give these robots more advanced ways of being aware of changes in external circumstances and of responding to these changes appropriately.

M. W. THRING

FORMATION OF ANTIBODIES

Molecular and Cellular Basis of Antibody Formation

Edited by J. Šterzl, with the co-workers of the Immunological Department. (Proceedings of a Symposium held in Prague on June 1–5, 1964.) Pp. 683. (Prague: Publishing House of the Czechoslovak Academy of Sciences, 1965.) 82 Kčs.

MOLECULAR and Cellular Basis of Antibody Formation records the proceedings of an international symposium held in June 1964 and organized by the Immunology Department, Institute of Microbiology, Czechoslovak Academy of Sciences. The meeting was devoted mainly to chemical and biological problems related to antibody formation. The communications are arranged in five sections: (1) nature of immunogenicity (five papers); (2) characterization of antibodies (sixteen papers); (3) primary response in antibody formation (nineteen papers); (4) secondary response in antibody formation (five papers); (5) theories of antibody formation (two papers). The discussion which followed each paper is briefly recorded and each section concludes with a more extended account of exchanges among the eighty-two participants in the symposium.

The first in this series of meetings was held in Prague and Liblice in 1959; the proceedings were published in 1960 and re-issued 2 years later in a book entitled *Mechanisms of Antibody Formation*. Comparison of this first book with the present volume reveals how rapidly the understanding of many immunological processes has advanced during the intervening 5 years. This is especially obvious in regard to knowledge of the chemical and physical heterogeneity of immunoglobulin molecules, the structure

and complexity of their constituent peptide chains and the development and functions of the complex lineage of lymphoreticular cells which are involved in antibody synthesis. Other more refractory problems such as the fate of antigenic molecules, the mechanisms activating immunoglobulin synthesis and the chemical basis of antibody specificity continue to be investigated in many laboratories and are discussed by several contributors to this volume.

It is clearly not the purpose of these proceedings to review comprehensively the topics indicated by the sectional headings. The book provides a wealth of experimental detail and informed comment from those most actively engaged in advancing their subject. Although much of the work described has now appeared in the literature, the publication in a single volume of these contributions and the discussion which they provoked provides an absorbing record of present-day immunological research; the book will be of value and interest to all who are involved in the study of immunological processes.

S. COHEN

CANCER CHEMOTHERAPY

Cancer Chemotherapy

By Prof. L. F. Larionov. Translated from the Russian by A. Crozy. Translation edited by W. J. P. Neish. Pp. xx + 544. (London and New York: Pergamon Press, Ltd., 1965.) 140*s*. net.

PROF. L. F. LARIONOV is one of the leading experts on the chemotherapy of cancer in the U.S.S.R. He is also its strongest protagonist and *Cancer Chemotherapy* is intended not only as a text-book for those "actively participating in the development of chemotherapy" and "for teaching and the further training of medical personnel in the new practical branch of oncology" but also as a piece of propaganda directed against the sceptics. He campaigns trenchantly against those surgeons and radiotherapists who "still have not realized that, in many cases of cancer disease, chemotherapy can and must be incorporated into the arsenal of therapeutic measures".

How well has Prof. Larionov succeeded in his overt aim? It would be interesting to know whether, since the Russian edition was published in 1962, things have improved in the U.S.S.R. and whether the criticism would be accepted as valid in Britain in 1966. Clinicians will search in this book to see how chemotherapy can add to their ability to treat neoplastic diseases. There is much of interest here for them, but it is not always easy to find.

The most interesting section is that in which the author states the "General Principles of Cancer Chemotherapy". The first principle is quite obvious—that the sensitivity of tumours of different organs to a given drug varies within extremely wide limits. The second is more controversial—that the sensitivity of tumours of the same organ to different drugs also varies within very wide limits. From the clinical section of the book it is clear that, for those neoplastic diseases that in general respond to chemotherapy, any of a wide range of drugs may be chosen. Prof. Larionov also asserts quite definitely, when he presents the technique of treating Hodgkin's disease, that "the technique of use of the drug is decisive—even more important than the properties of the individual compounds". A 'two-and-a-half' principle might well have been included here that would point to the wide range of sensitivity of tumours of the same organ to the same drug.

The third principle states that the effect of a particular drug is inversely proportional to the mass of tumour tissues. The relevance of this to use of chemotherapy in the treatment of small metastases while leaving the primary tumour for surgical resection is developed in a later section. The validity of the 'fact' on which this principle is based is a little doubtful. The lower therapeutic result from the treatment of large tumour masses is more likely to result from the failure of drug penetration than from any mass action effect.

The fourth principle states that, as a rule, metastases are more sensitive to drugs than primary tumours, and the fifth repeats the first principle but applied to metastases.

The author then draws two conclusions: (1) that chemotherapy with presently available drugs holds out no hope for patients with massive primary tumours and large metastases; (2) that the most promising approach seems to be to use drugs in the treatment of small metastases and at an early stage in the disease.

But all these principles derive from the inadequacy of the antineoplastic drugs that are in use, and the subsequent section in the book that deals with the reasons for the low selectivity and minimal therapeutic efficiency of these drugs should have been stated first; so, too, should the basic justification for chemotherapy—that surgery and irradiation are powerless in the face of disseminated disease. In the management of cancers, clinicians have had to rely on inadequate drugs and only by great experience and maximum supportive therapy have significant results been obtained. One of the best examples, which has developed since the book was written, is the Acute Leukaemia Task Force sponsored by the National Institutes of Health in the United States.

The section of the book in which the limited capacities of the drugs are clearly in the author's mind is also one of the best. His carefully thought-out protocols for clinical trials of new drugs are excellent and written with an evident scientific scepticism.

It is difficult to be similarly enthusiastic about the other two sections of the book, however. Part 1, which deals with experimental screening of new compounds, and Part 2, entitled "Pharmacology", fall heavily between two stools. They are neither comprehensive nor are they critically selective. It is of no service to the clinician or the experimentalist to include a rambling account of so many transplantable tumours, when, in one sentence, the author puts the whole problem of screening in perspective with his comment "that there are still no good models of the most widespread and important human tumours".

For Part 2, "Pharmacology" is not the best-chosen name, for here we have a miscellany of the chemistry, biochemistry and clinical applications of too many drugs, as well as some of their pharmacology. Since study in depth of a few drugs is so clearly the way to obtain beneficial results at present, far too many substances which failed in clinical trials are included in this list.

Although the Russian text is probably 5 years old now, much of the book has aged very little. This does not perhaps suggest a very strong basis for the engaging optimism of Prof. Larionov's foreword. Progress will surely come in this field as fundamental research in molecular biology probes deeper into the nature of malignancy, but it will not be on the basis of relatively non-selective cytotoxic drugs.

As a final comment, the author has not been nearly so well served by his translator and editor as he deserves. 'Near English' and abundant errors remain in the text.

W. DAVIS

REGIONAL FARMING HISTORY

The Agricultural Revolution in South Lincolnshire

By David Grigg. (Cambridge Studies in Economic History.) Pp. xvi + 219. (London: Cambridge University Press, 1966.) 50*s*. net. = 10 dollars.

LOCAL history is in a flourishing state at the present time. The work carried out on the subject varies widely, both in the degree of scholarship it displays and in the skill with which the historian presents his conclusions.

The Agricultural Revolution in South Lincolnshire demonstrates painstaking research, mastery of English (though 'number' is a singular noun: Dr. Grigg uses it as plural) and an inclusive grasp of the different factors that affect the agricultural economy of a region.

South Lincolnshire is divided sharply by categories of soil, and slight but marked changes in elevation though little of it is above the 500-ft. level. From the coast to the Heath there are the marsh and the fen, divided into two parts, the silts and the peats, covering nearly half the area; then a low plain; then the Heath, beyond which the land declines again to the basins of the rivers Witham and Brant. The soils and the lack of efficient drainage and flood control made the task of the farmer one of great difficulty in the late eighteenth century, in spite of the long work of centuries on the rivers and flood areas. These natural impediments were not eased by the system of land holding, many acres of open fields being retained until the 1790's. Flood waters, too, restricted agricultural activities, some areas being known as 'half-year lands' because they could not be safely used for crops sown in autumn. Dr. Grigg has come to the conclusion that during the French Wars South Lincolnshire farming was "essentially extensive and technologically backward". Perhaps so ! Nevertheless, numerous and varied crops were grown on the drier fen and marsh, and the four-course rotation had been adopted, to some extent, on the Wolds and Heath by about 1790.

After victory in war at Waterloo, and even a year or so before, there was a drop in prices which hit farmers pretty hard. Many took the facile line of reducing production cost by neglecting drainage, reducing cultivations, economizing by letting land fall down, and so on. The South Lincolnshire farmers took the opposite line. They intensified their farming, introduced more fodder crops, roots and clover, where the soil was suitable, and generally adopted mixed farming, the most advanced system of the time. Between 1815 and 1850 this area, with suitable variations in the different geographical regions, became an example of how to meet financial handicaps by adopting a more intensive system giving a higher yield of crops and producing better breeds of livestock. Incidentally, the new methods made a greater demand for labour so that the whole system was more expensive although it was very much more productive. Dr. Grigg has collected evidence that seems to show that the average yield of wheat in South Lincolnshire was between 28 and 32 bushels an acre round 1850. This is very high and equal to the national average between the two World Wars. The sheep and cattle, too, were by then of outstanding quality. No significant change in the size of farms seems to have taken place during this period of development.

It remains to be seen whether other farmers in other districts recovered their financial stability by reason of adopting this risky and drastic procedure, or whether the recovery that took place by 1850, and lasted to the 1870's, was caused by general circumstances outside the farmer's control. I tend to the latter opinion, but the point is not yet clear. Meanwhile, Dr. Grigg's work deserves the closest attention, not only because it presents a novel but well-substantiated point of view, but also because it is an excellent example of how such studies should be carried out.

G. E. FUSSELL

PHYSIOLOGY OF VISION

Vision and Visual Perception

Edited by Prof. Clarence H. Graham. Pp. vii+637. (New York and London: John Wiley and Sons, Inc., 1965.) 180*s*.

THE number of experimental psychologists to have made notable contributions to the physiology of vision is small in Britain, yet there seem to be very many in the United States. Six of them have clubbed together and, encouraged by Clarence Graham, have produced the best book on visuology since Helmholtz. The comparison may seem unfair because Helmholtz did it all on his own and *Vision and Visual Perception* is a smaller volume, contributed to by six workers. However, the six had more to chew, digested it well, and the balance seems just about right: no man could be expected to deal authoritatively with all the ramifications of this bewildering topic and too many writers might have wrecked the script.

The contents are orthodox. By way of prolegomenon, we are treated to a description of the nature of light, photometry, basic optics, and—most welcome of all—to an account of how to set up the sort of apparatus used by the authors and many of those whose work they discuss. The outline of the structure of the visual system is helpful, although a comparison of Figs. 2·5 and 2·6 is irritating, and the description of the crystalline lens and its function outdated. To say categorically that there is no evidence of binocular fusion within layers of the lateral geniculate body is an admission of ignorance of the work of Bishop and his collaborators. The electrophysiology of vision is dealt with competently and fairly, but—as is inevitable in the tornado of data production that has built up—is beginning to assume the patina of history. The account of cortical activity is a masterpiece of assimilation and more useful than the more pretentious offerings of many an earlier author. But it is sad to have to report that the poor quality of the chapter on photochemistry is not due solely to the contrast it unfortunately offers to the rest of the book. The writer of this chapter has no 'feel' for the subject. There is nothing presumptive about difference spectra being unaffected by light-insensitive impurities, and the opinion that they consist of two portions is like saying that my overdraft consists of two parts: the money I owe to my bank and the money it is owed by me. The endless data on thresholds are well presented, and it is interesting to learn that frequency-of-seeing curves were used nearly 40 years ago. Brightness discrimination and contrast are related to electrophysiological data, and the modulation aspect of flicker is brought out with great clarity. However, the comparison of electro-retinographic fusion curves as obtained for cats, guinea-pigs, and pigeons (Fig. 10·15) would have gained in significance with an attempt to relate the stimulus to the retina, not to say the receptor types involved.

Visual acuity is discussed by a master although the omission of any reference to the transfer function of the eye is the most surprising gap to be found in the book: when was this chapter written? Colour vision is covered in five excellent chapters leaning perhaps a little heavily on concepts not readily subscribed to by physicists and physiologists, yet competent, searching, and fair. Vociferous authors, adulated by less critical writers, are dismissed irreverently though with justice. The discussion of the convergence of confusion loci leaves something to be desired, especially as it is going to be of decisive import in our acceptance or rejection of recent data on cone pigments. While the detailed treatment of the line-element is welcome, the account of Hecht's theory and the repetition of Laurens and Hamilton's data are surely prompted by feelings of piety. Contrast and related subjects, and after-images, have never been discussed in such detail and with such a sure touch. Again, the chapters on binocular vision, and perception of form and movement, while brief, are free from the noise of grinding axes.

Text-books are becoming more and more expensive and all but pricing themselves out of the student's wallet. However, this book is a must. It puts in the shade much of what went before and, if statistics is anything to go by, what the near future is going to bring.

R. A. WEALE

A Fundamental Survey of the Moon

By Dr. Ralph B. Baldwin. (McGraw-Hill Series in Undergraduate Astronomy.) Pp. viii+149+8 plates. (New York: McGraw-Hill Book Company, Inc.; Maidenhead: McGraw-Hill Publishing Company, Ltd., 1965.) 4.95 dollars; 40*s*.

BALDWIN has undoubtedly succeeded in presenting, in this work, a concise and readable account of a good deal of our knowledge of the Moon; as an introductory work for those not particularly specializing in lunar physics, it suffers little from the lack of mathematical analysis in certain places. However, the complete lack of mention in the text of the now well-established principles of lunar tectonics is regrettable. It is true that if the references given in the preface are consulted most of the existing points of view will be encountered. Readers who do not do this, however, will be left ignorant of much important recent work.

In particular, some of the conclusions drawn about the nature of the maria and marial features will, almost certainly, have to be modified in the future as a result of the work of Dobar and others on the physical features of igneous structures caused by the inherent lunar surface conditions, which receives only passing reference in the text. The concluding statement that "The 136-year-old argument (concerning the origin of the craters) is over", based only on the evidence of the *Ranger* photographs, is far too emphatic.

The errors in this book are usually those of omission as indicated here, and many of these may be intentional to keep the length of the work to a minimum; but attention should be directed to the paragraph on faults in Chapter 6 in which it is specifically, and incorrectly, stated that there is no evidence for lunar strike-slip faulting. Printing errors are virtually non-existent, but it is unfortunate that the reference chart and contour map given are in astronautical and astronomical convention respectively, making comparison unnecessarily tedious.

L. WILSON

Basic Bacteriology

Its Biological and Chemical Background. By Dr. Carl Lamanna and Prof. M. Frank Mallette. Third edition. Pp. xiv+1001. (Baltimore, Md.: The Williams and Wilkins Company, 1965. Distributed in the U.K. by E. and S. Livingstone, Ltd., Edinburgh.) 140*s*.

DR. CARL LAMANNA and Prof. M. F. Mallette, in the third edition of their *Basic Bacteriology: Its Biological and Chemical Background*, have aimed at bridging the gap between the elementary text-book and the advanced monograph. Chapters are included on the taxonomy, microscopy and staining, structure, growth, nutrition and metabolism of bacteria with a final, somewhat fragmentary account of chemical sterilization. The book retains its rather excessive coverage of physical and chemical principles which are not invariably related to bacteriological problems. Since the publication of the second edition, the developments in bacteriology have necessitated considerable revision and amplification, and genetics, protein synthesis, enzymology, photosynthesis and permeability have received greatest reappraisal. Unfortunately, but perhaps understandably in such a large work, the revision has been uneven. Thus, the discussion of numerical taxonomy, while adequate, is not exemplified and classifications derived from such analysis have not been compared with those based on the *a priori* hierarchy of characters. Such omissions are made the more conspicuous by the amount of space devoted to speculations on bacterial origins and phylogeny. Chapter 6 examines the structure of bacteria: here the presentation tends to be disjointed, sometimes confused, for example, the discussion of the cytoplasmic membrane and membranous organelles; or restricted, for example, the fine structure and division of nucleoids are not considered. More than one-third of the book is concerned with bacterial metabolism and variation, subjects which are treated with thoroughness and care, although inorganic nitrogen and lipid metabolism deserve a fuller exposition. The authors have omitted text references deliberately but provide extensive bibliographies and a good index. There are very few errors of fact and immense care has been taken with proof reading. This book will remain an important source of information on bacteria.

A. T. BULL

Lexicon Allergologicum

Allergologie Worterbuch. Herausgegeben von Knud Wilken-Jensen. (Allergology Dictionary.) Pp. xii+119. (London and New York: Pergamon Press; Leipzig: Johann Ambrosius Barth Verlag, 1965.) n.p.

WITH increasing interest in the clinical aspects of immunology, the amount of allergological literature has been growing fast. The European Academy of Allergology has played a leading part in facilitating communication in this field and now presents a six-language dictionary of terms most frequently used in the practice of allergy.

This dictionary is in three parts. The first part consists of an alphabetical list of 142 such terms, with language equivalents in German, English, French, Italian, Russian and Spanish. In the second part, 64 of these terms are explained in the form of brief sentences; these are given in six languages and will minimize errors in communication between practising allergists. The third part consists of language equivalents of 100 common allergens, in alphabetical order, with Latin terms where appropriate. The whole volume is of a convenient size for work in clinic and library.

Recent advances in immunology may soon permit more precise descriptions of allergological phenomena. That the European Academy of Allergology has been able to publish this lexicon is surely a pointer towards the eventual standardization of nomenclature in this field.

D. C. DUMONDE

Ultrastructural Plant Cytology

With an Introduction to Molecular Biology. By Prof. A. Frey-Wyssling and Prof. K. Mühlethaler. Pp. x+377. (Amsterdam, London and New York: Elsevier Publishing Company, 1965.) 130*s*.

ULTRASTRUCTURAL Plant Cytology sets out the personal views and the results of the extensive work of the authors on the fine structure of plant cells. The approach is direct, almost dogmatic, but this makes the text very readable and simplifies many complex and difficult concepts.

The first section of the book deals with the elementary organic and physical chemistry of large molecules and leads up to the modern theoretical discussions of the molecular basis of heredity and protein structure and synthesis. Rather surprisingly, no mention is made of the possible mechanisms of the control of cell differentiation and very little attention is paid to this very important branch of cytological research where work on plant material has made very significant contributions.

The second section describes the ultrastructure of the plant cell and attempts to relate these investigations to the account of the chemistry and molecular biology given in the first section of the book. The authors and their colleagues have contributed much to the subject matter of this section and a large amount of the factual information is taken from their many publications in the field. As a consequence, it is superbly illustrated with electron micrographs and with pictures from the new freeze-etch technique developed at the Zurich laboratories.

It is a good though expensive text-book for a course in cell biology.

D. H. NORTHCOTE

CHARTER CENTENARY OF THE ROYAL MICROSCOPICAL SOCIETY

THE Royal Microscopical Society will celebrate the centenary of the granting of its Royal Charter this year by holding international meetings in London (July 18–22), Chicago (August 22–26), and elsewhere.

It is now nearly 127 years since seventeen gentlemen sat down in a drawing-room in Wellclose Square, near the Royal Mint, London, "to take into consideration the propriety of forming a society for the promotion of microscopical investigation, and for the introduction and improvement of the microscope as a scientific instrument"[1]. The almost immediate result was the foundation of the Microscopical Society of London, with the famous anatomist, Richard Owen, as first President. The foundation of the Society set an example which was followed in many countries.

At its second meeting, in January 1840, an event occurred that has probably affected every biological laboratory in the world, namely the fixing of 3 × 1 in. as the standard size of the microscopical slide. A special instrument was provided on this occasion, which cut slides of the standard size for the members present. One of the most useful functions of the Society has been the standardization of microscopical equipment. Almost all microscope manufacturers in the world have accepted the standard R.M.S. thread for objectives and the standard R.M.S. diameters for eyepieces, so that the parts of the microscope are readily interchangeable from one instrument to another.

It had been anticipated that the Society would concentrate at the start chiefly on the improvement of the instrument itself, since achromatism and the effective correction of spherical aberration had only recently been introduced into microscopy, and further rapid advances might have been expected. Instead, the members turned mainly to the use of the improved instrument in biology. The celebrated John Quekett, one of the early members of the Society and its secretary for 19 years, was soon making full use of it in histology. Some of the best-known British biologists of the time joined the Society, among them Edward Newman, Bowerbank, Thomas Bell, George Busk, and T. H. Huxley; but those who were interested in the optics of the instrument and in the physical aspects of microscopy in general also became members. Among the most eminent of these were J. J. Lister and Faraday. Others again, like William Carpenter, were authorities on the construction and optical principles of the microscope, and also made full use of it in biological studies. The Society was an active and happy mixture of professionals and amateurs.

It was during the presidency of James Glaisher, famous as a meteorologist rather than as a microscopist, that the Royal Charter was granted, largely as a result of Glaisher's representations in the right quarters[2]. The grant was announced by him at a meeting of the Society on October 10, 1866, and in the following month it was announced that Queen Victoria had been graciously pleased to command that the Society should be styled the "Royal Microscopical Society"[3].

Some of the most important contributions to microscopy made in the succeeding decades by Fellows of the Society were those of J. W. Stephenson, who not only introduced a very successful erecting binocular microscope[4,5], the fore-runner of the dissecting microscopes of to-day, but also suggested the system of homogeneous immersion[6] for objectives that was adopted by Abbe[7] and brought to practical perfection by Carl Zeiss.

During the long stable period from the introduction of apochromatic objectives to the marvellous outburst of invention in the thirties and forties of the present century, the Society functioned largely by supporting and guiding the practical use of the microscope in various sciences, particularly biology. Certain of its Fellows contributed important general works on the instrument that retain their usefulness to-day, among them Dallinger's edition of Carpenter's *The Microscope and its Revelations*[8] and Spitta's *Microscopy*[9]. The example of E. M. Nelson (a President of the Society) contributed to the attainment in practice of the highest resolving power of which the instrument was at that time capable. The period was one of consolidation, with progress in limited fields, such as ultramicroscopy and the increase of resolving power by the use of ultra-violet light for illumination.

With the ever-increasing use of the microscope in the universities, the proportion of professional microscopists in the Society grew steadily, until in 1941 the President announced that "the amateur has practically disappeared"[10]. This, luckily, is not quite exact even to-day, for the fellowship of the Society is not exclusively professional, though amateurs are in a small minority.

The sudden advances that followed the quiet period changed the whole outlook of the Society, and interest was focused for a time on the instrument rather than on its applications. Only a quarter of a century ago the President had said that the electron microscope "may at first glance appear beyond the interest of microscopists"[10], and indeed the potentialities of the new instrument could not at that time be realized. Then came phase contrast and interference microscopy and the practical application of electron microscopy, and later the field-ion microscope. From these advances the Society received an enormous stimulus, and the fellowship increased rapidly. A new section of the Society was formed for the study and development of electron microscopy. Section membership, not confined to Fellows, became an established feature of the Society, spreading its usefulness much more widely. The applications of microscopy were not overlooked, and the institution of a Histochemical and Cytochemical Section brought another large group of active workers into the Society as Fellows or section members. The Society had long had a considerable fellowship in the United States, and in 1963, for the first time, a meeting of the Society was held outside Great Britain. This meeting, at the National Institutes of Health, Bethesda, under the presidency of Dr. V. E. Cosslett, was a great success, and resulted in a large increase in the American fellowship.

In recent years the Society has instituted courses of advanced instruction in optical and electron microscopy. These have been attended by enthusiastic groups of students, eager to obtain knowledge and skills that are not readily acquired at most universities.

The Society has also extended its general usefulness by starting a series of 'Survey Lectures' at its monthly general meetings. In these lectures a subject of wide interest is discussed by a recognized authority in the field, who gives an essentially personal account of recent developments. The Survey Lectures have attracted large audiences and will probably be a regular feature of the Society's activities.

The Society's chief ambition for the future is to found a permanent 'microscope centre', where instruction and research could be carried out in the same building. The intention would be to have optical and electronic instruments of the most advanced design in the centre, under the control of highly qualified research workers and technicians. The former, who would be free to plan their own researches, would be responsible for the instruction, for it is thought that teaching is best done by those actively engaged in research. There would be regular courses of general instruction, as well as facilities for obtaining information and practical advice on particular branches of microscopy.

Fellows of the Society have the necessary knowledge and experience to set up and run a centre of this kind, and it is earnestly hoped that means of financing it may be found. There is urgent need for such a centre, which would serve the interests of both science and industry. It may be mentioned that the total value of the electron microscopes already installed in Britain, with their accessory apparatus, probably exceeds £5 million, and it is very doubtful whether there are enough highly trained research workers and technicians to make full use of them.

The principal meeting to celebrate the granting of the Royal Charter will be opened by the President of the Society, His Royal Highness the Duke of Edinburgh, at 10 a.m. on July 18, in the lecture hall of the Royal Geographical Society, Kensington Gore, London. An elaborate programme has been arranged for this international meeting, the subject of which is "The Role of the Microscope in Scientific Investigation". There will be seven Survey Lectures addressed to the whole membership, and numerous group meetings to deal with the problems of the eighteen subjects into which microscopy has been divided for the purposes of more specialized discussion.

There will be important exhibitions, and in particular a very large display by British and foreign manufacturers of optical and electron microscopes and accessories. This, and the group meetings, will be held in the Imperial College of Science and Technology. A special feature of the manufacturers' exhibition will be a large display of functioning electron microscopes. There will also be receptions and visits to places of scientific interest in London, Cambridge and elsewhere.

The programme of the meeting and particulars of membership can be obtained on application to the General Secretary of the Society at Tavistock House South, Tavistock Square, London, W.C.1. J. R. BAKER

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A BIOCHEMICAL INTERPRETATION OF SOME TAXONOMIC DIFFERENCES BETWEEN YEASTS*

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SINCE the pioneer work of Wickerham[1,2] and Kudriavzev[3,4], taxonomists have been testing thousands of strains of yeasts on each of more than thirty compounds to determine which of these compounds can be a sole source of carbon for aerobic growth for each yeast (see, for example, ref. 5). This article proposes a method for examining associations between the results of such tests. The method is used to deduce the routes by which yeasts convert the test compounds into intermediates of the main metabolic pathways.

The method proposed for scrutinizing these results is analogous to that by which information on biosynthetic pathways has been obtained from auxotrophic mutants. The principle of the latter technique is that, if the requirements of two mutant strains are satisfied by the same substrate x, and of only one of the strains by substrate y, then y is the precursor of x (ref. 6). Even if such work was often incomplete in terms of its detailed enzymology, nevertheless it opened up new fields of intermediary metabolism[7].

Accordingly, this article considers biochemical schemes which follow logically from the results of taxonomic tests and which may explain these results. It discusses, particularly: (i) enzymes the presence of which in some yeasts and absence in others lead to correlations between the results of many of the tests; and (ii) how analysis of the results of large numbers of tests could be used to make evident the biochemical features that are likely to underlie these correlations. Such considerations may not only give a better understanding of the tests themselves, but may also help taxonomists to decide when they can legitimately treat nutritional characters as characters of 'equal weight'[8]. Such an analysis also provides a basis for generalizations about the initial catabolic steps for large numbers of yeasts with many substrates. Both these generalizations and also exceptions to them, which may be of particular interest, must eventually be subjected to experimental verification by the enzymatic examination of selected yeasts.

The results of the tests considered in this article are from two separate surveys which are part of a new major taxonomic study of yeasts being carried out under the direction of Dr. J. Lodder. The two surveys are: (i) Kreger-van Rij's recent investigation of the genera *Endomycopsis*, *Pichia* and *Debaryomyces*[5]; and (ii) unpublished observations of van Uden and his colleagues on *Candida* and *Torulopsis*[9]. The results discussed here are those of growth-tests with carbohydrates and their derivatives. Responses to such nutritional tests are generally presented (see ref. 5) in an all-or-none fashion (+ or −) and that is how they are treated here.

Reasons for failure to use substrates. A yeast fails to grow aerobically on a substrate either (i) because the substrate does not enter the cells[10,11], or (ii) because the yeast lacks one or more enzymes necessary to convert the substrate into an intermediate metabolite of a main pathway. Generally, only minor differences of carbohydrate catabolism seem to be concerned in the tests, not differences in major metabolic pathways (see refs. 12–14, ref. 15, p. 62, and refs. 16, 17).

It is not possible by growth-tests alone to distinguish between these two alternative reasons for failure. Caution is, therefore, necessary in drawing any conclusion as to the nature of a metabolic pathway from the results of growth-tests. Thus the existence of specific translocation mechanisms[18,19] governing the entry of substrates into the cells gives rise to a difficulty, which could interfere with analyses of the sort proposed. The absence of such mechanisms in certain yeasts can mask the presence of functional catabolic routes within the cells. Nevertheless, in this article it is assumed that failure to grow on a particular substrate reflects the absence of a catabolic enzyme, rather than the failure of a substrate to penetrate the cells.

Alternative catabolic routes for a single substrate. Every yeast that uses a given substrate does not necessarily do so by means of the same metabolic route. Sorbitol, for

* Substance of a talk given at the symposium on "Techniques for the Identification of Fungi" of the Society for General Microbiology and the British Mycological Society, held in London on January 6.

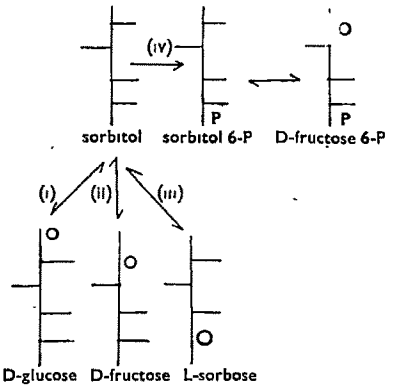


Fig. 1. Four alternative initial steps in the catabolism of sorbitol

example, could be oxidized to (i) D-glucose, (ii) D-fructose or (iii) L-sorbose; each oxidation requires a different dehydrogenase (usually coenzyme-linked)[20,21] (Fig. 1). The glucose and fructose thus produced would be metabolized through the Embden–Meyerhof pathway or the pentose cycle[22]. Sorbose, after phosphorylation[23,24], might be converted to glyceraldehyde and dihydroxyacetone phosphate[25]. There is, theoretically, a fourth possible route for sorbitol, namely, phosphorylation followed by dehydrogenation to, say, D-fructose 6-phosphate. Thus different yeasts perhaps use sorbitol in different ways. Consequently, when a taxonomist writes 'sorbitol +' for some yeasts and 'sorbitol −' for others, any one of four enzymatic mechanisms could be responsible for this distinction.

Interpretation of correlations of responses to different substrates. Taxonomists often find that most organisms which can utilize a certain substrate, *A*, can also use another, *B*, and conversely. Three alternative conditions may be responsible for such an association of responses to different substrates: (i) a catabolic enzyme, often that responsible for the initial attack on the test-substrate, may have a wide specificity; (ii) substrate *A* is converted into *B* as a step in *A*'s catabolic path; (iii) both *A* and *B* may be converted into *C*, a common metabolite of the route to the main pathway for both substrates.

Catabolic enzyme of wide substrate specificity. The polyol dehydrogenases[20] and the glycosidases[26] are well known for their wide specificities. An example is given by the β-glucosidases, a number of which have long been thought able to hydrolyse more than one substrate[27]. According to Herman and Halvorson[28], the β-glucosidase of a strain of *Saccharomyces lactis* hydrolyses arbutin, salicin and aesculin. The results of Kreger-van Rij's survey of the genera *Pichia*, *Debaryomyces* and *Endomycopsis* support the view that a single enzyme is responsible for hydrolysing various β-glucosides. Table 1 shows that, with only two exceptions, the yeasts which use arbutin also use cellobiose and salicin; and yeasts negative for one of these substrates are also negative for the others.

Interconvertibility of substrates. An example of substrate *A* being converted into substrate *B* is provided by D-ribose, which can be reduced to ribitol (adonitol) reversibly. Two possible routes (*X* and *Y*) for the catabolism of these two substrates are given in Fig. 2 (see ref. 29).

Table 1. RESULTS OF TESTING FIFTY-ONE SPECIES WITH THREE β-GLUCOSIDES

		Cellobiose		Salicin	
		+	−	+	−
Arbutin	+	33	0	32	2
	−	0	14	0	14

Entries in the table are numbers of species. +, Substrate utilized; −, substrate not utilized. From the observations of Kreger-van Rij (ref. 5) on *Endomycopsis*, *Pichia* and *Debaryomyces**.

* Of the fifty-one species, certain of them gave variable results from strain to strain: these results have been omitted.

Kreger-van Rij's survey[5] confirms, for the genera which she studied, that the utilization of ribitol is associated with that of D-ribose (Table 2). Were the two tests independent, the likelihood of obtaining the figures in Table 2 is less than one in a thousand (applying Yates's correction for continuity[30], $\chi^2_c = 16$, $n = 1$, therefore $P < 0{\cdot}001$). If (i) the initial step in D-ribose catabolism were always its reduction to ribitol (that is, route *X*, and not the formation of ribose phosphate); and (ii) the substrates are effectively unimpeded by cell membranes, then no yeast could be [D-ribose +, ribitol −]. If, on the other hand, ribitol were always first oxidized to D-ribose (route *Y*), then no yeast would be [ribitol +, D-ribose −]. The figures shown in Table 2 are consistent with the statement that D-ribose is converted initially to ribitol as in route *X*. They are inconsistent with route *Y*. Hence, analysis of the results of growth-tests with *Pichia*, *Debaryomyces* and *Endomycopsis* indicates that the first step in the catabolism of D-ribose is its conversion to ribitol.

Table 2. RESULTS OF AEROBIC GROWTH-TESTS WITH RIBITOL AND D-RIBOSE

		Ribitol	
		+	−
D-Ribose	+	18	0
	−	7	18

Explanation as for Table 1.

Table 3. RESULTS OF AEROBIC GROWTH-TESTS WITH L-ARABINOSE AND D-XYLOSE

		D-Xylose	
		+	−
L-Arabinose	+	17	0
	−	9	17

Explanation as for Table 1.

Common route to main pathway. The scheme in Fig. 3 (based on work with *Penicillium chrysogenum*[31] as well as with yeasts[29]) exemplifies the conversion of two substrates into a common metabolite. Thus, according to this scheme, both L-arabinose and D-xylose are converted, by way of xylitol, to D-threo-pentulose. In addition, the enzyme responsible for the initial reduction of L-arabinose may also catalyse the reduction of D-xylose[29,32]. There is evidence for the occurrence of such reactions in the yeasts *Candida utilis*[33], *Candida albicans*[32], *Rhodotorula glutinis*[34], *Saccharomyces rouxii*[35] and *Geotrichum candidum*[36].

Although the relationship between L-arabinose and D-xylose clearly differs from that described for D-ribose and ribitol, the figures from the taxonomic tests should be strictly comparable for both pairs of substrates. Provided that the scheme in Fig. 3 is true for yeasts, and that the substrates can penetrate the cells, a yeast able to use L-arabinose can also use D-xylose. As can be seen from Table 3, this proposition is true for species of *Endomycopsis*, *Pichia* and *Debaryomyces*[5]. It is also true for many other yeasts: Santa María[37] has found only one exception among 1,399 strains of yeasts of different genera isolated mainly from living insects and frass.

A method of handling the taxonomic data. For a satisfactory analysis, it is necessary to have more information about the results of the growth-tests than has usually been accessible hitherto. The results of the survey by Kreger-van Rij[5] are given only by species, and not by strain; certain species, therefore, have had to be omitted from the present analysis. The description[5] of *Pichia pinus*, for

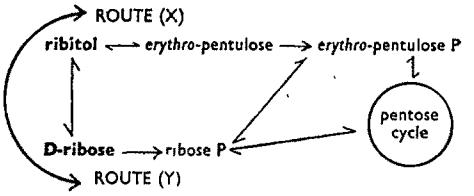


Fig. 2. Scheme for catabolism of D-ribose and ribitol

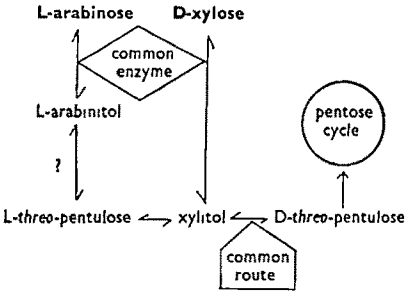


Fig. 3. Scheme for catabolic route of L-arabinose and D-xylose

example, indicates that the strains of this species differ in their reactions to D-xylose, L-arabinose and D-ribose; but it does not indicate whether a strain that uses, say, xylose can also use arabinose or ribose. Consequently, there is special interest in van Uden's observations on the ability of 293 strains (66 species) of *Candida* and 96 strains (32 species) of *Torulopsis* to grow aerobically on ten substrates[9]. These observations on individual strains have been arranged as tables of the kind already considered (Tables 1, 2 and 3).

The results of tests using L-sorbose and D-mannitol are given in Table 4. Again, these are not two independent tests ($\chi^2_c = 74$, $P < 0{\cdot}001$). The special interest of this table is that only eight strains out of 388 (that is, about 2 per cent) use L-sorbose but not D-mannitol. In other words, with only one exception in fifty (these perhaps impermeable to D-mannitol), if a strain of *Candida* or *Torulopsis* is able to use L-sorbose, it will also use D-mannitol. This relationship can be written L-sorbose ——◈ D-mannitol: in a more generalized form, so that A ——◈ B means that when substrate A is used, B is usually used, too, but not vice versa.

Table 4. RESULTS OF AEROBIC GROWTH-TESTS WITH L-SORBOSE AND D-MANNITOL

		D-Mannitol +	D-Mannitol −
L-Sorbose	+	208	8
	−	105	67

Entries in the table are numbers of strains of *Candida* and *Torulopsis* species. Data supplied by Dr. B. L. Brady (ref. 9). +, Substrate used; −, substrate not used.

The tables may be generalized in the form:

	$P+$	$P-$
$Q+$	a	b
$Q-$	c	d

The significance of the eight atypical yeasts (Table 4) found to be $Q+$, $P-$ (that is, b) is a function of (i) the total number of strains that are L-sorbose+ ($a+b=216$), (ii) the total number that are D-mannitol− ($b+d=75$) and (iii) the total number of strains ($a+b+c+d=388$). As the proportion in each case is small, namely, (i) $\frac{b}{a+b}=0{\cdot}037$, (ii) $\frac{b}{b+d}=0{\cdot}11$, it is reasonable to think of these eight strains as exceptions to a general rule.

It follows that it may be necessary to weight b according to the size of $(a+b)$. To take an extreme case, if in the above example $a+b=12$ and not 216, then $b=8$ would give little information about the relationship between P and Q. As a first attempt at weighting, a table of $\frac{100b}{a+b}$ was made for all the strains and substrates of van Uden's survey[9]. All pairs of substrates with $\frac{100b}{a+b} \leqslant 10$ (an arbitrarily chosen figure) are shown in the following system:



With not more than 10 per cent of exceptions, the system shows that (i) strains using any of the substrates, except xylose, used mannitol too; (ii) strains using any substrate listed in the left-hand column also used sorbitol; (iii) strains using D-arabinose or galactitol could use D-xylose. Certain examples of these interrelationships are discussed below.

L-Arabinose. L-Arabinose provides the first example: with six or seven exceptions, the strains that used L-arabinose also used sorbitol and mannitol (Table 5). Assuming that L-arabinose is in fact catabolized by way of xylitol (Fig. 3), then this relationship between L-arabinose, sorbitol and mannitol could be explained if (i) the enzyme responsible for xylitol ⇌D-threo-pentulose also catalyses sorbitol⇌D-fructose and (ii) a single enzyme is responsible for both D-mannitol⇌D-mannose and L-arabinose⇌L-arabinitol, as suggested by Horecker[29] with reference to his work on *Candida utilis*[33] and *Candida albicans*[32].

Table 5. RESULTS OF AEROBIC GROWTH-TESTS WITH L-ARABINOSE, SORBITOL AND D-MANNITOL

		Sorbitol +	Sorbitol −	D-Mannitol +	D-Mannitol −
L-Arabinose	+	166	6	165	7
	−	72	48	87	34

Strains of *Candida* species only, otherwise explanation as for Table 4.

Ten strains provide the exceptions to the L-arabinose/sorbitol/D-mannitol relationship (Table 5), of which six were described as '±' for L-arabinose[9]: that is, their ability to use L-arabinose is in doubt. None of the remaining four yeasts used D-mannitol, and only two of them used sorbitol. The behaviour of these four yeasts may be due to their impermeability to the polyols. There is evidence for such impermeability in a strain of *Candida utilis* which has been investigated in some detail. It contains an active dehydrogenase capable of converting sorbitol to D-fructose[34], but, in common with others of this species[9], this strain cannot utilize sorbitol for growth or for respiration, though it can use D-fructose[38]. Experiments with isotopically labelled sorbitol confirm that it does not enter the cells of this yeast[38].

L-Sorbose. The association discussed earlier between L-sorbose and D-mannitol (Table 4) may be explained by the hypothetical scheme shown in Fig. 4. Two transformations, namely, (i) L-sorbose⇌sorbitol and (ii) D-mannitol ⇌D-fructose, could be under the control of one enzyme, such as the NADP-linked dehydrogenase described for *Acetobacter suboxydans*[39–41]. There is evidence that extracts of *Candida utilis*[42] and of *Torulopsis candida*[38] can form L-sorbose reversibly from sorbitol. If yeasts that utilize L-sorbose always do so by the route shown in Fig. 4, then (ignoring any effects of permeability) such yeasts should use D-mannitol, too. The converse, however, would not be expected: first, the catabolism of sorbitol would be a necessary condition for L-sorbose utilization, though not for D-mannitol; secondly, D-mannitol may be oxidized by enzymes of other specificity, such as the one that converts it to D-mannose[29]. Were sorbose to be phosphorylated initially, there would be no obvious reason for its association with D-mannitol.

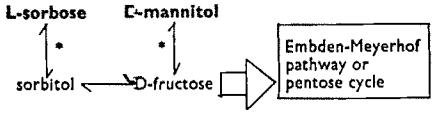


Fig. 4. Scheme for the catabolism of L-sorbose and D-mannitol. * The same enzyme is responsible for two transformations

Metabolic connexions between other substrates listed in this system will be discussed elsewhere. It is, however, worth noting that the position of D-mannitol in the scheme accords with Cochrane's observation[43] that "mannitol is the most generally utilizable of the hexitols, and is equivalent to glucose for many fungi".

Not enough has yet been done to determine the extent or nature of any metabolic differences between the groups of yeasts considered here. However, the mechanisms by which many test-substrates are utilized are clearly linked with each other. Shared enzymes and shared catabolic routes must both contribute to the correlations in the ability of yeasts to use different substrates: these associations are shown up by analysis of the taxonomic observations. The underlying mechanisms are linked, for instance, in the case of the polyols, pentoses and L-sorbose, which constitute about one-third of the test-substrates at present used for classifying yeasts. These substrates cannot be supposed to provide 'independent' tests. An appreciation of the underlying metabolic interrelationships, as well as of the statistical associations between the tests, is essential for a proper evaluation of the numerical taxonomists' practice of 'equal weighting', if that is to be considered as other than an *ad hoc* procedure.

In the past, a number of workers have proposed rules of associations between substrates based on the nutritional tests used for yeasts[44] or bacteria[45]. Many attempts have also been made to interpret the tests in metabolic terms, again both for bacteria[46] and for yeasts[12,47]. In this article a method has been described, which it is hoped to extend and to refine, and by which it is possible to scrutinize the behaviour of large numbers of strains. The increased use of carbohydrates and their derivatives in taxonomy has made this kind of investigation possible; and the yeasts, with their relative metabolic uniformity, are peculiarly suitable for such an analysis.

The method described here directs attention to (i) interrelationships between the results of nutritional tests, and (ii) exceptions to these interrelationships. Certain enzymatic or membrane characteristics have been proposed to explain these interrelationships and their exceptions. These proposals can be checked very readily by the enzymatic analysis of selected individual strains of those yeasts that have provided the general rules and also of those that have provided the exceptions. The method must stand or fall on its ability to predict the results of such analyses.

I thank Dr. B. L. Brady for compiling the observations on strains of *Candida* and *Torulopsis*, Dr. J. C. Gower for sorting these data by computer, and for his advice with Dr. R. M. Needham on handling the numbers, and Prof. S. R. Elsden for his valuable criticisms.

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MECHANOCHEMICAL ENGINES

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THE isothermal conversion of chemical metabolic energy into mechanical work underlies the motility of all living organisms. During the past two decades, synthetic models have been developed which have made it possible to investigate mechanochemical conversion on simpler systems amenable to a fuller theoretical interpretation. Polyelectrolyte fibres played a special part in this respect, since with them reversible contraction and relaxation could be easily obtained, for example, by simply changing the *p*H of the medium[1]. However, the operation of these synthetic fibres was generally slow and of poor reproducibility, so that attention has of late been directed to other materials, such as reconstituted collagen fibres, which undergo reversible 'chemical melting and crystallization' with concomitant contraction and relaxation. Rapid, reversible and reproducible contraction of partially cross-linked collagen can be obtained by treating the fibres with various reagents, such as aqueous solutions of lithium bromide, potassium thiocyanate or urea[2]. The contraction is strongly marked—at zero force a fibre would contract to about half its length, or if a force were applied, the fibre would lift a weight several thousand times its own.

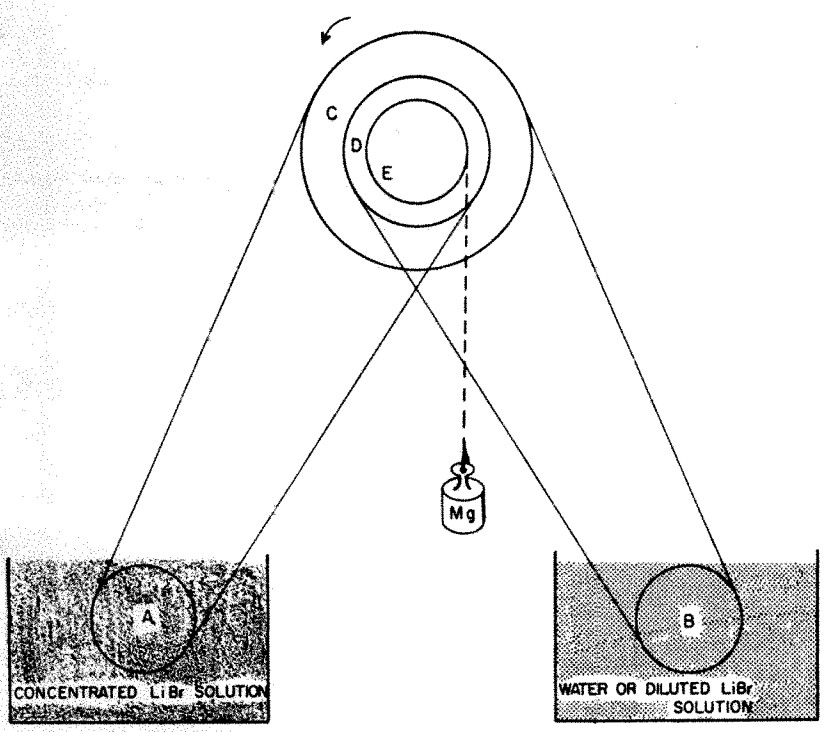


Fig. 1

Washing the fibres with water or a dilute solution reverses the process and leads to recrystallization and elongation, or relaxation, of the collagen. The contraction–relaxation cycle can be repeated many times by bringing the fibre alternately into contact with two liquids, such as a dilute and a concentrated solution of lithium bromide. During this process the solutions are mixed and the net mechanical work obtained in such a cyclic operation results from the free energy of mixing of these solutions. It was considered to be of interest to utilize this alternating contraction–relaxation process of contractile fibres in the construction of a continuously operating engine in which a difference in chemical potential would produce mechanical work in a direct manner. Such an engine should be capable of automatically carrying out the transfer of the fibre from one environment to another. Furthermore, rotary motion seemed to be preferable as the mechanical output of the engine. This article describes some isothermal mechanochemical engines which convert chemical energy directly into a continuous, self-regulating, rotary mechanical performance.

Fig. 1 shows the principle underlying the construction of a family of engines built in this Institute[3].

A closed loop of collagen fibre is wound around the pulleys as indicated in the drawing. When pulley A is dipped into a strong salt solution (such as 8–10 M aqueous lithium bromide) the collagen bathing in the solution contracts and exerts equal forces on the rims of pulleys C and D, which are rigidly connected. Since the radius of C is larger than that of D, a net rotary moment will act on the compound pulley C–D and will cause a counter-clockwise rotation. The rotation will bring a new part of the collagen belt into the salt solution, which reproduces the same effect. It should be noted that at each revolution of the pulley C–D, a longer stretch of fibre is brought in by pulley C than is taken out by pulley D. For a steady-state operation of the engine the net length of fibre introduced by C–D into the salt solution should be counterbalanced by the contraction due to the chemical interaction of collagen with salt. Correct matching of contraction and excess length introduced by C–D is achieved by a proper choice of the radii of C and D, as will be shown later.

The reverse process occurs at pulley B, which dips into water or a dilute salt solution which washes out the salt and relaxes the collagen.

Mechanical work may be obtained by, for example, attaching a suitable weight to the end of a cotton thread wound in the proper direction around pulley E, which has a common axis with C–D. While the engine is working, salt is transferred from the concentrated solution to the dilute one. It is the free-energy change due to this transfer, and to the associated water flow, that is converted partly into the mechanical work.

A useful modification of the model shown in Fig. 1 is represented schematically in Fig. 2.

Here a closed loop of collagen fibre E passes through a salt solution in the lower bath F and through water in the upper bath G. The transmission disks, A and B, rigidly coupled with the main wheels C and D, respectively, are connected by a loop of an inelastic string (a cotton fibre in the machine constructed). As the lower bath is filled up with salt solution, a contractile force develops in the part of the collagen which is immersed in the solution. This force acts on wheels C and D and tends to rotate the former counter-clockwise and the latter in a clockwise direction. The system of wheels will rotate in the direction of the algebraic sum of the moments acting on C and D. For the sake of simplicity, let us assume transmission disks A and B to be of equal radius. If wheel C has a larger radius than that of D, the wheels in the salt bath will turn counter-clockwise. Considerations similar to those presented for the model described in Fig. 1 dictate the choice of the ratio of the various pulleys in relation to the extent of contraction of the fibre.

Fig. 3 is a photograph of an experimental engine of the type drawn in Fig. 2. The upper two pairs of wheels were added to facilitate the entrance of the collagen loop into the water bath.

Let us now consider the mechanical work performed by a unit mass of collagen fibre when it undergoes a complete cycle in the engine depicted in Fig. 2. The specific length, l, that is, the length per unit mass of the collagen fibre, depends on the extent of the interaction of the fibre with salt as well as on the acting force. Hence, l will assume different values at different points on the belt. For the sake of simplicity we shall make several assumptions:

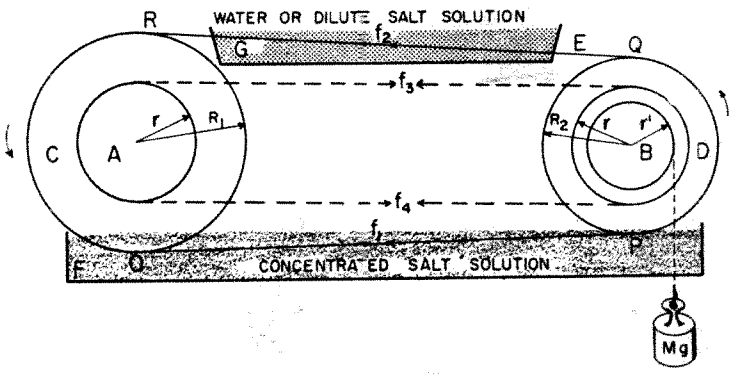


Fig. 2

(*a*) That the force f_1 in bath F between points O and P is constant. Similarly, the force f_2 in bath G between points Q and R is constant.

(*b*) The baths will be regarded as long enough to allow the collagen at P to assume an equilibrium length $\bar{l}_P$ corresponding to the salt concentration in the bath and to the force f_1. Similarly, the passage of the fibre through the water bath will allow the specific length of the collagen at point R to be the corresponding equilibrium length $\bar{l}_R$.

(*c*) We shall finally assume that the fibre firmly adheres to the pulley D between the points P and Q, the specific length remaining there at the value $\bar{l}_P$. A similar assumption is made for the interval R–O where the specific length retains a constant value $\bar{l}_R$.

The mechanical work performed by the engine when a point on the collagen fibre makes a full cycle is given by:

$$W = 2\pi r' \nu M g \tag{1}$$

where r' is the radius of the wheel carrying the mass M lifted by the machine, ν is the corresponding number of revolutions of wheel B, and g is the gravitational constant. Since B and D are rigidly coupled, the wheel D will transfer at the same time a mass m of collagen:

$$m = \frac{2\pi R_2 \nu}{\bar{l}_P} \tag{2}$$

Hence the mechanical work performed per unit mass of collagen transferred, $\overline{W}$, is:

$$\overline{W} = \frac{2\pi r' \nu M g}{2\pi R_2 \nu \bar{l}_P} = M g \bar{l}_P \frac{r'}{R_2} \tag{3}$$

When the machine runs at a constant velocity, the net moment acting on each of the pulleys A–C and B–D is evidently zero, that is (neglecting frictional forces at the bearings and in the solutions):

$$R_1(f_1 - f_2) + r(f_3 - f_4) = 0$$
$$R_2(f_1 - f_2) + r(r_3 - f_4) + r'Mg = 0$$

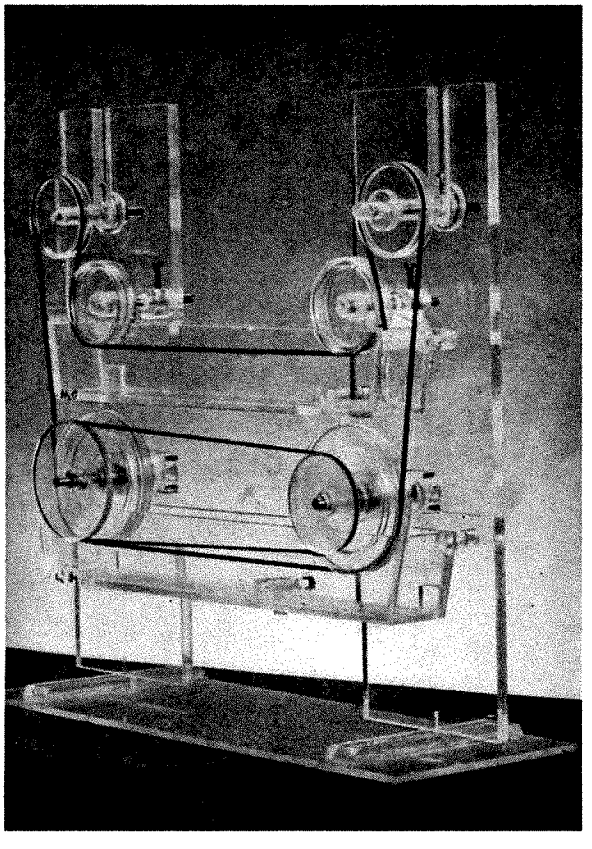


Fig. 3

or

$$(R_1 - R_2)(f_1 - f_2) = r'Mg \tag{4}$$

Introducing equation (4) into equation (3), we get:

$$\overline{W} = \frac{R_1 - R_2}{R_2}(f_1 - f_2)\bar{l}_P \tag{5}$$

For an engine to operate steadily, the amount of collagen entering the salt bath for each rotation of wheel C should equal the amount which leaves the bath with wheel D, or:

$$\frac{2\pi R_1}{\bar{l}_R} = \frac{2\pi R_2}{\bar{l}_P}$$

which dictates the choice of the ratio of the radii:

$$\frac{R_1}{R_2} = \frac{\bar{l}_R}{\bar{l}_P} \tag{6}$$

Introducing equation (6) into equation (5), we obtain the result:

$$\overline{W} = (f_1 - f_2)(\bar{l}_R - \bar{l}_P) \tag{7}$$

For mechanical work to be gained, both terms on the right-hand side of equation (7) should have the same sign.

The efficiency η of the conversion of chemical into mechanical energy is given by:

$$\eta = \frac{\overline{W}}{\Delta n_s \Delta\mu_s + \Delta n_w \Delta\mu_w} = \frac{(f_1 - f_2)(\bar{l}_R - \bar{l}_P)}{\Delta n_s \Delta\mu_s + \Delta n_w \Delta\mu_w} \tag{8}$$

where Δn_s and Δn_w are the number of moles of salt and water, respectively, transported by a unit mass of collagen from the concentrated to the dilute salt solution, whereas $\Delta\mu_s$ and $\Delta\mu_w$ are the corresponding differences in the chemical potentials in the two baths. Since we have neglected frictional losses in the derivation of equations (7) and (8), the real values of W and of η should evidently be smaller than those given by the last two equations.

The mechanical power and the chemical free energy involved have been examined in an engine of the type shown in Fig. 2. The fibre used was a formaldehyde-tanned 4/0 reconstituted collagen tape, which has been pretreated with an 11·0 M lithium bromide solution at 60° C for 10 min and then washed in water and dried in air overnight. The weight of the fibre was 44 mg. The machine had the following dimensions: the distance between the axes of the wheels was 20·0 cm; R_1 = 3·44 cm; R_2 = 3·05 cm; R_A = 3·55 cm; r = 3·15 cm. 11·25 M and 0·3 M lithium bromide solutions respectively were pumped through split tubes which served as the two baths. The mechanical power output was measured by means of a friction belt on an auxiliary wheel coupled to the machine. The measurements were carried out until the concentration of the concentrated solution changed by about 10 per cent. During this period (about 1 h) both the power and the chemical flows were practically constant. At a temperature of 23° C, wheel B made 40 r.p.m. under zero load. The maximum power obtained was about 0·03 W/g collagen. This value is practically identical with the maximum power, per g contractile proteins, of the sartorius muscle in the frog (calculated from the data of ref. 4).

Pryor[5] described a device in which a wheel with spokes made of collagen fibres was suspended in a trough filled to the level of the wheel axle with a concentrated solution of lithium bromide and above that with water. Contraction of the lower spokes placed the wheel in imbalance, thus effecting rotation. That device produced a complete rotation of the wheel in about 20 min. Obviously, spontaneous degradation of the chemical free energy due to diffusion was an inherent defect in that apparatus.

It is most probable that the machines described can be operated by other fibres that undergo reversible contractions and relaxations under the influence of suitable reagents. Furthermore, a continuously working thermal engine of the same design could be envisaged with

materials which develop varying contractile forces with changes in temperature. For example, rubber strips could replace the collagen fibres and two compartments at different temperatures could replace the chemical baths.

We thank Mr. M. Feld for his excellent work in the construction and development of mechanochemical engines; the Ethicon Co., Somerville, New Jersey, for the supply of collagen fibres and help in various stages of our research; Mr. J. Yonath for helpful discussions and Dr. G. Goldman for providing his preliminary experimental results before publication.

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SCIENCE IN CHEMICAL ENGINEERING*

By PROF. P. V. DANCKWERTS

President, Institution of Chemical Engineers

CHEMICAL engineering was not originally science-based in the same sense as electrical engineering. Although the chemical industry was firmly based on the science of chemistry, the role of the chemical engineer was originally merely to provide vessels, pipes, pumps and so on to enable a reaction to be carried out under the conditions specified by the chemist. It is true that there are early examples of where the engineering interacted with and influenced the process—for example, the lead-chamber sulphuric acid process, the Solvay ammonia-soda process and the Haber-Bosch ammonia synthesis. In general, however, the chemical engineer had little influence on the process, and there was very little science involved in chemical engineering. The most difficult part of the job was the choice of the right materials of construction—but until quite recently science has been of little help in this respect.

The type of chemical engineer I have just described—typical, perhaps, of the year 1900—has given way to someone who occupies a very different position. The scientific and unspecialized nature of the training of the chemical engineer fits him for employment in many industries apart from the chemical industry—for example, combustion engineering, food processing and extraction metallurgy. The proportion of chemical engineers employed in non-chemical industries in Britain has risen steadily in the past decade, and now amounts to about 25 per cent. This wider dissemination of the philosophy of chemical engineering is an important feature of the development of the profession, and is likely to be of great benefit to certain industries which have been somewhat isolated from the main stream of technological development.

I shall use the phrase "chemical engineering science" to mean the science deployed by chemical engineers in their various activities. It is clear that the chemical engineer does not himself always have to develop scientific methods for solving the problems which he encounters. For example, although he is professionally very deeply concerned with the properties of materials, he is not usually thought to be responsible for developing the science and technology of materials—metallurgy, corrosion, refractories, and so on. Other technologists have assumed this responsibility, which on the whole requires a different scientific background from that of the chemical engineer. Chemical engineering science is, therefore, the body of applied science developed by chemical engineers for their own purposes, in fields not covered by other branches of technology. It would be a great mistake to think of the content of chemical engineering science as permanently fixed. It is likely to alter greatly over the years in response to the changing requirements of industry and to the occasional technological 'break-through'.

The functions of chemical engineering science are mainly economic. One is the development of quantitative design procedures, so that full-scale plants can be designed by calculation, if necessary with the help of laboratory-scale experiments, but if possible without the need for expensive and time-consuming pilot-scale experiments. The more precise the design procedures which can be developed, the more precisely is it possible to optimize the design of plant, and the narrower becomes the wasteful margin of safety imposed by ignorance. There is also the need to improve the efficiency of processes and of the plant in which they are carried out—for example, to obtain a higher yield in a chemical reaction, a higher plate-efficiency in a distillation column, or a machine which will produce granular material of a more nearly uniform size. There is also the matter of true invention, leading to quite new processes and devices. Although science may not always provide the inspiration for inventions, it must usually be called in to develop them properly. Finally, there is the need to develop not only automatic but also self-optimizing processes and plants; in the chemical factory of the near future we shall have replaced not only the workman but the management by instruments.

The field of automation, incidentally, is a good example of the way in which the relative importance of different parts of the field of chemical engineering science can rapidly alter. Ten years ago most of us did not see beyond the simple feed-back control loop, and now a considerable part of the literature is concerned with much more sophisticated topics in the field of automation.

Pilot-scale experiments are of course not only expensive, but also occupy much time, and in the chemical industry it is often of vital importance to bring new processes into production quickly. The smaller the scale on which experimental work is done, the cheaper and quicker it will be. Much of chemical engineering science is therefore concerned with the problem of scaling-up. How does one design small-scale experiments which will give the information required for the design of full-scale plant? To what extent is it possible or necessary to build a true model of the plant? Is it possible to abstract some essential features and model these, while relaxing other criteria of similarity? Unfortunately, the type of plant used in industry for the sake of its efficiency or convenience is often very difficult to represent by a small-scale model. For example, in a fluidized-bed reactor we know that the effectiveness of the catalyst is likely to be influenced by the possibility that some of the reactant stream passes through the bed in bubbles, and does not come into contact with the catalyst. It is most unlikely that the effectiveness will be the same in a small model, even if we try to make all the operating parameters the same. Can we, therefore, derive any useful conclusions from observations on a small fluidized-bed reactor? In order to do so, we need a real insight into the

* Based on a draft for the Presidential Address to the Institution of Chemical Engineers, April 26, 1966.

mechanics of fluidized beds, on both the small and large scales; this may not enable us to make design predictions by means of calculations based on first principles, but without it we shall not know what experiments to carry out, or how to apply the results to design. One of the principal objectives of chemical engineering science is to provide methods for the design of experiments which will yield information of direct value to designers of processes which are too complicated to be modelled exactly or to be subjected to precise mathematical analysis.

Chemical engineering science, like other branches of science, is of course being profoundly influenced by the use of high-speed automatic computers. For one thing, the formulation of the problems is altering; no longer is it so important to be skilful in constructing conceptual models which bear an adequate resemblance to the problem under consideration and which can also be described in tractable mathematical terms. One can present the problem to a computer in a much more realistic form, and expect to get a much more accurate answer. This trend towards realistic calculation also creates a demand for more accurate knowledge of the physical behaviour of the system and is likely to make further experimental work rewarding rather than render it unnecessary. For example, trial-and-error plate-to-plate calculations for multi-component distillation equipment can now be carried out with great facility; the bottleneck in optimizing the design of such equipment lies in the limited knowledge of the factors which determine plate efficiency, and in the limited availability and accuracy of vapour-liquid equilibrium data.

In the department of physical chemistry, the laws governing chemical and phase equilibrium are among our most important tools. It is, of course, not enough to understand the principles—one must have thermodynamic data for calculation. I feel that an important field of activity for chemical engineers is the development of scientific methods for estimating thermodynamic quantities, both chemical and physical, in systems where they have not been measured.

The physical chemistry of interfaces has become of increasing interest to chemical engineers in recent years, particularly in relation to the formation and behaviour of drops, bubbles, foam, in mass-transfer equipment. In many cases we have a qualitative insight into the phenomena, but quantitative prediction is usually difficult—indeed, the profound effect of very small quantities of impurities sometimes renders it impossible to foresee the behaviour of a system even approximately. We may find that foaming is the limiting factor in the operation of a distillation column or an evaporator, or emulsification in the case of solvent extraction, and that the conventional problems of mass and heat transfer become of quite secondary importance.

Still in the department of physical chemistry, a topic of supreme importance for the chemical industry is that of catalysis. In general there seems to be a very wide gap between fundamental scientific understanding of the behaviour of heterogeneous catalysts, on one hand, and industrial practice, on the other hand.

A sub-department of chemical engineering science, sometimes called chemical reaction engineering, has grown up to deal with the design and optimization of various types of chemical reactor. The reaction kinetics themselves are usually taken as given, and attention is concentrated on such aspects as mass-transfer of reactants and products, back-mixing of reactant-streams and residence-time distribution, the control of temperature and the coalescence of liquid drops.

The next department to consider is that of fluid mechanics Many of the aspects which are of particular interest to chemical engineers are still in a state of obscurity, although promising work is in progress. Much remains to be done in the flow of liquid-gas, liquid-solid and gas-solid mixtures in pipes; the mechanics of fluidized beds; the settling of dense suspensions; the mechanics of bubbles, froths and foams; the flow of non-Newtonian fluids; and the mixing of fluids. The industrial importance of all these fields is not in doubt, yet I have the impression that real progress is extremely slow.

Turning to transfer processes, one finds a similar situation. Industrial mass-transfer equipment, for example, is designed to be effective, not to be simple for chemical engineering scientists to analyse. It is still not possible (in spite of the recent concentrated research programme of the American Institute of Chemical Engineers) to predict plate-efficiencies of distillation column for novel mixtures with any confidence. Much of the published work in this field has been of rather academic interest, and there are many problems of outstanding practical importance still to be solved.

The department of logic and control theory is perhaps the most active part of chemical engineering science these days. The subjects dealt with include the application of computers to standard design procedure (for example, the calculation of the number of equilibrium stages required in a distillation column); the development of procedures for optimizing the design of single items of plant and of complete factories; methods of feed-back and feed-forward control, control by on-line digital computer, and optimal and adaptive control systems. It is in these directions that the most striking and rapid developments in the chemical and petroleum industries are coming about at the moment, and the department is naturally one which is attracting a great deal of attention from chemical engineering scientists both in industry and in academic life. In fact, it may be claiming more than its fair share of attention. It brings some of the glamour of the space-age to the down-to-earth job of the chemical engineer, but no amount of automation will make a success of a process which is not sound from the engineering point of view.

The last department I should like to mention is the design of chemical plant and machinery, in the structural and mechanical sense. The influence of science in this important field seems to be extremely small, in any event in Britain. It is probably a weakness of the British system that scientifically trained chemical engineers tend to be process designers rather than designers of equipment and machinery, while scientifically trained mechanical engineers have too little insight into chemical engineering operations to make a great contribution to the development of equipment and machinery. It is true that there are certain fields, such as the design of pressure vessels, where scientific method has had an influence; and there are others, such as the design of pumps, where the chemical engineer has benefited from developments in general engineering. On the whole, however, the design of chemical engineering equipment and machinery as such seems to have attracted relatively little scientific attention.

There are some welcome signs that chemical engineering science is moving away from its excessively physical standpoint of the past few decades, to one in which more interest is taken in chemical phenomena. In the universities, both in teaching and in research, there may be advantages in placing more emphasis on applied chemistry and the chemical phenomena which form the basis of the chemical industry. The McLeod Report exposed the lack of research in chemical technology in our universities, and recently certain steps have been taken which will help to remedy this situation. As for teaching, I am sure that if we could turn out a chemical engineering graduate with a much deeper insight into chemical phenomena than we do at the moment, he would prove to be invaluable in process development work in the chemical industry. I must admit, however, that within the limits of our present university course, we can do little in this direction without a reduction in the attention paid to other topics. Perhaps the solution is to have degree courses which vary considerably in flavour from one university to another—a possibility envisaged in the *Scheme for a Degree Course*

which the Institution of Chemical Engineers has recently produced.

Surveying the field of chemical engineering science as a whole, therefore, there are certain fields, such as chemical thermodynamics and the logical-mathematical field, which can be developed rigorously from known principles. There are others, such as multiphase fluid mechanics and the technology of granular materials, which are of great technical importance but which are much less highly developed in principle, and require a combination of physical insight and experimental flair if progress is to be made. I feel that chemical engineering science should not become exclusively identified with the 'rigorous' subjects, and that we should be ready to use science to solve any of the problems of chemical engineering, however ill-formulated they may be. Those of us who work in universities or research institutes, and who claim to be chemical engineers, should be guided in our work by the genuine difficulties and economic requirements of industry, and not develop subjects for the sake of their intellectual interest alone. There is, I believe, sufficient intellectual interest in identifying the real problems and formulating them and trying to solve them (if only partially).

NEWS AND VIEWS

New Members of the National Academy of Engineering

THE following have been elected to membership of the academy: H. JULIAN ALLEN, director, Ames Research Centre, National Aeronautics and Space Administration; L. L. BERANEK, president, Bolt, Beranek and Newman, Incorporated; PROF. D. S. BERRY, Department of Civil Engineering, Northwestern University; H. G. BUSIGNIES, general technical director, International Telephone and Telegraph Corporation; PROF. A. CASAGRANDE, professor of soil mechanics and foundation engineering, Harvard University; PROF. T. H. CHILTON, visiting professor, University of Virginia; E. E. DAVID, JUN., executive director, Communications Systems Research Division, Bell Telephone Laboratories, Incorporated; PROF. H. E. EDGERTON, Department of Electrical Engineering, Massachusetts Institute of Technology; J. B. FISK, president, Bell Telephone Laboratories, Incorporated; E. G. FUBINI, vice-president for research and development, International Business Machines Corporation; W. M. HAWKINS, assistant secretary for research and development, United States Department of the Army; W. R. HIBBARD, director, Bureau of Mines, United States Department of the Interior; A. A. H. KEIL, technical director, David Taylor Model Basin, United States Department of the Navy; R. KOMPFNER, associate executive director of research, Communications Sciences Division, Bell Telephone Laboratories, Incorporated; H. LANDSBERG, director, Environmental Data Service, United States Department of Commerce; PROF. W. K. LEWIS, professor emeritus and honorary lecturer, Massachusetts Institute of Technology; F. K. MCCUNE, vice-president, Business Studies Service, General Electric Company; PROF. R. D. MINDLIN, professor of civil engineering, Columbia University; B. M. OLIVER, vice-president for research and development, Hewlett-Packard Corporation; E. R. PIORE, vice-president and chief scientist, International Business Machines Corporation; C. F. PRUTTON, director, FMC Corporation; J. A. RAJCHMAN, director, Computer Research Laboratory, RCA Laboratories, Radio Corporation of America; PROF. H. ROUSE, director, Institute of Hydraulic Research, University of Iowa; J. W. SIMPSON, vice-president and general manager, Electric Utility Group, Westinghouse Electric Corporation; PROF. V. SUOMI, Department of Meteorology and Soils, University of Wisconsin; PROF. O. G. VILLARD, JUN., professor of electrical engineering and director of Radioscience Laboratory, Stanford University; J. B. WIESNER, dean of science, Massachusetts Institute of Technology.

Dr. Kaysen to head Institute of Advanced Study

WHEN Dr. J. Robert Oppenheimer retires on July 1 after 19 years as director of the Institute for Advanced Study at Princeton, New Jersey, he will be succeeded by Dr. Carl Kaysen. Kaysen is professor of political economy at Harvard University and since 1961 has been associate dean of the Harvard Graduate School of Public Administration.

Dr. Kaysen received an A.B. in 1940 from the University of Pennsylvania and an A.M. in economics in 1947 from Harvard. He was a Junior Fellow of Harvard's Society of Fellows from 1947 until 1950. He received a Ph.D. from Harvard in 1954 and was appointed a full professor in 1957. In 1956 he was a visiting Fulbright professor at the London School of Economics and a Guggenheim Foundation fellow. From 1961 to 1963 he was on leave of absence from Harvard to serve as President Kennedy's Deputy Special Assistant for National Security Affairs. As an adviser to both Kennedy and Johnson, his work has centred on problems of nuclear disarmament, the military budget, and planning; he has served as a consultant to the Departments of Defense and Justice.

Oppenheimer, now 61, has been head of the Institute since 1947, after being in charge of the Los Alamos atomic bomb project during the Second World War. He will remain at Princeton as senior professor of theoretical physics, the post once held by Albert Einstein.

The fact that Kaysen is a social scientist is not expected to change the emphasis of the Institute. His three predecessors include Abraham Flexner, an educator and one of the founders of the Institute, who was director from 1930 to 1939. Frank Aydelotte, president of Swarthmore College for 19 years, directed the Institute from 1939 to 1947, when Oppenheimer was appointed.

The permanent faculty consists of thirteen research workers in historical studies, ten mathematicians, and five natural scientists. In addition, about a hundred temporary members are invited for one or two years.

Defence Studies in the United Kingdom

IN a written answer in the House of Commons on March 2 regarding the development of higher defence studies in the United Kingdom, the Secretary of State for Defence, Mr. Healey, stated that one of the primary objectives of the Government was to stimulate the interest of the universities in this field, and to encourage them to play a more active part as well as to foster general intellectual awareness of the problems of defence. As already announced, the Government proposed to award up to six fellowships annually to enable senior serving officers to study or carry out research in the atmosphere of a university. The Fellows themselves were expected to gain substantially. The Services and Ministry would have the benefit of the greater capacity for analysis which the students would acquire, and the universities would benefit from contact with experienced students of defence. The Government has also announced its intention of creating, at public expense, in each of a few selected universities,

an additional post of lecturer or senior lecturer in an existing faculty to specialize in the defence aspects of its discipline. Mr. Healey did not envisage initially any restrictions as to the faculties in which these posts should be set up. Teachers in any of the social or physical sciences or in technology with an interest in defence problems would be encouraged to specialize in their study. Eventually it was hoped that defence studies would emerge as a discipline in its own right in the universities concerned. The Defence White Paper also referred to the organization of short, intra-mural courses in the widest possible variety of subjects. Some of these would be organized in collaboration with the lecturers specially appointed under the arrangements mentioned. Some would be concerned with specific subjects of present and impending interest in the defence field. Others would deal with the techniques of management, and it was hoped to collaborate with the business management schools recently established at London and Manchester. These proposals represented a modest step forward on an experimental basis and at the same time the Government intended to create, within the Ministry of Defence, an organization to undertake, in an atmosphere free from day-to-day problems, research and long-range planning in the defence field. This organization would be designed to enable Britain to carry out research and to attempt to identify problems before they arose and to make available the results of the various studies to all those concerned with the day-to-day formulation and conduct of British defence policy. This activity would be carried on in association with the new university posts of lecturer or senior lecturer which were to be created, as well as with other universities or research centres which could help. Close liaison would also be maintained with Government departments concerned.

The Countryside and Leisure

THE White Paper, *Leisure in the Countryside: England and Wales*, amplifies slightly the statement on Countryside Parks which the Minister of Land and Natural Resources made to the 'Countryside in 1970' Conference in November 1965. However, it does not meet the real criticism of his proposals (Pp. 15. Cmnd. 2928. London: H.M.S.O. 1*s*. 6*d*. net). Parliament is to be invited to give elected local bodies the powers and the financial help they need to ensure that the resources of the countryside match the needs of the nation and to convert the National Parks Commission into a Countryside Commission. This Commission will be responsible for promoting and co-ordinating action and advising the Government on the measures needed for full enjoyment of the countryside and protecting its natural beauty. While reconnaissance, planning and research are proposed locally and nationally pending the introduction of an appropriate Bill, there are no proposals to provide the Commission itself and the Park authorities with either the financial resources or the powers they need for effective discharge even of their past responsibilities. On administration, the Government is content to hope that for Parks covering more than one county, the planning authorities concerned will consider very seriously the possibility of a change to joint planning boards, although the Government will consider exercising its power to revoke existing arrangements if further experience shows this to be required. For the future there will be a 75 per cent grant towards appropriate items of current expenditure and towards loan charges in respect both of National Parks and Areas of Outstanding Natural Beauty, and it is also proposed that an Exchequer grant at the rate of 75 per cent should be payable towards the additional administrative expenses of National Park Planning Authorities in creating new facilities or improving the landscape. The main instrument for creating County Parks would be the County Councils, and grant will be payable on approved expenditure. Other proposals relate to access to water, both gathering grounds and rivers and canals, to open country, to trees and woodlands. A reference to long-distance routes, however, reads strangely in the light of the comment in the National Parks Commission that progress here has been limited by the financial restrictions the Government itself imposed on the Commission.

Problems of Peace

IN his 1965 Alex Wood Memorial Lecture, *Dilemmas of Peacemaking*, Prof. E. G. Rupp concentrates, like R. Ullmann, on what are called 'East–West relations', and from a Christian and historical point of view (Pp. 10. New Malden, Surrey: The Fellowship for Reconciliation, 1965. 2*s*.). Indeed, Prof. Rupp describes his work as an afterthought to Ullmann's pamphlet *Dilemmas of a Peacemaker*. The experience of the Christian Peace Movement has been to discover men of great understanding, Christian spirit and integrity, and with more common ground than would have been conceivable if the confrontation between East and West had not been attempted. The confrontation has led to other positive gains, such as the realization of the deep underlying appreciation of the futility and evil of war, the dissolution of the problem of peace into its component parts and the demonstration of its indissoluble links with social justice. Prof. Rupp emphasizes the risk of misunderstandings through the use of such terms as peace, democracy and freedom when different meanings are attached to them, and he urges that the response to appeals to renounce war must be supported with intelligence and determination by millions. He agrees with Lincoln that these great concepts derive their meaning not from intellectual debate and discussion but from life itself.

New NASA Tracking Antenna

A NEW automatic tracking antenna, 210 ft. in diameter, was dedicated on April 29 at the Goldstone Communications Complex in the Mojave Desert, California. When it becomes operational in May or June it will be used by the National Aeronautics and Space Administration's Deep Space Network for tracking unmanned vehicles in space. The new antenna just completed by the Jet Propulsion Laboratory is a parabolic aluminium dish measuring 234 ft. from the desert floor to the peak of its apex. The entire structure, including the pedestal, weighs 8,000 tons. Rohr Corporation of San Diego, the prime antenna contractor, and dozens of other firms have taken 6 years to complete this 14 million dollar project.

The enormous size and nearly perfect contour of the "210" gives it a greater range and sensitivity than even larger antennae now in operation, such as the 250 ft. at Jodrell Bank. Its 715-ft. circumference forms a circle with an accuracy within 0·05 in., and is contoured to an accuracy of less than 0·25 in.

The Goldstone station is the primary station of the Deep Space Network, with other stations at Johannesburg, South Africa; Woomera and Canberra, Australia; and Madrid, Spain. This network is used only for tracking and two-way communication with unmanned vehicles, such as the *Pioneer* and *Ranger* series and the Venus and Mars probes of the *Mariner* spacecraft. It is distinct from the other NASA networks such as the Manned Space Flight Network, which tracks the *Gemini* and *Apollo* spacecraft, and the Scientific Satellite Network, which tracks Earth-orbiting scientific, meteorological and communications satellites.

NASA plans to use the "210" for continued tracking of the *Pioneer VI* spacecraft now in orbit around the Sun. With the 85-ft. antennae at present being used, the *Pioneer* could be followed for 6 months, but the more sensitive "210" should increase the useful life of the spacecraft to 14 months.

The reflector and its supporting structure rotate on a hydrostatic bearing. The base of the antenna is a pedestal

housing the control room, machinery and equipment rooms and the communications systems. It is anticipated that antennae the size of the "210" will be constructed eventually at other sites of the Deep Space Network.

Electrical Biology Laboratory

A LABORATORY for biological research for the electrical industry has been established by the Central Electricity Generating Board at Leatherhead, Surrey. The Laboratory was opened officially on May 6, 1966, by Sir Cyril Hinshelwood. It is intended to be a centre for the biological research of all kinds carried out by electricity authorities in all parts of Britain. Among the problems within the scope of the new Laboratory are the study of the growth of barnacles and mussels in power station cooling water. Mussels can detract from the operating efficiency by lodging in condenser tubes, thus causing corrosion. The same tubes are ideally suitable for the growth of many other marine organisms, which must be eliminated without, at the same time, so contaminating the water as to make it a public hazard. The Laboratory is also concerned with biological problems arising from the increased temperature of water discharged from power stations. The facilities of the Laboratory will include special tank rooms for the study of freshwater invertebrates. It will have X-ray machines which will, in particular, be able to study the effect of temperature on the shipworm *Teredo*—a pest of wooden marine structures. It is also intended to study the biological problems arising from the use of pulverized fuel ash in the reclamation of agricultural land.

Modern History

IN his presidential address to the Diamond Jubilee Conference of the Historical Association, Geoffrey Barraclough, Fellow of St. John's College, Cambridge, stressed that historians must offer the general public something more than technical controversies if they wished to compete against "the attractions of modern science".

Speaking on the subject "History and the Common Man", Barraclough said that the study of history has lost its sense of purpose and is suffering from what he termed a *malaise*; to the ordinary educated mathematician, nuclear physicist, and natural scientist, "history is a form of entertainment; . . . relaxation for the intelligent scientist or technologist who is a cut above the telly and does not take to novels". He added that there was "a sharp cleavage between history written for the public, which is not scholarly, and scholarly history which is not read by the public".

The two aspects of current historical teaching and research that Barraclough felt must change are its lack of relevance to present events and its fundamental postulate that history is the key to the explanation of events. He deplored the fact that the teaching of history was only slowly changing from the study of minute investigations of obscure details of British history to leave more time for the broader issues of European and world history.

Barraclough strongly attacked the Germanic approach that searched for the causes and motivations of events rather than their consequences. He noted that the Danish theologian Kierkegaard said that history should concern itself with results, while motives and intentions are the business of ethics. Barraclough expressed the belief that this historical guesswork is being replaced by a more scientific approach to history, in spite of a "suicide squad fighting a stubborn rearguard action". This means, he added, that "historians, at last, are breaking away from their vain pre-occupation with motives and causes and surmise, and getting down to facts".

It will be interesting to see if this hopeful prediction is fulfilled, and, if so, whether history will become less entertaining in the process.

University News: Glasgow

THE following have been appointed to chairs: H. B. Sutherland (civil engineering); Dr. I. A. Boyd (physiology); Dr. R. M. S. Smellie (biochemistry).

London

SIR THOMAS CREED, principal of Queen Mary College, has been elected vice-chancellor as from September 1, for the university year 1966–67. Prof. C. T. Ingold, of Birkbeck College, has been appointed deputy vice-chancellor as from September 1 for the university year 1966–67. The title of professor has been conferred on the following: Dr. R. H. Gorrill (bacteriology, in respect of his post at Guy's Hospital Medical School); Dr. E. Jean Hanson (biology, in respect of her post at King's College); Dr. C. W. Kilmister (mathematics, in respect of his post at King's College).

Announcements

DR. V. BUSH was awarded the first Founders' Medal of the National Academy of Engineering during the second annual meeting of the National Academy of Engineering on April 27. The Founders' Medal was established in April 1965 to honour outstanding contributions by an engineer both to his profession and to society.

PROF. B. EDLÉN of Lund has been awarded the Mees Medal of the Optical Society of America for distinguished work over a period of more than thirty-five years on the light emitted by the vapours of highly ionized elements.

A SYMPOSIUM on "Simulation Techniques and Languages" will be held in Brunel College during May 10–11. Further information can be obtained from the Academic Registrar, Brunel College, Woodlands Avenue, Acton, London, W.3.

THE second international congress on "Hormonal Steroids" will be held in the University of Milan during May 23–28. Further information can be obtained from Dr. L. Martini, Istituto di Farmacologia, Via Andrea del Sarto 21, Milan.

A SHORT course on "High Temperature Resistant Polymers" will be held at the Bradford Institute of Technology during May 13–14. Further information can be obtained from the Registrar, Bradford Institute of Technology, Bradford 7.

THE fifth international congress of the precast concrete industry will be held in London during May 21–27. Further information can be obtained from the Organizing Secretary of the Congress, Terminal House, Grosvenor Gardens, London, S.W.1.

THE third annual national colloquium on "Information Retrieval" will be held in the University of Pennsylvania during May 12–13. Further information can be obtained from A. W. Speakman, E. I. du Pont Company, Centre Road Building, Wilmington, Delaware.

A SYMPOSIUM on "Physico-Chemical Processes in Mixed Aqueous Solvents" will be held at Bradford Institute of Technology during May 23–24. Further information can be obtained from Dr. F. Franks, Department of Chemical Technology, Bradford Institute of Technology, Bradford 7.

A COURSE on "Cancer Chemotherapy" will be held in the University of Texas during May 9–21. Further information can be obtained from the Division of Continuing Education, University of Texas Graduate School of Biomedical Sciences at Houston, 102 Jesse Jones Library Building, Texas Medical Center, Houston.

THE sixth international symposium on "Condensation Nuclei" will be held in Albany, New York, and University Park, Pennsylvania, during May 9–14. Further information can be obtained from the chairman of the Organizing Committee, Office of the Director, Atmospheric Sciences Research Center, State University of New York, Post Office Box 7112, Albany, New York.

PLANS FOR HIGHER EDUCATION

IN opening a debate on technical education in the House of Commons on March 3, Mr. David Price drew attention to the increase in the number of students attending sandwich courses. This had risen from 4,000 in 1956 to 22,000 in 1964, and the number of day releases had risen by 196,000 to 574,000 in the same period. The number of students taking advanced courses leading to recognized qualifications at grant-aided establishments had risen from 90,000 in 1958 to 158,000 in 1964. After asking for a much greater breakdown of the figures given in the National Plan, Mr. Price urged the need for management studies in technical colleges. He thought there should be, first, a general type of course aimed especially at the established manager whose educational background might be poor; secondly, he recommended courses to keep current management in touch with new management thinking and techniques; and, thirdly, courses giving instruction in particular techniques. Sir Edward Boyle hoped that the Government would make every effort to keep the figures given in the National Plan suitably up to date, and thought there was probably a need in the new business schools for a good 2-year postgraduate university course in business education of a high intellectual level, though it should not be too academic.

In replying on the debate, the then Minister of State, Department of Education and Science, Mr. R. E. Prentice, said the National Plan hoped by 1970 to achieve a total of 70,000 students taking higher education courses compared with the figure of 50,000 recommended in the Robbins Report for 1973. Some 5,000 students were taking more specialized courses in management and about 8,000 were taking other professional examinations which included management as part of the course. In November 1964, about 276,000 people were getting day release, about 31 per cent of the boys and 7 per cent of the girls. The programme for 1966–67 was to cost £27 million and this would also be the figure for the 1967–68 programme. The National Advisory Council on Education in Industry and Commerce had been making four relevant studies; a study into the size of classes; another into buildings; one into the administrative methods of procedures; and a study of the organization of courses. These were at different stages, but the first study was complete and the report was being discussed with the local authority associations and others concerned. The position would also be improved by the raising of the school-leaving age in the 1970's, the impact of the Industrial Training Act, the expansion of the system of further education and the expansion and improvement of the youth employment service. In a written answer in the House of Commons on March 8, the Minister of Housing and Local Government, Mr. R. Crossman, stated that new arrangements were urgently needed to give an impetus to research in the environmental field. The task of reshaping existing towns, building new cities and meeting the needs of a fast-growing, mobile population demanded a vigorous, comprehensive and sustained programme of research. The failure in the past to sponsor research in this field meant that the data needed for sound policy decisions were lacking. It had been decided, therefore, to establish a Centre for Environmental Studies where practitioners and research workers, both at home and abroad, could discuss problems and settle research needs. The Centre would also be an important channel through which research contracts could be placed with universities and institutes. The Centre would be established as an independent educational trust. It was intended that it should be financed partly out of money voted by Parliament and partly from outside sources, and the Ford Foundation had agreed to make a substantial donation to the Centre over the next 5 years. Lord Llewelyn-Davies, professor of architecture in the University of London, had accepted an invitation to become chairman of the Governing Body of the Centre.

On March 9, the Prime Minister announced in the House of Commons that after careful examination of the issues involved the Government proposed that the closed period for public records should be reduced from 50 to 30 years. In reaching this conclusion they had had full regard to the recommendation of the Advisory Council on Public Records that the period should be reduced only to 40 years. The Council had found it difficult to decide between 40 and 35 years and had coupled its recommendation in favour of a reduction with a further recommendation that scholars undertaking research relating to matters falling within the period should have more liberal access to official records, including Cabinet records. The Government concluded, however, that a clean-cut closed period was to be preferred and that the period of 30 years would be reasonable. While the Leader of the Opposition agreed that a specific closed period was preferable in principle, he was not satisfied that reduction to 30 years was justified, mainly because of possible embarrassment to politicians and civil servants. The Government, however, did not think that the risk was significant.

EDUCATION AND THE AVIATION ESTABLISHMENTS

IN December 1964 the Electronics Research Council established a working party, under the chairmanship of Sir Nevill Mott, to examine the educational role of the Ministry of Aviation's establishments. Previously the Council's Working Party on Solid State Devices had recommended that the functions of the Ministry's establishments should be reviewed in order to determine what aspects of the special knowledge of their scientific staff could possibly be made available to students in higher education.

Obviously the establishments have much to offer in physics, electronics and related subjects, and the talents of the staff could be made available through secondment to universities and through students working at the establishments. The working party was therefore also asked to examine and consider the role of the universities in ensuring that students receive education which fits them for work in research, development and production in the subjects mentioned.

The earlier working party had also concluded that the establishments could—and should—play a greater part in helping civil developments in the electronics industry. It had recommended, in particular, that the possibility should be explored of establishing viable applied research units at selected universities. Such units, it was hoped, would provide incentives and opportunities for a few university departments to contribute in the field of solid-state devices, and would encourage students and post-graduates to make careers in applying solid-state physics to industry.

The working party surveyed the present position in the United Kingdom and at the same time compared it with the contribution which such establishment staff make to formal teaching in The Netherlands. It was impressed by the educational effort already made within the present mandates, and it concludes in its report, entitled *The Educational Role of the Ministry of Aviation Establishments**, that, as regards general scientific education, some encouragement and the removal of certain obstacles are all that are required.

On the other hand, as regards training which leads to design and development, the position is less satisfactory and it is here that the main responsibility of the establishments lies. The working party recommends that any establishment concerned with research and development in a given branch of technology should be encouraged to accept certain responsibilities for advanced training in this field and for seeing that educational potential resulting from its own work is properly utilized. This teaching mandate should be exercised not only as already in sandwich courses but also in postgraduate training in technology, and occasional teaching in postgraduate courses at the universities. To this end, the working party recommends that universities should be encouraged to offer selected members of establishment staffs part-time teaching positions with responsibilities particularly for postgraduate teaching similar to those in Holland.

The working party also believes that university and technical college staff should be encouraged to use research facilities in establishments and given in establishment programmes. Furthermore, it considers that the Ministry of Aviation should discuss with the Science Research Council ways to take account of this possibility in allocating research grants—provided that use of the facilities is more or less guaranteed. It also suggests that establishments should continue to place research contracts in universities, especially for work likely to lead to military or commercial development.

Finally, it recommends that special relations between the establishments and neighbouring universities should be developed where appropriate and that location of neighbouring research establishments should be taken into account when starting new universities or colleges, and vice versa.

* Ministry of Aviation: Electronics Research Council. *The Educational Role of the Ministry of Aviation Establishments—Working Party Report.* Pp iv+24. (London: H.M.S.O., 1966.) 2*s* 3*d* net.

EXOTIC ACACIAS IN SOUTH AFRICA

A NUMBER of Australian phyllode-bearing acacias introduced into the Cape Peninsula of South Africa are now conspicuous members of the local vegetation. They include *Acacia melanoxylon*, *A. longifolia*, *A. cyclops* and *A. cyanophylla*. At first cultivated, they have for some decades been spreading by natural means, and the tendency of the different species to occupy particular habitats is evident. On the assumption that the salt content of soil could influence distribution, a greenhouse investigation has been made by Prof. E. A. Roux of the tolerance of these plants to sodium chloride[1].

Young plants were grown in water culture in washed river sand. A full nutrient solution of low osmotic pressure (0·5 atmospheres) was used as the basic medium, to which was added pure sodium chloride in varying amounts. Aeration was achieved by draining the containers daily. After the seedlings were established in the basic medium, they were subjected to increasing concentrations of sodium chloride until they died. The limits of tolerance were found to rise if the increases in salt concentration were made at longer as opposed to shorter intervals. Plants subjected directly to certain high concentrations were killed immediately.

With slow increases of concentration, *A. melanoxylon* would tolerate up to 2·4 per cent sodium chloride, *A. longifolia* up to 3·6 per cent and *A. cyclops* and *A. cyanophylla* up to 4·0 per cent. Other experiments indicated that *A. cyclops* was slightly less tolerant to salt than *A. cyanophylla*.

As a result of all the experiments, the species were finally placed in the following order of increasing salt tolerance: *A. melanoxylon*, *A. longifolia*, *A. cyclops*, *A. cyanophylla*. This sequence bears a direct relation to the altitudes at which the species normally grow on the Table Mountain range. *A. melanoxylon* grows above the 2,000-ft. contour. The upper limit indicated for *A. cyclops* is about 1,000 ft. and, for *A. cyanophylla*, slightly less. *A. longifolia* grows up to the 1,000-ft contour.

Prof. Roux plans soil tests so as to determine the average salt content of the soils in the different regions, but general physiographic considerations would point to an increase in salinity with decreasing altitude. The two species most highly tolerant of sodium chloride, *A. cyclops* and *A. cyanophylla*, are dominant on the Cape Flats where altitudes are generally less than 100 ft. above sea level. *A. cyclops* is the dominant and often the only species on dunes near the sea, and it was interesting to discover that it is rather less tolerant of salt than *A. cyanophylla* which tends to dominate on the more consolidated and level areas. It seems probable that drainage is more efficient on the dunes and that the consolidated and less-well-drained soils have a higher salt content.

T. H. HAWKINS

[1] *South African J. of Sci.*, 61, No. 12 (1965).

AN INVESTIGATION OF MICROPULSATIONS AT MIDDLE LATITUDES

By M. J. USHER and W. F. STUART*

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RUBIDIUM magnetometers were set up at the three United Kingdom observatories (Lerwick, geomagnetic latitude 62·5° N., Eskdalemuir 58·4° N., Hartland 54·6° N.) and simultaneous recordings of micropulsations occurring in the total field were obtained for the second half of 1964. The three stations are close to the same geomagnetic longitude and are about equally spaced at intervals of 400 miles.

The rubidium magnetometer is an atomic oscillator producing a frequency proportional to the scalar value of the ambient magnetic field. Details of its method of operation have been given earlier[1,2]. The magnetometer frequency of about 200 kc/s in the total Earth's field was counted by a commercial 1-Mc/s unit and decoded into an analogue step record. The sensitivity was 1/20γ with a sampling rate of about 1 per sec, which ensured that the frequency response of the system was flat from very long periods to 5 sec: a chart speed of 0·25 in. per

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min was used. The instrumentation was operated by the staffs of the observatories.

Records were obtained from the three stations together for the period August 15–December 20, and from Hartland from April 25–December 31. They were analysed in the first instance by a hand-scaling technique, as the quantity of data obtained and the general nature of micropulsation activity made other approaches unrealistic. The characteristic periods of events occurring in hourly intervals were noted together with the average amplitude and a 'continuity' figure giving the number of 5-min intervals in 1 h for which a particular period was present. A 'continuity index' figure having a value 0, 1 or 2 was adopted as a general measure of degree of *Pc* activity. Events were designated *Pc* or *Pt* and good events were specially noted. Values of total field energy, proportional to the product of continuity and the square of the amplitude, were computed. The significance of energy is somewhat doubtful as it does not include components in the plane perpendicular to the field direction. A large number of tracings of good or interesting events occurring simultaneously or otherwise at the three stations were taken.

Occurrence diagrams were constructed from the data by representing any event by a dot on a plot of period versus time of day, producing a variable-density sonogram effect. The period intervals were in seconds from zero to two hundred and the time intervals in hours. Graphs of occurrence versus time of day for selected period ranges and of occurrence versus period for selected hourly intervals were then obtained by summing the dots in the required areas of the occurrence diagram. This was done for each station at intervals of 4 weeks.

This method of analysis is such that errors in measurements are adequately distributed. It is important to realize that the method of analysis and the results described here are of a very general nature. This procedure was thought to be advisable before a more detailed investigation was attempted.

Figs. 1, 2 and 3 are typical of the results obtained. Fig. 1 shows the seasonal variation of the *Pc* spectrum

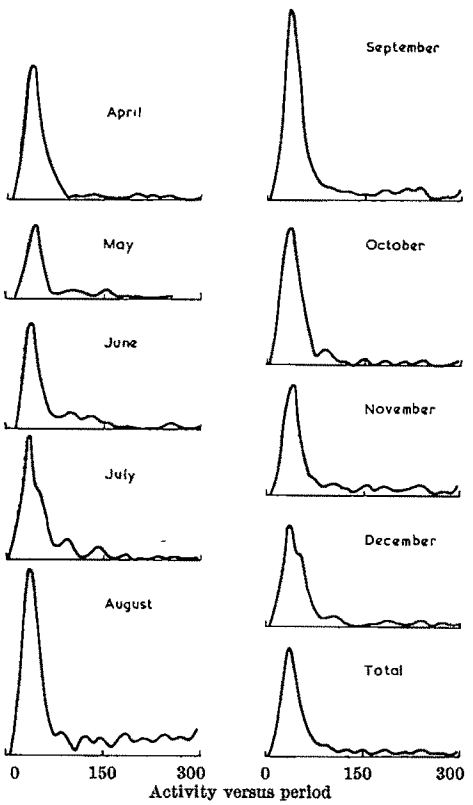
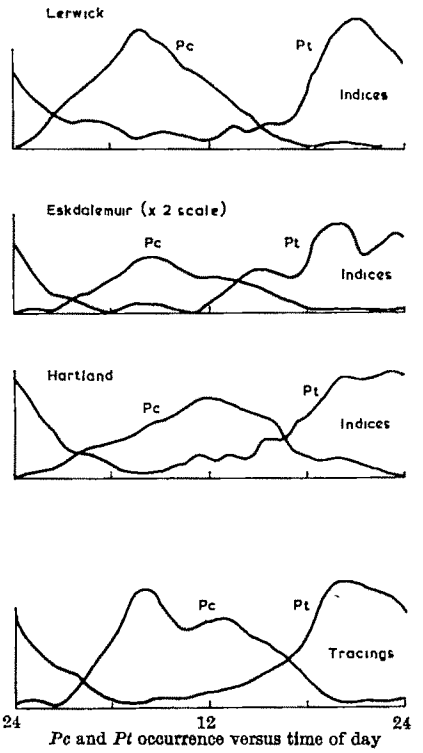


Fig. 1. Seasonal variation of the *Pc* spectrum at Hartland for the hours 07.00–12.00



Pc and *Pt* occurrence versus time of day

Fig. 2

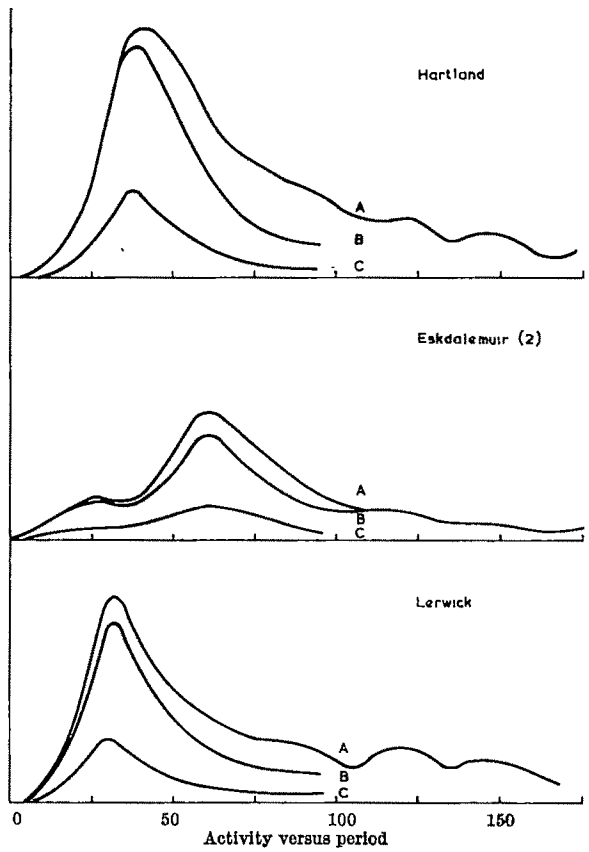


Fig. 3. *A*, Total; *B*, 03.00–17.00; *C*, best *Pc* hours

at Hartland (for the hours 07.00–12.00). A minimum in May/June and a maximum at the autumnal equinox are apparent. A suggestion of a decrease in peak period at times of increased activity is evident.

Fig. 2 is a plot of *Pc* and *Pt* occurrences at each station taken from the data sheet notations. A striking difference

between the times of maximum *Pc* occurrence at Hartland and Lerwick is evident, being 08.00 at Lerwick and 12.00 at Hartland. The *Pt* curve is similar at each station.

Fig. 3 shows period spectra for the three stations for periods up to 200 sec over the whole 24 h. The three stations contrast sharply, the peaks being 30 sec at Lerwick, 40 sec at Hartland and 60 sec at Eskdalemuir, where a subsidary peak occurs at 25 sec. This behaviour is not in agreement with the increase of period with latitude suggested by most theories.

Several distinct features arise from this general analysis of micropulsation activity:

(1) *Pc* occurrence times are radically different at the three stations, the maxima being 08.00 at Lerwick and 12.00 at Hartland. Related to the rotation of the Earth in a steady stream of solar wind, this represents 60° and is clearly an important feature in any theory of the origin of micropulsations.

(2) The general shape of the *Pc* spectrum is distinctly different at the three stations. The maxima are 30 sec at Lerwick, 60 sec at Eskdalemuir and 40 sec at Hartland, with a secondary peak of 25 sec at Eskdalemuir. This is not in agreement with theory but can be rationalized if harmonics are assumed to occur at the different stations. Harmonic activity is clearly evident on the records and apparently exists to a greater degree at the most northerly station. The period versus latitude variation shown by these curves does not agree with that found by Siebert[3] for stations at the same latitude in Germany.

(3) There is a seasonal change in both *Pc* and *Pt* activity. Hartland shows a maximum for *Pc* and *Pt* at the autumnal equinox, but Lerwick has a maximum in July/August for *Pc* and September/October for *Pt*. The statistical values are rather low, however, for *Pt* events.

(4) The peak *Pc* period decreases in times of increased activity, being shorter in the summer months. At such times *Pc* activity occurs earlier, especially for the shorter periods which then exhibit greater intensity. A similar diurnal variation occurs.

(5) Energy differences between stations are apparent, Eskdalemuir having only about half that at Lerwick and Hartland. This may be due to a crustal anomaly thought to exist in the region of Eskdalemuir, or could be due to the different proximity to the sea of the three stations. Measurements with a vector version of the magnetometer have shown that the total field record agrees closely with that obtained in the horizontal direction, since the vertical component is, in general, small.

This work is being extended to lower latitudes and an empirical investigation of the differences between Eskdalemuir, Lerwick and Hartland is being made with particular reference to inductive effects. A more detailed analysis of possible harmonic structure of good *Pc* events is being carried out using a vernier microscope, and *Pt* events are being measured for analysis by computer.

This work was supported by the Science Research Council and the British National Committee for Space Research. We thank the staffs of the various observatories for their attention to the instruments, and Miss Ann Clifford for compiling and presenting the analysis.

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LOCAL GROUND-LEVEL AIR CONCENTRATIONS OF LEAD-210 AT WINCHESTER, MASSACHUSETTS

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LEAD-210 concentrations in ground-level air are of interest because it has been estimated by Holtzman[1] that about half the activity of this isotope present in human bones is acquired through inhalation. Lead-210 occurs naturally and, due to its long half-life (22 years), is not restricted to the vicinity of its origin. Burton and Stewart[2] estimated the mean atmospheric residence time for lead-210 to be approximately 4 weeks, a period sufficient to allow atmospheric transport and mixing to occur. Barreira[3] has found ocean air low in radon-222 and it has been assumed that this type of air mass would also have a low concentration of lead-210. Holtzman[4] attributed lower lead-210 values in the bones of island dwellers to the lower atmospheric concentration of lead-210 in ocean air. The purpose of the present investigation was to obtain information on lead-210 concentrations in ground-level air and to investigate the relationship between airborne lead-210 and air masses.

The sampling site located at Winchester, Massachusetts, is in a suburban area approximately 15 km from the Atlantic sea-coast north-west of Boston. Daily air particulate samples were collected on a 47-mm diameter membrane filter (0·8μ pore size). Tests of this filter media indicated it was highly efficient for collection of radon daughter aerosols. The collector was located approximately 2 m above the ground and sampled air at a rate of 28 l./min. Each sample represented approximately 30 m³ of air. The daily collections were composited on a monthly basis from July 1964 to June 1965.

The samples were stored for one month after collection to allow equilibrium between lead-210 and its daughter, bismuth-210. They were then solubilized and the lead-210 separated from fission products and bismuth-210 by an ion exchange technique developed by Hahn[5]. The method converted the lead and bismuth present to the chloride by addition of hydrochloric acid. Bismuth and lead carriers were added and the solution passed through an anion exchange resin in the chloride form. The bismuth and lead were absorbed from solution. The lead was eluted with dilute hydrochloric acid and the bismuth with sulphuric acid. The lead was further purified by precipitation as a sulphate and converted to the chromate for counting; the bismuth was precipitated as the oxychloride for counting.

After a 30-day delay (to allow the daughter product, bismuth-210, to grow into secular equilibrium with lead-210), the lead-210 activity was determined by β counting its daughter bismuth-210. Determination of bismuth-210 activity initially separated served as a check of the values obtained.

Wind speed and direction data were obtained with a recording anemometer and wind direction transmitter mounted on a 50-ft. tower. Temperature and rainfall were concurrently recorded on appropriate instruments at ground level. Daily United States Weather Bureau maps provided information on the type of air mass over the sampling site.

Monthly lead-210 concentrations in ground-level air are presented in Table 1. Monthly results are averaged together in cases where the individual results are within the counting error at the 95 per cent confidence level. These results are consistent with those reported by Patter-

son and Lockhart[6] for Washington, D.C. For purposes of comparison with meteorological data, the values of lead-210 and the respective meteorological parameters were averaged over the time periods indicated in Table 1.

Based on data obtained from U.S. Weather Bureau maps, the predominant air mass over the sampling site was continental polar air (cP). Continental arctic (cA) air, which originates farther north than does continental polar air, was present over Winchester, Massachusetts, during the winter months. Intrusions of maritime tropical air (mT) occurred for the most part during the summer months, while maritime polar air (mP) was infrequently present. Maritime air masses may be expected to have a lower lead-210 content than continental air masses because of their origin over ocean areas. The effect of maritime air was indicated by lower lead-210 air concentrations during July–August–September 1964, and March–April–May–June 1965, when maritime tropical and maritime polar air was present over the sampling site 29 and 21 per cent of the time, respectively. During November–December 1964, the period of maximum lead-210 concentration, these types of air masses were present only 5 per cent of the time.

Table 1. LEAD-210 CONCENTRATIONS IN GROUND-LEVEL AIR

Month	Lead-210 (pc./m³ ± E*) Monthly composite	Mean for period
July 1964	0·0140 ± 0·0011	0·0125 ± 0·0025
August	0·0100 ± 0·0011	
September	0·0135 ± 0·0021	
October 1964	0·0232 ± 0·0031	0·0232 ± 0·0031
November 1964	0·0328 ± 0·0041	0·0370 ± 0·0066
December	0·0412 ± 0·0052	
January 1965	0·0222 ± 0·0052	0·0231 ± 0·0061
February	0·0240 ± 0·0033	
March 1965	0·0150 ± 0·0037	0·0157 ± 0·0057
April	0·0162 ± 0·0023	
May	0·0172 ± 0·0026	
June	0·0149 ± 0·0026	

* Counting error at 95 per cent confidence level.

A relationship between local lead-210 air concentration and the prevalence of particular air masses is indicated in Fig. 1. The concentration of lead-210 varies negatively with the time that maritime tropical (mT), maritime polar (mP), and continental arctic (cA) × (0·3) were over the sampling area. This relationship has a linear correlation coefficient of 0·93. The 68 per cent (one standard deviation) confidence interval for this value is 0·74–0·98.

Fig. 1 indicates a line of best fit based on regression analysis. Extrapolating this linear relationship indicates that lead-210 would approach zero if the indicated air masses were present 40 per cent of the time. Such a lead-210 concentration is physically unlikely. The concentration of lead-210 in continental arctic air was assumed to be lower than that for continental polar air, because ice cover as well as smaller land surfaces in the area of its origin would tend to reduce concentration of this radionuclide. A correction factor of 0·3 for continental arctic air proved to fit best the linear relationship shown in Fig. 1.

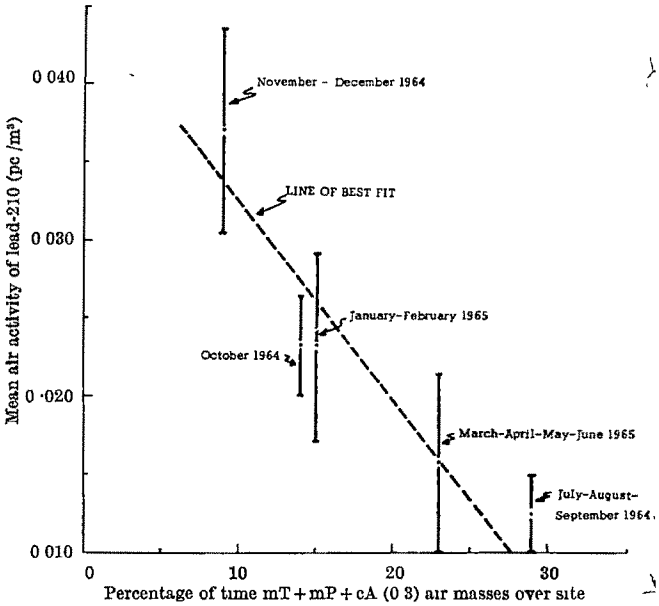


Fig. 1. Percentage of time that maritime tropical, maritime polar, and continental arctic × (0 3) air masses lay over sampling site versus the lead-210 concentration in ground-level air during selected sampling periods

Local meteorological parameters such as mean temperature, mean wind speed and direction, and precipitation, when averaged over the time periods indicated had a significant correlation with lead-210 except for easterly winds. A strong dilution effect by easterly winds, that is, winds carrying ocean air, was indicated by a correlation coefficient of 0·86 (0·53–0·97 for the 68 per cent confidence interval) for an inverse relationship between lead-210 concentrations and easterly winds. However, any relationships between local weather factors and lead-210 may have been lost in averaging the data over extended time periods.

The data on lead-210 in ground-level air collected at Winchester, Massachusetts, indicate that maritime air contains lower lead-210 concentrations than does continental air. It appears to support the hypothesis that lower values of lead-210 in the bones of island dwellers can be attributed to the lower atmospheric concentration of lead-210 over oceans.

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CAESIUM-137 AND STRONTIUM-90 IN NORWEGIAN MILK, 1960–1964

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MEASUREMENTS of caesium-137 and strontium-90 in Norwegian milk since 1956 have revealed seasonal variations and variations from district to district[1–5].

Since 1960, milk has been sampled at the eleven places shown on the map in Fig. 1. At sites 1–10 the samples are collected from dairies which receive milk from farms spread over a relatively large area. At site 11, the samples are from a single farm. Samples are normally collected about twice a month.

Quarterly average concentrations of caesium-137 in milk for sites 1–10 from 1960 to 1964 are shown in Fig. 2,*A*. A marked seasonal variation is apparent with a maximum during the quarter July–September, that is, during the grazing season. This indicates higher uptake from slow-growing grazing fields than from the better-cultivated fields supplying winter fodder. The highest value in curve *A*, 640 pc./l., found in 1964, is about four times greater than in 1960 and 1961.

The results also vary from site to site, both in concentration level and in seasonal pattern. The concentrations at site 11 have been influenced by an unusual composition of the fodder at this site, containing, for

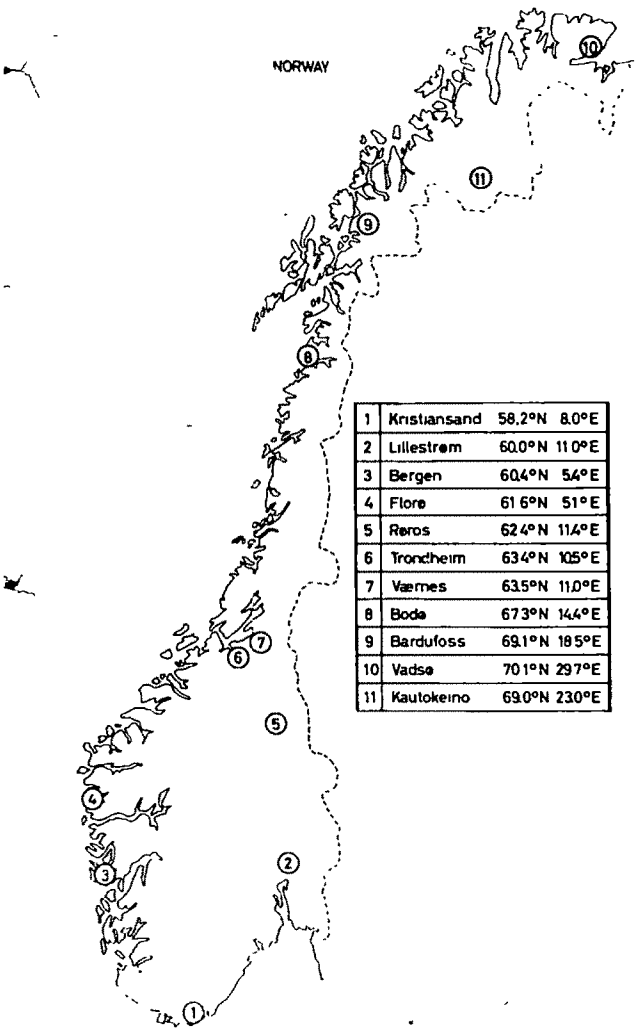


Fig. 1. Milk sampling sites

example, varying amounts of reindeer-lichen. This has sometimes given a maximum concentration during the first quarter.

The variations from district to district are evident in Fig. 3,*A*, which shows yearly average values of caesium-137 in milk at each of the eleven sites during the period 1960–64. The variations between sites follow the same general pattern from year to year, the highest concentrations being about eight times the lowest.

The variations do not follow the precipitation. As precipitation and caesium-137 deposition are to a first approximation proportional, the concentration in milk therefore depends on other factors in addition to deposition. These factors may be expected to involve feeding conditions, including quality of grazing fields and fodder composition, but there may be other less obvious influences. Based on the approximate proportionality between precipitation and caesium-137 deposition, the ratio of caesium-137 concentration in milk to yearly precipitation in the various districts gives an indication of the variations in these factors. Fig. 4,*A* shows average values of this ratio over the period 1960–64, in units of the ratio at site 2, with the sites arranged in order of increasing ratio. The highest value is about eleven times the lowest. The values are based on precipitation measurements made near the sample collection point. This represents an additional uncertainty, as each dairy receives milk from a large district within which there may be significant variations in yearly precipitation. The first four sites in Fig. 4 are inland, lowland districts, and show increasing ratios from south to north. The next five sites are coastal, largely mountainous districts, and also show increasing values from south to north. The last two sites are inland, mountainous districts, again showing increasing ratios

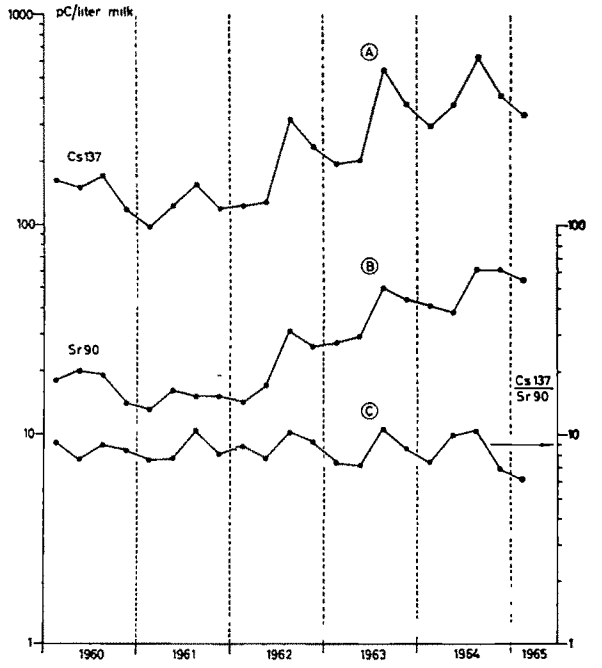


Fig. 2. Quarterly values of (*A*) the caesium-137 concentration, (*B*) the strontium-90 concentration, and (*C*) the caesium-137/strontium-90 ratio in milk, averaged over sites 1–10

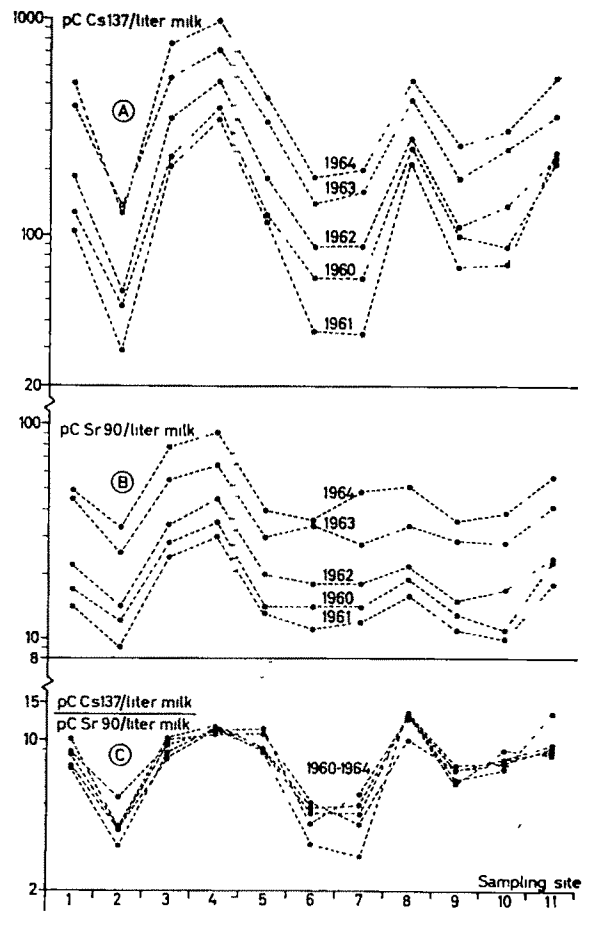


Fig. 3. Yearly average values at sites 1–11 of (*A*) the caesium-137 concentration, (*B*) the strontium-90 concentration, and (*C*) the caesium-137/strontium-90 ratio in milk

from south to north. These results indicate that inland, lowland districts have better feeding conditions than coastal districts, which in turn are better than inland, highland districts. This trend seems reasonable, as the growth conditions for vegetation in Norway normally deteriorate from lowland to highland and from south to north. Site 11 has the highest value, whereas it has the lowest precipitation. It is thus conceivable that the high uptake at this site reflects the local dry weather. However, results of other investigations carried out at this Establishment indicate that this is not necessarily the case. Sites with poor growth conditions, but with about ten times the precipitation at site 11, also have about ten times more caesium-137 in milk than site 11 (ref. 6). It was also shown that the uptake depends on the method of farming. At two neighbouring farms, one with modern, intensive farming, the other old-fashioned, the caesium-137 concentrations in milk at the latter were about ten times higher.

The dependence of caesium-137 milk concentrations on deposition can thus be expected to vary from district to district and from season to season as well as from year to year within the same district, depending on feeding conditions, which involve many factors of varying importance. A formula taking into account all factors, including delays or half-lives of the various stages, would be extremely complicated. For prediction purposes, a simplified model, even if less accurate, would still be very useful. Assuming that the caesium-137 concentration in milk, m, depends linearly on the rate of caesium-137 deposition, r, and on the cumulative deposition, c, a relationship can be written $m = a \cdot r + b \cdot c$. Owing to the difference between summer and winter feeding, the factors a and b have been determined separately for the two seasons. For the grazing season, that is, the quarter July–September, it has been assumed that the concentration, m, depends on the rate, r, during the same quarter and on the cumulative deposition, c, up to the middle of the quarter. For the winter-period the quarter January–March has been taken to be most representative, as during October–December the grazing season will be phasing out and during April–June it will be phasing in. For January–March it has been assumed that m depends on r and c for the quarter July–September of the previous year.

The rate and the cumulative deposition of caesium-137 for the various districts and years have been calculated from precipitation data and measurements of caesium-137 in soil samples and in air and precipitation since 1956.

The factors a and b determined for the average values over sites 1–10 are given in Table 1, together with the difference, in percentages, between the concentrations measured and those calculated using the expression $m = a \cdot r + b \cdot c$.

Table 1. RATE FACTOR a AND CUMULATIVE FACTOR b FOR UPTAKE OF CAESIUM-137 IN MILK

Periods	a	b	Max. deviations from measured values (%)
July-Sept. 1960–1964	14	3·3	11
Jan.-Mar. 1960–1965	6·5	2·5	26

Average values for sites 1 to 10.

$$a = \frac{\text{pc } ^{137}\text{Cs/l. milk}}{\text{mc. } ^{137}\text{Cs/km}^2\text{. 3 months}}$$

$$b = \frac{\text{pc. } ^{137}\text{Cs/l. milk}}{\text{mc. } ^{137}\text{Cs/km}^2}$$

The larger deviations for the first quarter are probably due more to the larger variations of fodder (hay, silage, turnips, lyed straw, processed cattle food, etc.) than to the inclusion of the first quarter of 1965.

The caesium-137 concentration in the diet will not always be directly related to the deposition in the district. For example, hay, straw and other fodder may be imported from other districts. The deviations will therefore be larger when the model is applied on the results from the separate sampling sites. The factors a and b for each of the sites 1–10 are shown in Fig. 4,*B*. The deviations from the measured concentrations in milk at any one site are, on average, 13 per cent for July–September and 22 per cent for January–March, with maximum values of 35 per cent and 46 per cent, respectively, for single quarters.

Because of the uncertainties involved in the model and the calculations, the curves in Fig. 4,*B* may be seen more as indicating the tendencies than as giving proportional relationships. The results indicate that the variations from district to district in caesium-137 concentration in milk relative to deposition are due more to variations in the cumulative factor than to change in the rate factor.

The concentrations of strontium-90 in milk during the years 1960–64 are shown in Fig. 2,*B* as quarterly average values over sites 1–10. The curve shows seasonal variations, but these are smaller than those for caesium-137. This difference between strontium and caesium is an indication of their different behaviour in the food chain.

The highest value in curve *B*, 60 pc./l. during 1964, is about four times greater than in 1961.

The variations from district to district, evident in Fig. 3,*B*, follow the same general pattern from year to year, the highest concentrations being about three times the lowest. The variations are thus smaller than for caesium-137. The ratios of strontium-90 concentration in milk to yearly precipitation are shown in Fig. 4,*A*. The highest ratio is about five times the lowest.

The districts, as for caesium-137, may be divided into three categories: inland lowlands, coastal mountains and inland highlands. Within each category, the ratio of strontium-90 to precipitation usually increases from south to north. But, in contrast to the caesium-137 ratio, not all ratios in the coastal districts are higher than in the inland, lowland districts. This shows that the strontium-90

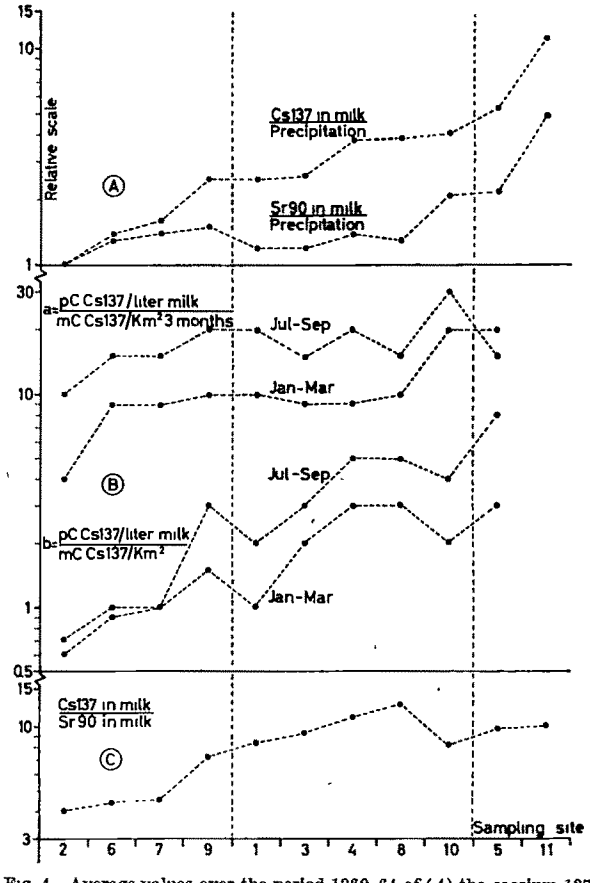


Fig. 4. Average values over the period 1960–64 of (*A*) the caesium-137 and strontium-90 in milk/yearly precipitation ratio, (*B*) the rate factor a and cumulative factor b for uptake of caesium-137 in milk, and (*C*) the caesium-137/strontium-90 ratio in milk at sites 1–11, arranged in order of increasing caesium-137 in milk/precipitation ratio

Table 2. RATE FACTOR a AND CUMULATIVE FACTOR b FOR UPTAKE OF STRONTIUM-90 IN MILK

Periods	a	b	Max deviations from measured values (%)
July–Sept. 1960–1964	2·1	0·56	14
Jan.–Mar. 1960–1965	1·5	0·53	17

Average values for sites 1 to 10.

$$a = \frac{\text{pc. } ^{90}\text{Sr/l. milk}}{\text{mc. } ^{90}\text{Sr/km}^2\text{. 3 months}}$$

$$b = \frac{\text{pc. } ^{90}\text{Sr/l. milk}}{\text{mc. } ^{90}\text{Sr/km}^2}$$

uptake in milk depends on other factors than the caesium-137 uptake, for example, the availability of calcium in soil.

The rate factor a and the cumulative factor b in the expression $m = a \cdot r + b \cdot c$ for uptake of strontium-90 in milk have been determined for the average values for sites 1–10, assuming the ratio of caesium-137 to strontium-90 deposition to be 1·7. The results are given in Table 2.

Values of the ratio of caesium-137 to strontium-90 in milk during the years 1960–64 are shown in Fig. 2,*C* as quarterly average values over sites 1–10. The ratio shows a seasonal variation with a maximum during July–September. This reflects the smaller seasonal variation of strontium-90.

There does not seem to be any systematic variation from year to year of these quarterly ratios. This may indicate that the ratio of rate uptake to cumulative uptake on the average has been about the same for caesium-137 and strontium-90. However, as is shown in Fig. 4,*B*, this may not be the case at the separate sites.

Fig. 3,*C* gives yearly average values of the ratio of caesium-137 to strontium-90 for each of the eleven sampling sites for the period 1960–64. It appears that the variations from district to district follow the same general pattern from year to year, the highest ratios being about three times the lowest, indicating that the differences are related to regional growth conditions rather than to other factors.

The average ratios over the period 1960–64 for each site are shown in Fig. 4. The lowest value is about 4, the highest about 13. The ratio increases from south to north, and is highest at the coastal sites, implying that the caesium-137 uptake in milk is more sensitive than the strontium-90 uptake to the growth conditions for vegetation.

Summarizing, the uptake of caesium-137 and strontium-90 in milk shows seasonal variations and variations from district to district. The variations seem to be more dependent on feeding conditions than on deposition. On the basis of the results for the period 1960–64 it seems feasible to make approximate predictions of milk activity from data on rate and cumulative deposition.

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ELECTRON PARAMAGNETIC RESONANCE SPECTRA OF BONE AND ITS MAJOR COMPONENTS

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A PRELIMINARY paper reported the detection of electron paramagnetic resonance (EPR) signals in non-irradiated human and amphibian bone[1]. Whole cortical bone was used in that investigation and the signals were described as multiple and anisotropic. The study was undertaken to obtain evidence to confirm the theory that some properties of bone result from a semiconducting *P-n* diode structure formed by the association between the mineral crystals and the collagen fibres of the matrix[2,3]. While the preliminary results neither confirmed nor refuted this thesis we wish now to report on a much more extensive study from which several interesting conclusions may be drawn.

While the literature is replete with reports of EPR studies on irradiated biological materials, we have found only scanty references to work on non-irradiated bone, collagen or mineral. Slager and Zucker[4] found no resonances in bone prepared for use in bone banks, and Cole and Silver[5] reported no resonances in a single deciduous tooth. Both these investigations were concerned primarily with the effects of irradiation and non-irradiated samples were employed only as controls. In discussing our preliminary communication, Commoner[6] stated that the resonance in whole bone was most likely due to the bone mineral, presumably apatite. Vinokurov and Zaripov[7] reported the presence of EPR signals in geological apatite. However, we have been unable to find any reports of similar studies on bone mineral. Likewise we have found no mention in the literature of EPR signals from non-irradiated collagen, derived either from bone or from soft tissue, except for the report by Abagyan[8] of complex signals from gelatine. Commoner and Ternberg[9,10], Swartz and Molenda[11] and Mallard and Kent[12] have all reported signals arising from whole, non-irradiated animal tissues of various types. However, none of these reports dealt with bone or with any type of collagen *per se*.

Bone samples. In the present investigation, powdered samples of human bone, collagen and mineral were used exclusively to avoid the complication which accompanied the previously reported anisotropic signals from whole bone. General sample procurement and treatment was as reported in detail in a previous publication, including the use of similar techniques for preparation of bone mineral and bone collagen. It is important to note that the extractions of collagen and mineral involve the use of aqueous solutions and subsequent washing in water. Once the basic samples were prepared they were stored in Petri dishes under normal laboratory conditions relative to temperature, humidity and illumination. In the present experiments these basic samples were reduced to powder by scraping with a sharp glass fragment (microscope slides were routinely used and discarded as soon as they showed signs of wear). The bone samples were held by hand in a paper tissue and no metallic objects were used in any part of the preparation procedure. This method resulted in a fine granular powder, uncontaminated by metallic or glass fragments, which was used for all samples except for the rat tail tendon. In this case the

fibres were obtained by stripping away from the bony structures; the portion of the tendon which had been grasped by the metallic clamp was then cut off and discarded. The fibres were permitted to equilibrate to room conditions, then sectioned into pieces of less than 1 mm length using sharp scissors. Dehydrated samples of all materials were prepared by vacuum drying at a pressure of 10μ of mercury for a minimum of 2 weeks. During the first few days of this period the vacuum chamber was repeatedly flushed with dry nitrogen. This procedure was primarily intended to produce the maximum water loss without producing heat denaturation of the protein components.

EPR techniques. Samples were examined in 4 mm optically dense quartz tubes; each tube was previously checked for resonances utilizing the same instrument settings to be subsequently applied to the sample. Tubes with any major resonances in the $g2$ vicinity were discarded. In order properly to compare signals of the two components with the whole bone signal, tubes were filled according to a 2 : 1 ratio of mineral to collagen. This resulted in the whole bone sample tubes containing 150 mg of material, the mineral tubes 100 mg and the collagen tubes 50 mg of material. A Varian '*X*-band' spectrometer with 100 k.c. field modulation and phase detection with a time constant of 0·3 sec was used. Variable temperature studies were done using a Varian variable temperature attachment utilizing a constant flow of dry nitrogen at the desired temperature; g values were calculated from a determination of the klystron frequency using a Hewlett–Packard wavemeter, type '*X*532*B*'. The first derivative of the absorption signal was recorded.

(*A*) *Whole bone powder*

Hydrated. At room temperature all samples showed a clearly defined single resonance at $g2{\cdot}001$ ($\pm 0{\cdot}005$) with a line-width of 10 ± 1 gauss. This resonance saturated at about 100 dB (Fig. 1). The total signal amplitude decreased very slowly with storage under dark conditions. In general a decline to 30 per cent of the original value was apparent after 90 days. The other signal parameters, however, remained unchanged.

Dehydrated. Vacuum drying resulted in approximately a four-fold increase in signal amplitude, but again the other parameters of the signal remained unchanged.

Resonance signal variations with temperature. The amplitude of both hydrated and dehydrated samples had the same temperature dependence in the interval −150° C–+20° C (Fig. 2). In this region the curve was found to be reproducible. On heating, however, hydrated bone showed a discrete shift to a smaller amplitude in the vicinity of +65° C (Fig. 3), while dehydrated bone showed a more gradual transition to smaller amplitudes over the range +20° C to +95° C. In both cases, the decrease is much greater than one would expect on the basis of a simple Curie dependence. The decrease in signal amplitude at +65° C appeared to be irreversible. Each sample demonstrated this phenomenon one time only and, provided a sample was not carried beyond +100° C (with resultant loss of volatile components), when returned to +20° C did not regain the original signal amplitude. Further heating to +200° C caused the hydrated sample signal to increase in magnitude while the dehydrated sample signal remained essentially constant. Heating of a hydrated sample in a +800° C flame for 3 min caused the signal to increase by a factor of 100. In all heating experiments including the burning in a +800° C flame, the g-value and line-width of the observed resonance remained constant.

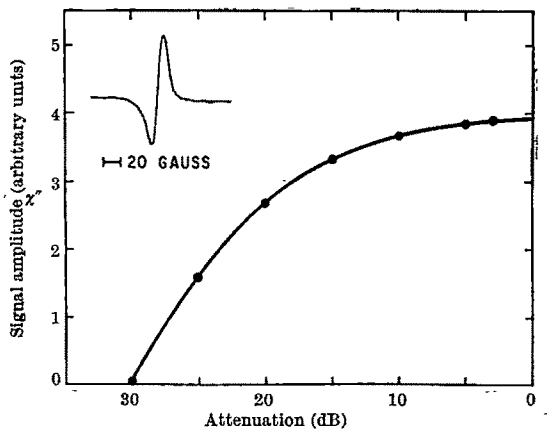


Fig. 1. Saturation of resonance from hydrated whole bone powder. The insert shows the standard resonance observed from this material

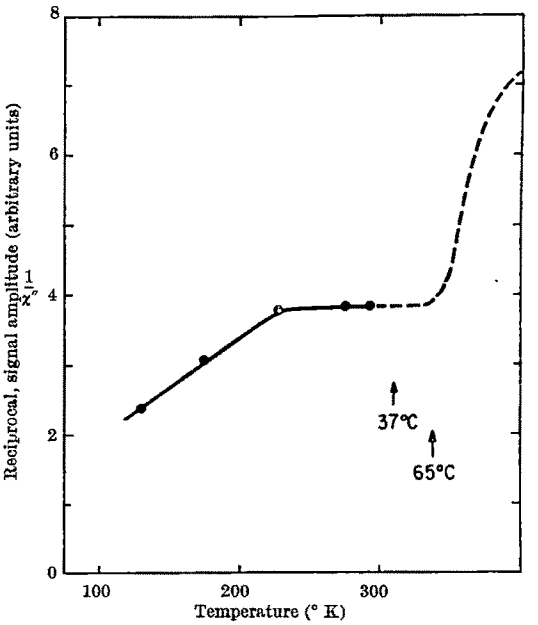


Fig. 2. Temperature dependence of reciprocal of signal amplitude from hydrated and dehydrated whole bone powder. The solid portion of the graph is stable and reproducible. The large sample temperature gradient (10° C) and temperature controller 'dead band' at present preclude assigning definite points to the curve above +300° K, that is, slope of dotted line depends on details of heating experiment. However, the abrupt signal change in vicinity of +65° C was routinely observed and is approximately as depicted

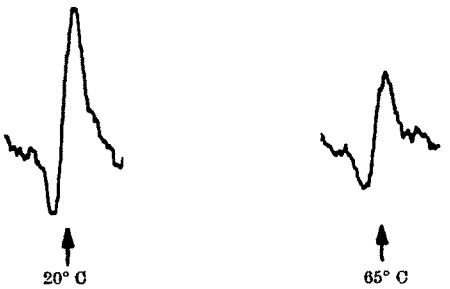


Fig. 3. Comparison of resonance signal amplitude from whole bone powder at room temperature and at +65° C. The shift to a lower amplitude appears to occur rather abruptly in the vicinity of +65° C

(*B*) *Bone mineral powder*

Hydrated. At room temperature a complex resonance (Fig. 4*A*) was observed, composed of several unresolved resonances with the largest slightly to the low field side of $g2$. In addition three equally spaced, low amplitude resonances have been found farther to the low field side. The low amplitude resonances are relatively independent of temperature, and disappear when the apatite is heated above +150° C. Storage in the dark for 90 days did not result in any appreciable decline in signal amplitude.

Dehydrated. At room temperature there was a slight increase in total resonance amplitude compared with hydrated apatite, but there was considerable improvement in resolution indicating the presence of three separate resonances in the $g2$ area (Fig. 4*B*). These are subsequently

identified as apatite resonances I, II and III with resonance III having a g-value very close to $g2$ and I and II being displaced towards the low field side of III. A careful inspection of the resonance observed in the hydrated sample reveals that the same three resonances are probably present in the one complex signal but with such low resolution as to be practically indistinguishable. The three low amplitude, low field resonances are still present but reduced in amplitude.

Resonance signal variations with temperature. (Fig. 5.) Both hydrated and dehydrated samples displayed the same series of changes with temperature. On cooling from room temperature to $-50°$ C there was a slight increase in total signal amplitude showing roughly an inverse dependence on temperature. In the vicinity of $-100°$ C a decrease in signal amplitude occurs and continues until at $-154°$ C the total signal amplitude is approximately 50 per cent of that at room temperature. These changes between room temperature and $-154°$ C are completely reversible. Heating the samples from room temperature to $+150°$ C results in a monotonic decrease in amplitude of the total resonance. At $+150°$ C, resonance I is no longer observable or combined with resonance II. The amplitude of resonance II is reduced sufficiently to render resonance III quite prominent. Between $+150°$ C and $+250°$ C there is a further reduction in the resonance representing the combination of I and II while resonance III demonstrates a real increase in amplitude. This same process (decrease in amplitude resonances I and II, increase in resonance III) continues up to $+300°$ C. If the heating process is halted at this point, and the sample cooled to room temperature and examined, a signal much different than the previous room temperature signal is seen.

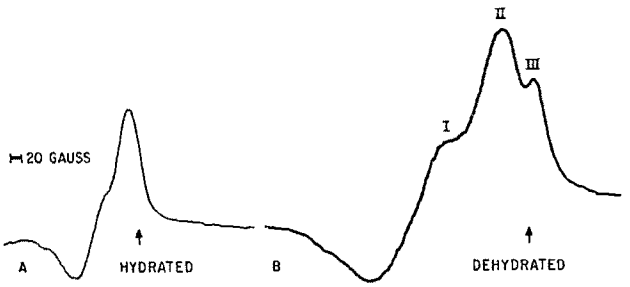


Fig. 4. Resonance signals obtained at room temperature from extracted bone mineral, hydrated (A) and dehydrated (B). The three low amplitude resonances towards the low field side of $g2$ are not pictured. There appear to be three resonances in the vicinity of $g2$; the numbers assigned to them on the dehydrated resonance (B) correspond to the numbers in the text. The scale of the horizontal axis of B is twice that of A

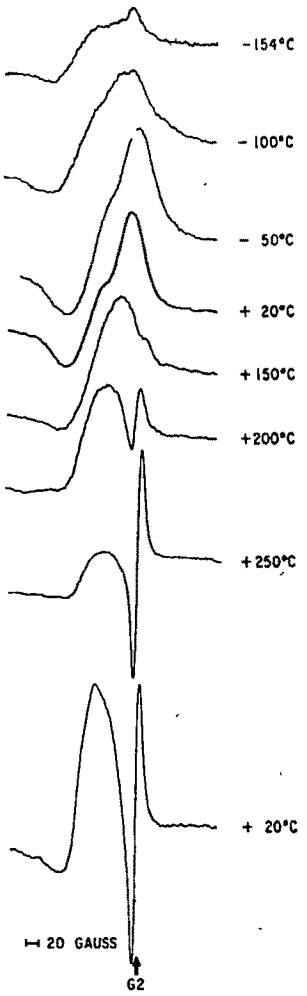


Fig. 5. Variations in the resonance signals from bone mineral (dehydrated in vacuum) with temperature changes. Instrument gain was reduced 50 per cent for the $+250°$ C temperature recording

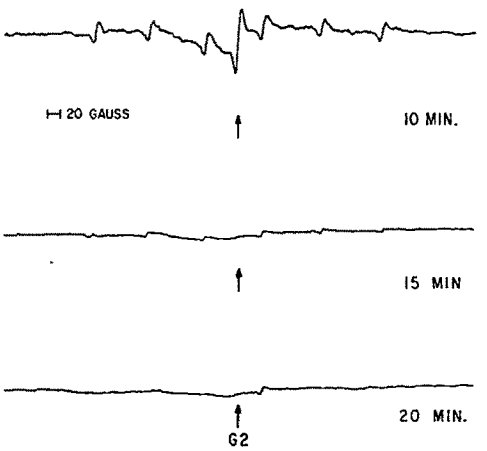


Fig. 6. Alterations in the resonance from bone mineral with exposure to $+800°$ C temperature for varying periods of time

Detailed experiments on bone mineral in the temperature range $+300°$ C to $+800°$ C using a muffle furnace are in process. However, the following preliminary observations have been made (Fig. 6).

(1) By heating to constant weight at $+400°$ C the broad low field resonance may be eliminated leaving essentially resonance III with a line-width of 10 gauss; g-value determinations were within the same range as the $g2$ signal from whole bone.

(2) By equilibrating mineral showing only a $g2$ resonance in distilled water for 24 h, the low field resonance may be partially restored.

(3) Transient heating in a $+800°$ C flame causes a decrease in the $g2$ resonance and the appearance of six satellite lines.

(4) Further flame heating causes the complete disappearance of the $g2$ line and a diminution of the satellite lines.

(5) Heating to constant weight at $+800°$ C causes the satellite lines to disappear completely with the exception of the first on the high field side.

The 'resolving' of the $g2$ resonance in apatite by heating to $+400°$ C enabled studies of its temperature dependence in the range $-154°$ C to $+200°$ C to be made (Fig. 7).

(C) *Collagen*

All samples of collagen, whether from rat tail tendon or human bone, that were immersed in water as a routine part of the preparation procedure displayed approximately the same complex signal: a broad peak towards the low field side of $g2$, a slightly narrower asymmetric peak closer to $g2$ and a partially resolved peak at $g2$. These resonances are hereafter referred to respectively

as I, II and III (Fig. 8*A*). Samples dehydrated in vacuum were essentially the same except for a slight increase in amplitude and resolution of the *g*2 peak (Fig. 8*B*). The only samples of collagen not immersed in water during preparation were those from rat tail tendon. These were found to have only the *g*2 peak (Fig. 8*C*). If these samples were immersed in water and air dried they displayed a resonance identical to hydrated bone collagen (Fig. 8*D*). Subsequent vacuum drying produced a resonance similar to that of vacuum dried bone collagen. Bone collagen stored under dark conditions for 90 days demonstrated approximately a 30 per cent loss in signal amplitude.

Signal variation with temperature

Bone collagen. Over the temperature range −154°–+150° C, resonance I appeared to increase slightly. Resonance II displayed a slight increase below −50° C, remained constant in amplitude between −50° C and +65° C, and decreased between +65° C and +150° C. Resonance III exhibited a constant decline from −154° C to +150° C. Since this resonance is poorly resolved, we cannot be certain whether it follows a strictly Curie dependence.

The signal complex composed of peaks II and III displayed a rather abrupt decrease in signal amplitude in the vicinity of +65° C. This phenomenon is somewhat easier to observe in the dehydrated bone collagen samples than in the hydrated. When samples that had been carried to +150° C were returned to room temperature, signals I and III were found to be relatively unchanged while signal II remained diminished in amplitude.

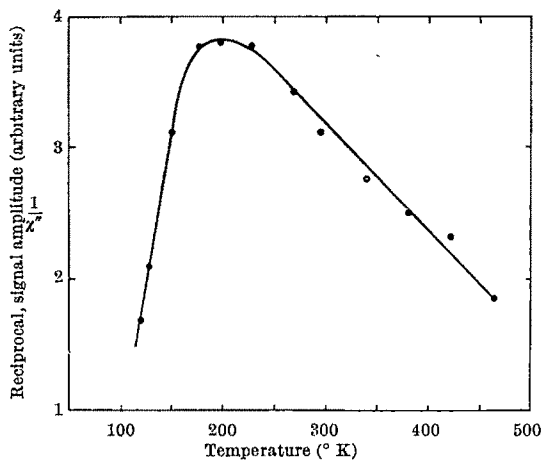


Fig. 7 Temperature dependence of reciprocal of signal amplitude from bone mineral treated to show only the *g*2 signal (heated to +400° C)

Rat tail tendon. The *g*2 signal was relatively independent of temperature between −154° C and +65° C. At the latter temperature an abrupt shift to a smaller amplitude is observed. Heating over +100° C produces an increase in signal amplitude that may well be ascribed to the loss of volatile materials.

In general, on an equal weight basis the signal amplitude from human bone collagen is approximately three times as great as that from rat tail tendon

Bone mineral. The resonance signals obtained from bone mineral appear to have considerable significance both in lending support to the thesis that the material is hydroxyapatite and in substantiating some concepts of its structure.

In order to explain the low calcium–phosphorus ratio in many hydroxyapatites it has been postulated that $2H_3O^{++}$ may be substituted for Ca^{++} in the crystal lattice, up to a maximum of four hydronium ions per unit cell. One of the chief successes of this theory is in explaining the observed water loss of apatite as a function of temperature. Experimentally, it is found, when heating apatite to constant weight, that weight is lost in a series of three

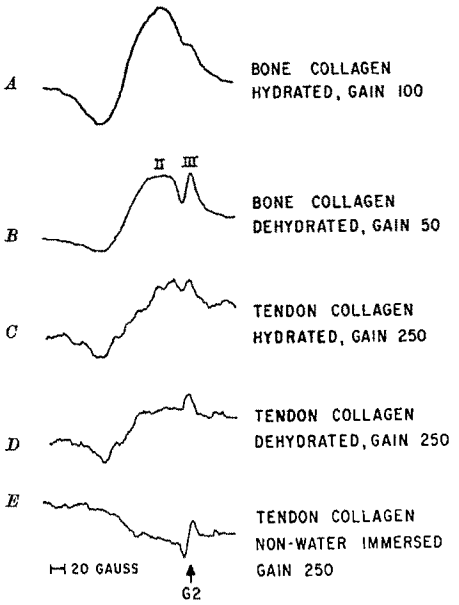


Fig. 8. Resonance signals from various types of collagen at room temperature The numbers on resonance *B* (dehydrated bone collagen) correspond to those in the text. Resonance I, the broad low field signal, is not pictured in the figure

discrete steps terminating at temperatures of +110° C, +400° C and +600° C (ref. 13). These changes have been attributed to an initial loss of surface absorbed water, a loss of substituted hydronium ions and, thirdly, to liberation of hydroxyl radicals from the unit cell.

A strikingly similar series of changes takes place in the EPR signal with temperature. Heating to +150° C eliminates the three low amplitude resonances and diminishes resonance II. Heating to +400° C almost completely eliminates I and II, leaving only the *g*2 resonance (III), and heating from +400° C to +800° C reveals the complex series of changes previously described. Prolonged heating at +800° C eliminates all resonances except the first high field satellite line.

Substituted hydronium ions (or similar type units) can produce a resonance signal. If it is postulated that the 'surface absorbed' water is somewhat similarly structured and capable of producing a resonance, then a high degree of correlation may be noted between the weight changes with temperature and the EPR signal changes with temperature, resonance II arising from surface-absorbed water and resonance I from H_3O^{++}. Further evidence for attributing the resonances in apatite, with the exception of the *g*2 resonance, to some form of water is provided by the work of Siegel *et al.*[14] on γ-irradiated ice. Among the resonances they found is one that is strikingly similar to the one we have found, showing resonance I (including the low field resonances) and resonance II, and differing only in that the unresolved resonance at *g*2 appears to be missing. While Siegel attributes the resonance to water the possibility remains that it is due to H_3O^{++} (or, alternatively, that water and not H_3O^{++} is substituted in the crystal lattice). In addition it should be noted that the broad low field resonance I may be enhanced by immersing the apatite in water for 24 h.

Vinokurov[7], investigating the EPR signal of blue geological apatites, finds in addition to Mn^{++} lines a relatively intense line at *g*2 of width 10 gauss, with six satellite lines symmetrically displaced of lesser intensity. He finds that the lines disappear at about +500° C, and attributes them to *F*-centre electrons localized at halogen ion vacancies (the position normally occupied by hydroxide in hydroxyapatite). As previously described, the same spectra with the notable absence of Mn^{++} lines are found in bone apatite in transient heating, and could presumably be produced by heating to constant weight in

the temperature range +400° C to +800° C. Significantly, the temperature at which the seven line spectra in bone apatite disappear, +800° C, is also the temperature at which the hydroxyl ion is driven off, collapsing the unit cell, and converting bone apatite into tricalcium phosphate. Commercially purchased tricalcium phosphate is found to have no resonance Thus, the $g2$ resonance in apatite is tentatively identified as being directly associated with either the hydroxyl radical itself, or electrons localized in hydroxyl vacancies (F-centres).

It is difficult to explain the observed anomalous temperature dependence of the $g2$ signal (Fig. 7) on the basis of strictly localized electrons as postulated by Vinokurov[7]. If one assumes that the trapped electrons do not participate in the resonance an alternative model that does fit the observations would be an excess of conduction electrons in equilibrium with the traps at +200° K (the observed signal minima), all traps would be considered filled, the resonance arising from the excess free electron population. Heating above +200° K would release increased numbers of electrons from the traps, thereby augmenting the resonance. Cooling below +200° K also leads to augmentation of the signal, derived in this case from the population of excess free electrons by virtue of the classical Curie dependence. On the basis of this picture, one may expect that there exists a temperature range in which all the traps are emptied, yet which lies below the temperature at which the onset of conversion to tricalcium phosphate begins (+600° C). In such a range, the resonance amplitude would be independent of temperature, except for the Curie dependence.

From a consideration of the data we conclude that resonance II (and the three low field resonances) results from surface-absorbed water possessing a structure capable of producing a resonant signal. Resonance I is tentatively identified with either H_3O^{++} ions or water in substitution positions within the lattice. The $g2$ resonance would appear to be derived from hydroxyl radicals, F-centre electrons, or conduction electrons. The similarity of the high temperature spectra sequence to that observed in geological apatite is striking but obviously not conclusive.

Collagen. All samples of tendon collagen initially demonstrated only the $g2$ resonance and within the confines of the experimental procedures did not indicate the presence of paramagnetic ions either as functional constituents or as impurities. Following exposure to aqueous solutions and subsequent drying (in air or vacuum) the resonance became practically identical with that of bone collagen, except in magnitude.

The resonance produced in rat tail collagen by water immersion (II) could be removed by heating to +150° C, while with bone collagen it was only partially removed. The line shape intensity and position of the remaining resonance (II), in +150° C heated bone collagen, together with spectroscopic data[15], strongly indicate that it is a result of residual apatite. Since the preparation of bone collagen involves prolonged exposure to aqueous solutions it must be considered possible that its unaltered, *in vivo*, resonance is also a similar single $g2$ peak. The data indicate that the broad resonance, II, observed after such exposure results from water structured on or within the collagen fibres which is structured in such a fashion as to be capable of producing an electron resonance. Since the water is apparently quite tightly bound to the collagen structure one may well question the relationship between structural or electronic data determined on collagen prepared in such a fashion with native unaltered collagen *in vivo*. In addition the data lead to the conclusion that both bone collagen and rat tail tendon collagen are not in free equilibrium with the water of the extracellular fluid. This is scarcely surprising for the type of bone collagen utilized (which is probably representative of 'unexchangeable bone'), but it is quite unexpected in the case of the tendon collagen.

The abrupt decline in signal magnitude in the vicinity of +65° C is indicative of a corresponding alteration in electronic structure. This phenomenon may, however, not be as abrupt as it would appear to be at first glance. For example, the curve of signal variation against temperature for bone (Fig. 2) shows a similar abrupt change at +65° C, but this transition terminates a temperature sequence from −50° C to +65° C within which the signal amplitude does not change with temperature. Below −50° C the signal follows a Curie dependence quite exactly. One has, therefore, rather than an abrupt anomaly occurring at a single temperature, an anomaly spread over a rather wide temperature range which is terminated abruptly at +65° C. It is interesting to reflect on the fact that the collagen *in vivo* exists in this anomalous state and that the temperature at which the state is terminated coincides quite well with the temperature at which the well-known 'shrinkage' phenomenon occurs.

Bone. The previously reported resonance signal at $g2$ is confirmed by the present investigation. The signal described in the present article is much greater in amplitude and much better defined than that previously reported because the total mass of specimen present in the powdered form is many times greater than the mass of the intact specimens used in the previous experiments.

The temperature dependence of the signal amplitude parallels that of collagen so well that the signal must be considered to derive, at least in part, from collagen. It is certainly not due solely to the presence of the apatite. While we cannot state with certainty the state of hydration of the apatite present in whole bone, all available evidence[13] indicates that at least that portion of the hydration represented by the substituted hydronium ions is present. One might therefore expect to see at least some of apatite resonance I in addition to the $g2$ resonance. However, none is found. In consideration of these facts it is surprising that the whole bone signal observed was consistently a relatively narrow, symmetrical intense line at $g2$. In general, the results are in accord with the thesis that the apatite–collagen relationship in bone produces a new unit with properties that are more than the simple additive sum of the properties of both components. If one may somewhat arbitrarily compare the single $g2$ signal of whole bone, with the corresponding signal of rat tail tendon collagen and that of apatite which has been heated to +400° C, few differences can be seen on superficial examination. However, the saturation curve for whole bone (Fig. 1) shows the onset of saturation at 10 dB, while a similar curve for both collagen and apatite reveals saturation in the vicinity of 15 dB, indicating some basic dissimilarity. In addition, the temperature dependences of bone powder and heated apatite are quite different.

To sum up, EPR observations have been made on powdered samples of whole human bone, bone collagen, bone mineral and rat tail tendon collagen.

(1) All substances examined revealed resonance signals.

(2) The resonance signal from bone mineral is quite similar to that derived from geological apatite within a certain temperature range and is attributed to certain types of structured water as well as F-centre electrons localized at the hydroxyl ion position and possibly conduction electrons.

(3) There is a distinct difference between the signals exhibited by water immersed and non-water immersed collagen. This is at present attributed to the ability of the collagen fibre to 'structure' absorbed water to a high degree.

(4) Collagen and bone exhibited an anomaly in the curve of signal against temperature at +65° C. The possible relationship between this and the shrinkage phenomenon is indicated.

(5) No paramagnetic species were found in any of the materials, either as functional components or as contaminants.

(6) The resonance signal in bone is not attributable to either apatite or collagen alone or to a simply additive sum of the resonances of both of these substances in combination.

This investigation was supported by a grant from the National Institutes of Health, U.S. Public Health Service.

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ELECTRON SPIN RESONANCE IN SURVIVING RAT TISSUES

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IN a previous communication[1] we presented some preliminary electron spin resonance results on normal tissues and tumour tissues from rats, confirming the earlier results of Commoner and Ternberg[2]. We have now made a more thorough investigation of these and other tissues with the addition of a continuous averaging technique system to our spectrometer[3]. A number of measurements have been made to determine more accurately the concentration of spins, the g-value and the line width observed from normal rat tissues. Samples of liver, kidney, heart, spleen, lung muscle and whole blood were investigated. In addition, several rat tumours were also studied. The results for the normal tissues are shown in Table 1 and those for the tumours in Table 2.

Table 1. CONCENTRATIONS OF FREE RADICALS IN VARIOUS TISSUES, g-VALUES AND LINE WIDTHS MEASURED BETWEEN THE POINTS OF INFLEXION OF THE ABSORPTION LINES

Also shown are the standard errors of the mean for each result, which, in the case of spin concentration, includes the calibration error

Tissue	Concentration of spins (moles/g) 10^{-9}	g-Value	Line width between points of inflexion (G)
Liver	8·58 ± 0·62	2·004 ± 0 0003	13·6 ± 0 2
Kidney	5·63 ± 0·33	2·004 ± 0·0002	13·7 ± 0·4
Heart	4 74 ± 0·42	2 003 ± 0·0002	14 0 ± 0·9
Spleen	0·99 ± 0 12	2·002 ± 0·0005	13·3 ± 0 5
Lung	0·79 ± 0·09	2·001 ± 0·0005	14 4 ± 0 5
Muscle	1·26 ± 0·40	2·002 ± 0·001	13 8 ± 1 4
Blood	0·1	—	—

Table 2. CONCENTRATION FOUND IN VARIOUS RAT TUMOURS OF FREE RADICALS AND OTHER PARAMAGNETIC SYSTEMS, g-VALUES AND LINE WIDTHS BETWEEN THE POINTS OF INFLEXION OF THE ABSORPTION LINE (TURNING POINTS ON FIRST DERIVATIVE SPECTRA)

Tumour type	Concentration of spins (moles/g)	g-Value	Line width between points of inflexion
Metastases of liver tumour	$(4·7 \pm 0 6) \times 10^{-6}$	2·011 ± 0 005	51·8 ± 0·8
Liver carcinoma	$(2·4 \pm 0·7) \times 10^{-6}$	2·016 ± 0·005	50·5 ± 2 6
Kidney carcinoma	$1·7 \times 10^{-9}$	2·002 ± 0·002	14 1
Radiation induced tumour	$2 4 \times 10^{-10}$?	2·004 ± 0 002 ?	10 ?
Sarcoma from congenital spina bifida	$3 2 \times 10^{-10}$?	2·004 ± 0 002 ?	11 ?
Eye tumour	$< 10^{-10}$	—	—
Breast tumour	$< 10^{-10}$	—	—

Measurements without standard errors indicate that only one sample was used. The maximum uncertainty here must be the concentration of impurity signal noted in the measuring system, which is of the order of 2×10^{-10} moles/g or giving a signal equivalent to that concentration. As a result, samples with signals of this order are in doubt as indicated.

The measurements on normal tissues were made on three samples of 100 mg of tissue from each of six rats in 5 per cent glucose solution; they show a surprisingly small standard error between samples. The results are shown diagrammatically, including the various standard errors, in Fig. 1. In this, the thick horizontal line represents the standard error of the mean of the sample measurements only on the inside edge, the outside edge being with the addition of the errors due to the calibrating standard sample used, which was an aqueous solution of 10^{-5} moles/l. manganese chloride. The narrow horizontal lines of wider spacing are the standard deviations of the tissue samples from the mean.

All measurements were made at 37° C cavity temperature and were averaged over 100 sweeps each of 8-sec duration. As was reported[1,2], the signals decay with time so that these figures must eventually be corrected for the decay occurring in that time. They will thus be slightly higher than reported here.

The results differ from those of Commoner and Ternberg[2] by a factor of approximately two. This may arise solely in the comparison of the experimental samples, which we assume have a spin of 1/2, with that of the calibrating standard, Mn^{++}, which has a spin of 5/2; the relative areas beneath the total absorption line shapes of samples of each of these spins with equal molar concentration we take to be not 5 : 1 but 35 : 3, due to the unequal population distribution in each of the six spin states of the Mn^{++}.

The following observations about the results from each tissue should be pointed out. Background spectra were subtracted as described previously[3], by recording the result of 100–300 sweeps, with glucose alone in the sample cell, on magnetic tape in digital form. In order to preserve, as far as possible, the advantages of the continuous averaging technique system with regard to improvement in signal-to-noise ratio, the background spectra were taken over a greater number of sweeps than that of the sample. They were then multiplied by a suitable constant when subtracted.

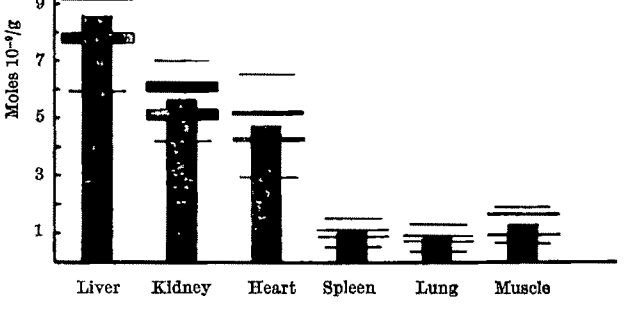


Fig. 1. Concentration of free radicals in a number of rat tissues measured at 37° C

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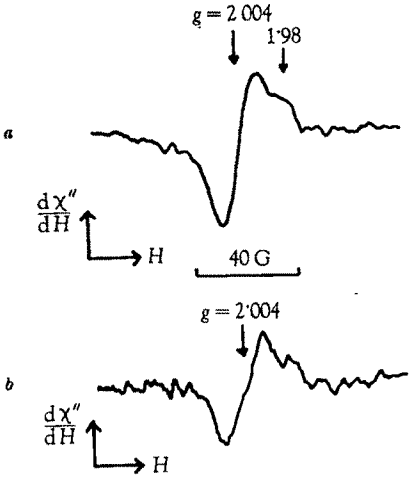


Fig. 2. (*a*) Fifty sweeps of rat liver after background spectrum subtraction. Modulation amplitude ~10 G; analyser display 10^5 counts full scale. (*b*) Twenty sweeps of the same sample with low modulation (~3 G). Analyser display 2×10^4 counts full scale

(*a*) *Liver.* For normal rat liver, the integration technique was a manual one. This explains the larger standard error for these particular results. In addition to the free-radical line, other less intense lines were observed around $g \sim 1{\cdot}98$ and 2·01 (Fig. 2), when the modulation amplitude was reduced to about 3 G. These lines were not accounted for in the integration, the results here being for a single free radical line only. A definite asymmetry was also observed and this will be discussed later.

(*b*) *Kidney.* The integration procedure, again for the free radical line observed at $g = 2{\cdot}004$, was semi-digital, the first derivative signal being integrated once only with the continuous averaging technique and the absorption line being plotted on homogeneous paper and weighed. The general appearance of the signal was similar to the liver, bearing the asymmetry observed before but to a lesser degree (Fig. 3).

(*c*) *Heart.* The integrations in this case were carried out in a completely digital manner using the continuous

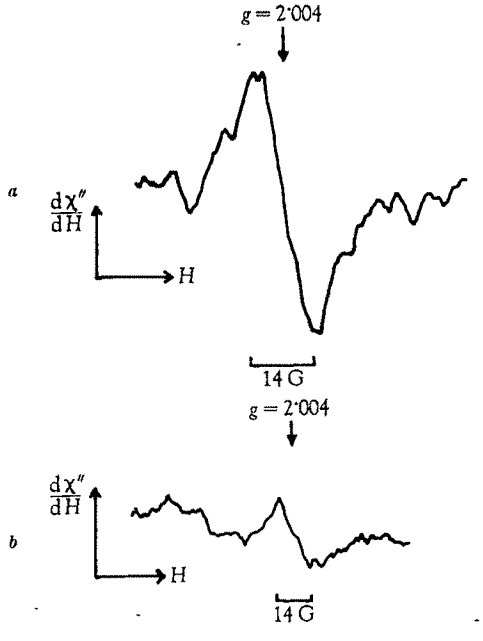


Fig. 3 (*a*) 100 sweeps of normal rat kidney. Signal represents $\sim 5 \times 10^{-9}$ moles/g on the analyser display scale of 10^5 counts full scale. The magnitude of the field sweep was ~90 G. (*b*) 300 sweeps of spontaneous rat kidney carcinoma. Signal represents $\sim 2 \times 10^{-9}$ moles/g on the analyser display scale of 5×10^5 counts full scale. The field sweep was ~130 G

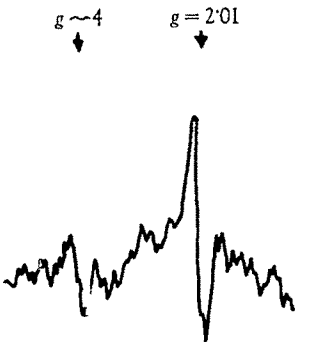


Fig. 4. Single sweep over 3·000 G of metastasis of liver tumour showing presence of signals at $g \sim 4$ and $g = 2{\cdot}01$

averaging technique. There again appeared to be at least one less intense line at $g \sim 1{\cdot}99$, contributing to the general asymmetry, but no others could be seen.

(*d*) *Spleen.* A very low concentration of spins was found in samples of this organ. It was noted that the apparent signal-to-noise ratio was improved by the integration technique[3]. The *g*-value of the signal found (2·002) appears to differ significantly from that of the other tissues mentioned so far. We could not detect any other lines in this spectrum, nor was there any obvious asymmetry.

(*e*) *Lung.* The low concentration of free radicals measured in this organ is the lowest mean value of the normal tissues measured in this series (with the exception of whole blood) and again the *g*-value appears to be significantly different from the others.

(*f*) *Muscle.* Only three results were taken for this tissue, but they were within the usual standard deviation of 25 per cent as found for other tissues.

Some tumour samples have also been examined (see Table 2). The majority of these show very little or absence of free radical signal above the limit of detection of our present system. They are thus smaller than our previously reported limits[1].

(*a*) *Metastasis of liver tumour in abdominal lymph nodes.* Samples from this tumour caused some surprise when they gave a large broad resonance line at $g = 2{\cdot}011$ and the suggestion of a low field line in the region of $g = 4$ (Fig. 4). No such signal had been reported before in any tumour at our normal cavity temperature of 37° C, although recent work by Brennan, Cole, Singley and Hodgkinson showed a complex spectrum in cancerous tissue at liquid nitrogen temperature (77° K)[4].

This signal was again found in other samples from the same metastasis and also for samples from an identical metastasis in another rat, the same result being obtained for the $g = 2{\cdot}011$ line but the low field signal being less evident.

(*b*) *Liver carcinoma induced by butter yellow (di-methyl aminoazobenzene).* After the discovery of the large broad lines in the metastases, samples of liver carcinoma, induced by butter yellow from two rats, were investigated for similar lines. No signals were observed from the apparently necrotic tissue of these tumours, but from the viable tissue this large resonance was again observed (Fig. 5). The low field resonance was not detected. The *g*-value and line width (2·016; 50 G), and the fact that no observable decay in intensity over a period of 24 h occurred at room temperature, seem to indicate that the signal is due to a paramagnetic ion rather than a free radical associated with the metabolic processes. It was not thought that the signal arose from some contamination in the carcinogen which induced the tumour, since the tumour had been transplanted through many generations of rats. Neither was any impurity suspected, since, as noted, no signal occurred in the necrotic tissue.

A search was made for similar signals in the viable tissue of the other tumour types investigated here, but no such lines were found.

This signal was not apparent in previous measurements[1] because of two factors. First, the line width is large and could possibly be missed if the field sweep was relatively small or of the same order, and secondly, the paramagnetic absorption from our early sample cells, which were of 'Pyrex' and not pure silica[1], appears to have hidden this line. However, the observation made before, that there appeared to be no free radical present in any detectable amount, seems to be further verified. It would, however, be difficult to resolve the comparatively small free radical signal that would be expected, from the large, broad resonance reported here.

(*c*) *Spontaneous kidney carcinoma.* This tumour was the only one in which an apparent free radical signal was observed. Although this signal was small, it was sufficiently large to make us confident of its existence (Fig. 3). It was not observed, however, in all the samples taken of this tumour type, but this may have been due to larger uncertain amounts of necrotic tissue present.

(*d*) *Other tumours.* A variety of other rat tumours (see Table 2) were investigated but yielded very small or no detectable signals, so that if free radicals are present as one would expect, their concentration is less than 10^{-7} moles./l.

The observations on free radical signals in normal tissues for the greater part merely establish facts about these tissues that previously had been observed only with liver, kidney and heart. The usefulness of these results is apparent when comparisons are made between normal and abnormal tissues of the same type.

The different g-values found for different tissues indicate that the source of the signals is most probably different in each tissue.

Beinert and Sands[5] and Bray, Petterson and Ehrenberg[6] have investigated the signals from the types of enzyme-substrate complexes found in rat liver and have shown the presence of both free radical and paramagnetic ion signals. The former is generally attributed by all to the formation of a semiquinone free radical in equilibrium, and the latter to molybdenum and iron of suitable valency, associated with the redox system. Since the mitochondria contain many such enzyme systems and since heart muscle mitochondrial particles have been shown

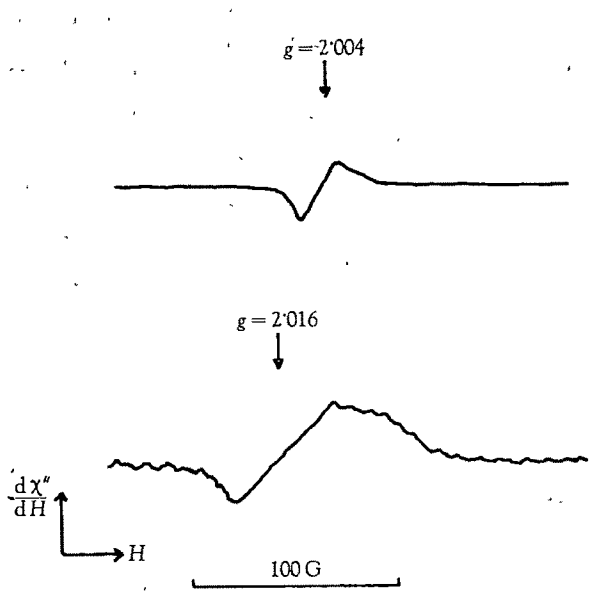


Fig. 5. Signal observed from liver carcinoma with (above) a representation of the normal rat liver signal on the same scale

to exhibit a similar signal[7], it would be expected that the signal observed would possibly be the envelope of a number of lines produced by the different active centres. The obvious conclusion is that this may account for the observed asymmetry in some of the signals discussed here. The problem is now to obtain the best fit to the envelope from linear combinations of a number of different free radicals of different g-values. At present the information is obviously too limited for such a process, but an assessment of the amount of each active system, associated with a measurement of g-values, might resolve these signals if this is the cause of the asymmetry. Other less intense lines are observed in fact as the modulation amplitude is reduced (see Fig. 2), but the resolution is poor. No change in overall line width or shape was noticed as the microwave power was reduced.

Noticeable, perhaps, is the apparent difference in g-values between the different tissues and also that those with the lowest g have been found to be least asymmetric. More precision in g-value measurements is required here.

The presence of the large paramagnetic absorption at $g = 2{\cdot}01$ (and possibly $g \sim 4$) from the rat liver tumours investigated is most interesting. (This was not observed in a recent communication by Vithayathil, Ternberg and Commoner[9], published after the preparation of this article.) It was thought that the presence of this signal in these tumours might have been due merely to an increase of a paramagnetic element in the system. Since this signal is not detectable in the normal liver, only a free radical signal being present, the signal obtained from tumour tissue is at least 100 times greater than any that might be present in the normal tissue. However, other methods of analysis on normal liver and liver tumours[8] show that for a number of typically paramagnetic species (in an ionized state), that is, copper, iron, molybdenum, manganese, the absolute concentration of these in the tumour is increased by only a factor of 2 above normal. The changes found in molar concentration from normal to tumour tissue by these workers are $4–8 \times 10^{-3}$ moles/l. for iron and $4–8 \times 10^{-4}$ moles/l. for copper. For molybdenum there is no change, the level remaining at about 2×10^{-5} moles/l. and likewise for manganese which remains at 4×10^{-4} moles/l.

Thus both iron and copper are present at levels several orders of magnitude greater in concentration than the paramagnetic species found in signals from rat liver tumours, and, if this signal is associated with the increase in concentration of one of these elements, then at least 1 part in 100 of this increase must be in a suitable valency state to give rise to this signal, as compared with only 1 part in 10^{-4} in the normal liver. In this case, the principal cause of the difference of signal between tumour and normal tissue need not be related to the actual increase in concentration of that particular element but more to a change in valency state. The implication of this is that some bound transition element in the normal liver cells experiences a valence change in the malignant cells (or perhaps is less tightly bound). The increased concentration of both copper and iron in these cells may or may not be related to this.

The g-value of the broad signal is similar to that found for Fe^{2+} and Mo^{5+} in various biological systems as described by other workers[5,6], and may be due to the presence of these. Since no hyperfine structure was detected and since the line was only 50 G wide, Mn^{++} was not thought to be contributing.

A programme of work is now in progress to separate the components of the cells contributing to the various signals in the overall pattern and to carry out activation analysis studies on the same samples measured in the electron spin resonance spectrometer.

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ALPHA-RADIOACTIVITY OF HUMAN BLOOD

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MUCH attention has been paid in recent years to the problem of the natural radioactive content of human tissues. The development of a simple low-level α-counting technique[1] and a sensitive α-spectroscopic technique[2] by workers at the Royal Cancer Hospital has resulted in the accumulation of a large number of data by this group. The results most pertinent to the present discussion have been summarized by Mayneord *et al.*[3] and by Hill[4]. The total α-activity of normal human soft tissue was reported[3] at a level of about 7 pc./kg of wet tissue for most tissues, although values ranged from as low as 2 pc./kg in brain tissue to as high as 33 pc./kg in hair. The α-spectroscopic data[4] indicated that in many cases a large fraction of this activity was due to the nuclide polonium-210. Other workers have tended to concentrate on the measurement of specific nuclides rather than of total α-activity, but the results are in general agreement with the data quoted. Thus, polonium-210 concentrations in soft tissues ranging from 1 to 13 pc./kg have been reported[4–8], while radium-226 values of about 2 pc./kg[9,10] and less[4,11] are on record. Measurements of the activity due to the thorium series or to specific nuclides therein have sometimes been attempted. Such activity is generally detectable, but appears to contribute less than 50 per cent of the total α-activity[12].

The data referred to in the preceding paragraph were obtained from a variety of soft tissues such as lung, liver, kidney, spleen, muscle and hair. In addition, a great many data have been obtained from bone specimens, which tend to have an α-radioactivity of the order of twenty times that of soft tissues[3]. An important constituent of the human body which does not appear to have been investigated from the point of view of α-radioactivity is blood. A search through the literature revealed data by Gofman[13], who reported both uranium and thorium in human blood serum at his limits of detectability between 0·00 and 0·10 p.p.m., and a solitary value from a single blood sample by Hoffman[14], who quoted a uranium concentration of 10^{-10} g/ml. Several workers[1,15–17] have measured α-radioactivity in the blood of abnormally exposed persons (for example, radium dial painters and thorotrast patients), but we have not been able to find a record of any general survey of the α-radioactivity of normal human blood. The purpose of this article is to report the preliminary results of such a survey.

We made use of the α-counting technique of Turner *et al.*[1]. This involves a sealed sample-phosphor system of great sensitivity and convenience. The particular set-up used is essentially the same as that described in an earlier report from this laboratory[18]. The preparation of biological samples for thick-source α-counting usually requires some method of artificially concentrating the activity, and the method used must involve a process which results in (*a*) no contamination, (*b*) minimum loss of volatile trace elements, and (*c*) an end-product which is homogeneous (or easily homogenized by grinding). After investigation, the well-known freeze-drying technique[19] was found to be suitable for the blood samples used in the present investigation. In this technique the blood is initially frozen, and the process essentially involves the sublimation of moisture from the sample under vacuum. The blood comes into contact only with the surfaces of the containers in which it is stored and those in which the freeze-drying takes place (1·5-l. round-bottomed 'Pyrex' flasks in the present work); the risk of contamination is very small. The drying is usually performed at temperatures between $-10°$ and $-40°$ C (at most the temperature might rise to room temperature), and loss of trace elements by volatilization is thus minimized. The end-product is a dry, homogeneous powder which is easily ground in an agate mortar before packing on the phosphor for counting. Interpretation of results is simple and follows the usual 'thick-source' α-counting formula[1,20,21].

The blood samples used in this investigation consisted, in most cases, of expired blood from normal donors to the local blood transfusion service. As such, they contained additives in the form of standard anticoagulant chemicals, and it was necessary to correct for the α-radioactivity in these materials. This was measured, and the necessary small correction was made to all the results.

Checks on the results were made by:

(*A*) Sub-dividing eight samples into halves, and freeze-drying the two halves of each sample in two different containers. The total α-activities of the two halves of each sample were then measured separately. The average deviation from the mean was 8 per cent, and in no case was the deviation more than 20 per cent. Random contamination from containers would appear to be unimportant.

(*B*) Sub-dividing one sample into six batches, and adjusting the *p*H of each batch to values varying from 1·4 to 12·2 (compare a 'normal' *p*H of about 6·5 for citrated whole blood). The *p*H variation appeared to have no definite observable effect on the recorded α-activity, and we concluded that a *p*H dependent adsorption mechanism was unlikely to be a serious problem.

(*C*) Sub-dividing seventeen samples into two halves, then using one-half of each sample for a whole-blood measurement and centrifuging the other half to provide separate 'red cell' and 'plasma' volumes. The α-activities of the 'red cells' and the 'plasma' were then measured separately; these two results, combined together after suitable allowance for concentration factors, gave a whole-blood α-activity measurement which could be compared with the direct measurement. The results of this check are shown in Table 1. The errors quoted are those due to counting statistics only, and to each must be added an arbitrary error (of the order of 10 per cent of the activity) to allow for non-reproducibility in sample preparation. If this is taken into account, comparison of columns four and five of Table 1 shows that the agreement is reasonably good for most of the samples.

We feel that it is highly unlikely that we would have obtained such agreement if sample contamination were a major problem.

(*D*) As a final check, four of the samples (Nos. 14–17 in Table 1) were measured without the prior addition of anticoagulant. The activity values obtained for these samples do not appear to be appreciably different from those obtained for the other samples after applying the small anticoagulant correction.

The salient features of the results can be summarized as follows:

(1) The results shown in Table 1 represent our most carefully checked set of data, and they give a mean α-activity for the 17 samples of 54 pc./kg. The range of activities encountered covers a factor of about two both above and below this figure. In addition to the data shown in Table 1, we have measured the total α-activities of some forty other whole blood samples by the same technique. These have not been as carefully checked as the results in Table 1, but they confirm the relatively high value for the total α-radioactivity of blood which is quoted above.

(2) Our apparatus incorporated a second long-dead-time electromechanical register to record α-particle 'pairs'[1] due to successive decays from emanation-220 to polonium-216 to lead-212. This is made possible by the 0·16 sec half-life of polonium-216, and makes it possible to estimate the percentage of the total α-activity which is due to the thorium series. For all the whole blood samples measured, a striking feature was the high thorium-series percentages observed. In fact, if it is assumed that either radium-228 or thorium-228 is supporting the thorium series activity, then our results indicate that this series accounts for more than 90 per cent of the total α-activity in thirteen of the seventeen samples listed in Table 1, and for between 55 and 90 per cent of the activity in the other four samples. Because of the low 'pairs' rates involved the errors in the thorium series percentages are large as far as individual samples are concerned, but the overall picture of a high thorium series contribution seems to be unambiguous.

Table 1. TOTAL α-ACTIVITY OF HUMAN BLOOD IN PC./KG

Sample No	Red cells	Plasma	Whole blood: Derived from red cells and plasma	Whole blood: By direct measurement
1	82±6	77±4	80±5	61±5
2	82±6	31±2	56±4	55±5
3	70±5	31±2	50±4	54±2
4	93±6	26±2	59±4	55±5
5	62±6	20±2	37±4	44±5
6	43±4	29±1	34±2	40±4
7	75±6	26±1	44±4	66±5
8	77±5	29±2	44±4	45±4
9	114±7	42±2	70±5	82±5
10	55±5	83±2	72±4	55±4
11	125±7	42±2	81±5	109±5
12	55±5	36±4	40±4	79±7
13	33±6	29±2	32±4	39±4
14	43±5	35±2	39±4	69±4
15	34±5	33±2	33±4	34±4
16	36±5	40±2	38±4	42±4
17	47±4	49±4	48±4	40±2

(3) Comparison of columns two and three of Table 1 shows that the activity present in the red cells was greater than that in the plasma in fourteen of the seventeen cases—the average ratio (red cell activity)/(plasma activity) being equal to 1·71.

(4) Within the limits of reproducibility of our experimental technique, and of the number of samples analysed, we have not found any significant variation of α-activity level with either the blood group or the age of the donor.

Our present results are sufficiently different from those recorded on human soft tissues to require some comment. Most noteworthy, of course, is the fact that they indicate a blood α-activity level which is about seven or eight times larger than the average on record for most soft tissues. There are three possible explanations of this feature:

(*a*) Our results are substantially too high. We feel this is unlikely, in view of the care taken experimentally, the satisfactory checks carried out and the inherently non-contaminatory nature of the freeze-drying process. In view of the very low levels of activity involved, it is none-the-less highly desirable that our data be checked by other workers.

(*b*) The α-activity of blood is, in fact, substantially higher than that of muscle, liver, kidney, spleen, etc. It is difficult to see why this should be so, but our present lack of detailed knowledge concerning the metabolic fate of the α-emitting trace elements in the human body makes it impossible to rule out this possibility at this stage. Our results might even be construed as indicating that some nuclide supporting the thorium-series activity is, for some reason, retained in the blood rather than being transported to other tissues. Once again, such a conclusion can only be regarded as speculative at this stage.

(*c*) The results of previous workers are substantially too low. We intend to repeat measurements on various soft tissues utilizing the freeze-drying process; meanwhile, we can offer the following evidence as indicating that the possibility of previously reported values being too low does, in fact, have some experimental support: examination of the references quoted earlier shows that nearly all the data in the literature concerning the natural α-radioactivity of soft tissues were obtained from samples that were either ashed (at about 600° C) or wet-ashed (presumably at about 250° C). These processes were used either for the purpose of concentrating the activity to measurable levels or for chemical separations. They do, however, involve the risk of loss of volatile components, and several workers[13,23,24] have emphasized the serious nature of this risk where trace elements are involved; McDaniel *et al.*[23], in fact, particularly recommend that sample treatment be limited to 'mere lyophilization' (freeze-drying). We tested this possibility by unpacking three of our freeze-dried blood samples after counting and heating them for set periods at temperatures ranging from 100° C to 700° C. After determining the concentration factor involved at each temperature, the material was packed in the standard sealed sample-phosphor system and then counted after allowing the usual three to four week period for the samples to come back into a degree of radioactive equilibrium. The activity was then calculated, due allowance being made for the fact that the change in sample composition affects the α-particle range in the sample. It was immediately apparent that substantial losses (by a factor of two or more) occurred even on heating to 100° C, while those samples treated at the ashing temperatures in the vicinity of 600° C gave activity values which were lower than the freeze-dried values by factors as large as nine. It was also obvious that the degree of loss depended on other factors besides temperature—notably length of heating time—and that quantitative experiments would be difficult to reproduce accurately.

Also worthy of comment is the predominance of thorium-series activity which we have observed. This must in turn imply that the nuclide polonium-210 accounts for only a very small fraction of the total blood α-activity. Once again, this result is not in accord with the published data for human soft tissues.

The present work presents the preliminary results of a survey of the α-radioactivity of normal human blood. The activity values observed are surprisingly high, being several times higher than those previously reported for human soft tissues. Furthermore, they indicate a surprisingly high percentage of thorium series activity. If substantiated, these results raise intriguing problems concerning the disposition and metabolism of the α-radioactive trace elements in the human body. We hope that other workers in this field will check our results, and we commend to them the freeze-drying technique as a satisfactory method of sample preparation.

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INTERLOCUS SPECIFICITY FOR CHEMICAL MUTAGENS IN *Aspergillus nidulans*

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THE present paper reports preliminary observations of the apparent specificities of certain chemical mutagens towards various related gene loci in the fungus, *Aspergillus nidulans*. The mutagenic effects of two types of chemical compound are recorded, the monofunctional ethylating agents (diethyl sulphate and ethyl methanesulphonate), and the pyrolizidine alkaloids (heliotrine and lasiocarpine).

The mutational system used for study is one in which a methionine dependent strain of Aspergillus (*meth*$_1$; *bi*: requiring methionine and biotin) mutates by forward mutation to methionine independence after mutation at any of several independent suppressor gene loci[1]: the *meth*$_1$ suppressor mutations which have so far been tested are found not to be genetically linked to *meth*$_1$ (ref. 2).

Uninucleate and haploid conidia of the *meth*$_1$; *bi* strain give rise with a low frequency to methionine independent colonies when plated on a medium lacking methionine. Three of the revertant types (types *A*, *B* and *C*), which occur with the highest frequencies, and which are phenotypically distinguishable from one another, have been chosen for investigation. The type *A* suppressor forms a thinly growing green colony, the type *B* suppressor produces a brown pigment, and the type *C* suppressor forms a thick green colony with a hyaline edge; illustration of these three *meth*$_1$ revertant types is found in refs. 1 and 3. It is not known whether reversion occurs by back-mutation at the *meth*$_1$ locus, but if this occurs, the revertants are likely to show the essentially wild-type phenotype of the *meth*$_1$ type *A* suppressor class.

While genetical analysis[1,2] of both the *meth*$_1$ type *A* and *C* suppressor effects has shown that each phenotype is produced at either of two independent (and not closely linked) gene loci, technical difficulties have prevented the genetic resolution of the *meth*$_1$ type *B* suppressor effect. The *meth*$_1$ suppressor system thus covers the mutational response of several (at least five) gene loci, and allows the frequency of the three *meth*$_1$ suppressor phenotypes to be scored in one and the same experiment. However, as it is not possible to assign a mutant to a particular gene locus by phenotype alone, the *meth*$_1$ suppressor system is technically limited to scoring individual suppressor phenotypes, and these may, of course, show differential interlocus mutational specificities within themselves. Nevertheless, the results reported in the present investigation demonstrate a general differential specificity for mutation at the *meth*$_1$ suppressor loci after treatment with chemical mutagens.

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The techniques used are those at present in use in studies on *Aspergillus nidulans*[4].

Conidia for treatment were collected from 5-day-old plates and suspended in 0·2 M phosphate buffer (*p*H 7·0) containing 1 ml. of 0·1 per cent 'Tween-80' (a wetting agent) per 10 ml. of buffer, shaken in a Vortex mixer to break up conidial chains, and filtered through sterile cotton wool to remove mycelial debris.

Treatments were carried out at 37° C on a suspension of about 1–5 × 10^6 conidia per ml., either by pipetting in the reagent directly (as with diethyl sulphate, and ethyl methanesulphonate), or by re-suspending in the appropriate concentration of buffer plus reagent (as with lasiocarpine and heliotrine). After treatment, the conidia were washed by centrifuging and re-suspending in fresh buffer. Controls were handled in the same way. Viable counts were obtained by plating appropriate dilutions of the conidial suspension on to a medium supplemented with methionine and biotin; direct conidial counts were by haemocytometer. Mutants were assayed by pipetting samples of the conidial suspension into a methionineless-medium supplemented with biotin (at 45° C to prevent gelling) and poured on to Petri dishes barely to cover the surface of the plate. All cultures and plates were incubated at 37° C. The number of plates used for the assay of mutants is recorded for each experiment (Tables 1 and 2) to give an indication of the density of mutants per plate.

Total II (Tables 1 and 2) gives a corrected distribution for the type *A* and *B* suppressor phenotypes arising from an initial misclassification of a proportion of the type *A* revertants. A small number of apparent type *B* revertant colonies appeared atypically type *B*, in that, although they are darkly pigmented they conidiate green, unlike the majority of the type *B* suppressors. On transfer to a medium containing methionine, the pseudo-*B* type gives the type *A* phenotype, yet gives the pseudo-*B* phenotype when re-transferred on to a methionine-less medium. The proportion of pseudo-*B* phenotypes among type *A* varies between treatments (see Tables 1 and 2); the only mutagenic chemical which so far has not produced the pseudo-*B* phenotype is hydroxylamine[5].

Total III (Tables 1 and 2) is the correction for spontaneous mutation.

Spontaneous mutation. The rate of mutation from methionine dependence to methionine independence of the *meth*$_1$; *bi* strain is found to be about 1 × 10^{-6} (Table 1), with relative frequences of the type *A*, *B* and *C* phenotypes of 0·64, 0·36 and 0·09 × 10^{-6}, respectively, and overall percentages for the respective type *A*, *B* and *C* phenotypes of 59, 33 and 8.

It should be noted, however, that the spontaneous mutation rate and the overall percentages of *meth*$_1$ suppressor types reported by other workers for the *meth*$_1$; *bi* stock are at variance with the figure reported here. Thus Siddiqui records[3] (his Table 1, our calculation) a spontaneous mutation rate of $8{\cdot}2 \times 10^{-6}$, and overall percentages of *A*, *B* and *C* suppressor phenotypes of 34, 31 and 35, respectively. Furthermore, Lilly records a spontaneous mutation rate of 16×10^{-6} (ref. 1), with distributions of suppressor types[6] of 66 per cent type *A*, 31 per cent type *B*, and 3 per cent type *C* (based on 233 revertants). Roper reports[7] a spontaneous *meth*$_1$ suppressor mutation rate for the *meth*$_1$; *bi* stock held in his laboratory as about 10×10^{-6}, and this latter figure has been confirmed by us on his stock when plated under our conditions; a spontaneous mutation rate of $9{\cdot}2 \times 10^{-6}$, and a percentage distribution of the respective type *A*, *B* and *C* suppressor phenotypes (based on 199 revertants) of 35, 65 and zero.

Although there are pronounced disparities in the behaviour of the various *meth*$_1$; *bi* stocks, which, so far as is known, originated from a common stock in the Department of Genetics, Glasgow, our spontaneous *meth*$_1$ suppressor mutation rate is consistent (Table 1, and others). However, there is a statistically significant heterogeneity for the distribution of suppressor phenotypes between the controls in Table 1 ($\chi^2_{(12)} = 39$, $P < 0{\cdot}001$).

The monofunctional ethylating agents

Table 1 illustrates the mutagenic response of the *meth*$_1$ strain to the induction of type *A*, *B* and *C* suppressor phenotypes after treatment of conidia with diethyl sulphate and ethyl methanesulphonate; both treatments have been repeated several times over a range of conidial survival levels.

The ethyl methanesulphonate treatments (1–6) show a highly significant heterogeneity for the distribution of suppressor phenotypes ($\chi^2_{(10)} = 229$, $P \ll 0{\cdot}001$), as might be expected in treatments covering varying levels of conidial survival. However, there is still a significant heterogeneity in the distribution of suppressor phenotypes at similar levels of conidial survival; for example, the ethyl methanesulphonate treatments 1, 2 and 3, which are all at about the 50 per cent survival level, give a $\chi^2_{(4)} = 22$, $P < 0{\cdot}001$. The overall distribution of *meth*$_1$ suppressor phenotypes after ethyl methanesulphonate treatment shows a mutational response predominantly to the type *A* phenotype (92 per cent), with low responses to the type *B* (5 per cent) and type *C* (3 per cent) phenotypes.

The diethyl sulphate treatments (1–5) similarly show a highly significant heterogeneity in the distribution of the type *A*, *B* and *C* phenotypes ($\chi^2_{(8)} = 405$, $P \ll 0{\cdot}001$) over the recorded conidial survival levels.

At similar levels of conidial survival, the ethyl methanesulphonate (experiments EMS-5 and -6) and the diethyl sulphate treatments (experiments DES-2 and -3) show a significant difference in the distribution of suppressor phenotypes ($\chi^2_{(2)} = 22$, $P < 0{\cdot}001$).

The overall distribution of *meth*$_1$ suppressor phenotypes is significantly different for the control and diethyl sulphate ($\chi^2_{(2)} = 26{\cdot}5$, $P < 0{\cdot}001$), control and ethyl methanesulphonate ($\chi^2_{(2)} = 235$, $P \ll 0{\cdot}001$), and ethyl methane-

Table 1. MUTAGENIC EFFECT OF ETHYL METHANESULPHONATE (EMS) AND DIETHYL SULPHATE (DES) ON THE REVERSION (BY SUPPRESSOR MUTATIONS) OF THE METHIONINE DEPENDENT (*meth*$_1$) STRAIN OF *Aspergillus nidulans*

Experiment	Molarity of treatment and duration	Percentage of survival	No. surviving conidia plated ($\times 10^6$)	No. plates used	No. and class of revertant types *A*	*B*	*C*	Frequency of mutants $\times 10^{-6}$ ($A+B+C$)	Relative frequency of revertants $\times 10^{-6}$, and (in parentheses) the overall percentages of suppressor phenotypes *A*	*B*	*C*
Control-1	—	73	3 2	5	1	—	—	0·30	0 3	—	—
Control-2	—	64	7 7	10	8	3	2	1·70	1·0	0·4	0 3
Control-3	—	69	8·3	4	5	5	2	1·40	0 6	0 6	0 2
Control-4	—	66	37·0	20	56	7	5	1 80	1·5	0 19	0 14
Control-5	—	69	41·0	12	18	30	4	1·26	0 44	0·73	0·1
Control-6	—	70	41·0	12	10	5	—	0 36	0·24	0·12	—
Control-7	—	61	46·0	10	20	17	3	0·87	0·43	0 37	0·07
Total I		67	184·2	73	118	67	16	1·10	0·64 (59)	0·36 (33)	0 09 (8)
Total II*		67	184·2	73	126	59	16	1·10	0 68 (63)	0·32 (29)	0·09 (8)
EMS-1	0·1 M for 30 min	51	4·0	16	391	6	—	99	97 5	1 5	—
EMS-2	0·1 M for 30 min	50	5 8	10	120	2	—	21	20 7	0·3	—
EMS-3	0·1 M for 30 min	50	13·0	18	547	35	4	45	42·0	2·7	0·3
EMS-4	0·2 M for 30 min	26	1·7	52	268	59	44	218	157·6	34·7	25·9
EMS-5	0·2 M for 30 min	4	1·3	57	1,597	214	74	1,450	1,228·5	164·5	57 0
EMS-6	0 2 M for 30 min	3	0 8	18	821	89	7	1,159	1,026·0	124·0	9·0
Total I			26·6	171	3,744	415	129				
Total II*			26·6	171	3,951	208	129				
Total III*			26 6	171	3,933	200	129	160	148·0 (92·0)	7 0 (5 0)	5 0 (3·0)
DES-1	0·05 M for 20 min	6	3 20	13	876	63	16	298	274·0	19·0	5·0
DES-2	0·05 M for 20 min	5	0·87	12	390	60	26	547	448·0	69 0	30·0
DES-3	0 05 M for 20 min	3	0·60	13	513	118	22	1,088	855·0	196·5	36·5
DES-4	0·05 M for 20 min	1·4	0·22	48	97	120	19	1,073	441 0	545 4	86·6
DES-5	0·05 M for 30 min	0·5	0·19	61	1,335	582	48	10,342	7,026·3	3,063·1	252·6
Total I			5 08	147	3,211	943	131				
Total II*			5 08	147	3,284	870	131				
Total III*			5·08	147	3,280	868	131	842	645 5 (76·6)	170 8 (20·3)	25·7 (3·1)

* Total II: corrected for pseudo-*B* phenotypes (see text).
Total III: corrected for spontaneous mutation.

Table 2. MUTAGENIC EFFECT OF LASIOCARPINE (LASIO) AND HELIOTRINE (HELIO) ON THE REVERSION (BY SUPPRESSOR MUTATIONS) OF THE METHIONINE DEPENDENT (*meth*$_1$) STRAIN OF *Aspergillus nidulans*

Experiment	Molarity of treatment and duration	Percentage of survival	No. surviving conidia plated ($\times 10^6$)	No. plates used	No. and class of revertant types *A*	*B*	*C*	Frequency of mutants $\times 10^{-6}$ ($A+B+C$)	Relative frequency of revertants $\times 10^{-6}$, and (in parentheses) the overall percentages of suppressor phenotypes *A*	*B*	*C*
Lasio-1	0 02 M for 6 h	75	29·0	10	32	273	1	10 5	1·10	9 40	0 03
Lasio-2	0·02 M for 18 h	27	19·0	12	46	421	18	25 5	2·40	22·15	0 95
Lasio-3	0·02 M for 18 h	11	6·0	98	151	382	46	96·5	25·1	63·7	7·7
Lasio-4	0·02 M for 24 h	0·6	0 5	56	19	139	6	328·0	38·0	278·0	12 0
Total I			54·5	176	248	1,215	71				
Total II*			54·5	176	296	1,167	71				
Total III*			54 5	176	258	1,150	66	27·0	4·7 (17·5)	21 1 (78·0)	1 2 (4 5)
Helio-1	0·02 M for 4 h	40	11·2	6	86	61	3	13·4	7·7	5·4	0 3
Helio-2	0·02 M for 5 h	35	16·0	12	29	35	2	4·1	1·8	2 2	0 1
Helio-3	0·02 M for 18 h	30	13·6	14	543	246	7	58·5	39·9	18·1	0 5
Helio-4	0 02 M for 18 h	8	2·6	20	124	109	13	94 6	47·7	41·9	5 0
Total I			43·4		782	451	25				
Total II*			43·4		1,061	172	25				
Total III*			43 4		1,031	158	21	27 9	23·8 (85·0)	3·6 (13·0)	0·5 (2·0)

* Total II: corrected for pseudo-*B* phenotypes (see text).
Total III: corrected for spontaneous mutation.

sulphonate and diethyl sulphate treatments ($\chi^2_{(2)} = 477$, $P \ll 0.001$).

The diethyl sulphate treatments show an overall distribution of *meth*$_1$ suppressor phenotypes which is different from the ethyl methanesulphonate treatments, that is, a 15·4 per cent decrease of the type *A* phenotype, and a corresponding increase in the type *B* phenotypes; there is no difference in their mutational response to the type *C* phenotype, but treatment with either ethylating agent shows a lower response to the type *C* phenotype that is found in the control experiments.

The pyrolizidine alkaloids[8]

Treatment of conidia with lasiocarpine and heliotrine induces two quite distinct and unrelated distributions for the *meth*$_1$ suppressor phenotypes. Lasiocarpine is found to give predominantly the type *B* suppressor phenotype (78 per cent) compared with the relatively low mutational response to this phenotype (13 per cent) produced by heliotrine. Correspondingly, heliotrine gives 85 per cent of the type *A* phenotype compared with the figure of 17·5 per cent produced by lasiocarpine; heliotrine also produces about half the percentage of the type *C* phenotype as lasiocarpine. Both distributions show highly significant heterogeneities, lasiocarpine ($\chi^2_{(6)} = 110$, $P \ll 0.001$), and heliotrine ($\chi^2_{(6)} = 50$, $P < 0.001$), with obviously significant deviations between their overall *meth*$_1$ suppressor distributions ($\chi^2_{(2)} = 122$, $P \ll 0.001$).

While the *meth*$_1$ suppressor distribution induced by lasiocarpine is clearly different from the other treatments recorded here, the distribution given by heliotrine deviates significantly from that of ethyl methanesulphonate ($\chi^2_{(2)} = 111$, $P \ll 0.001$), diethyl sulphate ($\chi^2_{(2)} = 70$, $P \ll 0.001$), and the control ($\chi^2_{(2)} = 67$, $P \ll 0.001$).

The various recorded mutagenic treatments obviously show striking interlocus specificities for the *meth*$_1$ suppressor phenotypes, despite the impracticability of assigning the mutant types to a particular gene locus; and despite the statistically significant heterogeneities of the *meth*$_1$ suppressor distributions after individual treatments. Further, the distinct variations shown in *meth*$_1$ suppressor phenotype distributions after treatment of conidia with such structurally similar chemicals as ethyl methanesulphonate and diethyl sulphate, on the one hand, and lasiocarpine and heliotrine[8], on the other, demonstrate the difficulties involved in assigning a common mutational mechanism to even closely related and assumedly functionally similar chemical mutagens. This consideration is heightened, if, as is claimed[8], the pyrolizidine alkaloids, in common with the ethylating agents, act by alkylation.

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PREPARATION AND PROPERTIES OF AN ENZYME SYSTEM FOR ETHYLENE PRODUCTION

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PREVIOUS studies[1–3] with several organisms have shown that ethylene is evolved from subcellular particles that have been partially disintegrated by such means as ageing *in vitro*. This paper reports the preparation and properties of enzyme powders capable of catalysing ethylene production, and such information, along with that obtained from experiments with enzyme inhibitors[4,5], makes it possible to propose a biosynthetic pathway for ethylene production.

Crude enzyme powders were prepared from subcellular fractions from oat seedlings, *Avena sativa*, wax bean seedlings, *Phaseolus vulgaris*, or wax bean cotyledons. Immediately before planting, the seeds were soaked for 10 min in 50 per cent sodium hypochlorite solution, washed in cold water, soaked for 5 min in 50 per cent ethanol solution and then rewashed. They were allowed to germinate and grow in the dark in previously sterilized flats of 'Perlite *R*' covered with wet tissue, or in 'California soil mix'. The seedlings were gathered at 8–10 days after planting and the cotyledons at 7–8 days. The latter tissue was used for most experiments because ethylene production was found to be maximal when the cotyledons were senescent (Fig. 1), and because mitochondria might be expected to undergo a decrease in activity and number at this stage[7]. (Disintegration of mitochondria in intact fruits[8] coincides with the period of maximum ethylene evolution, and *in vitro* production of the gas has been shown to be associated with mitochondrial disruption[1].)

For preparation of the enzyme powders, the whole seedlings were frozen at −15° C overnight, then ground for 15 sec in ice-cold buffer (0·5 M sucrose, 0·2 M potassium hydrogen phosphate, *p*H adjusted to 7 with sodium hydroxide) at low speed in a Waring blender, strained through cheese cloth and centrifuged at 0° C at 4,080*g* for 10 min. The supernatant was then recentrifuged at 35,000*g* for 15 min. When only cotyledons were used, they were crushed in a hydraulic press at 20,000 p.s.i. in ice-cold sucrose–phosphate buffer of the above composition, then strained and centrifuged in the same manner as the crushed seedlings. The method used for tissue grinding was found to be very critical. If the treatment with the blender was too severe, activity was greatly reduced and sometimes completely lost. Crushing the tissue hydraulically, as was done with the frozen cotyledons,

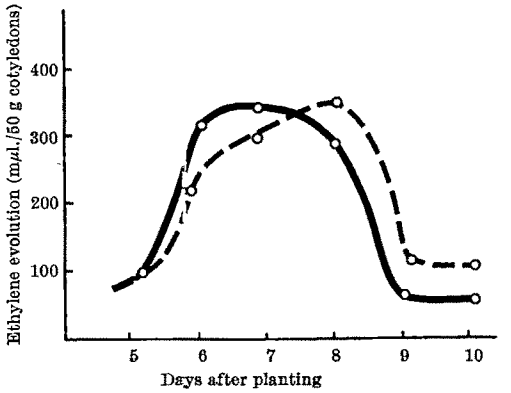


Fig. 1. Ethylene production by bean cotyledons: 0–2 h after harvest (————); 2–4 h after harvest (– – – –)

Table 1. ETHYLENE-PRODUCING ACTIVITY OF CRUDE AND PARTIALLY PURIFIED ENZYME POWDERS FROM BEAN COTYLEDONS

Collection time (h)	Treatment	Ethylene (mμl.)			
		Crude powder	0–20 per cent saturation with ammonium sulphate	20–65 per cent saturation with ammonium sulphate	65–100 per cent saturation with ammonium sulphate
0–2	none	28	*N*	62	28
2–20	none	141	*N*	124	203
20–21	none	17	*N*	28	*N*
21–24	frozen and thawed	34	*N*	56	124
Total		220	*N*	270	355
Powder (mg/ml.)		25	22·6	9·6	1·8

Crude powder was from a different batch preparation than the fractions.
N = not detectable.
Reaction mixtures: all samples contained 0·5 M sucrose–0 125 M phosphate buffer *p*H 7·0, 1 5 mM ATP, 50 mM β-alanine, 2·0 mM TPP, 1 0 mM Mg^{2+} added as $MgSO_4$, 0·17 mM Co*A*. The 65–100 per cent fraction contained 0·5 mM pyridoxal phosphate and 0·2 mM Fe^{3+} added as $Fe_2(SO_4)_3$. (Other experiments have shown that the 0–20 per cent fraction is inactive on addition of pyridoxal phosphate and Fe^{3+}.)

has been found to be a less drastic treatment giving a greater yield of structurally intact mitochondria. With both preparations, the pellet obtained from the second centrifugation was suspended in cold deionized water, then frozen in a dry ice–acetone bath and thawed in a 40° C water bath three times., This suspension was then subjected to ammonium sulphate ($(NH_4)_2SO_4$) fractional precipitation. Cold saturated ammonium sulphate solution, brought to *p*H 8·0 with sodium hydroxide, was used, and the fraction that separated after 1·5 h at 5° C at 66 per cent saturation with ammonium sulphate was lyophilized, and in the presence of β-alanine and ATP was active in ethylene production. (The supernatant fraction was only slightly active under these conditions.)

Lipids were then removed from the crude powder by a butanol extraction. To achieve this, the powder was suspended in cold absolute *n*-butanol (50 ml./g of powder) by use of a Servall 'Omnimixer' set at low speed, for 30 min; the suspension was centrifuged at 18,800*g* for 10 min at 0° C and the supernatant discarded. The residue was again treated with butanol and centrifuged as above. The precipitate was partially dried on filter paper under suction, in an atmosphere of nitrogen.

Further purification was obtained by intensive ammonium sulphate fractionation. The material on the filter paper was suspended in cold deionized water (10 ml. water/g of initial crude powder) for 5 min before addition of ammonium sulphate. All ammonium sulphate fractions were allowed to aggregate at 5° C and were subsequently spun down at 18,800*g* for 10 min at 0° C. The 0–20 per cent fraction was inactive in ethylene production and the 20–65 per cent fraction was active. The supernatant, after dialysis against deionized water for 24 h, was lyophilized, yielding the 65–100 per cent fraction. It is evident from Table 1 that this fraction was considerably more active with respect to ethylene evolution than the original crude powder. In subsequent experiments, a 35–100 per cent fraction was used in order to incorporate a large portion of the activity.

Collections and determinations of ethylene were conducted as previously described[1,6] except that in the gas chromatographic analyses for ethylene, 50 × 0·35 cm columns of activated alumina coated with silicone ('Burrell 341-116-02') were sometimes used instead of columns of squalane-coated firebrick.

When ^{14}C-labelled ethylene was used, it was collected in mercuric perchlorate and the radioactivity determined with a 'Nuclear-Chicago' liquid scintillation unit, Model 725, operated at 5° C. Mercuric perchlorate, containing the labelled gas as a mercury–ethylene complex, was mixed with anthracene and 'Tween 81' in the ratio 3 g anthracene : 2 ml. perchlorate : 1 drop 'Tween 81'. The mixture was stirred to a dry paste and counted.

β-alanine, together with the cofactors pyridoxal phosphate and ferric ion (Fe^{3+}) for transamination, and thiamine pyrophosphate (TPP) and magnesium ion (Mg^{2+}) for decarboxylation, when added to the enzyme powders yielded ethylene. Table 2 illustrates typical results obtained with crude powders. β-alanine augmented ethylene production by approximately 35 per cent relative to a control of crude powder and ATP. β-alanine, TPP and Mg^{2+} increased ethylene production by approximately 18 per cent relative to the same control containing crude powder and ATP. Addition of nicotine adenine dinucleotide (NAD) to β-alanine, TPP, Mg^{2+}, ATP and crude powder, inhibited production by approximately 25 per cent; hence it appeared that a transaminase rather than a deaminase was active. Pyridoxal phosphate did not in itself extensively augment emanation. It is possible that substantial amounts of this cofactor remained tightly bound within the system throughout the preparation of the crude enzyme powder. A system of β-alanine, TPP, Mg^{2+}, coenzyme *A* (Co*A*) and ATP increased ethylene production by approximately 41 per cent relative to a control containing crude powder and ATP.

Table 2. EFFECT OF ADDED COFACTORS AND SUBSTRATE ON ETHYLENE PRODUCTION BY CRUDE ENZYME POWDERS FROM OAT SEEDLINGS

Collection time (h)	Treatment	Ethylene (mμl.)					
		1*A*	1*B*	1*C*	1*D*	2*A*	2*B*
0–2	none	62	141	84	*N*	28	90
2–20	none	90	*N*	141	56	248	129
20–21	none	68	107	56	79	*N*	91
21–24	frozen and thawed	79	214	84	113	*N*	158
Total		299	462	365	248	276	468
Powder (mg/ml.)		25	25	25	25	25	25

Nos. 1 and 2 indicate different batches of powder.
N, not detectable.
Reaction mixtures: 1*A* and 2*A*: 0·5 M sucrose, 0·125 M phosphate buffer *p*H 7, 1·5 mM ATP. All other samples had 50 mM β-alanine in addition; 1*C*, 1*D* and 2*B* contained 2·0 mM TPP, 1·0 mM Mg^{2+} added as $MgSO_4$; sample 1*D* was 0 005 mM in NAD and 2*B* 0·17 mM in Co*A*.

The purified powder, in the presence of appropriate cofactors and substrates, produced a substantial amount of ethylene, but in the absence of these materials, the same weight of powder was essentially inactive (Table 3). No ethylene was produced by a mixture of substrates and cofactors in the absence of enzyme powder. Addition of α-ketoglutarate (αKG) to a system containing ATP, β-alanine, TPP, Mg^{2+}, pyridoxal phosphate, Fe^{3+} and Co*A* augmented production by 40 per cent, and addition of malonic acid caused an 83 per cent increase in ethylene evolution. Lipoic acid decreased production by 73 per cent, suggesting that the decarboxylation was probably non-oxidative.

When β-alanine-2-^{14}C was added to a complete reaction mixture, labelled ethylene was obtained as indicated in Table 3. Other experiments showed that production of ethylene was not affected by the presence or absence of sucrose.

The ethylene-producing activity of the enzyme preparations decreased markedly with time, and 2 weeks storage in a desiccator at 5° C rendered the powders essentially inactive. Furthermore, the system was found to be sensitive to small differences in *p*H, being virtually inactive outside the range 6·7–7·2.

In purification of the enzyme powders, it was necessary, before lyophilization, to dialyse the 35–100 per cent ammonium sulphate fraction. To replace losses during dialyses, in addition to required substrates and cofactors, αKG was introduced as an amino group acceptor.

Table 3. EFFECTS OF ADDED COFACTORS AND SUBSTRATES ON ETHYLENE PRODUCTION BY PARTIALLY PURIFIED ENZYME POWDERS FROM BEAN COTYLEDONS LABELLED ETHYLENE OBTAINED FROM ADDED β-ALANINE-2-^{14}C

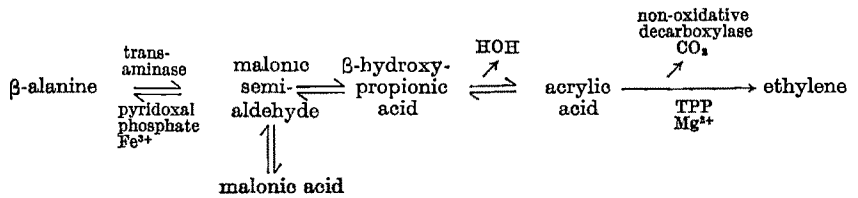
Collection time (h)	Treatment	Ethylene (mμl.) 1A	1B	2A	2B	3A	3B	4A	4B	5	5 d.p.m.
0–2	none	N	90	51	112	N	8	90	45	96	2,050
2–20	none	N	68	12	28	23	23	68	15		
20–21	none	N	62	22	34	56	100	62	15		
21–24	frozen and thawed	15	57	33	23	96	960	57	N	73	225
Total		15	277	118	197	175	1,051	277	75	169	2,275
Powder (mg/ml.)			1·25		1·75		0·65		1·25		2

Nos. 1, 2, 3, 4, and 5 indicate different batches of powders with differing activities.
N, non-detectable.
d p.m., c.p.m. corrected for 20 per cent efficiency of the counting procedure.

Sample No.	Reaction mixtures: 0·5 M sucrose 0·125 M phosphate pH 7·0	0·125 M phosphate pH 7·0	1·5 mM ATP	50 mM β-alanine	2·0 mM TPP	1·0 mM Mg^{2+} added as $MgSO_4$	0·5 mM pyridoxal phosphate	0·2 mM Fe^{3+} added as $Fe_2(SO_4)_3$	0·17 mM CoA	50 mM α-k-to-glutarate	70 mM malonic acid	0·02 mM lipoic acid	10 mM β-alanine	90 μc. β-alanine-2-^{14}C
1A	+	−	−	−	−	−	−	−	−	−	−	−	−	−
1B	+	−	+	+	+	+	+	+	+	+	−	−	−	−
2A	+	−	+	+	+	+	+	+	+	−	−	−	−	−
2B	+	−	+	+	+	+	+	+	+	+	−	−	−	−
3A	+	−	+	+	+	+	+	+	+	+	−	−	−	−
3B	+	−	+	+	+	+	+	+	+	+	+	−	−	−
4A	+	−	+	+	+	+	+	+	+	+	−	−	−	−
4B	+	−	+	+	+	+	+	+	+	+	−	+	−	−
5	−	+	+	−	+	+	+	+	+	+	+	−	+	+

(When α-ketoglutarate or malonate was added, it was necessary to adjust the reaction mixture to pH 7·0 with sodium hydroxide).

A possible pathway for the conversion of β-alanine to ethylene is the following:



Malonic semi-aldehyde is very unstable and could quickly go to either β-hydroxypropionic acid or malonic acid. It is suspected that the large boost in ethylene production on addition of malonic acid arises from a shift in equilibria favouring production of β-hydroxypropionic acid.

Addition of 90 μc. of labelled β-alanine to purified powder, substrates and cofactors yielded labelled ethylene. In this experiment, unlabelled β-alanine was reduced to 20 per cent of its normal molarity to lessen the dilution of the label. The lower specific activity of the 21–24 h collection compared to the initial production (Table 3) may implicate adjustment of the equilibria allowing conversion of malonic acid to malonic semi-aldehyde, rather than β-alanine to malonic semi-aldehyde. It is significant that the per cent conversion of label (0·001 per cent) was comparable to the molar conversion of β-alanine to ethylene; about half the ethylene produced was labelled. Preliminary identification of intermediates further supports the proposed pathway.

Additional evidence that β-alanine may be a precursor of ethylene came from other experiments, when production of the gas was obtained non-enzymatically by a heat-catalysed Snell reaction among β-alanine, pyridoxal hydrochloride and cupric ion.

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LOCATION OF THE MECHANISM OF ACTIVE TRANSPORT OF SODIUM ACROSS THE FROG SKIN

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THE past 10 years, since Koefoed-Johnson and Ussing[1] introduced a model of ion transport, have seen a considerable increase in our knowledge of the properties of the epithelial membranes. This model assumes that net Na^+ transport across frog skin consists of two main steps: a passive diffusion through an outer barrier and an active transport across an inner barrier. The model also assumes that the outer barrier is almost impermeable to K^+. Since Meyer and Bernfeld[2], as well as Ussing[3], suggested that the seat of the skin electrical potentials and salt-transporting mechanism was located in the cells of the stratum germinativum, the inner and outer barrier of the model would be identified as the inner and outer facing membranes of these cells. However, Kidder, Cereijido and Curran[4], on the basis of electrical potentials changes following a change in Na^+ concentration in the outer solution, demonstrated that an Na^+-sensitive barrier appears to be located at the outer border of the skin. This information, together with the observation of Curran and Cereijido[5] that all the K^+ in the skin exchanges with potassium-42 in the inside solution with apparently a single time constant, would suggest that the outer

barrier of the Koefoed-Johnson and Ussing model is located near the anatomical outer border of the skin. With respect to the mechanism by which Na^+ crosses that barrier Cereijido, Herrera, Flanigan and Curran[6] have clearly shown that it is not entirely by simple diffusion; instead, Na^+ interacts with the membrane. The present article deals with the location and properties of the outer barrier as well as the location of the Na^+-active transport mechanism. It will be shown that Na^+ entrance to the cell across the outer barrier involves an active mechanism and that there exists at that level, and across the whole epithelial layer, a sodium–potassium activated adenosine-triphosphatase (Na–K ATPase) which may constitute the molecular basis of the Na^+ active transport.

The abdominal skin of *Leptodactylus ocellatus* was mounted as a flat sheet between 'Lucite' chambers (3·14 cm^2 area) and bathed with Ringer's solution containing 1–10 mM Na^+ (sodium sulphate–sodium bicarbonate) on the outside and 120 mM on the inside. Both Ringer's solutions contained 2 mM K^+ (potassium sulphate), 250 mg/l. calcium sulphate, 2·4 mM HCO_3^-. Sucrose was added to the outside Ringer's solution in order to adjust the osmolarity to 188 mosm, and on both sides air was bubbled through the Ringer's solutions to give a *p*H of 8·2. In this preparation the short-circuit current measures the Na^+ net flux when sulphate Ringer's solutions are used[7]. In nine experiments the Na^+ net flux was 0·50 ± 0·07 (mean ± 1 standard error) μmol. h^{-1} cm^{-2}, which indicates that the skin is able to transport Na^+ from a solution with a concentration of 1–10 mM Na^+. In a second group of skins treated in the same way, ^{14}C-inulin was added to the inside solution to measure the inner extracellular space. After an hour the skins were removed from the chamber, blotted, and 2·67 cm^2 of the exposed area in the chamber cut out with a cork borer and weighed. The portions of skin were then allowed to dry to a constant weight at 90° C and the difference between the wet weight and the dry weight taken as the total water. Na^+ and ^{14}C-inulin were extracted with 2·0 ml. of 0·1 N nitric acid and measured. With these data and the concentration of Na^+ and ^{14}C-inulin in the bathing solutions the concentration of Na^+ in the cell was computed. Since in another set of skins with ^{14}C-inulin added to the outside solution the outer extracellular space was found to be between 0·1 and 0·3 μl. cm^{-2}, and because the Na^+ concentration in the outside solution never exceeded 10 mM, no correction was made for the amount of Na^+ contained in this space. Table 1 shows the values of the wet weight, the extracellular space, the percentage of water and the Na^+ content. In fifteen experiments in which these parameters were measured in the same piece of tissue, the Na^+ concentration in the cells was 97·0 ± 4·8 mM. It was possible to compute a similar value from data published by Huf, Doss and Wills[8]. Using *Rana pipiens*, they found 346 μmoles of Na^+ per gram of dry skin and a water content of 74·6 per cent. Since the Na^+ concentration in the Ringer's solution was 119 mM, assuming that, as in our experiments, 28·2 per cent of that water was in the epithelial cells, the concentration of Na^+ in the cells was 114 mM. Therefore the Na^+ net flux from the outer solution to the cells occurs against a concentration gradient. However, Cereijido and Curran[9], using microelectrodes, demonstrated that under short-circuited conditions in sulphate Ringer's solution the skin maintained an electrical potential difference of −18 mV (cell negative), that is, was oriented in the direction which 'helped' Na^+ to enter the cell compartment. Then, if Na^+ was to have entered this compartment by a passive process, its concentration in the cell water should have been at the most $10.e^{-18\ F/RT} = 20{\cdot}4$ mM, which is far below the actual concentration measured. It is concluded that an active mechanism must be operating at the outside solution-cell boundary.

An Na–K ATPase associated with ion transport was found in many tissues[10]. Bonting and Caravaggio[11] demonstrated the presence of a sodium–potassium-dependent, ouabain-sensitive ATPase activity in homogenates of frog skin. Farquhar and Palade[12] demonstrated histochemically that ATPase activity was associated with all cell membranes facing the intercellular spaces, including the cell membranes of the outermost epithelial layer. This ATPase, however, is not necessarily the sodium–potassium-dependent one. The relationship between these ATPases was therefore studied in an effort to determine whether there is any Na–K ATPase activity near the outermost cell layer of the skin where an active mechanism was found. The skin was placed on the stage of a freezing microtome with the outer surface facing up; it was then frozen and a cylinder of 2·00 cm^2 base area was cut by means of a cork borer. The cylindrical piece of tissue was then sliced, and an effort was made to obtain slices covering the whole area. The ATPase activity and proteins were measured in each slice. Determination of ATPase activity was based on the method of Lowry *et al.*[13]. The assays were performed in triplicate at 40° C using two incubation media the only difference in which was that one had 5 mM K^+ and the other had 10 mM ouabain. The difference in activity was taken as ouabain-sensitive (or sodium–potassium-dependent) ATPase. Since the Q_{10} of this enzyme is 1·9 (see ref. 11) the values were corrected for 20° C. Proteins were measured according to the method of Lowry *et al.*[14], and the amount of protein per unit area was thus calculated. Table 2 shows the distribution of Na–K ATPase specific activity expressed as μmoles of inorganic phosphate liberated per mg of protein and per hour across the 'normalized depth' at which the slice was obtained; the outer border of the skin was taken as the zero reference. The 'normalized depth' was obtained as follows: the amount of protein measured in a given slice was added to the amount measured in all previous slices (that is, between the outer border and the given slice). This value was divided by the total amount of protein which in turn was obtained by adding together the amounts contained in all the individual slices. The fraction obtained was considered to be a measure of the depth. The slices came from eight different skins. Each of the figures in the column on the right represents a mean value of all those slices obtained at the same range of normalized depth. Na–K ATPase seems to be evenly distributed across the whole skin with a mean value of 0·182 μmole/h/mg of protein. Since 3·38 mg cm^{-2} of protein was present, the total amount of Na–K ATPase activity was 0·72 μmole h^{-1} cm^{-2}.

The stoichiometry between the active transport of Na^+ and the hydrolysis of ATP due to the Na–K ATPase was found to be close to three Na^+ ions actively transported per energy-rich phosphate bond split[11,15–17]. If the active transport mechanism is to pump three Na^+ ions per molecule of ATP split, 0·167 μmole of ATP would be needed per hour and per square centimetre in order to account for the net flux. From the data presented in Table 2, it is clear that this amount of ATP can be hydrolysed by the ATPase contained in a band covering the

Table 1

		n
Wet weight	26·1 ± 0·6 mg cm^{-2}	60
Percentage of water	72·7 ± 0·86 per cent	41
Extracellular space	13·6 ± 0·9 ml. cm^{-2}	34
Sodium content	2·14 ± 0·04 μmol. cm^{-2}	16
Sodium concentration in the cell	97·0 ± 4·8 mM	15

Table 2

'Normalized depth' %	Protein mg cm^{-2}	Na–K ATPase specific activity μmole h^{-1} mg^{-1}	*n*
0–10	0–0·33	0·182 ± 0·042	14
11–25	0·33–0·82	0·189 ± 0·066	12
26–40	0·82–1·30	0·168 ± 0·058	11
41–65	1·30–2·12	0·166 ± 0·053	12
66–100*	2·12–3·38†	0·203 ± 0·050	10
	Mean	0·182	

* Total thickness.

† Total amount of protein per square centimetre of membrane.

outer 28·2 per cent of the total thickness. If we take the relation cell water/total water as an equivalent to the relation cell/total tissue, then the cells occupy 28·2 per cent of the total thickness—a figure which agrees remarkably well with the area needed to satisfy the Na–K ATPase requirement (see Table 1). This estimate rests on the assumption that the assay method measures the whole enzyme activity in the tissue and that the proteins are homogeneously distributed. If this were not so, the amount of Na–K ATPase needed for the net sodium transport would be contained in a narrower band of tissue and only the outermost layers of the epithelium would actually participate in the process.

Recent investigations by Snell and Chowdhury[18] suggest that the electrical profile of the frog skin is a linear function of distance across the epithelial cell layers, and that the contralateral effect of Na^+ and K^+ on the potential is mediated by their primary effects on the Na^+ transport system. The homogeneous distribution of Na–K ATPase through the epithelium, together with recent observations that the active sodium transport mechanism is electrogenic[5,19], lend strong support to the suggestion made by Snell and Chowdhury. This is a somewhat puzzling conclusion: while the electrochemical potential gradient between the sodium in the outer solution and in the cells is not explained on the basis of a passive distribution and requires an active step, the pumping mechanism is distributed over the whole epithelium. An alternative explanation would be a 'compartmentalization' of sodium. This agrees with the observation[20] that only a small fraction (less than 10 per cent) of the total sodium in the cells participates in the transport of this ion.

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FINE STRUCTURAL LOCALIZATION OF NORADRENALINE IN VESICLES OF AUTONOMIC NERVE ENDINGS

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SEVERAL electron microscopic investigations[1–3] have confirmed the usefulness of the histochemical reaction by which primary glutaraldehyde fixation and subsequent exposure of adrenal medullary cells to one of several metal-containing oxidizing agents, such as dichromate[4], ammoniacal silver[5] or osmium tetroxide[6], can be used for the selective production of electron density in noradrenaline-containing granules. In medullary tissue subjected to this histochemical reaction[7], nerve endings on chromaffin cells were found to contain granular material of slight density within large synaptic vesicles. The latter are in the size range of similar structures reported in previous fine structural studies of hypothalamus, brain stem, spinal cord and pineal gland, after a variety of preparative techniques. The failure of the semi-dense material in the medullary nerve ending vesicles to react equivalently to the chromaffin cell noradrenaline-containing granules after the above reactions jeopardizes the possible correlation of so-called 'dense core' synaptic vesicles with the storage site of noradrenaline in nerve endings.

In our experiments we have utilized an oxidative histochemical reaction[4] in combination with the specific noradrenaline-depleting action of α-methyl metatyrosine (AMMT)[8] and the less specific depletory action of reserpine[9], to compare and characterize the reactivity of rat medullary chromaffin noradrenaline-containing granules with the nerve ending 'dense core' vesicles in both adrenal medulla and vas deferens. The latter structure has nerve fibres which are strongly fluorescent for noradrenaline[10], and extensive pharmacological data suggest a functional role for noradrenaline in the vas[11]. Richardson, who utilized this organ for his original description of autonomic nerve ending fine structure[12], observed a high percentage of 'dense core' vesicles in these endings after fixation with osmium tetroxide.

Normal, untreated, adult albino rats and litter mates treated with either AMMT (150 mg/kg), given intravenously 12–14 h previously, or with two intra-peritoneal injections of reserpine (2·5 mg/kg), given 24 and 4 h before killing, were anaesthetized with 'Nembutal' (25 mg/kg). One vas deferens and both adrenal glands were quickly excised and immersed in 5 per cent glutaraldehyde buffered to *p*H 7·4 with phosphate[13]. While immersed, the adrenal cortices were removed by trimming, and 1–2-mm thick longitudinal, transverse sections of vas were prepared. After a total fixation of 2 h, half the tissue blocks were incubated in 2·5 per cent potassium dichromate[4] in the phosphate buffer (*p*H 7·0) for 2 h and then refixed in phosphate-buffered 1 per cent osmium tetroxide for 1 h. The other half of the blocks were refixed in the buffered 1 per cent osmium tetroxide for 4 h directly after the glutaraldehyde, without an intervening dichromate oxidation. Routine ethanol dehydration and embedment in maraglass—'*D.E.R.* 732' (ref. 14)—followed the fixation; thin sections, cut on an '*LKB*' ultratome, were examined in an *RCA* '*EMU*' 3-*F* electron microscope. Some of the thin sections were exposed to saturated uranyl acetate to enhance the contrast of cellular membranes.

In confirmation of the previous results, both of the foregoing reaction protocols (oxidation with dichromate or osmium) revealed two types of chromaffin cell granules on the basis of their electron density. The majority of cells had membrane-bounded granules of only slight or moderate electron density and corresponded to adrenaline-containing cells. Fewer cells, scattered in groups, con-

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tained membrane-bounded granules which were extremely electron-dense after either oxidative procedure, and these were considered to be the noradrenaline-containing cells. Nerve endings, which contained two types of synaptic vesicles, abutted on both types of cells. The majority of the vesicles, 300–500 Å in diameter, contained clear contents, while a few were larger (400–800 Å) and contained a core of material which showed only slight density after reaction with oxidizing agents (Fig. 4). For comparison, the density of the contents of the larger vesicles, which were usually observed at the perimeter of the axoplasm of the endings, was closer to the density developed in the epinephrine-contained granules and was distinctly less dense than the noradrenaline-containing granules of chromaffin cells. This material contained in vesicles of a synaptic ending was, therefore, considered unreactive for noradrenaline.

After fixation in glutaraldehyde and subsequent oxidative reaction, the nerve endings of the vas deferens were found to contain 'dense core' vesicles 400–900 Å in size, uniformly distributed throughout the ending (Fig. 1). The contents of these vesicles were intensely reactive histochemically and, therefore, electron-dense, and this type constituted about 80 per cent of the vesicles observed in normal endings. The remainder were electron-lucent vesicles, which were of the same size range as the above-described, reactive 'dense core' vesicles. The material made dense by oxidation in the vesicles was roughly equivalent in density to that of the noradrenaline-containing granules of chromaffin cells. Following either the reserpine or AMMT treatments, the nerve ending of the vas deferens contained few or no 'dense core' granules within the synaptic vesicles (Figs. 2–3). In micrographs of more than 100 nerve endings of the treated animals, only 1–7 per cent of the vesicles contained sparse, dense-

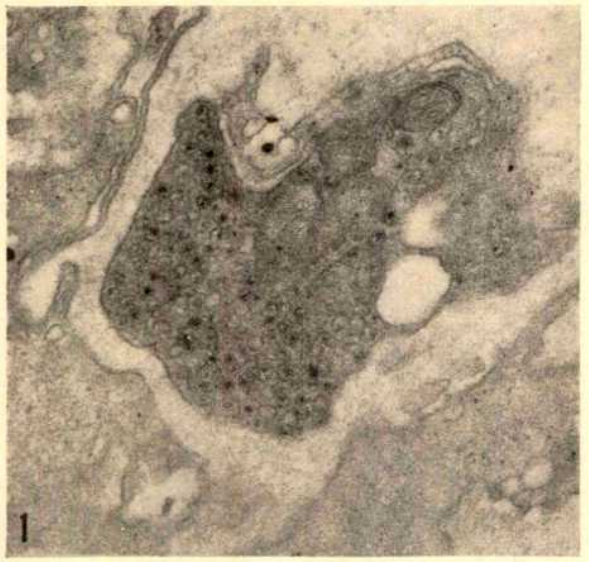


Fig. 1. Autonomic nerve ending in rat vas deferens. Contents of most synaptic vesicles appear dense due to dichromate oxidation (×50,000)



Fig. 2. Autonomic nerve ending in vas deferens of rat tested with reserpine. Despite prolonged osmium oxidation, little or no dense material occurs in synaptic vesicles (×50,000)

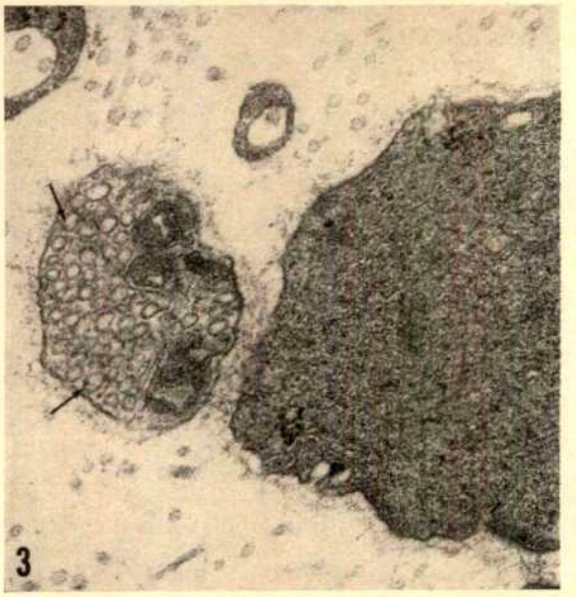


Fig. 3. Autonomic nerve ending in vas deferens of rat treated with α-methyl meta-tyrosine. Despite dichromate oxidation, only little dense material occurs in synaptic vesicles (arrows) (×50,000)

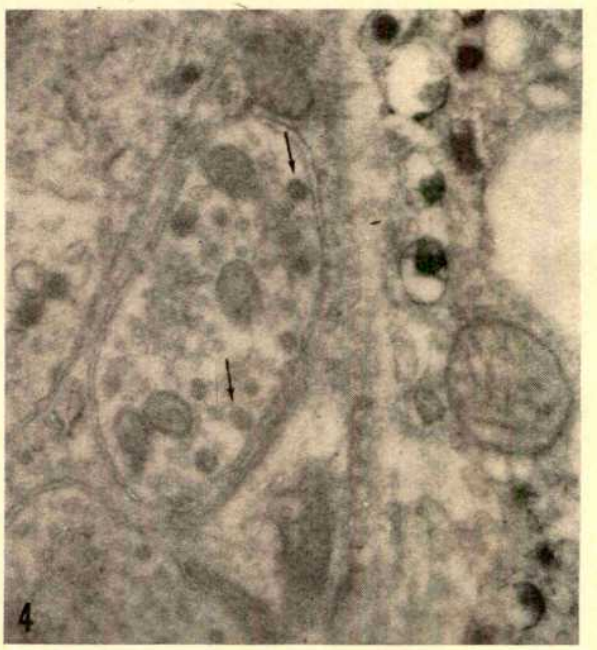


Fig. 4. Autonomic nerve ending on norepinephrine-containing cell of adrenal medulla of rat treated with α-meta-tyrosine. Although drug treatment markedly decreased granular content made dense by oxidation with dichromate, no such effect was noted on large synaptic vesicles (arrows) whose contents were not reactive with dichromate (×25,000)

staining contents, usually distributed in a spotty fashion in relation to the membranous envelope. However, the total number of vesicles was similar to that found in the vas of untreated animals. In the nerve endings of the treated animals the few vesicles containing some dense material were found surrounded by vesicles with clear contents, as though depleted of their reactive material. Although the depletor drug treatments caused fragmentation and partial or complete evacuation of the dense noradrenaline-containing granules in the medullary cells, the unreactive, semi-dense core material in vesicles of nerve ending of the medulla were unchanged in either density or number (Fig. 4). There was also no apparent reduction in the total number of vesicles in these nerve endings as a result of drug treatment.

These results indicate that the 'dense core' granules contained within the synaptic vesicles in presumably adrenergic vas deferens nerve endings demonstrate, as a result of a selective histochemical reaction, an electron density equal to that of noradrenaline-containing chromaffin cell granules in the adrenal medulla. However, all granular material within synaptic vesicles does not give a positive reaction (increase in density after oxidation with dichromate osmium tetroxide), as shown by the 'non-reactivity' of those 'dense core' vesicles in the presumably cholinergic nerve endings on the same chromaffin cells showing reactive noradrenaline-containing granules. That this is a substantive difference and not one related to the concentration of the amine bound within the vesicles is confirmed by the depletion experiments. When the animals were given a drug (AMMT or reserpine) capable of depleting normal stores of noradrenaline, the 'dense core' granules in synaptic vesicles of the vas are depleted either partially or completely, while the semi-dense material with 'dense core' synaptic vesicles of the medullary nerve endings remained unchanged. Since the semi-dense material in the latter vesicles was not influenced by reserpine treatment, which also depletes serotonin and dopamine, an even wider histochemical-pharmacological separation between these two types of granular material contained within vesicles is likely. While either dopamine or serotonin is capable of reducing metallic-containing oxidants[15] to produce density, these amines, not present in the vas, could interfere with interpretation in other neural areas where all three biogenic amines are present. However, AMMT, which presumably depletes only noradrenaline after the interval of time used, is likely to be of greater utility in ultrastructural neuropharmacological studies by increasing the specificity of the noradrenaline identification by subtraction of stored, histochemically-reactive, granular material.

The present results are most compatible with those of Wolfe *et al.*[16], whose electron microscopic autoradiographic localization of tritiated noradrenaline in nerve endings of the rat pineal indicated that the dense material of 'dense core' granules within these synaptic vesicles was due to the amine itself. A logical extension of these data would be to include the macromolecular binding matrix within the granules as an additional possible source of density (on histochemical reaction), which might be expected to fluctuate with storage and release of the amine[17]. Recent suggestions that the 'dense core' granules within synaptic vesicles may represent forms of lysosomes[18], or that their internal density is due to a lipoprotein similar to that forming the membranous structures[19], need be less seriously considered. The identity and function of the semi-dense unreactive material in some synaptic vesicles remain to be explained. In addition, this finding suggests that a blanket association of dense material within synaptic vesicles with biogenic amines should be viewed with caution. Further details on the specificity of the histochemical reactions, with more extensive pharmacological experimentation, will appear separately. This combined pharmacological and fine structural histochemical approach is at present being applied to a variety of nerve-ending sites within the central nervous system.

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EFFECT OF SPORIDESMIN ON LIPID METABOLISM IN RABBITS

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SPORIDESMIN, a highly toxic metabolic product of the fungus *Pithomyces chartarum*, is considered to be the causative agent of facial eczema, a liver disease in sheep and cattle in New Zealand and Australia[1].

The pathological changes that occur as the disease progresses have been described by Mortimer[2], who also noted that similar pathological changes could be produced in rabbits, guinea-pigs and rats. One of the prominent biochemical features of the enterohepatic disturbances preceding biliary obstruction is an accumulation of liver triglycerides, which was interpreted as a malfunction of the hepatic triglyceride-secreting mechanism[3,4].

It was of interest to know whether this type of mechanism, as the result of sporidesmin intoxication, also occurs in non-ruminants. If this were the case it would, for reasons of animal accommodation and economy, be more expedient to use small laboratory animals in order to investigate, in greater detail, this type of deranged lipid metabolism. This article describes the changes in serum and liver lipids in rabbits during the period of 48 h following dosing with sporidesmin. In addition, consideration is given to the disturbances associated with biliary obstruction.

Crystalline sporidesmin-benzene complex was obtained from the Ruakura Animal Research Station, Department of Agriculture, New Zealand. Immediately before use the complex was dissolved in a small volume of ethanol, and this solution was diluted with distilled water. The final preparation contained 0·5 per cent ethanol and 0·25 mg sporidesmin per ml. Eighteen virgin female rabbits (2·1–3·4 kg, 6–7 months old) were divided into three groups of six animals. After fasting overnight, each animal in two of the groups received an oral dose of 1 mg/kg body-weight of sporidesmin by stomach tube

under light ether anaesthesia. Similarly, each animal in the third (control) group received an equivalent dose of distilled water. The fasting animals of one sporidesmin-dosed group, and those of the control group, were killed 24 h after dosing. Those of the remaining sporidesmin-dosed group were killed 48 h after dosing. The animals were bled under 'Nembutal' anaesthesia from a polythene cannula inserted into a carotid artery, and their livers excised immediately after death. Sera and livers were frozen as described[4].

Within 4 h after bleeding, non-esterified fatty acids were determined[5]. Serum lipid extracts[6] and liver lipid extracts[4] were prepared, and their cholesterol esters, triglycerides, cholesterol and phospholipids compositions determined by means of a quantitative thin-layer chromatography procedure[7]. The results of this investigation are summarized in Table 1.

Despite the wide range of compositions of the serum and liver lipids, certain general conclusions are justified which lend support to earlier suggestions[3,4]. The action of sporidesmin resembles that of other hepatotoxins[8,9] in producing a high elevation of liver triglyceride levels within 24 h, which then subsides to considerably lower levels at 48 h after dosing. However, no concomitant hypotriglyceridaemia is observed. It is conceivable, therefore, that sporidesmin poisoning inhibits not only the mechanism of triglyceride exit from the liver, but also, to a greater extent, the mechanism which regulates triglyceride uptake from the circulation into adipose tissue. The net result is that the serum triglyceride levels are elevated at the 24 h stage. Diminished lipoprotein lipase activity could possibly be responsible for this rise.

If the accumulation of triglycerides in the liver were due to an increased release of fatty acids from the adipose tissue (for references see Henderson[10]) a rise in serum non-esterified fatty acid level would be observed. Since sporidesmin induces not only a fall in the serum non-esterified fatty acid levels, but also a rise in the serum triglyceride levels, other factors, not directly associated with lipid transport, are involved which subsequently lead to the accumulation of liver triglycerides. In fact, there is evidence that the fatty liver, induced by the action of hepatotoxins, results from inhibited synthesis of the protein moiety of lipoproteins[11,12]. More specifically, Gallagher[13] has shown that, at this stage, the effect of sporidesmin in the rat resulted in decreased mitochondrial protein with decreased total activity of mitochondrial enzymes.

Probably because of a wider range of individual susceptibility, together with the highly cytotoxic action of sporidesmin, an even dose-response is difficult to obtain in a group of small animals, such as rabbits. Thus, unlike the response to a 1 mg/kg dose of sporidesmin in larger animals, the later symptoms of the diphasic clinical response[1] follow closely, or sometimes overlap, the early first-phase symptoms.

These first-phase symptoms are characterized histologically by severe hepatic necrosis and degeneration[2]. Simultaneously, mitochondrial respiration is decreased[13], followed by the changes in serum and liver triglycerides, as described here. Whatever these first-phase symptoms may be, they are transitory and reach their maximum, in the majority of animals, at about 24 h after dosing. Hepatic necrosis then subsides and cell regeneration takes place[2], whereas the liver triglyceride level declines rapidly with an equally rapid rise in serum triglyceride level (Table 1). This increase in serum triglycerides may be an 'overflow' effect of triglycerides from the liver.

The second-phase symptoms are characterized by progressive biliary cirrhosis and bile duct occlusion[2]. These symptoms are noted by gradual increases in the serum phospholipids, cholesterol and cholesterol esters. Early indications of these increases were already observed in this investigation 48 h after dosing (Table 1). In the animals which showed these increased serum lipids there were also considerable haemolysis and increases of serum bile acids and bilirubin. A rise in serum bilirubin levels has been recorded previously[14] in sheep under similar experimental conditions.

The gradual accumulation of bile acids (to be described more fully elsewhere) is of considerable interest since they may be related to the pathological and clinical changes that occur as the disease progresses[2]. Thus, the progressive bile duct occlusion and cirrhosis may be initiated by leakage of bile through injured bile channels during the early contact with sporidesmin and the subsequent process of undisciplined proliferation of biliary epithelium and fibrosis. The gradual obstruction of the normal flow of bile leads to a disturbance of the enterohepatic cycle by which bile acids, delivered to the small intestine, are largely reabsorbed and recirculated through the liver. As the result of this obstruction bile acids are retained in the liver and blood.

Pharmacologically, the bile acids are potent choleretic agents. They irritate mucous membranes, and, when injected into the circulation, they can produce severe nervous and cardiac depression and haemolysis[15]. Biochemically, bile acids are a major end-product of cholesterol metabolism, and cholesterol is probably the only precursor of bile acids. Also, their presence in the enterohepatic cycle exerts an important regulatory influence on cholesterol metabolism[16]. Thus, the feeding of bile acids, which should overload the enterohepatic cycle and depress cholesterol oxidation, causes an increase in serum cholesterol[17]. These investigators[17] also observed hyperplasia of the bile duct as a result of ingestion of lithocholic acid.

The information available at present regarding the biochemical changes induced by sporidesmin intoxication can be summarized as follows: (1) The early action of sporidesmin is similar in its effects to those of many other hepatotoxins. (2) Its effects in the rabbit (a non-

Table 1
Liver lipids (mg/100 g tissue)

	Control		24 h		48 h	
	Mean (± S.D.)	Range	Mean (± S.D.)	Range	Mean (± S.D.)	Range
Total lipids	4,347 (616)	3,787–5,432	7,255 (1,202)	4,220–14,406	5,029 (1,898)	3,315–8,055
Cholesterol esters	108 (37)	56–165	161 (83)	66–258	152 (91)	75–305
Cholesterol	230 (6)	220–238	210 (28)	170–246	221 (11)	209–235
Triglycerides	1,358 (649)	806–2,558	3,708 (3,652)	1,023–10,980	2,249 (2,044)	355–5,445
Phospholipids	2,714 (377)	2,264–3,228	3,009 (434)	2,480–3,512	2,571 (502)	2,061–3,229

Serum lipids (mg/100 ml.)

	Control		24 h		48 h	
	Mean (± S.D.)	Range	Mean (± S.D.)	Range	Mean (± S.D.)	Range
Non-esterified fatty acids	20·8 (3·7)	17·4–26·5	12·5 (1·9)	10·1–14·3	12·9 (2·6)	9·6–16·8
Cholesterol esters	22·6 (5·1)	15·2–30·9	25·9 (5·7)	15·6–31·9	47·3 (18·9)	21·8–64 4
Cholesterol	9·5 (3·0)	5·3–14·2	10·2 (4·3)	5·3–18·2	19·6 (7·1)	12·8–31·3
Triglycerides	18·0 (5·4)	13·0–27·8	45·2 (31·9)	17·1–92·3	136 (99)	42·4–276
Phospholipids	66·9 (12·9)	53·0–87·4	59·1 (18·0)	33·9–84·7	99 (26·6)	73·0–138

ruminant) are comparable to those observed in the sheep (a ruminant). (3) This early action of sporidesmin results in changes in the triglyceride equilibrium of serum and liver which are probably initiated by transitory disturbances of hepatic mitochondrial protein synthesis. (4) The pathological and biochemical changes which follow recovery from the cytotoxic action of sporidesmin may be the result of bile acid accumulation in the liver and circulation.

I thank Miss G. P. Ashton and Miss J. E. Simpson for their competent technical assistance.

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EFFECT OF NEONATAL THYMECTOMY ON THE INDUCTION OF PAPILLOMATA AND CARCINOMATA BY 3,4-BENZOPYRENE IN MICE

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PREVIOUS experiments in this laboratory using random-bred Schofield mice[1] and inbred *C57BL* mice[2] have shown that benign and malignant tumours arise earlier in animals whose homograft reactivity is impaired by thymectomy on the third day of life than in sham-thymectomized control mice. However, thymectomy on the third day of life has less effect on immunological function than thymectomy within 24 h of birth[3]. In some inbred strains neonatal thymectomy is associated with wasting disease if the animals are reared under conventional conditions[4,5]. However, random-bred Swiss *S* mice, thymectomized within 24 h of birth, are not affected in this way, yet show distinct impairment of immunological function as judged by their ability to form antibody in response to foreign antigens[6] and by the proportion of spleen cells capable of lysing sheep red cells when cultured with these cells using Jerne's technique[7] 4–5 days after an immunizing injection with these cells[8]. It was decided, therefore, to repeat one of the experiments previously reported in which thymectomized and sham-thymectomized mice had skin tumours induced by applications of 3,4-benzopyrene using Swiss *S* mice thymectomized during the first 24 h of life and a low weekly dose of the hydrocarbon.

Swiss *S* mice from the colony maintained at Pollards Wood Research Station were therefore thymectomized or sham-thymectomized within 24 h of birth, and 50 μg of 3,4-benzopyrene dissolved in 0·2 ml. acetone was applied to the shaved skin of the back once a week for 40 weeks beginning when the mice were approximately 6 weeks old. Papillomata and carcinomata which arose as a result of this treatment were recorded every week. Carcinomata were only so designated when they reached a diameter of 10 mm and when a biopsy taken a week after this size was first recorded showed that invasion of the panniculus carnosus muscle had occurred.

Thymectomized animals were examined after death for thymus remnants and no animals with macroscopic or microscopic fragments were included in the results. 25 thymectomized and 33 sham-thymectomized animals survived to 26 weeks of age, the time when the first papillomata appeared. The experiment was terminated 45 weeks after the initial application of benzopyrene when the survivors, 21 thymectomized and 28 sham-thymectomized mice, were 51–52 weeks old. Results are expressed in Figs. 1 and 2 in terms of the proportion of tumour-free survivors (that is, animals which have never shown the relevant tumour).

The statistical method[9] chosen to analyse this experiment is based on the hypothesis that for any animal in a given group the probability that it is tumour-free at x weeks can be written:

$$\exp\,[-b\,(x-w)^k]$$

The parameters w and k are the same for both groups (though, of course, different for papillomata and carcinomata), and the effect of the treatment is measured by the parameter b. This distribution is called the third extreme value distribution.

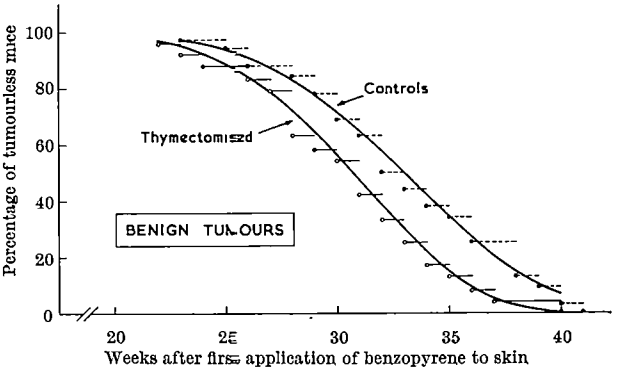


Fig 1. Percentage of tumourless mice, that is, mice without a benign tumour, at different stages of the experiment. The first appearance of a benign tumour on any animal was at 20 weeks, and (as explained in the text) what are plotted here are tumours appearing after this time. The parameters of the fitted curves stopping at 40 weeks are $k = 4\cdot34$, $w = 13\cdot97$, b (for control group) $= 1\cdot91 \times 10^{-6}$, b (for thymectomized group) $= 3\cdot35 \times 10^{-6}$, $\chi^2_1 = 3\cdot98$ for difference between b's ($P = 0\cdot05$)

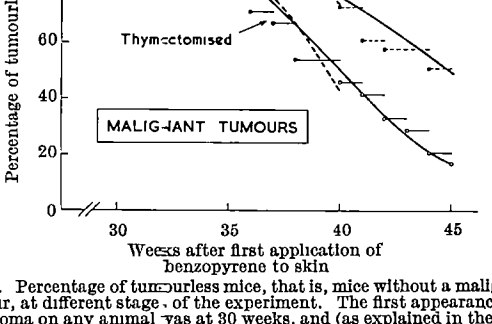


Fig. 2. Percentage of tumourless mice, that is, mice without a malignant tumour, at different stages of the experiment. The first appearance of a carcinoma on any animal was at 30 weeks, and (as explained in the text) what is plotted here are tumours appearing after this time. The parameters of the fitted curves stopping at 40 weeks are $k = 8\cdot66$, $w = 15\cdot57$, b (for control group) $= 2\cdot98 \times 10^{-13}$, b (for thymectomized group) $= 8\cdot10 \times 10^{-13}$, $\chi^2_1 = 4\cdot92$ for difference between b's ($P = 0\cdot03$)

For this distributional form there is a linear relationship between 'death'-rate (that is, the rate of getting the relevant tumour) and the logarithm of $(x-w)$; and this is precisely what is observed in many human cancers[10-12].

The parameters w, k and b are estimated by a maximum likelihood method[9] and the fits of this distributional form to the data are shown in Figs. 1 and 2. For technical statistical reasons it is convenient to consider in the figures only those animals free of tumours at the time of appearance of the first relevant tumour in any animal. The first papillomata appeared at 20 weeks and the percentage of the remaining mice without papillomata at different stages of the experiment is shown in Fig. 1. Similarly, the first carcinomata appeared at 30 weeks and the percentage of the remaining mice without carcinomata at different stages of the experiment is shown in Fig. 2. The series of short straight horizontal lines in Figs. 1 and 2 show the 'observed' proportions; for example, the papilloma results for the thymectomized group given in Fig. 1 show that 100 per cent are tumour-free from 20 weeks after the first application of benzopyrene until 22 weeks, 95·8 per cent are tumour-free from 22 weeks until 23 weeks, 91·7 per cent are tumour-free from 23 weeks until 24 weeks, 87·5 per cent are tumour-free from 24 weeks until 26 weeks, and so on. To calculate the 'observed' proportion of tumour-free survivors making allowance for intercurrent deaths an actuarial method is used[9,13].

The painting stopped at 40 weeks and, as we have previously shown[13,14] that very soon after painting ceases there is a definite break in the rate at which the first papillomata appear, we should not expect any smooth continuous curve to fit the complete experimental data. For this reason we consider here the papilloma results only up to 40 weeks (in this instance this means excluding only the final papilloma in the control group).

The fit of the suggested form of distribution is clearly good (Fig. 1) and the difference between the thymectomized and sham-thymectomized group is significant at the 5 per cent level ($\chi^2 = 3{\cdot}98$ on one degree of freedom).

The cessation of painting at 40 weeks also affected the incidence of carcinomata, so that we should not expect any smooth continuous curve to fit the complete experimental data. For this reason we consider first the results only up to 40 weeks.

The fit of the suggested form of distribution is satisfactory (broken line in Fig. 2) and the difference between the thymectomized and sham-thymectomized groups is significant at less than the 5 per cent level ($\chi^2 = 4{\cdot}92$ on one degree of freedom).

Fig. 2 also shows the third extreme value distribution fitted to the complete carcinoma data. This may be rationalized by noting that the experiment continued for only 5 weeks after painting stopped, and that the carcinomata recorded during the last five weeks were already present at 40 weeks but were too small to be counted.

If this reasoning is accepted, then the difference between the thymectomized and sham-thymectomized groups is significant at the 0·5 per cent level ($\chi^2 = 7{\cdot}94$ on one degree of freedom).

The results show that when random-bred mice are thymectomized during the first day of life so that the homograft rejection mechanism is slightly impaired benign and malignant tumours appear earlier in response to application to the skin of low doses of a chemical carcinogen. This confirms previous observations when a higher dose of benzopyrene was used and thymectomy was performed on the third instead of the first day of life.

The differences between the immunologically deficient group and the normal animals although statistically significant are not so great as to suggest that the immunological state of the host is the major factor in determining whether tumours grow or not. It may be that some tumours initiated by benzopyrene do not differ sufficiently from the host to evoke a homograft reaction against them even when the immunological competence is unimpaired. It may be that factors such as the growth-rate of the tumour allow it to progress in spite of a reaction against it, or it may be that under some conditions an animal may become specifically tolerant to a tumour which it bears.

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SEPARATION OF TWO KINDS OF POLYMERASE FROM *Alcaligenes faecalis**

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SINCE the studies of Kornberg and his colleagues on the deoxyribonucleic acid polymerase of *Escherichia coli*[1], similar enzymes have been demonstrated in various sources. In general, the physical state required for the template DNA varies with the species from which the enzyme is prepared. One is represented by the *E. coli* DNA polymerase which uses double- as well as single-stranded DNA as primer; polymerases of *Bacillus subtilis*[2], sea urchin[3] and regenerating liver[4] are identified as enzymes of this type. The other is the polymerase which requires single-stranded DNA preferentially as primer; the enzymes from calf thymus[5], bacteriophage *T*2-infected *E. coli*[6] and ascites tumour[7] belong to this group. It was considered that investigations of the utilization of DNA primer by several polymerases might throw some light on the mechanism of replication.

Meanwhile, Cairns[8] has inferred from his autoradiographic investigations that the chromosome is synthesized from a fixed point and that the synthesis systematically travels along the entire length of the chromosome. Other experiments have also added support to this inference[9,10]. However, recent investigations with the *E. coli* polymerase demonstrated that this enzyme cannot synthesize two new polynucleotide chains simultaneously in the same direction from one end of a DNA template along its two strands of opposite polarity[11]. Thus, attention has recently been directed to the possibility that more than one polymerase may be involved in the replication of DNA molecules.

This article presents evidence for the presence of two separate polymerases in extracts of *Alcaligenes faecalis*. Results of our investigations reveal that one of the enzymes prefers single-stranded DNA and the other double-stranded DNA as the primer for their polymerization reaction.

* A preliminary account of this work was given at the Twelfth Symposium on Nucleic Acids, Chiba, Japan (1965).

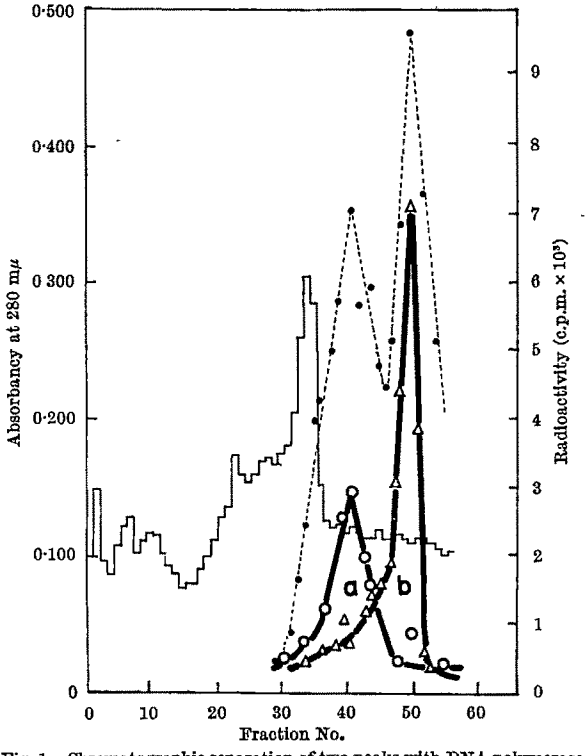


Fig. 1. Chromatographic separation of two peaks with DNA polymerase activity. A column of DEAE-cellulose (1 cm² × 27 cm) was prepared and equilibrated with 0·01 M potassium phosphate buffer, *p*H 7·0, containing 0·005 M 2-mercaptoethanol. The ammonium sulphate fraction dissolved in 10 ml. of the same phosphate buffer containing 2-mercaptoethanol was adsorbed to the column. After washing, a linear gradient of elution was applied with 0·02 M and 0·5 M sodium chloride, as limiting concentrations. Sodium chloride was dissolved in 0·01 M potassium phosphate buffer, *p*H 7·0, containing 0·005 M 2-mercaptoethanol and 150 ml. of each solution were used. The flow rate was 10 ml. per h, and 3-ml. fractions were collected. The activity of the eluted fractions was assayed by the standard assay system, with native (—△—), denatured (—○—), and 'activated' calf thymus DNA (- - - ● - - -) as primer, respectively. Absorbancy of each fraction at 260 mμ is also shown (———)

The strain used in these experiments was *Alcaligenes faecalis LB*, which was kindly provided by Dr. K. G. Lark, of Kansas State University. Fractions with polymerase activity were obtained from a frozen cell paste following a procedure of sonic disintegration, streptomycin precipitation, protamine precipitation, ammonium sulphate fractionation and DEAE-cellulose chromatography. Details of the preparation will be described in a subsequent publication[12]. In the routine assay of DNA polymerase activity, the conversion of tritiated thymidine triphosphate (^{3}H-TTP) into an acid-insoluble product was measured. The reaction mixture (150 μl.) contained 5 μg of DNA primer, 10 μmoles of glycine buffer, *p*H 9·0, 1 μmole of magnesium chloride, 5 mμmoles each of dATP, dGTP and dCTP, 3 mμmoles of ^{3}H-TTP ($1·6 \times 10^7$ c.p.m. per μmole), and various quantities of enzyme preparation. After incubation for 30 min at 37° C, the reaction was stopped by the addition of 0·3 ml. of cold 1 N perchloric acid and the acid-insoluble fraction was isolated by centrifugation as described before[13]. Radioactivity measurements were made in a window-less gas flow counter ('*Aloka*'). A unit of enzyme activity was defined as described by Richardson *et al.*[14]. Protein was determined by the method of Lowry *et al.*[15]. Tritiated TTP was prepared by enzymatic phosphorylation of ^{3}H-thymidine (Schwarz BioResearch, Inc.) by a crude extract of calf thymus and purified on a 'Dowex-1' (Cl^-) column. Unlabelled deoxyribonucleoside triphosphates were purchased from the California Corporation for Biochemical Research. Various DNA primers were isolated as follows: calf thymus DNA according to Kay, Simmons and Dounce[16]; bacterial DNA by the method of Marmur[17]; *T2* DNA as described by Mandell and Hershey[18]. 'Activated' calf thymus DNA was obtained by exposing the DNA

Table 1. REQUIREMENTS FOR REACTION

System	^{3}H-dTMP incorporated (μμmoles) Peak fraction *a*	Peak fraction *b*
Complete	665	260
Minus DNA	0	0
Minus magnesium chloride	0	<1
Minus dATP	34	17
Minus dGTP	36	13
Minus dATP and dGTP	28	7
Minus dATP, dGTP and dCTP	27	4

The complete system for the reactions is described in the text. For columns 1 and 2, respectively, 5 μg of heat-denatured DNA and 4·8 units of fraction *a* and 5 μg of native DNA and 0·7 unit of fraction *b* were used.

to crystalline pancreatic DNase (Worthington Biochemical Corporation) according to the method of Aposhian and Kornberg[6]. Heat-denatured DNA samples were prepared by maintaining a solution of DNA at 100° C for 10 min and then immediately cooling it in an ice water bath.

When fractions eluted from DEAE-cellulose column were assayed with 'activated' calf thymus DNA as the primer, polymerase activity was distributed in two separate peaks (peaks *a* and *b*) on the chromatogram as shown in Fig. 1. Investigations of the requirements for the reaction catalysed by these two peaks revealed fundamental similarities to the *E. coli* polymerase. The omission of one, two or three of the four deoxyribonucleoside triphosphates resulted in a marked reduction of the reaction. The presence of a DNA primer and a magnesium ion was essential for the reactions, as can be seen from Table 1. However, difference in the effectiveness of the primer was observed for two peaks. One of the peak fractions (*a*) eluted with a low concentration of sodium chloride exhibited an activity about ten times greater with heat-denatured DNA as primer than with native DNA. Also the single-stranded DNA from φ*X*174 phage was an effective primer without heating. On the other hand, the other peak fraction (*b*), eluted later with higher concentration of sodium chloride, was far more active with native DNA than with heat-denatured DNA (Table 2). Additional evidence for the dissimilarity in primer requirement was obtained from experiments in which the effects of heat-denaturation of native DNA on the priming activity were investigated with various DNA samples, in which were included calf thymus, *Escherichia coli*, *Alcaligenes faecalis*, *Pseudomonas aeruginosa*, *Aerobacter aerogenes* and bacteriophage *T2* (Fig. 2). With fraction *a*, priming activity of native DNA was dramatically increased by heating for 10 min at a temperature higher than 75° C, followed by quick cooling. Conversely, with fraction *b*, priming activity of native DNA was reduced in proportion to the grade of its denaturation, when heated at a temperature higher than 75° C, and DNA heated at 100° C had activity corresponding to 10–20 per

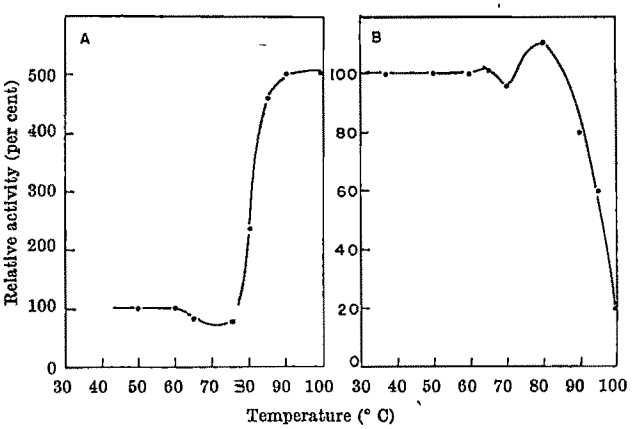


Fig. 2. The effects of heat-denaturation of DNA on the priming activity. A solution of DNA (100 μg per ml. in 0·01 M potassium phosphate buffer, *p*H 7·0, and 0·15 M sodium chloride) was kept at various temperatures for 10 min and then immediately cooled in an ice-water bath. Priming activity of these heated DNA samples was assayed in the standard incubation mixture described in the text. For experiment *A*, *P. aeruginosa* DNA and 1·1 units of peak fraction *a*; and for *B*, calf thymus DNA and 0·5 unit of peak fraction *b* were used, respectively

Table 2. ACTIVITIES OF VARIOUS DNA PRIMERS FOR TWO POLYMERASE PEAKS

Primer	Acid-precipitable radioactivity after incubation (c.p.m.) Peak fraction *a*	Peak fraction *b*
Calf thymus DNA, native	793	7,136
Calf thymus DNA, heated	5,814	916
Calf thymus DNA, 'activated'	9,217	9,676

Primer effectiveness for fractions *a* and *b* was studied by comparing acid-precipitable radioactivity after 30 min of incubation with different samples of calf thymus DNA as primer under the standard experimental conditions described in the text. The enzyme used was 0·8 unit of fraction *a* or 1·8 units of fraction *b*.

cent of that obtained with non-treated native DNA. The reduced level (Fig. 2*A*) or the augmented level (Fig. 2*B*) of priming activity observed at about 70° C may be due to the transition of a single-stranded part in native molecule of DNA to double-stranded structure. In the next experiments the two fractions were compared for affinity to native and heat-denatured DNAs, and for maximal velocity of the reaction with both primers. The results were plotted in Fig. 3, according to the method of Lineweaver and Burk[18]. Fraction *a* had a higher affinity for denatured DNA than for native DNA, whereas with fraction *b* the opposite was observed. Thus, the polymerase activities exhibited by fractions *a* and *b* were distinct and separable.

It could have been assumed that the polymerization fraction *b* with native DNA as primer was due to combined action of a nuclease persisting in the fraction and supplying native DNA to an 'activated' template, and a polymerase active for denatured DNA as primer—the same enzyme as that eluted in the fraction *b* fractions. However, there are several points against this. First, treatment of a native DNA sample with fraction *b* for various periods at 37° C, in the absence of the four deoxyribonucleoside triphosphates, did not improve its priming capacity (series 1 of Table 3). The primer effectiveness of the DNA thus treated was not elevated even when heated for 10 min at 100° C and quickly cooled after pre-incubation, and there was only a marked decrease due to heat-denaturation (series 2 of Table 3). Secondly, when two fractions were mixed in various proportions, polymerase activity with heated or unheated DNA as primer was closely equal to the sum of the activities assayed separately with each peak and neither stimulation nor inhibition was observed (Table 4). Finally, when a mixture of the two fractions was chromatographed on hydroxyapatite, there were again two discrete peaks of polymerase activity, one primed by single-stranded and the other by double-stranded DNA (Fig. 4). Therefore, it was suggested that different entities may be responsible for two polymerase activities.

One possibility is that artificial modification in the course of purification, for example, partial destruction of the conformation, attachment of unusual materials such

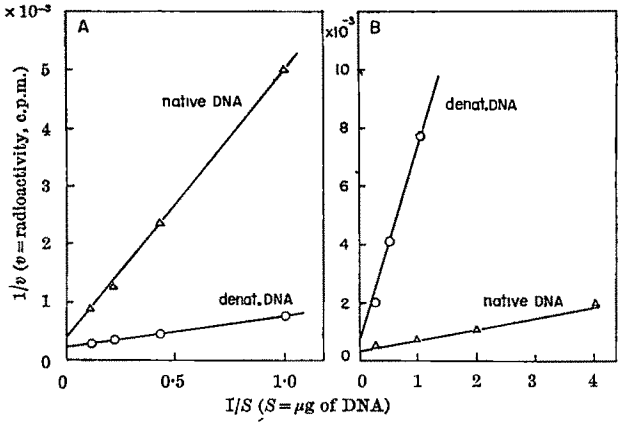


Fig. 3. The effects of primer concentration on the reaction rate. The standard assay conditions were used, except that the concentration of native and denatured DNAs added as primer was varied. The enzyme was 4 units of fraction *a*, and 0·5 unit of fraction *b* for experiments *A* and *B*, respectively

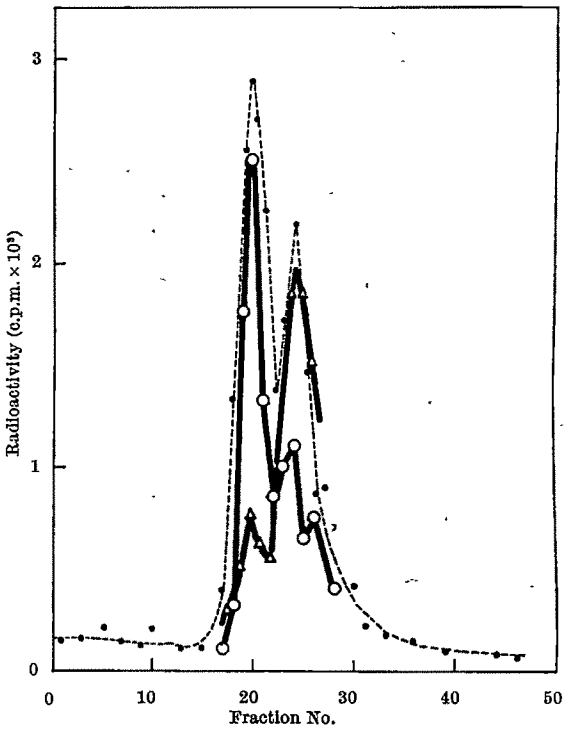


Fig. 4. Chromatography of a mixture of fractions *a* and *b* on hydroxylapatite. A column of hydroxylapatite (1 cm² × 7 cm) was prepared and washed with 0·001 M potassium phosphate buffer, *p*H 7·0, containing 0·005 2-mercaptoethanol. Two polymerase peak fractions from DEAE-cellulose chromatogram were combined (18 ml.), applied to the column, and elution was achieved with a linear gradient of potassium phosphate buffer ranging from 0·02 to 0·3 M at *p*H 7·0. The buffers contained 0·005 M 2-mercaptoethanol and 80 ml. of each buffer were used; fractions of 3 ml. were collected every 20 min and assayed for polymerase activity as described in the text, with native (—△—), denatured (—○—), and 'activated' calf thymus DNA (- - - ● - - -) as primer, respectively

as streptomycin or protamine, or interaction with naturally occurring components such as proteins or nucleic acids persisting in the fraction, is responsible for the heterogeneous character of polymerase activity on the chromatography. Both polymerase activities, however, were always detected, even when the streptomycin fraction was exposed to digestion with pancreatic DNase in

Table 3. EFFECTS OF PRE-INCUBATION OF NATIVE DNA WITH PEAK FRACTIONS *a* ON THE PRIMING ACTIVITY

Exp. series	Pre-incubation (min)	Acid-precipitable radioactivity after incubation (c.p.m.)
1	60	2,060
	30	2,421
	20	3,002
	10	3,064
	5	2,754
	0	3,009
2	60	411
	30	506
	20	786
	10	530
	5	587
	0	604

Five μg of calf thymus native DNA was incubated at 37° C for various intervals described in the table with 10 μmoles of glycine buffer, *p*H 9·5, 1 μmole of magnesium chloride and 0·2 unit of fraction *b* in a total volume of 80 μl. Then, in series 1, four deoxyribonucleoside triphosphates were immediately added to the reaction mixture, and priming activity of DNA thus treated was measured by counting the acid-precipitable radioactivity after a 30-min incubation at 37° C. In series 2, the reaction mixture after pre-incubation was heated for 10 min at 100° C and quickly cooled, then subjected to the assay for priming activity by the addition of four deoxyribonucleoside triphosphates and 0·2 unit of fraction *b*.

Table 4. POLYMERASE ACTIVITY OF THE MIXTURE OF TWO PEAK FRACTIONS *a* AND *b*

Enzyme (μl.) Peak *a*	Peak *b*	Acid-precipitable radioactivity after incubation (c.p.m.) Native DNA	Heat-denatured DNA
30	0	541	2,860
0	30	5,809	845
10	30	5,987	1,872
30	10	2,540	3,250
30	30	6,520	3,716

The standard assay system was used with indicated volume of fractions *a* (36 units per ml.) and *b* (20 units per ml.) added as the enzyme.

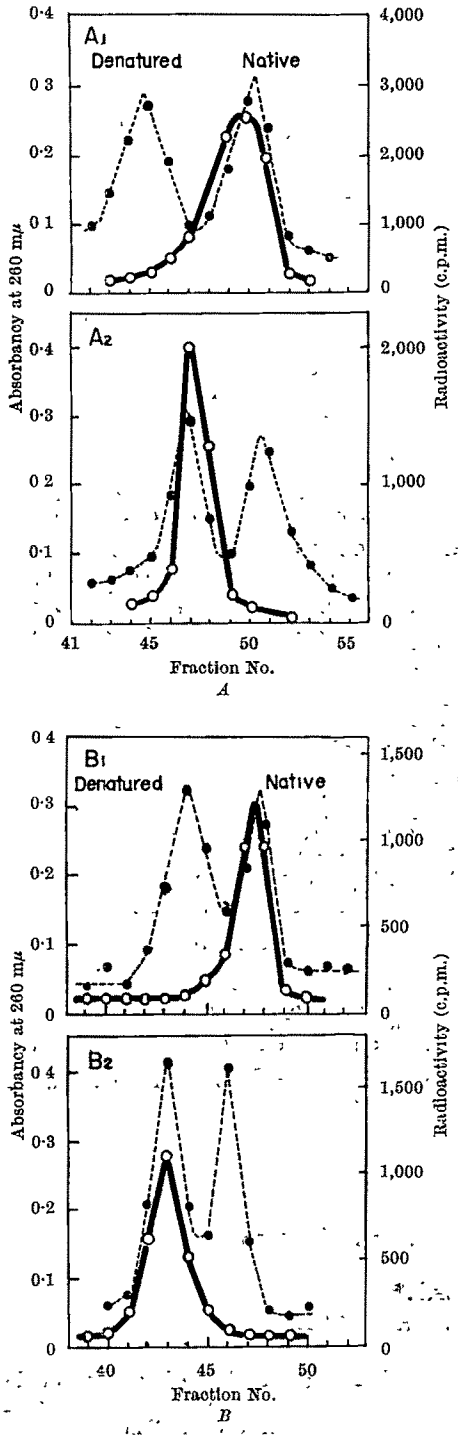


Fig. 5. 'Geon' zone electropherograms of reaction product. *A*, The incubation mixture (750 μl.) contained 22 μg of heat-denatured calf thymus DNA, 50 μmoles of glycine buffer, *p*H 9·0, 10 μmoles of magnesium chloride, 15 mμmoles each of dATP, dGTP, dCTP and ^{3}H-dTTP, and 20 units of the *S*-polymerase. After 3 h of incubation at 37° C, the reaction mixture was deproteinized by phenol, dialysed against 500 ml. of 0·15 M sodium chloride-0·01 M potassium phosphate buffer, *p*H 7·0, for 12 h at 4° C. Tritium-labelled product thus obtained was divided into two portions: one was heated for 10 min at 100° C and cooled quickly, and the other left untreated. To both portions were added 250 μg each of unlabelled native and heat-denatured DNA as references and 0·7 ml. of 0·03 M citrate buffer, *p*H 6·5, and electrophoresis was carried out on unheated product (A_1) and heat-denatured product (A_2) for 16 h at 4° C as described by Matsubara and Takagi (ref. 20). *B*, The incubation mixture (3·0 ml.) contained 100 μg of native calf thymus DNA, 200 μmoles of glycine buffer, *p*H 9·0, 40 μmoles of magnesium chloride, 100 mμmoles each of dATP, dGTP, dCTP and ^{3}H-dTTP, and 10 units of the purified *D*-polymerase. After incubation for 5 h, the reaction mixture was deproteinized by phenol and then dialysed against 500 ml. of 0·15 M sodium chloride-0·01 M potassium phosphate buffer, *p*H 7·0, for 12 h at 4° C. The other experimental conditions were the same as described in *A*. Mobility of native and denatured DNAs added as references was indicated by absorbancy at 260 mμ of each fraction (- - - ● - - -)

order to remove nucleic acids more extensively, and followed immediately by chromatography on a DEAE-cellulose column without protamine treatment and ammonium sulphate fractionation. The same results were obtained when the streptomycin non-precipitable fraction was applied to a DEAE-cellulose column. Therefore, it seems unlikely that the two polymerase activities are associated with a single protein. The fraction *a* enzyme will be tentatively referred to as polymerase primed by single-stranded DNA (*S*-polymerase), and the fraction *b* enzyme by double-stranded DNA (*D*-polymerase), respectively.

Three experiments were carried out in order to determine the structure of the reaction product. When the amount of acid-precipitable product, synthesized with *S*-polymerase and with denatured DNA as primer, had attained approximately 50 per cent of the amount of primer added, the product was subjected to the following procedures: (1) when tested by zone electrophoresis with 'Geon'[20], the reaction product was found in a peak with mobility similar to that of the double-stranded DNA, added as reference, whereas the same product after heat-denaturation represented a peak of identical mobility with that of DNA in single-stranded structure (Fig. 5*A*); (2) another aliquot of the product was applied on a methylated serum albumin column (1 × 3 cm), then eluted with sodium chloride using stepwise increase of the concentration[21]; the product was eluted quantitatively with 0·7 M sodium chloride, while the product after heat-treatment was scarcely eluted by sodium chloride up to 1 M; (3) the product was not hydrolysed by the action of exonuclease I of *E. coli* purified as described by Lehman[22]—an enzyme known to have a high specificity toward single-stranded DNA—whereas, after previously heated for 10 min at 100° C and quickly cooled, it was gradually degraded by the enzyme, as shown in Fig. 6. Similar results were also obtained with the product of *D*-polymerase reaction (Fig. 5*B*). Then it was found that both polymerases give product in a single-stranded structure, which is easily converted to single-stranded form by heating and rapid cooling.

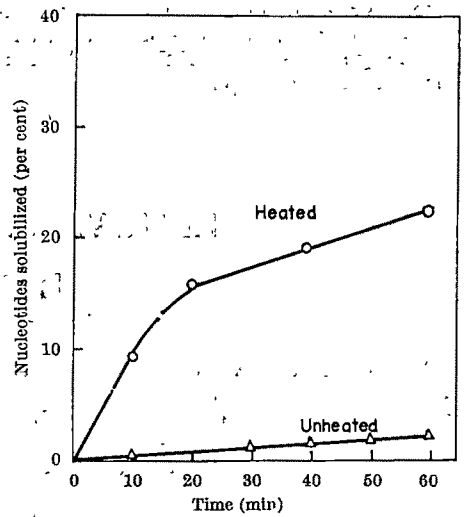


Fig. 6. Enzymatic digestion of reaction product. Tritium-labelled reaction product by *S*-polymerase was prepared according to the procedures described for Fig. 5. The incubation mixture (1·0 ml.) contained 250 μg of heat-denatured calf thymus DNA, 10 μg of tritium-labelled reaction product heated or unheated, 25 μmoles of glycine buffer, *p*H 9·5, 5 μmoles of magnesium chloride, and 25 μg of *E. coli* exonuclease I. After incubation at 37° C, aliquots were taken at intervals and the radioactivity rendered acid-soluble was measured

From the results described in this article, it would seem that *Alcaligenes faecalis* contains two separate polymerases; however, it is possible that the enzyme exists in the cell in an aggregate form but that under the conditions used for the extraction or purification it has become partly separated into sub-units resulting in dif-

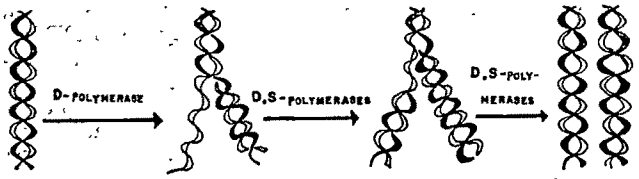


Fig. 7. Schematic mechanism of DNA replication

ferent chromatographic behaviour and different primer requirements.

There are several interpretations of the function of the two different polymerases present in one source. In order to explain the replication of chromosome in the cell as it is at present known, it is necessary to assume that two kinds of reaction proceed simultaneously from one end of a DNA template, along its two strands of opposite polarity. One is the interaction of the 3′-hydroxyl group of a deoxyribonucleoside triphosphate with the 5′-phosphate group of the terminal nucleotide of DNA strand (type I), and the other is the attack of the 5′-phosphate group of a deoxyribonucleoside triphosphate on the terminal 3′-hydroxyl group of DNA chain (type II). The *E. coli* polymerase is known to initiate its 'replicating' reaction from the 3′-hydroxyl end of the template strand, according to the mechanism of type II (ref. 11), but as yet the enzyme responsible for the other type of reaction (type I) has not been found in Nature. Another peculiarity is that the product of DNA polymerase from *E. coli* is unlike native DNA in two respects: (i) from electron microscope studies it seems to be a multi-branched form; (ii) it renatures fully after heating and quick cooling[23]. These may explain the failure to replicate biologically active DNA. Thus there is no enzymatic mechanism available to explain the replication process of chromosomes in the cell, and some doubts have been expressed as to whether the DNA polymerases we have are "only repair enzyme". At present, it is not possible to say whether or not these enzymes are involved in DNA replication or not, but there are two possibilities if this is so: (1) the enzymes are responsible for initiating two different types of reaction in the cell, but only one reaction in *in vitro* conditions; (2) the cell is provided with another enzyme for the type I reaction. Efforts are being made in many laboratories to explore polymerase activity, different from these polymerases, with regard to the direction of 'replication'. However, if the two distinct polymerases referred to in this article are not both repair enzymes, then it is possible that our observations indicate that polymerases—essentially related to DNA replication—are different in their primer requirement as well as in the type of reaction already mentioned. Furthermore, this suggestion fortifies a plausible hypothesis for DNA replication in the cell as indicated in Fig. 7. The *D*-polymerase may initiate the reproduction from one end of a DNA template along one of its two strands, leaving another strand free which will sequentially serve as a template for *S*-polymerase. Thus, the replication may continue until the two daughter molecules are completely formed. We are at present investigating this hypothesis.

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HEARTWOOD FORMATION IN TREES

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DESPITE the fact that wood has been used by man for countless ages, and that countries throughout the world are devoting an increasing amount of time to the planting and cultivation of trees, one of their most important features still is not understood, namely why trees form heartwood.

Only two basically different hypotheses concerning heartwood formation have been put forward, the first involving moisture–air relationships in the cells, the second fungal infestation. The former, which has received by far the greater support, has a number of variants, but neither hypothesis has been used or can be used to explain genetic and environmental differences in heartwood formation. It is the purpose of this article to present a third hypothesis and to illustrate its value by offering explanations for some of these genetic and environmental differences.

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Before embarking on this discussion of heartwood formation, I consider that a few introductory remarks on heartwood would be in order. There is no adequate definition of heartwood, but it is usually considered to be the wood within a tree which is distinct from sapwood, for example, is of a different colour, is usually of a lower moisture content, may contain a higher extractive content, may be of higher durability, may be tylosed, and in which the cells including ray parenchyma are dead.

In the first hypothesis to be put forward on heartwood formation, Priestley[1] contended that heartwood resulted from the accumulation of air in the closed vessel systems, and the resultant effect on permanent water content brought about secondary changes in the living parenchyma cells. It has been pointed out[2] that this view is supported by the relatively constant thickness of sapwood within a species and sometimes a genus; however, it was considered necessary to explain why the length of life of sapwood ray cells of various species differs so greatly

and also why the age at which sapwood becomes heartwood in roots, branches and stems is so different. A variant of this hypothesis is that of Zycha[3], who believed that the water requirements of the crown reduced the inner sapwood moisture content, so causing air to be drawn in through dead or broken limbs, and it has been suggested[4] that formation of 'false heartwood' brought about by artificial means supports this hypothesis—as does the absence of fungal infection[5].

In the second hypothesis, Münch[6], Büsgen and Münch[7], Jacquiot[8] and Chattaway[9] believed that fungal infection initiated the transformation. This hypothesis has not received wide support, and one of the major criticisms is that it is frequently not possible to isolate fungi from the appropriate wood. It has been suggested[2] that if this were the correct hypothesis it would have to be used to explain why normal heartwood is usually symmetrical in shape and position, and also why concentric rings of included sapwood are found.

The third, and new, hypothesis, which I shall present here, depends on the fact that: (1) Water requirements of the crown may reduce the moisture content of the inner parts of the tree at certain periods[8,10,11]. (2) The food economy of the young tree, if considered in the terms of Kramer and Kozlowski[12,13], which involves income (food manufactured by photosynthesis), expenditure (assimilation and respiration) and balance (food accumulated and used as a metabolic substrate at a later date), is efficient, such that an over-production of storage food does not occur. (3) Food economy efficiency decreases with age, so that the reserve food and the by-products of increased respiration are not completely re-utilized. (4) Hydrolysis of starch to sugar will occur as a result of water deficit[14,15]. (5) Redistribution of soluble carbohydrate will occur between living cells in both centrifugal and centripetal directions. (6) Xylem cell walls, even in dead sapwood cells, behave as semi-permeable membranes and will allow centripetal and centrifugal flow of water and certain solutes[16,17]. (7) Respiration will continue in inner ray parenchyma (because of the presence of carbohydrate and related compounds), even under conditions of reduced moisture content. Such conditions should favour reactions which increase the gas pressure (though the oxygen content may fall and the carbon dioxide content rise).

In the rapidly growing young tree little food is reserved, but after a few years the tree produces greater amounts of reserve foods. When, for example, the reserve starch is being reabsorbed by hydrolysis to sugar the reaction will proceed if the reaction product(s) is removed. If it is not removed, then equilibrium will be attained and the reaction will cease; however, the product should move in both a centrifugal and a centripetal direction in an attempt to reach equilibrium. Because large amounts of soluble reserve food are utilized at these times in those parts of the tree which are actively growing, we tend to consider that only centrifugal flow occurs and we have overlooked the fact that there is a competing centripetal flow in which the initial driving force is the need of the starving ray parenchyma and the normal diffusion laws. The rate of centripetal flow will clearly be dependent on the rate of centrifugal flow, and the absolute amount of material will vary according to the availability of soluble reserve food. It should be pointed out that centripetal flow of solute may occur concurrently with a centrifugal flow of water if the concentration (but not the absolute amount) in the inner sapwood is lower than in the outer sapwood. Centripetal flow of carbohydrate should cease if, and when, equilibrium is attained. But if the soluble carbohydrate is transformed into a further substance which for convenience we shall call an extractive, by an irreversible reaction such as may occur in a ray parenchyma cell, then equilibrium will not be attained and centripetal flow of the carbohydrate will continue—just as centrifugal flow continues. For similar reasons, centripetal flow of the reaction products will tend to continue. For convenience so far, only one food reserve material or extractive has been considered, but it will be quite clear that a series of extractives could be involved. For example, in an angiosperm it could be starch changing to a carbohydrate *A* changing in turn to an extractive *B*, which is again transformed to *C*. Ultimately an extractive *E* is produced which has a relatively low solubility in aqueous solution ('sap') and so is precipitated. Thus we have the beginning of heartwood formation, since further centripetal flow can now take place to maintain equilibrium. It is only by such centripetal flow that we can explain why heartwood may have a higher extractive content than the corresponding sapwood, and the increase in extractives of the heartwood type on the cambial side of insect holes in sapwood and their reduced concentration on the pith side.

It will be noted that it has not been necessary to consider the detailed mechanisms involved in cell transport and death, and the reader is referred to Frey-Wyssling and Bosshard[18] and Nečesany[19] for an explanation of cytological changes which occur in sapwood–heartwood transformation.

The position at which precipitation is occurring, and which we may term the heartwood–sapwood boundary, will be moving away from the pith if the moisture content falls during the year. Thus the extractive content of the heartwood at a specific position should be related to the tree's efficiency in producing cellular materials and its ability to re-use its own food reserves. It should be pointed out that if precipitation did not occur, extractives may also tend to move back towards the sapwood to maintain equilibrium, with the result that extractive contents would not vary radially.

This hypothesis offers satisfactory explanations for a number of phenomena, thus overcoming major obstacles to its acceptance in a general sense; some of these will now be discussed.

A species which is not efficient will produce heartwood when quite young, and as the tree increases in age, so its efficiency will decrease with the result that its sapwood thickness will decrease and its heartwood extractive content increase. A species which is efficient will not produce heartwood when it is young, and in fact may never produce normal heartwood. (The so-called pathological heartwood may develop at a later stage because of fungal attack.) In between the two limits we will have species with wide sapwoods and which produce heartwood late in life.

So far it has been suggested that a combination of a tree's inability to obtain sufficient water (and probably inorganic constituents) and the fall in efficiency governs the time of heartwood formation and subsequent sapwood thickness. If the change in efficiency is rapid, then this will contribute to an early increase in heartwood extractive content.

Let us examine its explanation of the fact that within a species trees which have grown slowly in general have a higher heartwood extractive content than trees which have grown quickly. It is known, for example, that starch reserves rise in dry weather in the northern hemisphere deciduous trees, and further proof of this association comes from the finding[20] that some heartwood evergreens (*Eucalyptus* species) in Australia exhibit two general rises and falls in starch content in each year which are associated with two periods of moisture shortage and slow growth. This starch, if inadequately re-absorbed, is free to produce heartwood extractives, so an association between moisture stress, slow growth and high extractive content is evident. The converse of this basic reasoning explains why trees which have grown quickly are found to contain low extractive contents; indeed the high growth rate in the very young tree is also associated with a low extractive content and it would appear that the faster the tree grows the fewer 'by-products' it produces.

It has been reported[21] that good dominant *Pseudotsuga menziesii* trees have a wider sapwood than the average tree, that the sapwood is wider on good sites for the top and middle of the stem, and that the sapwood is thickest at the base of the stem and least in the middle of the stem. Also that *Fraxinus excelsior* has a higher wood moisture content and later heartwood formation on moist sites and in milder regions, in fact is a facultative heartwood producer whereas in continental regions it is an obligate heartwood producer[22]. Conditions which favour high growth rate include an adequate rainfall, and then the demand for water from the wood nearest to the pith is low; thus there could be a low centrifugal migration of water in the stem, which would cause a later than usual precipitation of extractives, hence a wider sapwood. On the basis that the lower part of the stem is closest to the source of water (the roots) one would expect that the water stress would be low and thus the sapwood would be wide, whereas higher up the stem it would be narrower owing to the water stress being greater. It is tempting to suggest that the upper stem sapwood thickness would be greater because of its proximity to the crown, but this would be mere wishful thinking.

Related to these explanations is an explanation for the formation of heartwood at differing ages in roots and stems. For example, *Robinia pseudoacacia*, seldom contains more than a few annual rings which are free from tyloses, and is known for its narrow sapwood, whereas tyloses and heartwood formation are absent in even the twentieth ring in the roots[7].

On the basis of the present hypothesis, formation of a fairly symmetrical heartwood is not unexpected, since the branches are situated reasonably symmetrically around the upper stem and so the demands of the crown for water are transmitted fairly evenly around the stem perimeter. Likewise the positioning of the roots ensures a fairly even supply of water to the base of the stem. Nevertheless, it is known that small moisture content variations do occur around the stems, and minor unevennesses in heartwood formation are known. Whether these correlate should prove an interesting study.

It has been pointed out[2] that the heartwood periphery of a stem containing tension wood shows no relationship with the growth rings or age of the living cells. Also, any hypothesis should be able to explain why the heartwood extractive content of tension wood is considerably lower than that of the opposite wood. Tension wood is produced in response to an external stimulus and the tree is able to control its response.

Tension wood is wood of high growth rate, and on the basis of the previous assumption of increased efficiency being associated with high growth rate, then the extractive content of tension heartwood would be expected to be low. On the opposite side of the tree, though there is a reduction in growth rate, there is no marked change in the availability of water as may be the case in suppressed trees, so there is no need markedly to reduce the sapwood thickness.

The formation of concentric rings of included sapwood (target rings) must now be considered. This is clearly the result of variations in the amounts of reserve food available for centripetal flow. When the amount available is low, then a heartwood is produced which is so low in extractives as to appear to be sapwood. On the return of normal conditions, the centripetal flow is resumed, and proceeds to a position which is determined, in effect, by the availability of moisture within the tree, precipitation occurring when saturated solutions are obtained. The zones of low extractive content that stand out, usually as lighter coloured, including sapwood on the pith side of insect holes or scars, are also heartwood to which normal centripetal flow of extractives has been stopped. However, the sapwood side of the injury may also contain an area of low extractive content according to the rate of moisture loss into and through the hole. Next to this zone will be a zone of high extractive content, since this contains those extractives which were originally centripetally flowing to the included sapwood zone. Examination of such zones reveals the normal heartwood constituents, indicating that premature desiccation produced results comparable with the normal heartwood process.

Similar reasoning may be used to explain the failure to increase the proportion of obvious heartwood in oak and larch by girdling[23] because clearly the centripetal flow of extractives had been interrupted.

On the basis of the previous discussions it will be seen that species which tend to be referred to as sapwood species must no longer be considered to be anomalous, whether there is or is not a step-wise change in extractive content at some position across the radius of the stem.

It should be pointed out that in the sapwoods of hardwood species, the initially formed extractives are water soluble and frequently polysaccharides[24]. Any modification of these, particularly aromatization, decreases their water solubility. It seems understandable, therefore, that the heartwood extractives of many hardwoods include aromatic substances. Nevertheless, non-aromatic compounds, such as terpenes, which are relatively insoluble in water, will produce the same effect.

There are numerous other examples where this hypothesis can be successfully applied, and readers will be able to try for themselves a number of their own observations. Nevertheless, a cautionary remark may prove helpful and avoid the hypothesis being discredited on limited evidence. It would seem worth while to illustrate this by an example of the failure of the hypothesis to predict what has been reported, but which I believe does not discredit the hypothesis.

Pinus radiata grown in New Zealand has been investigated[25] and the conclusion drawn that physiological drought retards heartwood formation, but that with a deep root, adequate rainfall and low drying conditions, heartwood formation is at a maximum (compare this with the results for *Pseudotsuga menziesii*), though again dominant trees contained a wider sapwood than suppressed trees. Immediately, this unexpected result suggests that factors other than the availability of moisture are of some importance; for example, to what extent are mineral deficiencies involved in controlling what has been referred to as 'true efficiency'.

It is not my intention to imply that this hypothesis is perfect or complete; rather it is intended that it should stimulate thought and so provoke biochemists, botanists and foresters into planning further research into this most intriguing facet of Nature.

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LETTERS TO THE EDITOR

ASTROPHYSICS

A Search for Variations in the Flux of the Quasi-stellar Source *CTA* 102

THE source *CTA* 102 has evoked considerable interest following Sholomitsky's[1] announcement that the flux at 32·5 cm varied 25 per cent, with a period of about 100 days. Caswell and Wills[2], however, could find no evidence for variations at 1·7 m in their observations made during the same period. The determination by Schmidt[3] of a red shift for *CTA* 102 $\Delta\lambda/\lambda = 1{\cdot}037$, placing the object at a distance of about 3,000 Mpc, contradicts Sholomitsky's estimate of $\sim$ 2 Mpc, based on angular size and light travel time. Maltby and Moffet[4] have since examined results of measurements made by the California Institute of Technology at 31·4 cm, and for the period 1959·7–1961·7 found no significant variation, and concluded that Sholomitsky's result is probably in error.

Rees and Sciama[5] have attempted to reconcile Sholomitsky's result with the 178 Mc/s and red-shift data. They assume synchrotron self-absorption to account for the lack of variation at 178 Mc/s, and postulate a rather improbable geometry to resolve the difficulty of light travel time. On the basis of this model they predicted a variable component with a 25 per cent peak amplitude, for frequencies greater than 1,000 Mc/s.

We have undertaken measurements spanning a period of 75 days, from September 16 to November 29, 1965, to detect possible variations in the flux density of *CTA* 102 at 13 cm (2,295 Mc/s). Measurements were made with an 85-ft. polar mounted antenna at the National Aeronautics and Space Administration Deep Space Station at Hartebeesthoek, near Johannesburg, South Africa. A right-hand circularly polarized feed was used. The radiometer uses a travelling-wave maser, and peak-to-peak fluctuations are of the order of 1 flux unit, with a post-detection integration time of 5 sec. Full details of the equipment will appear elsewhere[6].

Observations were made at 2–4 day intervals. The sources 3*C*33 (S = 8 F.U.) and 3*C*353 (S = 38 F.U.) were chosen as control sources to check stability of the flux scale, since these have both been identified with *D* galaxies[7], and thus would not be expected to vary. From weekly averages for 3*C*33, the long-term stability of the flux scale, referred to an argon standard, was found to be constant to better than ± 1 per cent over the observing period. Day to day stability was checked for 12 days from September 16 to October 30 from observations of 3*C*33 and 3*C*353. Peak deviation from the average was ± 1·5 per cent with a median deviation of ± 1 per cent.

The results for *CTA* 102 are shown in Fig. 1. Each point is the average of nine measurements and the bars indicate the standard deviation for each set of measurements. The data indicate no evidence for the 100 day, 25 per cent variation. Of twenty-nine values, twenty-two are within ± 2·5 per cent of the mean, six between ± (2·5–5) per cent, and only one point deviates by 6 per cent. This scatter is no greater than would be expected from measurement inaccuracies, and there do not appear to be any day to day variations greater than 5 per cent.

Fig. 2 shows averages of flux density over approximately weekly periods, each point being the mean of twenty-seven observations. Peak-to-peak scatter about the mean is 6 per cent. Also shown is the expected variation, from extrapolation of Sholomitsky's result, and assuming the model proposed by Rees and Sciama. We conclude that any variation with a period of the order of 100 days has a peak-to-peak amplitude < 6 per cent, and hence the flux of such a variable component is < 0·3 F.U. If the source is still variable at 32·5 cm, then the spectral index of the variable component must be greater than 2, between λ = 32·5 and 13 cm.

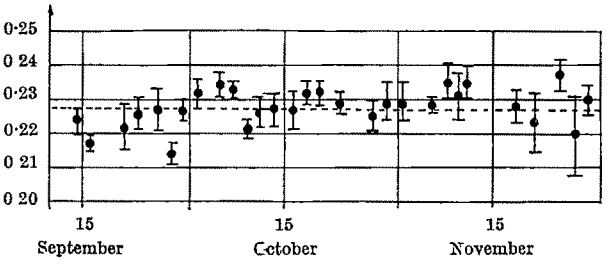


Fig. 1. *CTA* 102: argon noise tube

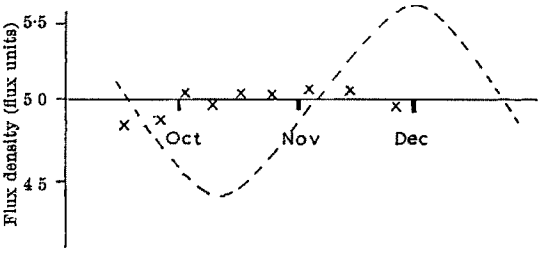


Fig. 2. *CTA* 102 flux density

The foregoing work has been made possible by a contract with the National Aeronautics and Space Administration, in terms of which the South African C.S.I.R. staffs and operates the Hartebeesthoek facility.

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Suggested Mechanism for the Anomalous Excitation of OH Microwave Emissions from H-II Regions

STRONG microwave emission lines at 18 cm have recently been reported from a number of sources[1–3], the frequencies being close to those of the hyperfine components of the Λ-doublet of the ground state ($X\ ^2\Pi_{3/2}$, J = 3/2) as measured in the laboratory[4]. Λ-doubling is due to coupling between the rotation of the nuclei and the orbital rotation of the electrons, which has a quantum number Λ = 1 in the Π state. The energies differ slightly

according to the relative orientation of the two angular momenta. Coupling with the nuclear spin of the proton leads to further hyperfine splitting (Fig. 1). Galactic absorption lines at these frequencies, detected in the past 3 years, have rather broad profiles similar to those of the 14-cm H-emission line; and, except for OH near the galactic centre, the intensities of the hyperfine components are compatible with populations of levels in thermodynamic equilibrium[5]. The emission lines, by contrast, show a number of remarkable features.

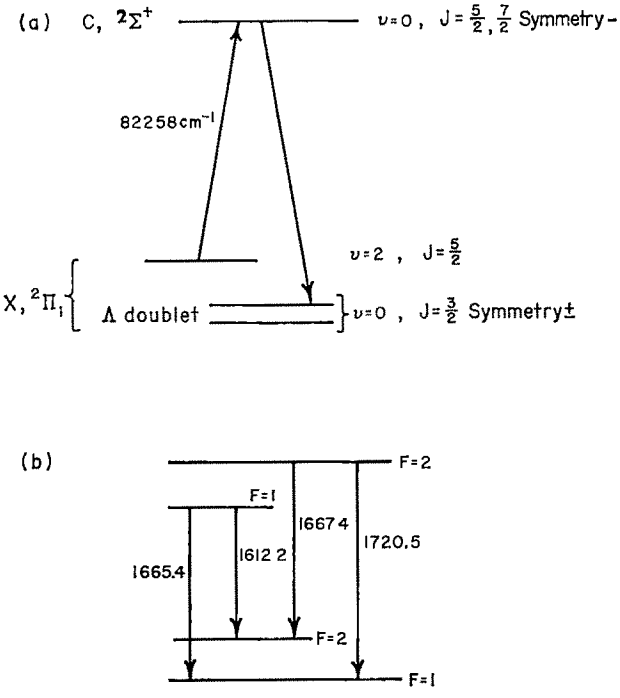


Fig. 1. Portion of level diagram of OH molecule: (*a*) Ground state and electronically excited state *C*. *J*, Total angular momentum quantum number (excluding nuclear spin), *v*, vibrational quantum number. (*b*) Hyperfine structure of Λ-doublet, frequencies in Mc/s. $F = J \pm \frac{1}{2}$

The lines are extremely narrow, the corresponding kinetic temperatures being often less than 50° K; the lines are multiple, as many as seven components separated by 10–15 kc/s being observed in one case[3]; the 1,665-Mc/s line from *W*.3 is linearly polarized by up to 37 per cent and is up to twenty-five times as intense as the 1,667-Mc/s line, whereas, if the populations of the hyperfine levels follow the Boltzmann distribution, the 1,667-Mc/s line should be 1·8 times as intense as the 1,665-Mc/s line; the emission lines are sometimes associated with absorption lines, the frequencies of the latter being less than those of the former by an amount corresponding to 10–15 km/s; the widths of the absorption lines are of the same order as those of the emission lines, and the intensities of the absorption lines are as predicted for a Boltzmann distribution; all the sources are closely associated with thermal continuum sources, some of which are known to be H-II regions.

Although Weaver *et al.*[1] left open the possibility that the emission lines may come from something other than hydroxyl ('mysterium'), Weinreb *et al.*[2] consider the lines to arise from OH excited in some anomalous way. Lines close to three of the four hyperfine components have now been observed in emission from radio sources, and it seems most unlikely that any other emitter should have so closely similar a spectrum.

An optical pumping mechanism for the anomalous excitation of OH is suggested in this communication. Zuckerman *et al.*[3] have observed that none of the frequencies of $n \rightarrow n-1$ transitions of excited hydrogen coincides closely with the frequencies of the OH lines. There is none that coincides with a transition between rotational levels of $X\ ^2\Pi_i$, $v = 0$; nor is there any coincidence with transitions between different vibrational levels of $X\ ^2\Pi_i$. There seems, however, to be an extremely close coincidence between $Ly - \alpha$ ($\lambda = 1216$ Å, wave number 82,259 cm^{-1}) and the transition $X\ ^2\Pi_i$, $v = 2$, $J = 5/2 \rightarrow C^2\Sigma^+$, $v = 0$, $J = 5/2$, 7/2, between a vibrationally excited state of $X\ ^2\Pi_i$ and the electronically excited state $C^2\Sigma^+$. The levels concerned are shown in Fig. 1. Unfortunately, this part of the spectrum of OH, which lies between 1000 Å and 1700 Å, has not so far been observed; the wave number of the transition (82,258 cm^{-1}), as calculated from the data given by Wallace[6], is within 1 cm^{-1} of $Ly - \alpha$.

It is therefore suggested that OH absorbs radiation from the thermal source, giving the $v = 2$ level of the X ground state an appreciable population, and that absorption of $Ly - \alpha$ then populates the $C^2\Sigma^+$, $v = 0$, $J = 5/2$, 7/2 state. (Carrington[7] has shown that OH can be formed in some circumstances in vibrationally excited states by irradiation of water with $Ly - \alpha$.) The symmetry selection rules permit an electric dipole transition from this C state to the upper, but not to the lower, of the Λ-doublet levels. Furthermore, it is found that H-α radiation from H-II regions can be polarized. It may be expected that there are magnetic fields in these regions and that $Ly - \alpha$ is also polarized; it would therefore be possible for the Zeeman sub-levels of the hyperfine levels to be unequally populated.

Conditions under which the suggested mechanism could produce the observed phenomena would seem to exist close to the ionization-shock front believed to form the boundary of an H-II region[8]. As such a front advances, at 10–20 km/s, into the surrounding cloud of cold atomic hydrogen, the gas suddenly becomes almost completely ionized and acquires a velocity of 10–20 km/s. OH in the shock front will be irradiated with both thermal and $Ly - \alpha$ radiation; in front of the shock, $Ly - \alpha$ will be absorbed and broadened by neutral atomic hydrogen and will not be so effective in selective optical pumping, while the cold OH will absorb thermal radiation at microwave frequencies differing from the mean frequencies of the emission lines by amounts corresponding to the shock speed. Behind the shock the temperature of OH will rise to about 10^4 °K, and the spectrum will merge with the thermal emission at that temperature.

To summarize, absorption of $Ly - \alpha$, followed by radiation from the excited C-state, can give abnormal populations in the upper states of the Λ-doublet; conditions in which narrow emission lines associated with slightly displaced narrow absorption lines can arise seem to occur in the ionization-shock front at the boundary of an H-II region. No other transitions of atomic hydrogen have frequencies that nearly coincide with any in the spectrum of OH. Details of the suggested mechanism are being investigated.

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PHYSICS

Buoyancy and Stable Levitation of a Magnetic Body immersed in a Magnetizable Fluid

THIS communication concerns the nature of some peculiar forces experienced by a body immersed in magnetic fluid when the body is a source of magnetic field. Prediction and experimental demonstration are given of the stable 'fluidmagnetic' levitation of a permanent magnet at an interior region of the fluid.

It is possible to make a permanent suspension of a finely divided ferro-magnetic solid in a carrier liquid to produce a fluid that responds strongly to a non-uniform applied magnetic field[1]; such fluids are produced and studied in our laboratory—the range of initial permeability $\mu=(\partial B/\partial H)_0$ so far obtained is $1 < \mu < 2$ corresponding to fluids whose ferric induction $M=B-H$ at saturation in units of gauss is $0<M<730$. The fluid is a colloidal dispersion in which the typical particle size is but 100 Å with the number density of the particles being of the order of 10^{17}/c.c. In situations of fluidstatics and fluiddynamics the fluid behaves like a homogeneous Newtonian liquid even though it has the additional characteristic of an appreciable initial permeability in response to an applied magnetic field; the viscosity of these fluids is essentially independent of the field.

The net force acting on a body immersed in stationary magnetic fluid under the influence of gravity, but in the absence of magnetic field sources external to the body, is found from the following integrations of force over the volume V and the surface S.

$$\mathbf{F} = -\mathbf{z}\int_v \rho_b\, g\,dV - \int_s p\mathbf{n}dS$$

Here $\mathbf{z}$ is the unit vertical vector, ρ_b is the density of the body which will be assumed constant hereafter, g is the acceleration of gravity, p is the fluid pressure, and $\mathbf{n}$ is the unit vector oriented outward from any differential surface element dS. Evaluation of the surface integral now requires an expression for the pressure and this is considered next.

A treatment has been given of the fluid mechanical relationships that govern the motion and pressure distribution of magnetic fluids in the presence of an applied magnetic field[2]. It was found possible to derive an augmented Bernoulli relationship which may be expressed as follows to apply at any point within the motionless magnetic fluid that surrounds the immersed body.

$$p + p_h - p_m = \text{constant}$$

In this relationship p is ordinary fluid pressure and p_h is a hydrostatic pressure, such that:

$$p_h = \rho\, gh$$

where ρ is density of the magnetic fluid and h is the height in the gravitational field, while p_m, termed the 'fluidmagnetic pressure', is given by the following expression:

$$p_m = \frac{1}{4\pi}\int_0^H M dH$$

M is the ferric induction, a property of the fluid which is dependent on the magnetic field H. It can easily be seen, since $M \geqslant 0$ for any H, that the magnetic pressure increases when H increases. The equation is written in cgs units wherein M is expressed in gauss and H in oersted, so the numerical value of p_m is expressed in units of dynes/cm^2.

Stated verbally the magnetostatic form of the augmented Bernoulli relationship tells that the sum of ordinary pressure plus a hydrostatic pressure minus the fluidmagnetic pressure is a constant anywhere within the motionless fluid.

Elimination of ordinary pressure p from the force expression, followed by application of Gauss's divergence theorem to convert the surface integrals to volume integrals for the case of uniform gravitational field g and fluid density ρ, gives on recognizing that the gradient of the Bernoulli constant is zero and $\nabla h=\mathbf{z}$ that:

$$\mathbf{F} = \mathbf{F}_m + \mathbf{F}_b$$

where

$$\mathbf{F}_m = -\int_s p_m \mathbf{n} dS = -\frac{1}{4\pi}\int_0^H\int_s \mathbf{n} M dH dS$$

$$\mathbf{F}_b = gV(\rho - \rho_b)\mathbf{z}$$

$\mathbf{F}_m$ above represents a general expression for the fluidmagnetic force experienced by the immersed body while $\mathbf{F}_b$ is the usual expression for the buoyancy (Archimedes' principle) minus the weight.

If the permeability of the fluid may be regarded as constant, then $M=(\mu-1)H$ and the expressions for fluidmagnetic pressure and force become, respectively:

$$\left.\begin{aligned} p_m &= \frac{\mu-1}{8\pi}H^2 \\ \mathbf{F}_m &= -\frac{1}{8\pi}(\mu-1)\int_s H^2 \mathbf{n} dS \end{aligned}\right\} \mu = \text{constant}$$

In this special case of constant permeability the fluidmagnetic pressure at a point varies quadratically with the local magnitude of magnetic field and thereby illustrates the more general relationship already noted. With $\mu=1{\cdot}94$ and $H=395$ oersted the computed value of p_m is 5,809 dynes/cm^2, which is appreciable compared with the ordinary hydrostatic pressure in a small vessel of liquid.

Due to the general property of the fluidmagnetic pressure to be high where the magnitude of the magnetic field is high the possibility exists that a distribution of field might be found giving $\mathbf{F}=0$ and thereby producing a fluid magnetic force equal and opposite to the buoyant weight with the end result that a material object is levitated. Fig. 1 is an X-ray photograph through the opaque fluid that demonstrates such a means of levitation; a ceramic permanent magnet in the shape of a disk, having density $\rho_b=4{\cdot}7$, and immersed in magnetic fluid the density of which is but $\rho=1{\cdot}2$, is levitated in the Earth gravitational field above the bottom of the container holding the fluid. The magnetic fluid has permeability $\mu=1{\cdot}94$. The disk is magnetized in the direction normal to its circular faces. Hall probe measurement shows that it produces a magnetic induction of 395 gauss at a point adjacent to the centre of a face.

Taken together, the plan and elevation views illustrate that the levitation is about an interior point of the fluid. It may be understood, due to the greater magnetic permeability of the fluid compared with the permeability of the non-magnetic surroundings, that the magnet field lines which emanate from and end on the magnet will be more compressed between the magnet and a nearby wall than between the magnet and a more distant wall. Then, due to the nature of the fluidmagnetic pressure and its dependence on field magnitude, it follows that there will be repulsion from any wall. The magnetic body, therefore, seeks an interior point equidistant from all parts of the boundary between fluid and its surroundings (considered to be non-magnetic) such that stable equilibrium attains. A small displacement of the magnet in any direction from its equilibrium position is then accompanied by a positive restoring force.

Further investigation of this and related phenomena, the relationship to Earnshaw's theorem[3], and detailed results of quantitative experimentation have been completed and

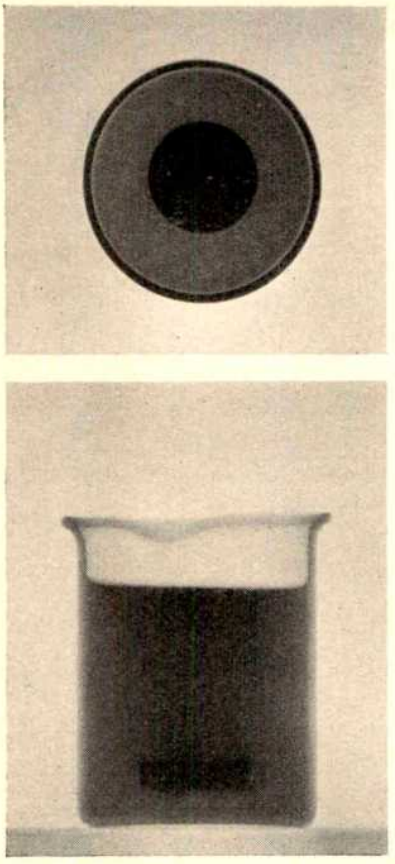


Fig. 1. Plan and elevation X-ray photographs of a disk-shaped permanent magnet immersed in a finite volume of magnetizable fluid. The fluid has a uniform density of 1·2 g/cm³ while the density of the magnet is greater and measures 4·7 g/cm³. The magnet is levitated against gravity and repulsed from the fluid's boundaries to a position of stable equilibrium at an interior region of the fluid space

will be described in another communication. This work is supported in part by the Fluid Physics Branch, National Aeronautics and Space Administration, Research Division, Office of Advanced Research and Technology.

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Meissner Effect in a Classical Electron Gas

M. v. LAUE[1] pointed out that thermodynamics can be applied to a superconductor only because it has, by displaying a Meissner effect, a unique state in a magnetic field. The classical electrodynamics of a perfect conductor, however, yields an ambiguous state[1], but only if acceleration effects are neglected. Classical statistics of a free-electron gas give a unique state of zero current (theorem of van Leeuwen[2]) independent of the external magnetic field. It has never been shown, however, how a magnetic field can enter the electron gas without the assumption of surface friction[3].

There are three possibilities: (1) The concept of a gas of classical free electrons is a contradiction *per se*; (2) classical thermodynamics in which the magnetic field does not enter because of van Leeuwen's theorem, and classical electrodynamics contradict each other; (3) van Leeuwen's theorem is not correct. It is the aim of this note to show that the third alternative is true.

The proof of the theorem[2] has neglected magnetic interaction. Including it, the energy law becomes

$$\sum_i \left\{ \frac{m_i}{2}\vec{v}_i^{\,2} + \frac{e_i}{c}\vec{v}_i\left(\frac{1}{2}\vec{A}_i^{\,\mathrm{int}} + \vec{A}_i^{\,\mathrm{ext}}\right) + e_i\left(\frac{1}{2}\varphi_i^{\,\mathrm{int}} + \varphi_i^{\,\mathrm{ext}}\right)\right\} = \text{constant} + \int^t \varepsilon\, dt \quad (1)$$

m_i = mass, e_i = charge, $\vec{v}_i$ = velocity of the particles, $\vec{A}_i$ = vector potential at the momentary positions of the particles defining the internal (int) or external (ext) magnetic field, φ_i = scalar potential. ε is the energy which has to be submitted per sec to the external current system in order to yield external fields which are constant in time in spite of the interaction with the particles.

In the equilibrium case, $\varepsilon = 0$. Evaluating the maximum of entropy for constant left side of equation (1) in the usual way (internal interaction terms are to be duplicated) one obtains a velocity distribution proportional to

$$\exp\left(-\frac{1}{2mkT}\left\{\left(m\vec{v} + \frac{e}{c}\vec{A}\right)^2 - \frac{e^2}{c^2}\vec{A}^2 + 2m\varphi\right\}\right)$$

with $\vec{A} = \vec{A}^{\,\mathrm{int}} + \vec{A}^{\,\mathrm{ext}}$; $\varphi = \varphi^{\,\mathrm{int}} + \varphi^{\,\mathrm{ext}}$

The mean velocity is found to be

$$\vec{v} = -\frac{e}{mc}\vec{A} \quad (2)$$

This is London's equation of the Meissner effect. This effect, therefore, is not a basic quantum phenomenon, as stated by several authors[4,5].

Equation (2) also holds in the case of Bose or Fermi statistics. This removes a further difficulty which has never been mentioned. The old proof of van Leeuwen's theorem can also be applied, word by word, to non-classical statistics. If the theorem was valid, the super-electrons which produce a Meissner effect would obey, apparently, no statistics at all.

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Measurement of the Absolute Disintegration Rate of Technetium-99*m*

TECHNETIUM-99*m* is becoming an important tracer for many diagnostic investigations; for example, brain scanning and thyroid scanning[1,2]. Its chief advantages are that it has a short half-life and emits an essentially mono-energetic γ-ray which is only slightly internally converted. Hence quite large activities of technetium-99*m* can be safely administered to patients. So far this nuclide has only been assayed by comparing it with a standard of some other nuclide—for example, cobalt-57 (ref. 3)—and no absolute measurements have been reported. However, as will be shown here, it is possible to make an absolute measurement by the $4\pi\beta$-γ coincidence technique[4]. (The National Physical Laboratory plans to distribute standards of technetium-99*m*, calibrated by this technique, in October 1966, to users within 12 h travelling time of Teddington.)

The decay scheme of technetium-99*m* is shown in Fig. 1. The internal conversion coefficients of the transitions

γ_1 and γ_3 are very large and that for γ_2 is approximately 0·1. If coincidences are observed between the transition γ_1 detected in the β counter and γ_2 in the γ counter, the relationship between N_0, the absolute disintegration rate, and the observed β count rate N_β, γ count rate N_γ and the coincidence count rate N_c is:

$$\frac{N_\beta N_\gamma}{N_c} = N_0\left[a + (1-a)\,\varepsilon_{\beta 3}\frac{\alpha_3}{1+\alpha_3} + \frac{1-\varepsilon_{\beta 1}}{\varepsilon_{\beta 1}}\left\{\frac{a\alpha_2\varepsilon_{\beta 2}}{1+\alpha_2} + \frac{(1-a)\alpha_3\varepsilon_{\beta 3}}{1+\alpha_3}\right\}\right]$$

multiplied by:

$$\left[1 + \frac{1-a}{a}\cdot\frac{1+\alpha_2}{1+\alpha_3}\cdot\frac{\varepsilon_{\gamma 3}}{\varepsilon_{\gamma 2}}\right]$$

also:

$$\frac{N_c}{N_\gamma} = \varepsilon_{\beta 1}$$

where $\varepsilon_{\beta 1}$ is the efficiency of the β counter to the 2 keV transition γ_1, $\varepsilon_{\beta 2}$, $\varepsilon_{\beta 3}$ are the efficiencies of the β counter to conversion electrons from the transitions γ_2 and γ_3, the total internal conversion coefficients of which are α_2 and α_3 respectively, and $\varepsilon_{\gamma r}$ is the efficiency of the γ counter for the rth γ ray. Since $(1-a)\approx 0{\cdot}01$, $\alpha_3 \approx 30$, $\varepsilon_{\gamma 3}\approx\varepsilon_{\gamma 2}$, and for the sources used $\varepsilon_{\beta 2}\approx\varepsilon_{\beta 3}=\varepsilon_\beta \geqslant 0{\cdot}95$, equation 1 can be reduced to:

$$\frac{N_\beta N_\gamma}{N_c} \approx N_0\left[1 + \frac{1-\varepsilon_{\beta 1}}{\varepsilon_{\beta 1}}\cdot\varepsilon_\beta\left\{\frac{a\alpha_2}{1+\alpha_2} + \frac{(1-a)\alpha_3}{1+\alpha_3}\right\}\right]$$

Hence if $\varepsilon_{\beta 1}$ is varied without altering ε_β, extrapolation to $\varepsilon_{\beta 1}=1$ gives the absolute disintegration rate N_0.

Using this method sources of technetium-99*m* were measured. The efficiency of the β counter to the conversion electrons from the γ_1 transition was varied by altering the counter voltage, and to ensure that this procedure did not affect the value of ε_β, all measurements were made within the range of the normal β plateau. A plot of the observed quantity $\frac{N_\beta N_\gamma}{N_c}$ per mg of solution used in source preparation against $\frac{1-\varepsilon_{\beta 1}}{\varepsilon_{\beta 1}}$ for six different sources is shown in Fig. 2, and the statistical error on the intercept at $\frac{1-\varepsilon_{\beta 1}}{\varepsilon_{\beta 1}} = 0$ is ± 0·3 per cent. The overall systematic error on the absolute disintegration rate was estimated as ± 3 per cent. This error includes an allowance for the assumed linearity of the extrapolation of the data shown in Fig. 2.

The technetium-99*m* used in this measurement was obtained from a molybdenum-99 generator supplied by the Radiochemical Centre, Amersham. The molybdenum-99 was absorbed on an alumina column as ammonium molybdate, and technetium-99*m*, the daughter of molybdenum-99, was eluted with an isotonic saline solution.

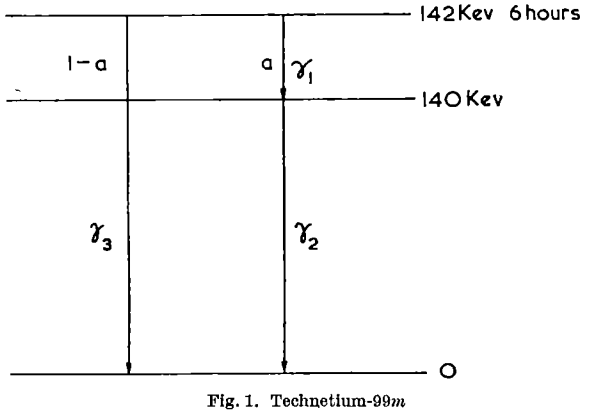


Fig. 1. Technetium-99*m*

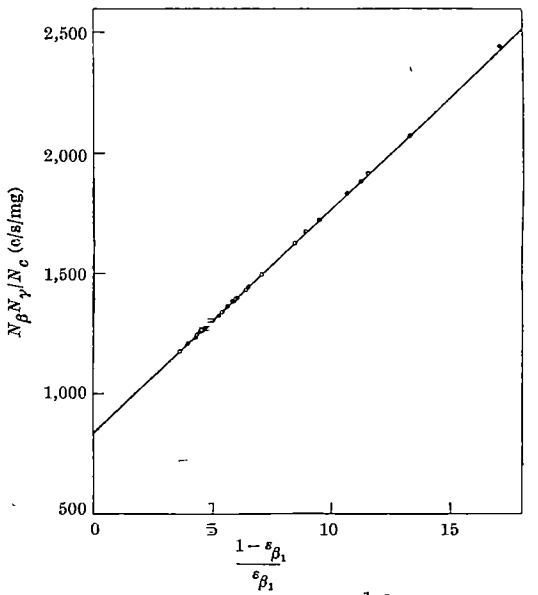


Fig. 2. Plot of $N_\beta N_\gamma/N_c$ against $\frac{1-\varepsilon_{\beta_1}}{\varepsilon_{\beta_1}}$

The purity of the technetium-99*m* was checked by γ-ray spectrometry. The molybdenum-99 content was less than 0·05 per cent and no other γ-emitting nuclides were detected. In subsidiary experiments it was shown that there was no adsorption of carrier-free technetium-99*m* as pertechnetate ion in isotonic saline solution on to the siliconed glassware used in the standardization.

The half-life of technetium-99*m* was measured using an ampoule of sodium pertechnetate in isotonic saline solution in a re-entrant high-pressure γ ionization chamber, the readings being taken over a period of five half-lives. The purity of the technetium-99*m* was again measured by γ-ray spectrometry at the end of the half-life measurements and at this time the molybdenum-99 contaminant was less than 0·005 per cent. The value obtained for the half-life was 6·006$_7$ h with a standard deviation of 0·01 per cent, and an estimated overall systematic error of ± 0·03 per cent.

We thank Dr. P. J. Campion for suggesting that technetium-99*m* could be measured by the 4πβ-γ coincidence technique and Miss R. Birdseye, Miss F. H. Hughes and K. Fordham for help with the experimental work.

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[1] McAfee, J. G., Fueger, C. F., Stern, H. S., Wagner, H. N., and Migita, T., *J. Nucl. Med.*, **5**, 811 (1964).
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A Silver-110*m* Contamination in Scintillation Detector Shielding

We have recently completed the third of three similar lead shielding assemblies for sodium iodide (thallium activated) γ-detectors, used in conjunction with multichannel pulse height analysers. These equipments are used for the assay of biological and other samples arising from statutory monitoring responsibilities connected with the disposal of radioactive waste from nuclear sites.

Many of the samples assayed contain low levels of radioactivity, and the latest detector assembly has been designed to have a low background count rate, and hence

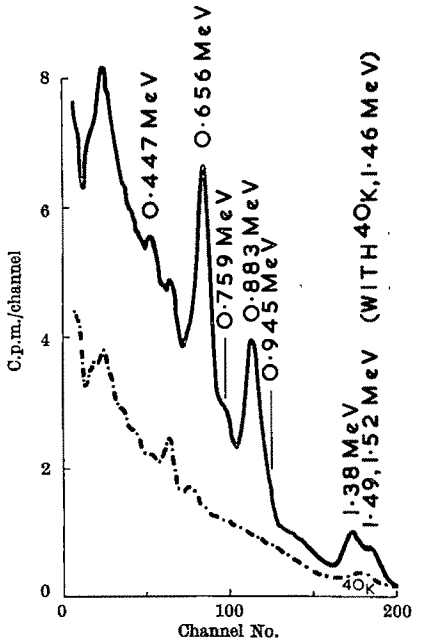


Fig. 1. Background spectra obtained from identical lead shields. ——, Lead contaminated with silver-110*m*; — · — · —, uncontaminated lead

greater sensitivity. Preliminary checks of the detector system in an existing shield were encouraging, indicating a reduction in background count rate from about 350 c.p.m. to 250 c.p.m. over the energy range 50 keV–1·6 MeV.

Completion of the assembly in a newly erected shield resulted in a surprisingly high background rate of 560 c.p.m., and a short spectrometric count of the roughly calibrated detector showed the presence of two photopeaks in the 500 keV–1 MeV region. Since this laboratory uses millicurie quantities of caesium-134 for research purposes, our first reaction was that, in spite of careful control of this nuclide, the detector had been contaminated with caesium-134, probably by a very small quantity, with very good counting geometry.

The detector assembly was dismantled, cleaned and re-assembled, but these measures failed to reduce the high background count rate, indicating contamination of the lead shielding. This shield was constructed with 4 in. × 4 in. interlocking lead bricks to Atomic Energy Research Establishment specification 420, and had internal dimensions of 18 in. × 18 in. × 22 in. Each wall of the shield was separately exposed to the detector, and this showed the contamination to be uniformly distributed over the bricks. Surface washing of the bricks indicated that the contamination was homogeneous within the lead.

After careful calibration of the energy response of the detector, and a long count, photopeaks due to the γ-emissions of silver-110*m* were identified. The suppliers of the bricks were most co-operative, but claimed not to use silver-110*m* within their plant, and it was therefore assumed that contamination was present before the bulk lead reached the brick makers. We concluded that radioactive silver was possibly used by the mining company in a tracer method of investigating the efficiency of silver extraction processes.

Whatever the source of contamination, a man-made nuclide was introduced which affected subsequent use of the material. The quantities involved are in no way hazardous, being below the threshold sensitivity of normal health physics instruments, and, with a half-life of 270 days, will have little effect in this particular application after about 6 years' decay time.

When encountered in γ-spectrometry shields, such contamination is easily identified and removed (by replacement of the shield), but when it occurs in the shield of a single-channel analyser system, or other γ-sensitive detector, the contamination might not be identified and remedied, and could result in reduced sensitivity. Moreover, the cause of the slowly falling background count, due to the comparatively long half-life of silver-110*m*, would probably not be recognized, and could be wrongly attributed to other factors such as electronic instability, thermal drift, etc.

γ-Spectra demonstrating the difference between contaminated and uncontaminated shields are shown in Fig. 1, and it is hoped that this report will help other users (and manufacturers) of similar shields to avoid the delays and expense incurred as a result of such contamination.

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Rotation and the Inverse Square Law

CONSIDER a cloud-like distribution of matter, of volume v, and with average density ρ which is roughly uniform; and which rotates with angular velocity Ω. The angular velocity may vary with time. Let C be the origin of a frame of reference fixed in the distribution and P any point of the cloud.

The acceleration of P will contain among others the term $\Omega \times [\Omega \times \vec{CP}]$. Writing this term as $\mathbf{f}$ we find curl $\mathbf{f} = 0$ and div $\mathbf{f} = -2\Omega^2$. From these we obtain $\mathbf{f} = -\nabla\varphi$, $\nabla^2\varphi = +4\pi\lambda\rho$, where $2\pi\lambda\rho = \Omega^2$.

This is Poisson's equation for uniform density with $\mathbf{f}$ taking the role of the usual newtonian gravitational force. Hence, adopting the usual mathematical arguments for Poisson's equation in reverse, we have:

$$\mathrm{div}\left(\mathbf{f} + \int_v \frac{\gamma\rho\mathbf{r}}{r^3}\,d\tau\right) = 0$$

where $d\tau$ is a volume element of v, $\mathbf{r} = \vec{PQ}$ and Q is any point other than P.

Integrating

$$\mathbf{f} = -\int_v \frac{\gamma\rho\mathbf{r}}{r^3}\,d\tau + \mathrm{curl}\,\mathbf{H}$$

So the acceleration term $\mathbf{f}$ is equivalent to the distribution being composed of gravitating material according to the inverse-square law, as well as subject to a field $\mathbf{H}$. Hence we may look on the distribution as gravitating matter subject to a possible additional field $\mathbf{H}$, instead of having the contribution due to $\Omega \times [\Omega \times \vec{CP}]$ from the acceleration.

These results are capable of considerable extension.

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GEOPHYSICS

Convergence and Divergence in Gravity and Magnetic Interpretation

A RECENT communication by Prof. B. S. R. Rao and I. V. Radhakrishnamurty[1] creates a misleading impression (more particularly for the case of a sphere) that the depth of a body can be easily interpreted from the gravity (or magnetic) data by finding out the critical radius of the circle beyond which average radial gravity (or magnetic) value diverges. In actual practice this interpretation is not possible because for a body of finite dimensions (at any depth) the average radial value can never be infinite (this is obvious from physical reasons) however large a circle we may choose.

This apparent contradiction in theory and practice has occurred because the authors have ignored the restriction inherent in the expansion of the function $\bar{g}(r)$. The expansion for $\bar{g}(r)$ is permissible only in the circle of convergence and the representation is no more valid beyond that region. Since this series is an equivalent form of Taylor's series, the radius of convergence of this series for $\bar{g}(r)$ is also the distance from the centre of the circle to the closest singular point. In the case of the sphere cited this would mean that the expansion (and the interpretation based on that expansion) is incorrect for all values of $R > Z$. The ratio test clearly shows that the given series for the case of the sphere is absolutely convergent for all values of $R < Z$ (instead of $R < 0{\cdot}88\ Z$). It can also be added that the convergence/divergence of the series inside/outside this region of convergence is not a function of the number of terms chosen.

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[1] Rao, B. S. R., and Radhakrishnamurty, I. V., *Nature*, **206**, 179 (1965).

DR. NEGI'S criticism seems to have been based on a misreading of the purpose of our communication[1]. What we actually meant to convey was that the average radial gravity $\bar{g}(r)$, expressed in Taylor's expansion or in any of its allied forms, cannot always be accepted in view of its oscillatory character. Thus such representation should be made with some caution and only below a certain range of radius, depending on the depth of the body and the location of the point of measurement.

Secondly, Dr. Negi's argument that the expansion for $\bar{g}(r)$ is permissible only in the circle of convergence and that the radius of this circle should be Z and not $0{\cdot}88\ Z$ is clearly untenable. If we extend this argument it means that Taylor's expansion is valid only for its closest singular point, which would result in the divergence of the series for all positive values of r, which is not correct.

Dr. Negi has further pointed out that the convergence/divergence of the series inside/outside this region of convergence is not a function of the number of terms chosen. One can easily disprove this argument by adding terms, one by one, and finding out the value of $\bar{g}(r)$.

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[1] Rao, B. S. R., and Radhakrishnamurty, I. V., *Nature*, **206**, 179 (1965).

Equatorial Ionospheric Drifts

A NUMBER of authors[1-3] have shown that near the magnetic equator ionospheric drifts occur, in both the E and F regions, which are mainly westwards by day and mainly eastwards by night. Results for F region drifts at Ibadan have been given by Skinner, Lyon and Wright[1] for the International Geophysical Year (IGY) (1957–58). These show that for quiet days during equinox periods at sunspot maximum, the median velocity of drift reached a value of about 100 m/sec eastwards at midnight and a value of about 76 m/sec westwards at noon. The changes in direction occurred rather rapidly at about 0700 in the morning and at about 2000 in the evening.

A similar series of observations were made at Ibadan during the International Years of the Quiet Sun (IQSY) (1964–65), when solar activity was at a minimum. The results for October 1964 are shown in Fig. 1 in the form of a mass plot of the diurnal variation. The circles indicate median values for each hour, and the smooth curve estimates the median diurnal variation. The form of the variation is very similar to that obtained during the IGY, with mainly westward velocities by day and mainly eastward velocities by night, and with the change-overs occurring at very nearly the same times. The magnitudes of the drift velocities are, however, little more than half those observed in the IGY, being about 55 m/sec eastwards at midnight, and about 41 m/sec westwards at noon. A comparison of the two variations is shown in Fig. 2*a*. Very similar results were obtained during the World Geophysical Interval in March 1965, and a comparison of the variation for this period with the IGY variation is shown in Fig. 2*b*.

For simplicity only the smooth diurnal curves, fitted by visual estimation, are shown in Fig. 2. The uncertain-

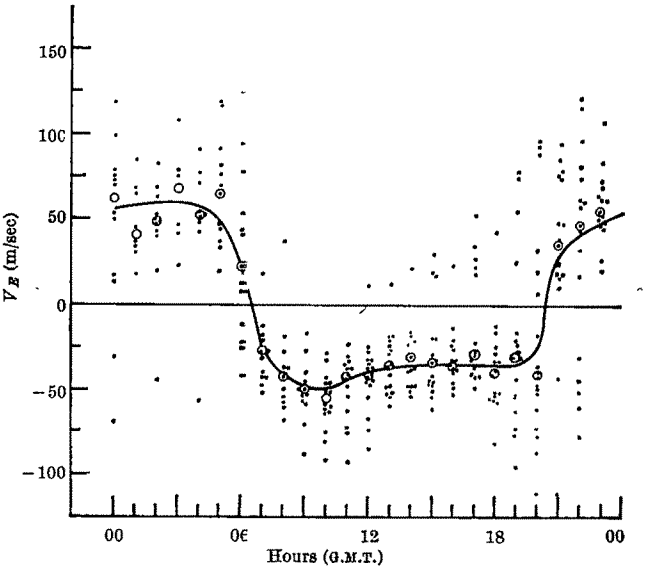


Fig. 1. Mass plot of V_E, the eastward component of F-region drift velocity observed at Ibadan during October 1964. Circles indicate hourly medians; the smooth curve is a visually estimated diurnal variation

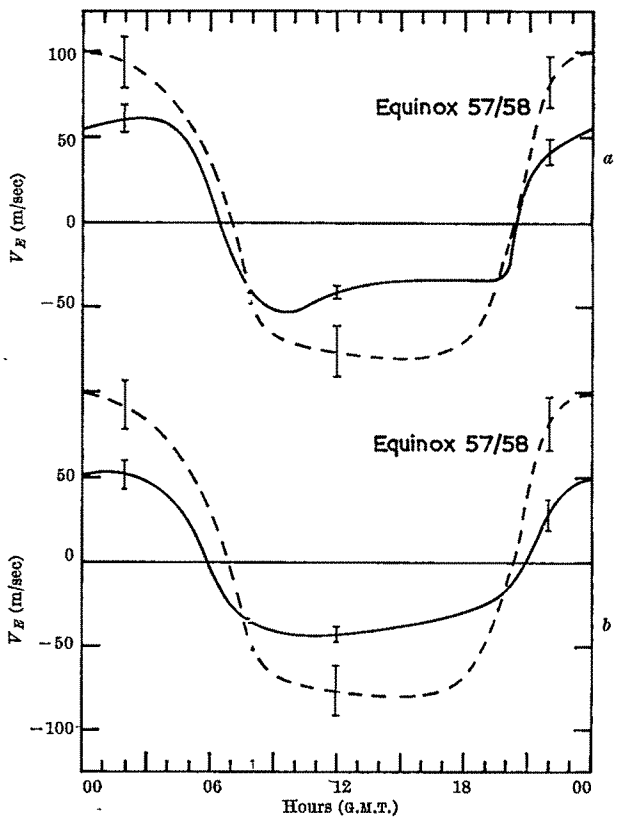


Fig. 2. Comparison of results for V_E obtained during the International Geophysical Year (broken lines) and the International Years of the Quiet Sun (continuous lines) at equinox periods. *a*, comparison for October 1964; *b*, comparison for March 1965. The error bars indicate the estimated uncertainty of values on the fitted curves shown

ties in these fitted curves are indicated by error bars, representing estimated standard errors. The large variability from day to day shown in Fig. 1 is believed to be mainly due to a real variability in the drifts rather than to measurement errors. For the IQSY results the standard error of a value on the fitted curve is estimated at ± 3 m/sec near noon, and at ± 8 m/sec near midnight. The uncertainty on the IGY curve is somewhat greater, and both near noon and midnight the standard error is estimated to be about ± 15 m/sec.

It thus appears that *F*-region drift velocities have decreased significantly and substantially from sunspot maximum to sunspot minimum: near noon, from about 75 ± 15 m/sec to about 41 ± 3 m/sec, and near midnight from about 100 ± 15 m/sec to about 55 ± 8 m/sec. The ratio of decrease is about 1·8 in both cases.

If the observed drifts are produced by electrodynamic forces in the manner suggested by Martyn[4], it is not obvious why such a change in drift velocities should occur. Although conductivities and electric currents vary with the solar cycle, tidal velocities and electric fields, and hence electrodynamic drift velocities, should, at least on a simple model, remain constant. From the present evidence it appears, however, that the *F*-region drift velocity varies almost as much as the daily range of the horizontal component of the Earth's magnetic field, ΔH. At Ibadan this decreased by a factor of about 2·1 between the solar maximum of the IGY and the solar minimum of the IQSY, which may be compared with the ratio of 1·8 for drift velocity. It is interesting also to compare this result with the evidence obtained by Osborne and Skinner[5] of a correlation between drift velocity and ΔH even on a day-to-day basis.

In the *E*-region we have found no evidence of any solar-cycle variation of drift velocity similar to that found in the *F*-region. Thus the drift velocity in the *E*-region near noon was 42 ± 3 m/sec in October 1964 and 39 ± 5 m/sec in March 1965. The IGY data are less numerous, but from eighteen values obtained between 10 and 14 h in equinox months at sunspot maximum the median value is 45 ± 10 m/sec. The median of seventeen values obtained between 10 and 14 h in winter months is similarly 51 ± 6 m/sec. It thus appears that if there is any change in *E*-region drift velocity with sunspot activity, it is considerably less than occurs in the *F*-region. This would seem to be consistent with the results reported by Rao and Rao[3].

It may be remarked in conclusion that the IGY data and the IQSY data were analysed by somewhat different methods. For the former the usual full correlation method was used, but for the more recent data a method originally suggested by Briggs and Spencer[6] has been used. Fuller details will be given elsewhere and here we shall only say that we have concluded from numerous test comparisons that there is no appreciable systematic difference between the two methods.

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Variation of the Ratio Intensity to Susceptibility in Red Sandstones

As the remanent intensity of magnetization, *I*, of rocks is dependent on the strength of the magnetic field in which it was acquired, an estimate of relative field strengths can be made by comparing the remanent intensities from different rock units which have supposedly acquired their magnetization in a comparable manner[1]. A statistical method that gives an effective mean for each unit would be expected to be capable of revealing broad changes in the strength of the palaeogeomagnetic field. It has been found that the distribution of remanent intensities generally seems to follow the log normal distribution. As the susceptibility, *K*, of red sandstones has been found to be proportional to the amount of iron oxide present[2] the ratio of intensity to susceptibility is partially compensated for variations in the amount and type of magnetic material present. This ratio should be more dependent on the field strength than would be the intensity alone. The geometric means of this ratio have been calculated for a collection of red sandstones obtained from the United States[3,4] mainly from flat lying sites. The results from eighty-two sampling sites in thirty formations are summarized in Fig. 1. It can be seen that the *I*/*K* ratio yields a smoother pattern with age than the *I* plot. It shows a palaeozoic low. The vertical lines give the standard deviation of the geometric site values for the given era.

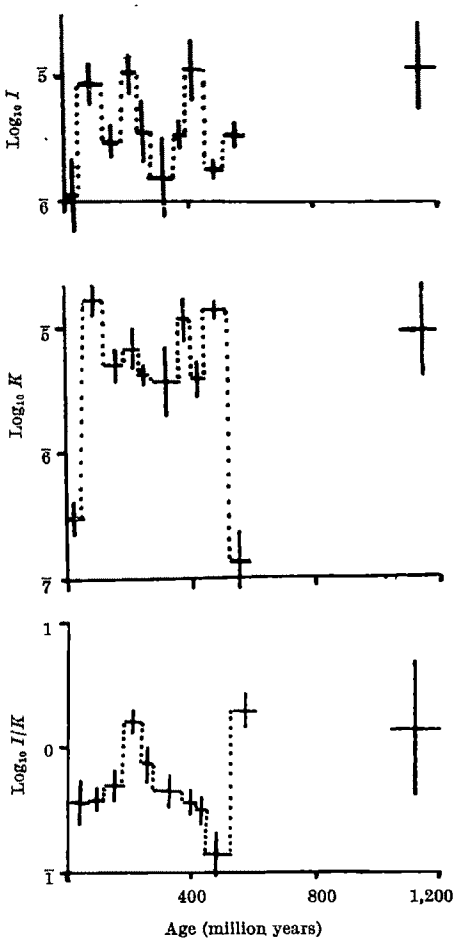


Fig. 1. Log_{10} plots of *I*, *K* and *I*/*K* against age of red sandstones

I thank Prof. S. K. Runcorn for access to his collection of red sandstones.

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Azimuthal Variation of U.S. Station Residuals

DEVIATIONS from the Jeffreys–Bullen tables of P times to North American stations in the distance range 32°–100° have been studied recently by Cleary and Hales[1]. The earthquakes on which the investigation was based fell into 3 groups: (1) from the Mid-Atlantic Ridge and Europe; (2) along the west side of South America; and (3) from Alaska to Formosa. The station residuals extracted from the data were observed to vary regionally, in the manner shown by the contours mapped in Fig. 1. After removing these systematic effects, it was found that the remaining residuals were azimuthally dependent for some stations. This is exemplified by the plots reproduced in Fig. 2; the stations concerned have also been mapped

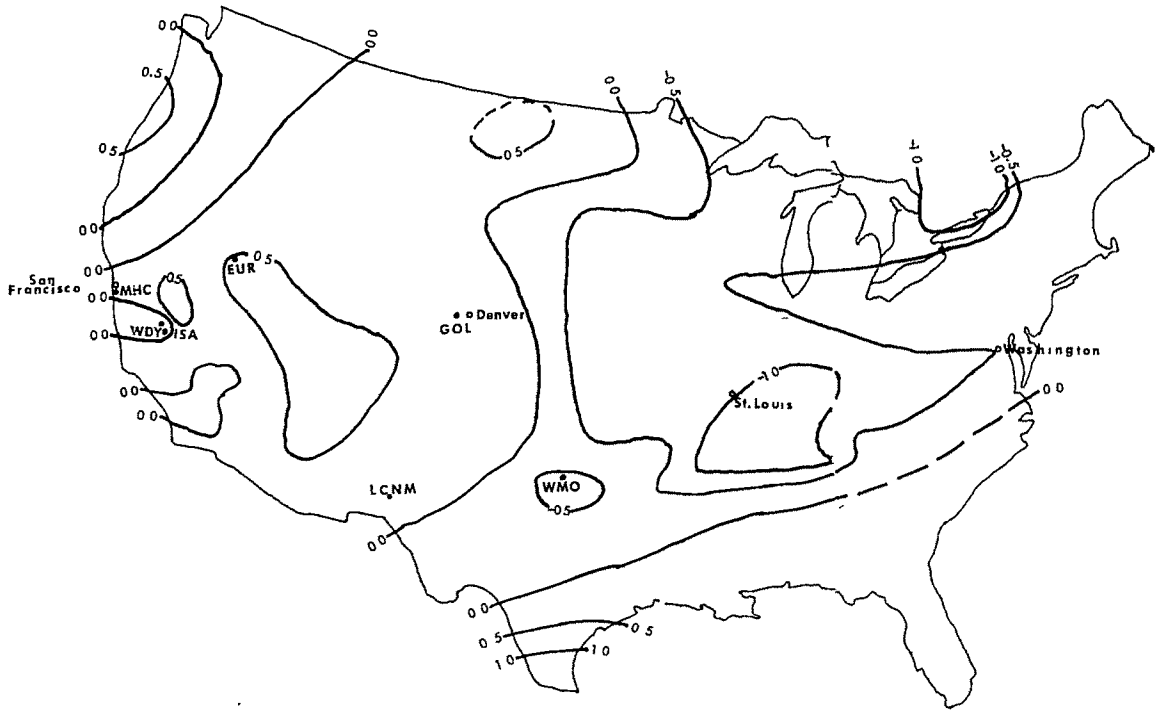


Fig. 1. Residuals for North American stations

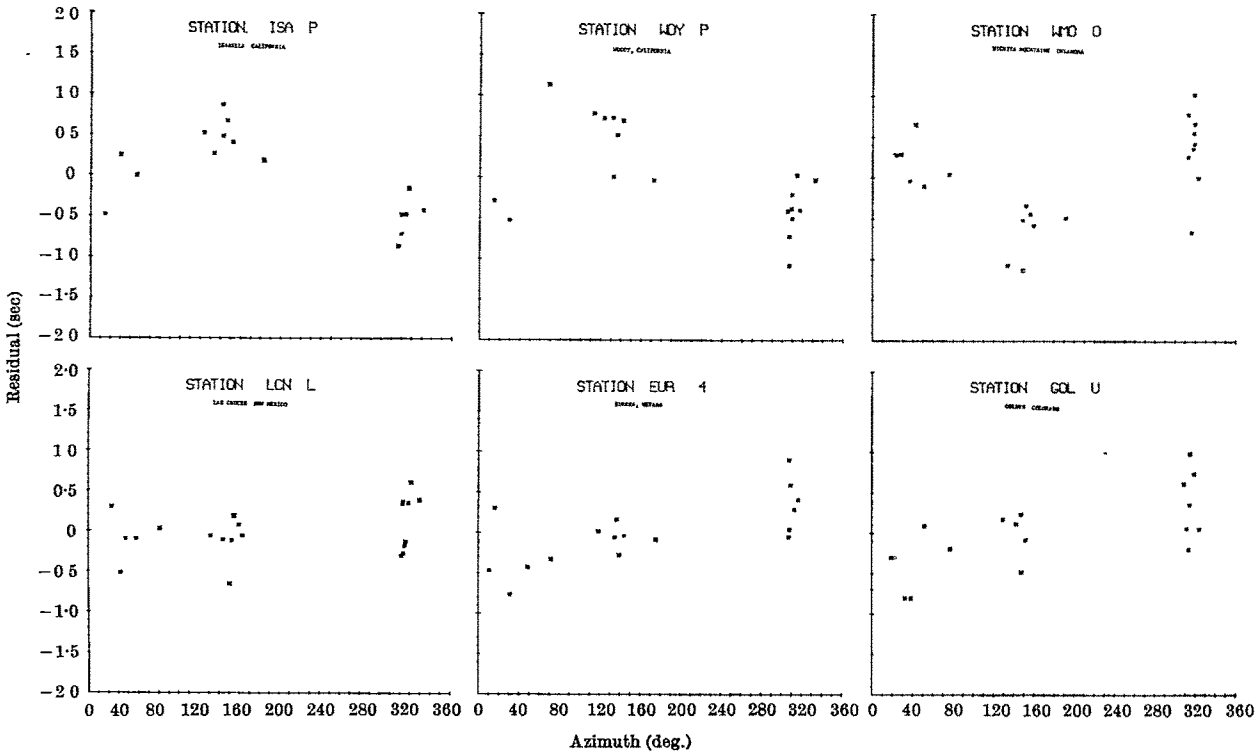


Fig. 2

in Fig. 1 (abbreviations used are those recommended by the U.S. Coast and Geodetic Survey). The residuals between azimuths of 0° and 80°, 100° and 200°, and 280° and 360° are from groups (1), (2) and (3), respectively.

The variation in azimuthal dependence from station to station precludes any possibility that the effect is due to errors in location of epicentres. See, for example, the marked contrast in the azimuthal effects at ISA and LCNM, even though the mean azimuths for each group are virtually the same at both stations.

Azimuthal variations appear to be most marked for stations situated in regions where the contours indicate a fairly rapid change in station residual (ISA, WDY, WMO), and weak or absent for stations in regions of gradual change (LCNM, EUR, GOL). The vectors of maximum azimuthal variation at the strongly affected stations are reasonably consistent with that to be expected from the disposition of the contours. Further investigations of this relationship should provide some indication of the depths at which station delays are introduced.

The work on which this letter is based was sponsored by the Air Force Cambridge Research Laboratories, O.A.R., under a contract for the Advanced Research Projects Agencies' Project VELA-Uniform.

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GEOCHEMISTRY

A Potassium–Argon Age for the Barwell Meteorite

A POTASSIUM–ARGON age has been determined for the olivine-hypersthene chondrite that fell at Barwell, Leicestershire, on December 24, 1965.

Determinations of the potassium content have been made at the British Museum (Natural History) and the Universities of Leicester and Oxford. The results are shown in Table 1, where the errors indicated are standard deviations of replicate analyses.

Table 1

Laboratory	Method	Potassium content (per cent)
British Museum (Natural History) (analysed by A. J. Easton)	Flame photometry using a buffering technique	0·10 ± 0·01
University of Leicester (analysed by M. L. W. Blackley)	Flame photometry using a buffering technique	0·11 ± 0·02
University of Oxford	Flame photometry using a buffering technique. (Triplicate analysis)	0 095 ± 0 002

A neutron activation analysis has also been carried out at the University of Oxford. This yielded a value of 0·096 ± 0·005 per cent potassium. These results are concordant among themselves. Potassium determinations by flame photometry at Cambridge, however, have given the following figures: First run, 0·163 ± 0·005 per cent potassium; second run, 0·162 ± 0·006 per cent potassium. This suggests either that the fragments analysed at Cambridge have been contaminated, or that there are very considerable variations in the potassium content of the Barwell meteorite, or that the fragments analysed in Oxford, Leicester and London have been leached.

It is clear that in any event the potassium content of the Barwell meteorite is rather larger than normal. It has been found that the potassium contents of chondrites (excepting carbonaceous chondrites) cluster quite closely around a value of 0·085 per cent potassium[1], although a few well-substantiated deviations have been noted; for example, the olivine-bronzite chondrite Beardsley which has a potassium content of about 0·1 per cent potassium[2]. It has been suggested that the departure of potassium determinations from the average value of 0·085 per cent potassium should be taken as a rough index of the reliability of the data[3]. Since it seems now, however, that significantly different values can occur, it might prove more profitable to re-examine deviant analyses, especially since analytical methods superior to those used by Edwards[1] or Edwards and Urey[4] are now available.

Argon analyses have been made at Oxford and Cambridge. At Oxford, the meteorite sample was fused with boric oxide flux at about 1,600° C for about 25 min. This produced complete fusion, as was proved by examining thin sections prepared from the fused mass. About 2·5 g of meteorite were used together with nearly the same weight of flux. The gas analysis was made with a Reynolds mass spectrometer. The gases evolved were purified by the usual techniques and measured with spike argon-38. The radiogenic argon-40 content of the meteorite was calculated by isotope dilution and found to be (6·20 ± 0·12) × 10^{-5} c.c. S.T.P./g. The error quoted here includes the uncertainty in the spike calibration. Contamination due to atmospheric argon-40 amounted to 6·5 per cent of the total argon-40 measured.

At Cambridge, the meteorite sample was heated by itself until fusion was complete. This was checked by reheating the sample: no more argon was evolved. After cleaning with a red-hot titanium sponge, the gases were measured with spike argon-38 in an Omegatron mass-spectrometer device. Two samples were run as a check, and gave values of 6·56 × 10^{-5} c.c. S.T.P./g and 6·58 × 10^{-5} c.c. S.T.P./g. There is a probable error of ± 1 per cent in these measurements due to uncertainty in the spike argon volume. The atmospheric contaminations amounted to 9·6 per cent and 10·2 per cent, respectively.

In calculating ages, the partial decay constants for potassium-40 were taken to be: $\lambda_\beta = 4{\cdot}72 \times 10^{-10}$ yr^{-1}; $\lambda_e = 0{\cdot}584 \times 10^{-10}$ yr^{-1}; potassium-40/potassium = 0·0119 atom per cent. With a value for the potassium content of 0·096 per cent, the Oxford results give an age for the meteorite of (4·19 ± 0·06) × 10^9 yr. With a potassium content of 0·1 per cent, the two Cambridge results give ages of (4·30 ± 0·05) × 10^9 yr and (4·31 ± 0·05) × 10^9 yr. Using the Cambridge potassium results instead, with a mean value of 0·163 per cent potassium, the two Cambridge argon determinations then give ages of 3·49 and 3·50 × 10^9 yr, respectively.

Several hypersthene chondrites have been found to give potassium–argon ages which are appreciably shorter than the usual chondrite range of 3·5–4·5 × 10^9 yr. These particular chondrites are also usually found to show signs of shock effects (that is, blackening, veining or brecciation)[5], which may well have caused argon loss. The Barwell meteorite shows no obvious signs of shock effects: the absence of such effects would appear to be supported by its normal age.

We are indebted to the British Museum (Natural History) for permission to quote potassium determinations made there.

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OCEANOGRAPHY

Mechanical Behaviour of Metals in Deep Ocean Environments

THE rapidly developing interest in deep ocean engineering makes it necessary to consider possible effects of deep ocean environments on the properties of engineering materials. The principal difference between a deep ocean environment and an environment just below the surface of an ocean is, of course, that in the former there exists a large hydrostatic pressure. It can easily be shown that if the depth from the surface, h, is measured in fathoms, the hydrostatic pressure, P, is given by:

$$P \simeq 3h \text{ lb./in.}^2 \quad (1)$$

This communication considers the changes in mechanical properties which might be expected to result from this hydrostatic pressure. We shall consider first reversible effects, that is, effects which should be observed only when a metal or alloy is stressed while submerged in a deep ocean, and, secondly, possible irreversible effects, that is, property changes which might be caused by deep submergence and persist after the metal or alloy has been removed from the deep ocean environment.

We shall restrict our discussion of reversible effects to metals and alloys which exhibit a ductile-brittle transition, that is, those the behaviour of which changes from ductile to completely brittle over a narrow temperature range. Examples are iron and most of the ferritic steels. For convenience, we shall focus our attention on the simplest possible case, the effect of a hydrostatic pressure on a transition occurring in a series of specimens subjected to uniaxial loading at a constant strain-rate over a range of temperatures embracing the transition. We define the transition temperature as the highest temperature at which completely brittle behaviour can be observed.

According to the present theories of the transition[1,2], the macroscopic yield stress and crack propagation stress are equal at the transition temperature. In the absence of a hydrostatic pressure, let the transition temperature in °K and the macroscopic yield stress at the transition temperature be T_0 and σ_0, respectively. For most materials, including all those such as iron and ferritic steels which have a body-centred cubic crystal structure, the yield stress, σ, and temperature, T, are related by the equation:

$$\sigma \simeq A + \frac{B}{T} \quad (2)$$

where A and B are constants characteristic of the particular material being considered. The parameters σ_0 and T_0 are therefore related by:

$$\sigma_0 = A + \frac{B}{T_0} \quad (3)$$

When a hydrostatic pressure P is applied, it is reasonable to expect that the macroscopic yield stress will be unaffected, since the glide and multiplication of dislocations are governed only by shear stresses. The applied tensile stress required to propagate the crack will, however, be raised in magnitude by P. Thus, a specimen tested at a temperature T_0 in the presence of the hydrostatic pressure P will not behave in a brittle manner. In order to obtain brittle behaviour, the temperature must be lowered to some value T_1 such that the resulting increase in σ is equal in magnitude to P. If this new value of σ is σ_1, we have:

$$\sigma_1 = A + \frac{B}{T_1} \quad (4)$$

$$\sigma_1 = \sigma_0 + P = \sigma_0 + 3h \quad (5)$$

Combining equations (3), (4) and (5) we obtain, putting $T_0T_1 = T_0^2$:

$$\Delta T \simeq \frac{3hT_0^2}{B} \quad (6)$$

where ΔT is the decrease in transition temperature, $T_0 - T_1$, due to the hydrostatic pressure.

Equation (6) predicts that the transition temperature should decrease linearly with increasing depth, and the insertion of typical values of B and a value of T_0 close to ambient temperature shows that for a deep ocean ($h \simeq$ 6,000 fathoms) ΔT should be several tens of degrees. No measurements of ΔT in an actual ocean environment have as yet been reported, but recently one set of laboratory measurements of the effect of hydrostatic pressures up to 20,000 lb./in.^{2} on the ductile-brittle transition temperature of molybdenum have been reported[3]. These showed that in a high strain-rate bend test the transition temperature decreased from about 50° C to 2° C as the pressure was increased from atmospheric to 20,000 lb./in.^{2} (see Table 1).

Table 1. EFFECT OF HYDROSTATIC PRESSURE ON THE DUCTILE–BRITTLE TRANSITION TEMPERATURE OF MOLYBDENUM (REF. 3)

Pressure (lb./in.^{2})	Transition temp.
15 (1 atm.)	50° C
10,000	25° C
20,000	2° C

Thus these experiments confirm the magnitude of the effect predicted in equation (6). They are also consistent within experimental area with the linear dependence of ΔT on h which this equation predicts. A second piece of experimental evidence which supports the existence of this effect in zinc has recently been published by Pugh[4]. He found that the application of a hydrostatic pressure of about 13,000 lb./in.^{2} changed the room temperature tensile behaviour of zinc from completely brittle to ductile. This can, as Pugh points out, be interpreted to mean that the transition temperature is lowered from above room temperature to below it by the hydrostatic pressure. Thus, the theoretical ideas expressed by equation (6), and these two pieces of supporting experimental evidence, leave little doubt that a hydrostatic pressure, such as exists in a deep ocean, can by itself drastically alter the mechanical properties of a metal or alloy.

It is more difficult to predict whether deep ocean submergence should produce irreversible effects on the mechanical properties of metals. Bullen *et al.*[5,6] have recently published the results of some laboratory experiments in which iron and chromium were subjected to pressures of up to 150,000 lb./in.^{2}. He found that the pressurization caused substantial irreversible increases in ductility and decreases in yield stress. At lower pressures comparable with those in deep oceans, few results were obtained, but they suggested that the effect was small or negligible for these pressures. A significant feature of the results of Bullen *et al.* on iron was the observation that the magnitude of the effects obtained were sensitive to the purity of the metal used. The larger effects found in impure iron were interpreted as in some way due to the generation of dislocations at the surfaces of the inclusions. Stresses are, of course, set up at an inclusion-matrix interface under a hydrostatic pressure because the inclusion and matrix have different bulk moduli. If this interpretation is correct, it seems reasonable to expect that the effects may be enhanced in engineering alloys which frequently contain high densities of inclusions. There therefore seems some chance that deep ocean submergence will be found to produce significant irreversible mechanical property changes in metals and alloys.

A programme of laboratory work to be carried out in collaboration with the Naval Applied Science Laboratory, Brooklyn, has recently been initiated to explore both reversible and irreversible changes in the mechanical properties of metals and alloys resulting from the applica-

tion of hydrostatic pressures of the magnitude found in deep oceans.

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CHEMISTRY

Complex Formation and Carcinogenicity of Aromatic Compounds

SEVERAL crystalline complexes of aromatic hydrocarbons and related compounds with 1,3,7,9-tetramethyluric acid (TMU) have been prepared[1,2]. These and similar complexes are of interest because of the possibility that *in vivo* complex formation between carcinogens and nucleic acids or their precursors may play a part in chemical carcinogenesis. This possibility has been explored also in investigations of the binding of aromatic hydrocarbons to nucleic acids[3,4].

Measurements of ultra-violet absorption spectra of solutions of the complexes revealed small shifts to longer wave-length but did not allow for conclusions about the nature of the complexes or their possible role in carcinogenesis[2].

In a recent report Pullman *et al.*[5] explored the possibility of other types of complex formation. These involve polarization and dispersion forces, rather than charge transfer earlier emphasized by them[6]. Work at present under way in this laboratory and reported in part[7,8] bears directly on this problem.

A survey of earlier work[9] left no doubt that the purine-aromatic complexes are largely or entirely dissociated in solution. Since the *in vivo* interactions may occur as solid-solid interactions between nucleic acid and carcinogen, rather than in solution, it was important to examine these complexes in the solid state. The fluorescence spectra of a number of aromatic and heterocyclic compounds were measured in a potassium bromide matrix in which they are dispersed as solid solutions or as crystalline solids depending on concentration[10]. The fluorescence excitation and emission spectra of the TMU complexes of three aromatic hydrocarbons[7], and more recently of heterocyclics and substituted aromatics, were also examined. All complexes examined were analytically pure materials; they vary, however, in their TMU content; the pertinent spectral data of these complexes are summarized in Table 1.

The first five complexes listed in Table 1 show shifts to shorter wave-length in comparison with the crystal fluorescence of the parent compounds and to longer wave-length with respect to their solution spectra. Noticeably, α-naphthol and β-naphthylamine do not show any fluorescence wave-length shifts when complexed to TMU and examined in the crystalline state. With the exception of pyrene[7] these fluorescence shifts are not observed, even in concentrated solutions.

The fluorescence emission maxima of the crystalline solids are shifted without comparable changes in excitation maxima; this means that the mirror-image symmetry of excitation and emission, observed for charge transfer complexes[11], is not maintained.

Although solubilization of aromatic hydrocarbons in water increases with increasing electron-donor properties

Table 1. FLUORESCENCE SPECTRA OF TMU COMPLEXES

		Excitation (mμ)*	Emission (mμ)*
Pyrene	(1)	305, 320, *335*	372, *385*, 392
	(2)	325, *366*	*463*, 485
(1 : 1)†	(3)	*373*	*430*
Benzo(*a*)pyrene	(1)	283, 295, 335, 363, *385*	*405*, 417, *428*, 457, 485
	(2)	*380*	*480*, 505
(1 . 2)	(3)	*367*, 380	*447*, 480
Coronene	(1)	*350*, 380, 400, 405	430, 436, *448*, *454*, 475, 485
	(2)	*375*	477, *505*
(1 : 2)	(3)‡	*375*	*490*
Dibenz(*a,j*)-acridine	(1)	*300*	*395*, 400, *416*, 440, 465
	(2)	*400*	*500*§
(1 . 3)	(3)	*417*	*450*, *475* (*doublet*)
Benzidine	(1)	*315*	*385*
	(2)	*350*	*385*
(1 · 2)	(3)	*350*	*370*
α-Naphthol	(1)	*292*, 322	*360*
	(2)	*307*, 322	*355 to 375*
(1 : 1)	(3)	*307*	*355 to 375*
β-Naphthylamine	(1)	*292*, 342	*380*
	(2)	*372*	*397*
(1 1)	(3)‡	*372*	*397*

(1) Dilute solution spectra: pyrene, benzo(*a*)pyrene and dibenz(*a,j*)-acridine in *cyclo*hexane, benzidine, coronene and β-naphthylamine in benzene, α-naphthol in methyl alcohol.
(2) Potassium bromide pellet spectra of crystalline compounds.
(3) Potassium bromide pellet spectra of crystalline complexes.
* Main peaks are italicized; all crystal fluorescence spectra were run with appropriate Corning glass filters.
† Ratio of compound to TMU in complex.
‡ The TMU complexes of these materials show pronounced increases of fluorescence intensities compared with the parent compounds.
§ Weak shoulders at shorter wave-length due to solid solution spectra.

of the purine[1] there is no correlation between the electron affinity of the hydrocarbons and the extent to which they are solubilized in water by TMU (ref. 7). These observations lead to the conclusion that the donor-acceptor complexes are not involved.

Liquori *et al.*[12] suggested that polarization bonding may be involved in these complexes. This type of bonding involves a polar component (TMU) and a component that can be polarized. The present findings are in agreement with this type of bonding and some of these conclusions were described in our earlier report[7]. Pullman's recent contribution on this problem[5] is also consistent with this type of bonding.

Since carcinogenic and non-carcinogenic hydrocarbons form complexes of this type and, presumably, interact with nucleic acids[3] the significance of this type of complex formation in chemical carcinogenesis remains to be determined. However, these complexes should be explored further because carcinogenic metabolites of aromatic hydrocarbons have not as yet been isolated.

Several investigators[13–15] have pointed to the formation of coloured charge transfer complexes of aromatic carcinogens with electron acceptors such as 1,3,5-trinitrobenzene as indicative of a relationship between carcinogenicity and π-complex formation. But, if the ionization potential or the energy of the highest filled molecular orbital of the hydrocarbon is plotted against wave-length of the charge transfer band, a straight line is obtained; this has been observed for many hydrocarbons with a series of acceptors[11,16,17].

The correlation drawn by these authors[13–15] is, therefore, another way of implying that there is a relationship between carcinogenicity and the ionization potential or the energy of the highest filled molecular orbital of the aromatic carcinogen.

Inspection of the values for the energy of the highest filled molecular orbitals of a number of aromatic hydrocarbons[18] reveals that the carcinogenic hydrocarbons generally have energy values in the range 0·37–0·52; some carcinogenic hydrocarbons fall outside this range and non-carcinogens fall within this range.

It is clear then that π-complex formation, *per se*, does not correlate directly with carcinogenicity. Other factors, such as molecular size and reactivity at particular sites of the molecule, undoubtedly are also important.

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Hot-wire Ignition of *Cyclo*propane/Oxygen Mixtures

EXPLOSION risks with certain anaesthetic gas mixtures can be increased in an operating theatre containing a pressurized oxygen atmosphere (hyperbaric oxygen operating theatre). The use of electrically heated wires for cauterizing raises particular problems as the hot wire can act as a continuous source of ignition.

Although the characteristics of ignition by a hot wire in a *cyclo*propane/oxygen system are complex, it is possible to predict with some confidence the upper limit of wire temperature that can be used.

The basis of the theory of thermal ignition is due to Van't Hoff[1]. According to this theory the condition for thermal ignition of an explosive mixture is the impossibility of thermal equilibrium between the reacting system and surrounding medium being maintained. The qualitative formulation of this condition, as contact between the curve of heat supply and that of heat removal, was first given by Le Chatelier[2].

For thermal ignition by a hot wire the heat supplied by chemical reaction (in a zone radius r_2 surrounding wire radius r_1) can be shown to be:

$$q = A' \exp -\left(\frac{E/R(T_1 + T_2)}{2}\right) \pi (r_2 - r_1) L.Q. \qquad (1)$$

where, taking the particular case of *cyclo*propane/oxygen, A' is the frequency factor[3], E is the Arrhenius activation energy[3], T_1, T_2, the absolute temperatures at r_1 and r_2, r_1, the radius of the wire, r_2, the radius of wire + quenching radius for the *cyclo*propane/oxygen system[4], L, the wire length, and Q, the heat of reaction for *cyclo*propane/oxygen[5].

The mechanism of heat removal for the zone is a complex combination of conduction and convection. For the purpose of these calculations the heat removal is considered to be purely by conduction. This is permissible as ignition occurs before convection currents are set up.

$$q = \frac{km 2\pi L (t_1 - t_2)}{Ln \left(\frac{r_2}{r_1}\right)} \qquad (2)$$

where km is the thermal conductivity of the system[6], and t_1 and t_2 are the temperatures at r_1 and r_2.

Solving equations (1) and (2) graphically gives T_2 for a fixed T_1; T_2 can be obtained from a transient heat transfer solution for the system. Gemant[7] gives:

$$\theta = 1 + \frac{2}{\pi} \int_0^\infty e^{-(\tau\nu^2)} \cdot \frac{J_0(\rho\nu) N_0(\nu) - J_0(\nu) N_0(\rho\nu)}{J_0^2(\nu) + N_0^2(\nu)} \cdot \frac{d\nu}{\nu} \qquad (3)$$

where $\theta = T/T_0$ (T is the temperature difference between any point in the medium and the surroundings, and T_0, the temperature difference between the wire and the surroundings), and ν, the dimensionless variable extending from zero to infinity; $K = \frac{\tau a^2}{r}$, the thermal diffusivity of the medium; $\rho = r/a$ (dimensionless distance ratio); r, distance from centre of wire to point at temperature T; a, the wire radius; J_0, the Bessel function of first kind (zero order) and N_0, the Bessel function of second kind (zero order).

Fig. 1 shows a graphical solution of equations (1) and (2) for a stoichiometric *cyclo*propane/oxygen mixture at 3 atm. using a wire of diameter 1 mm. The solutions of equations (1) and (2) for wire diameters 1, 0·5 and 0·25 mm at 1 and 3 atm. are given in Table 1. It can be seen from Table 1 that for a fixed T_1, T_2 is independent of wire diameter. This enables a plot of log (T_1) versus log $(T_1 - T_2)$ (Fig. 2) to be made. This holds for any wire diameter and predicts for any T_1 the temperature at the quenching diameter required for ignition. The solution of equation (3) for wire diameters 1, 0·5 and 0·25 mm allowing a maximum[8] of 5 sec for an explosion to be initiated are given in Table 2. The mutual solutions of equations (1), (2) and (3) which enable the maximum permissible wire temperature for no ignition to be predicted are given in Table 3.

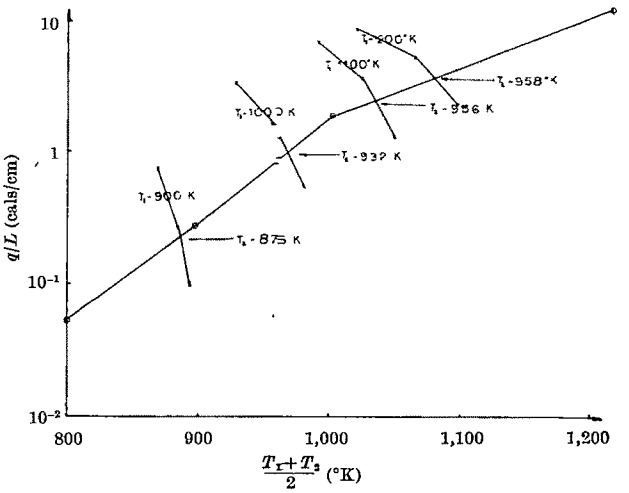


Fig. 1. q/L versus $\frac{T_1+T_2}{2}$ for stoichiometric *cyclo*propane/oxygen mixture at 3 atm. Diameter of wire, 1 mm

No reports are available for a direct comparison of these theoretically derived wire temperatures. However, Jones[9] has reported ignition temperatures of 450°–500° C for *cyclo*propane/oxygen mixtures in an evacuated bulb maintained at a predetermined temperature. These ignition conditions are similar to those that would be obtained using a thick heated wire ($\theta = 0{\cdot}99$). In the temperature range considered this corresponds to $T_1 - T_2 = 3°–5°$ C, which from equation (4) gives the wire temperature as 450°–500° C.

The experimental ignition temperatures obtained for the evacuated flask and the values predicted for a thick wire are in reasonable agreement. This suggests that the

Table 1

Pressure atm	d_1 (mm)	d_2 (mm)	T_1 (° K)	T_2 (° K)
1	1	1·3	1,200	960
	0·5	0·8		960
	0·25	0 55		952
1	1	1·3	1,100	944
	0·5	0·8		950
	0·25	0·55		948
1	1	1·3	1,000	928
	0·5	0·8		924
	0·25	0·55		928
1	1	1·3	900	868
	0·5	0·8		866
	0 25	0·55		876
3	1	1·1	1,200	958
	0·5	0·6		965
	0·25	0·35		965
3	1	1·1	1,100	956
	0·5	0·6		952
	0·25	0·35		952
3	1	1·1	1,000	932
	0·5	0·6		930
	0·25	0·35		930
3	1	1·1	900	875
	0·5	0·6		875
	0·25	0·35		875

Table 2

Pressure atm.	1	1	1	3	3	3
ϱ	1·3	1·6	2·2	1·1	1·2	1·4
ϱ_0	0·90	0·87	0·86	0·97	0·95	0 94

Table 3

Pressure atm.	1	1	1	3	3	3
d_2 (mm)	1	0·5	0·25	1	0·5	0·25
Wire ignition temperature (°K)	1,025	1,085	1,100	920	955	980

assumptions made in the theoretical derivations are reasonable.

This technique can be used to calculate the maximum safe temperatures for electrically heated wires in explosive atmospheres at pressures up to 3 atm.

The use of spark discharges in similar explosive atmospheres would have to be treated by a different analysis; however, in general, the energy dissipated in the type of sparks used in surgical practice is high enough to render the technique dangerous in oxygen atmospheres containing anaesthetic gases such as *cyclo*propane.

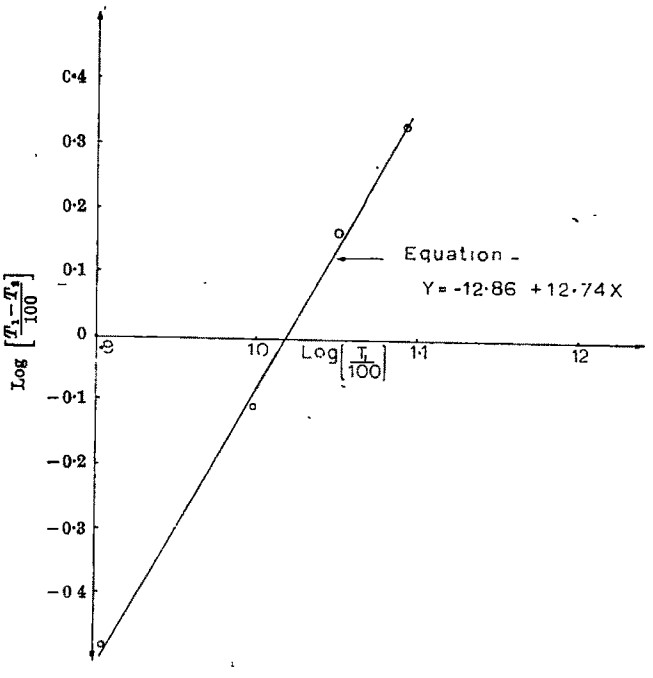


Fig. 2. Log $\left[\frac{T_1-T_2}{100}\right]$ versus log $\left[\frac{T_1}{100}\right]$ for stoichiometric *cyclo*propane/oxygen mixture at 1 atm

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Absorption Spectra of Cadmium and Zinc Atoms trapped in Noble Gas Matrices

THE absorption spectra of cadmium and zinc atoms trapped in argon, krypton and xenon matrices at low temperatures have been observed in the region λλ 1600–3500 Å. Spectra were recorded for various matrix/impurity (M/A) ratios, and at several temperatures. This communication reports our preliminary results.

The experimental procedure used for the preparation of inert-gas films doped with metallic impurities was similar to that described elsewhere[1–3]. Metal atoms were vaporized from a stainless steel oven and allowed to mix with a beam of the noble gas at the liquid helium-cooled deposition window. This window was of lithium fluoride, calcium fluoride, or quartz. No difference was noticed in the absorption spectra when the samples were deposited on different substrates. The matrix gases were British Oxygen Company spectroscopically pure grade, while the cadmium and zinc metals were 99·999 per cent pure. Spectra were recorded photographically with Ilford $Q2$ plates on a 1 m normal incidence vacuum spectrograph having a dispersion of 16·7 Å/mm in the first order.

The general features of the absorption spectra are: (1) A strong triplet within a few per cent of the free-atom $^1S_0$–$^1P^o_1$ transition energy; (2) one or two sharp doublets to the short wave-length side of the triplet, and of comparable intensity to the triplet; (3) diffuse broad bands to the red of the triplet; (3) weak, diffuse single absorptions to either side of the triplet.

If one correlates the triplet absorption with the $^1S_0$–$^1P^o_1$ transition of the free atomic impurity, the shift of this transition is to higher energy in argon and krypton, and to lower energy in xenon. This feature broadens quickly as the impurity concentration is increased. For a 1 per cent solution of cadmium in argon the 'triplet' absorption is approximately 200 Å wide. It is evident that the rapid increase in width with increasing impurity concentration is due to trapping of the foreign atoms at a multiplicity of sites. As a film containing a large concentration of cadmium or zinc atoms is allowed to warm up, this feature sharpens considerably.

The doublet absorptions were also found to broaden as the impurity concentration was increased, but to a lesser extent, and doublet structure could generally be resolved. In heavily doped samples, a sharpening of these features occurred as the temperature increased.

The broad absorption bands to the red of the $^1S_0$–$^1P^o_1$ transition appeared at medium and high concentrations of impurities. In the spectra of the systems cadmium–argon and cadmium–krypton one of these bands shows vibrational structure. These bands are probably due to weakly bound molecules formed between atoms trapped at nearby sites.

The absorption features were observed to shift to longer wave-lengths as the temperature of the sample increased.

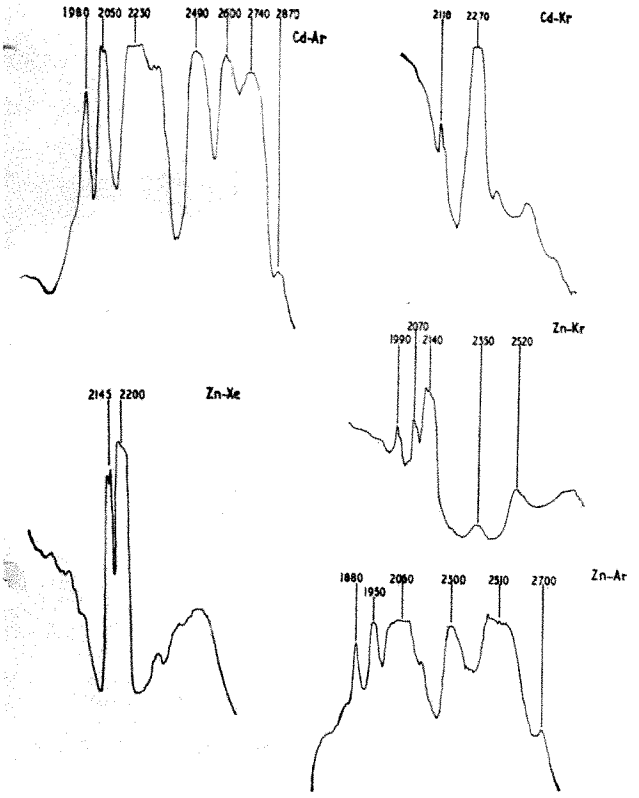


Fig. 1. Microdensitometer traces of the absorption spectra of zinc–argon ($M/A \simeq 100$), zinc–krypton ($M/A \simeq 300$), zinc–xenon ($M/A \simeq 200$), cadmium–argon ($M/A \simeq 100$) and cadmium–krypton ($M/A \simeq 1{,}000$) recorded at 4·2° K. Wave-lengths are in Å

The magnitude of this shift was similar to that reported for other systems[4–6]. At low impurity concentrations, when triplet structure was resolved, the components were observed to broaden considerably with increasing temperature.

Typical absorption spectra recorded at 4·2° K for the systems zinc–argon, zinc–krypton, zinc–xenon, cadmium–argon, and cadmium–krypton are shown in Fig. 1. A fuller description and interpretation of these results will be presented in a forthcoming publication.

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A Simple Micro-cuvette for Use in Standard Spectrophotometers

This apparatus was designed to enable optical density measurements to be made on very small volumes, using a standard spectrophotometer without further modification to the cell housing. A few small cuvettes are available commercially, but in these the advantages of small capacity are outweighed by short light paths. Several micro-cuvettes have been reported[1–3] but they are difficult to construct and require special techniques in handling[4]. The present apparatus is relatively simple in construction and use.

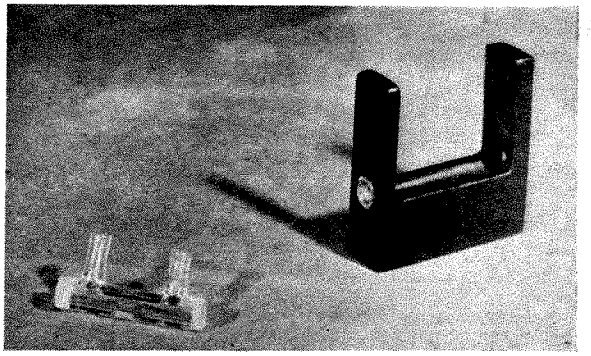


Fig. 1. Micro-cuvette and holder

The cuvette (Fig. 1) consists of a piece of thick-walled glass tubing of 3-mm bore, the ends of which are closed by disks of optically plane glass. These are cemented to the ends with 'Araldite' to avoid heat stresses or distortion which might be produced if they were fused. Two small side tubes are fitted, one at each end, and the cuvette is filled by means of a fine-bore Pasteur pipette placed inside one tube, the displaced air bubbling out of the other. After filling, minute air bubbles are dislodged by tilting towards each end in turn and gently tapping the sides.

A special holder (Fig. 1) has been designed for this cuvette for use with the Unicam '*S.P.* 500' spectrophotometer. This is made from a block of 'Darvic' (Imperial Chemical Industries, Ltd.) $1{\cdot}3 \times 4{\cdot}2 \times 4{\cdot}5$ cm which fits firmly inside the cuvette holder of the instrument. A 0·272 in. hole is bored lengthwise through the 'Darvic' block, the upper central portion of which is cut away leaving a semicircular trough in which the cuvette is placed. The remaining ends of the block help to keep it rigid in the metal cuvette holder and also serve as handles for inserting and removing the cuvette. The holes at either end of the trough are closed by brass plugs pierced by 0·086 in. holes, which are slightly smaller than the bore of the glass tube to eliminate stray light which might be refracted by the glass walls.

The cuvette has a light path of 3 cm and a total capacity of less than 0·3 ml. The universal cell housing of the instrument is used with the 12×2 mm shutter and cylindrical lens provided. Before readings are taken the cuvettes are allowed to equilibrate in the spectrophotometer for 10–20 min since small changes in temperature have a marked effect on the readings. It is essential for the light beam to pass accurately through the centre of the cuvettes so the spectrophotometer must be very carefully aligned. The carriage must be rigid in each of its set positions, and multiple readings should be taken to ensure it comes to rest in exactly the same position each time. It is necessary to match the cuvettes beforehand when filled with pure solvent and to subtract the small blank readings before calculation. Once matched, each cuvette should always be used the same way round in the same holder.

To test the reproducibility of readings in these cuvettes, two alcoholic solutions of gentian violet were measured in turn in two cuvettes. They were emptied and refilled twice and each time triplicate readings were taken. The results are shown in Table 1. Analysis of these data shows

Table 1. Optical Densities of Gentian Violet Solutions ($E \times 10^3$)

	Dilute solution		Concentrated solution	
	Cuvette 2	Cuvette 3	Cuvette 2	Cuvette 3
Read (1)	170, 172, 173	171, 172, 172	722, 722, 720	701, 701, 701
Refill (2)	174, 173, 174	174, 174, 175	722, 722, 727	721, 721, 721
Refill (3)	177, 175, 178	175, 174, 175	717, 722, 722	711, 711, 711
	Mean = 174 ± 2·0 *S.D.* Coefficient of variation = 1·2 per cent.		Mean = 716 ± 8·3 *S.D.* Coefficient of variation = 1·2 per cent.	

a coefficient of variation of 1·2 per cent for both a dilute and a concentrated solution.

This work was supported by a grant from the British Empire Cancer Campaign. I thank Mr. G. Marshall for skilful assistance in making the plastic holders and Mr. E. Roberts for the construction of the cuvettes.

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Electrodialysing with a Non-polarizing Membrane

IN the electrodialysis process for demineralizing brackish water, the feed is passed through parallel compartments separated by cation- and anion-exchange membranes arranged alternately. A direct current passed at right-angles to the long, narrow compartments impels the cations and anions to pass through the appropriate, selective membranes, so that alternate compartments become either depleted or concentrated in dissolved salts.

It has been found that when a critical current density is exceeded, the transport of ions to the membrane surface is insufficient to maintain the current, and hydrogen and hydroxyl ions pass through the membrane instead, at a higher voltage[1]. The excess energy is dissipated by the splitting of water molecules. Moreover, the *p*H change caused by hydroxyl ions passing through the anion-exchange membranes induces scale to form in the concentrate compartments, further increasing the electrical resistance and eventually blocking the membranes. In practice, therefore, the current density is maintained at a value of only about 50 per cent of the theoretically critical one. Since the output from an installation is proportional to the current density, this is a serious limitation.

During a recent investigation[2], a series of commercial ion-exchange membranes were tested in the following cell:

Silver/silver chloride | 0·01 N sodium chloride | Membrane | 0·01 N sodium chloride | Silver/silver chloride

Each membrane was clamped horizontally and the current was so arranged that the flow of counter-ions was upward, minimizing convection. The change of voltage with time was noted for constant current.

Fig. 1 is a chronopotentiograph of 'Selemion *DMT*', a weak-base anion-exchange membrane manufactured for dialysis purposes[3]. When the solution layer immediately below the membrane became depleted of salt, the resistance rose and hydroxyl ions began to carry an increasing proportion of the current upwards, towards the cathode. Above *p*H 8–9, however, there was practically no dissociation of the weakly basic groups and the membrane became non-selective. Electrolyte replenished the depleted membrane-solution interface and permeated the membrane, the current being transported by both sodium and chloride ions. The electrical resistance fell accordingly, as did the *p*H, the functional groups increased their dissociation and the cycle continued. Eventually a steady-state would have been reached.

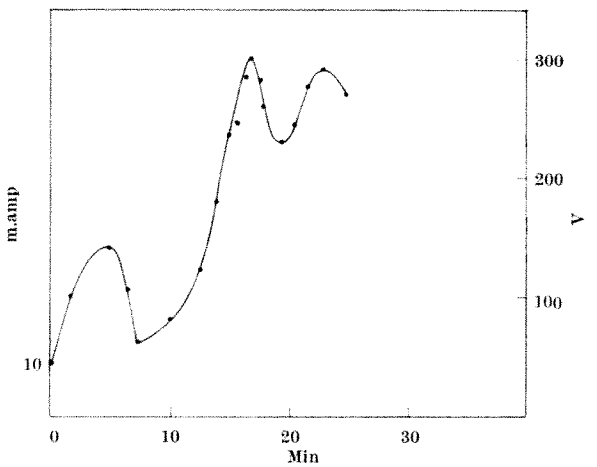


Fig. 1. Voltage-time curve for 'Selemion *DMT*'

Suppose such a membrane were used in electrodialysis. An increase in the current density above the critical value would not cause scale and stoppage of the process; instead, the current efficiency would simply decline locally until operating conditions were corrected. This could permit the operating current density to be raised much closer to the theoretically critical value. Only trials in a multi-membrane stack could test the validity of this approach.

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Combined Ion Exchange–Solvent Extraction: a New Dimension in Inorganic Separation Chemistry

MOST of the methods at present used for separation of inorganic ions by column chromatography are either based on cation and anion exchange or on partitition chromatographic procedures using cellulose, silica gel, 'Kel-*F*' and various other inert materials as supports for a stationary phase, mostly an aqueous solution, or, as with the reversed-phase techniques, with an organic phase which either acts as an extractant or as a cation or anion exchanger. Common to these methods is the fact that only one mechanism (either ion exchange or solvent extraction) is the decisive factor for effecting the separation. This is especially so in relatively strong mineral acid solutions. Seen from this point of view, these separation techniques are in practically all cases only one-dimensional, that is to say, only one of these principles can be utilized.

Obviously a two-dimensional separation system can be obtained by combining the ion exchange principle with liquid–liquid extraction. This can be achieved by using a solid cation exchanger as the support and a partly non-aqueous system as the mobile phase acting as an extractant for certain metal ions. Consequently, separations which can be achieved under such conditions may be termed "combined ion exchange-solvent extraction" separations (CIESE) because both the principles of ion exchange and solvent extraction are operative simultaneously. The following two separations of large amounts of iron from cobalt and nickel and of uranium from other elements are examples of this CIESE principle.

In the first case, the separation of iron(III) is effected on a column of a 'Dowex 50' cation exchanger using 80 per cent (tetrahydrofuran) (THF)–20 per cent 3 N hydrochloric acid as the eluent. Under these conditions, iron (III) (distribution coefficient $K_d \simeq 1$) is removed by washing the resin with this mixture and cobalt is then eluted with 90 per cent THF–10 per cent 6 N hydrochloric acid (v/v) leaving nickel adsorbed on the column. The nickel can finally be eluted with pure aqueous 6 N hydrochloric acid. This separation cannot be achieved on the same resin using pure aqueous 0·6 N hydrochloric acid (same overall acidity as in the THF–hydrochloric acid mixtures) because the K_d-values are 180 (iron), 45 (cobalt),

and 45 (nickel). This is also impossible using the conventional extraction method for iron (III), that is, extraction from 6 N hydrochloric acid by means of ethers, ketones or esters, because this only removes the iron (III) from the aqueous phase leaving the cobalt and nickel unseparated.

In the second case, the separation of uranyl nitrate from practically all other elements is effected on the same resin using 90 per cent THF–10 per cent 6 N nitric acid (v/v) as eluent for the uranium (K_d value, 40). Under these conditions, the K_d values of practically all other elements including di-, tri- and higher valent ions are either $> 10^3$ or ~ 1 so that a very effective separation is obtained. This separation cannot be achieved if a pure aqueous solution in nitric acid of the same acidity is used. Following elution of uranium with the above THF–nitric acid mixture, fractionation of the adsorbed elements can be achieved using the THF–hydrochloric acid eluents as in the separation of iron (III), cobalt and nickel. A fractionation of this kind is not achieved by the common extraction methods for uranyl nitrate with ethers, esters, or ketones from aqueous nitric acid solutions containing nitrates as salting agents.

These two examples of the application of the CIESE principle demonstrate that it is superior both to common ion exchange in pure aqueous solution and to the conventional liquid–liquid extraction method for the removal of iron (III) chloride and uranyl nitrate.

The main advantages of the CIESE principle over the one-dimensional separation principle (conventional column chromatography and solvent extraction) are:

(1) After removal of one or more components as with liquid–liquid extraction a further fractionation of other elements on the solid exchange material is possible.

(2) No salting agents or high concentrations of strong acids are necessary so that subsequent separations can readily be achieved without interference by these agents.

(3) The main constituent which is preferentially eluted (in our case iron and uranium) may be present in gram quantities without affecting the separations.

As a result of these advantages, the CIESE principle is of great use, especially when trace or minor constituents have to be separated from the bulk of the sample and further fractionation of these minor constituents is necessary. The aforementioned two examples can thus be used for the analysis of ferrous materials, such as steel and iron meteorites, and for the separation and preparation of highly pure uranyl nitrate.

To explain this CIESE principle it can be assumed that THF reacts with the mineral acid present to form an oxonium salt which acts as a liquid anion exchanger and thus reacts with the anionic complexes of the metal ions present. Consequently this liquid anion exchanger competes with the solid cation exchanger for the metal ions and according to the experimental conditions either the solid or liquid exchanger retains them preferentially.

The effect of the liquid exchanger, that is, the 'extraction' effect, was found to increase both with increasing THF and mineral acid concentration. Similar effects can be observed in ketone and ether–alcohol media (such as methyl glycol) containing hydrochloric acid but not when these contain aliphatic alcohols.

The CIESE principle can probably also be used for many separation purposes other than those already mentioned by suitable choice of the liquid organic component, complexing acid and solid exchange material.

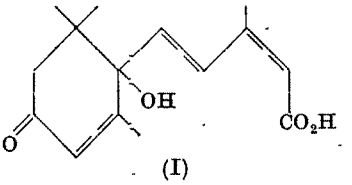
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BIOCHEMISTRY

Identification and Estimation of (+)-Abscisin II ('Dormin') in Plant Extracts by Spectropolarimetry

ABSCISIN II is a recently discovered[1,2] plant hormone which appears to inhibit the action of auxins, gibberellins and kinins[3]. Its structure[4] (I) contains one centre of asymmetry, and a comparison of its properties with those of synthetic[5] (±)-abscisin II suggested that the natural substance is a single enantiomorph. We have now measured the optical rotatory dispersion of abscisin II from sycamore leaves[2], and find that the hormone from this source is dextro-rotatory and exhibits a very intense positive Cotton effect with extrema at 287 mμ ([α] + 24,000°) and 245 mμ ([α] −69,000°):



(I)

All measurements of optical rotatory dispersion were made in acidified methanolic or ethanolic solutions (c = 1–50 μg/ml., l = 1 cm). Although the ultra-violet absorption spectrum of abscisin II is sensitive[2] to pH, it was found that λ_{max} and ε_{max} reach constant values (262 mμ and 21,400, respectively) when the solution is ⩾ 0·002 N in sulphuric or hydrochloric acid; the absorbance of such solutions can then be used to measure abscisin concentrations. The position and amplitude of the Cotton effect also become constant when the solution is ⩾ 0·002 N in acid.

The exceptionally high amplitude of the Cotton effect (corresponding to a swing of 250,000° in molecular rotation) and the availability of a sensitive spectropolarimeter (a Bellingham–Stanley 'Polarmatic 62') provide a method for detecting and estimating (+)-abscisin II in plant extracts. The limit of detection for the pure compound is 0·5 μg in the 0·65 ml. of solvent required to fill the polarimeter cell (a sensitivity similar to that of our bioassay[2] using excised wheat embryos). In the presence of other light-absorbing substances the performance of the polarimeter falls with decreasing light transmission by the solution, and crude plant extracts required cleaning-up before satisfactory measurements of optical rotatory dispersion could be made. Usually, isolation of the ether-soluble acidic components and their separation by thin-layer chromatography (kieselgel-G; propanol–butanol–ammonia–water, 6 : 2 : 1 : 2 v/v) sufficed. A Cotton effect of the correct shape and position, and inhibitory activity measured by bioassay[2], both confined to material at the same R_F (about 0·65) as that of a reference marker of (±)-abscisin II, were taken as positive identification of (+)-abscisin II, while the intensity of the Cotton effect provided a measure of the concentration which could be checked against the inhibitory activity. Because the optical rotation of (+)-abscisin II is so large, interference from other substances was negligible; for example, material eluted from other areas of the thin-layer plates showed no measurable rotation under conditions suitable for detecting (+)-abscisin II.

We have used the method described here to detect (+)-abscisin II in the following:

Species	Organ	Date of harvest
Sycamore (*Acer pseudoplatanus*)	Buds; debudded twigs (cf. leaves (ref. 2))	January 19, 1966
Birch (*Betula pubescens*)	Leaves	November 12, 1964
Rose (*Rosa arvensis*)	Achenes; pseudocarp	December 13, 1965
Cabbage (*Brassica oleracea*, hort. var.)	Leaves of the heart	December 13, 1965
Potato (*Solanum tuberosum*)	Tuber	November 10, 1965
Avocado (*Persea grantissima*)	Seed	—
Lemon (*Citrus medica*)	Fruit	—

Through the courtesy of Mr. H. W. B. Barlow of the East Malling Research Station we have also been able to examine an extensively purified inhibitor from plum twigs[6] (*Prunus domestica*, var. 'Myrobalan B'), and have identified the major component as (+)-abscisin II (ultra-violet and infra-red spectra, chromatographic behaviour, and optical rotatory dispersion).

The concentration of (+)-abscisin II found in the plant tissues examined has been of the order of one part in ten million, fresh weight.

Evidence is accumulating that (+)-abscisin II can account for most of the inhibitory activity found in the acid fraction of plant extracts[7]. However, its physiological function is still incompletely defined and would seem to be as complex as that of the other plant hormones. Abscisin II has already been implicated in abscission in cotton[1] and in dormancy in sycamore and birch[8]. It may also be one of the natural germination inhibitors in the rose studied by Jackson[9], because we find large quantities in the pseudocarp of rose hips and smaller amounts in the achenes.

We thank Mrs. P. Gajdatsy and Mr. D. T. Green for help with the extractions and measurements of optical rotatory dispersion respectively.

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Production of Cysteic Acid, Taurine and Cystamine under Primitive Earth Conditions

IT is now well established that most of the simple, biologically-important 'monomers' (such as amino-acids, pentoses, purines and fatty acids) are formed when various high-energy sources are applied to samples of the atmosphere of the primitive Earth[1-2]. This atmosphere is assumed to consist mainly of methane, water, ammonia, nitrogen and hydrogen (the last decreasing rapidly with increased age of the Earth)[3]. Although the amino-acids are among the most prominent products of the irradiations of 'primitive-Earth atmospheres', no report has yet appeared in the literature of the appearance of the sulphur-containing amino-acids. This has presented no problem, since it can safely be assumed that the primitive atmosphere contained at least traces of hydrogen sulphide—however, it is important to have laboratory experimentation to back up assumptions.

Primitive Earth atmospheres, in the presence of added hydrogen sulphide, have been studied by Heyns, Walter and Meyer[4]. These authors passed a spark through the mixture and found essentially that the hydrogen sulphide had no effect on the syntheses of non-sulphur-containing amino-acids. They tentatively identified one sulphur-containing product, ammonium thiocyanate. However, a search for sulphur-containing amino-acids (by the method of Stein and Moore[5]) was unsuccessful, although the authors did report the presence on their chromatograms of six ninhydrin-positive, but unidentified, products. These amino-acids may have been sulphur-containing, but their yield was too small to permit identification.

It seemed to us to be worthwhile to search for the 'primitive Earth' synthesis of sulphur-containing amino-acids by using $H_2{}^{35}S$ as one of the reactants, thus greatly increasing the detection sensitivity for an amino-acid product. In this way we have been able to establish the appearance of cysteic acid ($HO_3SCH_2CH(NH_2)CO_2H$) and the closely related compounds taurine ($HO_3SCH_2CH_2NH_2$) and cystamine ($SCH_2CH_2NH_2)_2$. Cysteine and cystine probably were formed (there was evidence for them in our early chromatograms), but they appeared to be oxidized to cysteic acid during subsequent chromatography. We found no evidence for methionine production.

In each of the three experiments reported here, 20 ml. of 2 N ammonium hydroxide, 200 mm of methane and 100 mm of $H_2{}^{35}S$ (containing 5 mc.) were placed in a 'Pyrex' tube and irradiated, with an electron beam, in the manner previously described[6]. No air was present during the irradiations. The electron beam was used for no reason other than that it was a convenient source of ionizing radiation. The radiation dose in each case was about 10^9 rads. This is a very high dose; however, our products, as they were formed, were removed from the electron beam (in the condensate dripping off a condenser—see ref. 6) and thus protected from radiation decomposition. The 10^9 rads were delivered during 45-min exposures to the electron beam.

After the addition of chromatographic quantities of unlabelled carriers (cystine, taurine, etc.), the non-volatile contents and washings from the irradiation chamber were transferred to a round-bottom flask and evaporated to a small volume at room temperature. Centrifugation was then employed to remove some polymeric material (in part, elementary sulphur). An aliquot portion (usually 0·1) of the concentrated solution was then placed on Whatman No. 1 chromatographic paper. The solvent in the first direction was *n*-butanol–acetic acid–water (100 : 22 : 50, v/v/v) and, in the second, isopropanol–methanol–water (7 : 1 : 2, v/v/v). The positions and shapes of radioactive spots were determined by autoradiography (Kodak 'Blue Sensitive' medical X-ray film). Radioactive spots with R_F values similar to those of added carrier compounds were cut out, eluted and rechromatographed in the following solvent systems: (1) secondary butanol–formic acid (88–90 per cent)–water (15 : 3 : 2, v/v/v); (2) secondary butanol–tertiary butanol–water (43 : 48·4 : 8·6, v/v/v). On the second chromatograms the positions and shapes of the radioactive spots were compared with those of the coloured spots revealed by spraying the chromatogram with ninhydrin solution. The amount of radioactivity in a given spot was determined either (1) directly on the paper by means of a Geiger–Müller counter or (2) after elution, by liquid scintillation counting. This radioactivity figure could then be compared with the total amount of ^{35}S (as $H_2{}^{35}S$) that was originally present in the irradiation flask. In this way, the yields of products from the hydrogen sulphide could be determined (due allowance being made for the half-life of ^{35}S).

The following ^{35}S-labelled products (yield from $H_2{}^{35}S$ given in parentheses) were positively identified: cysteic acid (0·01 per cent), cystamine (0·003 per cent), and taurine (0·01 per cent). We expected to find, and therefore intensively searched for, cysteine, cystine, and methionine; however, if these amino-acids are formed, their yields must be well below 0·001 per cent. As was mentioned, cysteine and cystine are probably formed but then disappear by oxidation to cysteic acid. Other compounds for which we searched were homocysteine and homocysteic acid, but we could find no evidence for their synthesis.

It is pleasing to record that at least one of the sulphur-containing α-amino-acids (cysteic acid) is formed under 'primitive Earth conditions'. We believe that others

are also formed (for example, the cysteic acid is probably formed by attack of the ·OH radical on cysteine). Probably, small changes in the conditions of the 'primitive Earth' experiments (such as longer times of irradiation at lower dose rates, presence of catalytic surfaces) will lead to the identifications of all the biologically important sulphur-containing amino-acids.

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Inhibition of 6-Methylsalicylic Acid Synthesis in *Penicillium patulum*

VARIOUS reports[1–3] have indicated that the biosynthesis of 6-methylsalicylic acid (2,6-cresotic acid; 6-MSA) may be patterned after fatty acids, involving the condensation of one acetyl and three malonyl derivatives (presumably derivatives of coenzyme *A* or of a protein analogous to the acyl carrier protein[4] in *E. coli*). Bu'Lock and Ryan[5] and later Tanenbaum and Bassett[6] showed that 6-MSA is metabolized by *Penicillium patulum* to form patulin and possibly other compounds as well. These compounds, which have been designated secondary metabolites[7], have no apparent function in the organism. While the formation of 6-MSA is limited to a few species, the phenomenon of secondary metabolite formation is widespread in micro-organisms.

Factors governing the appearance and metabolism of secondary metabolites are still obscure, although some evidence indicates the nutritional environment of the cells is very important[7,8]. Bu'Lock and Powell[7] have distinguished between a 'tropophase' or balanced phase of growth and an 'idiophase' (peculiar phase) of growth marked by carbon assimilation and secondary metabolite production in *P. patulum*. They briefly referred to an inhibition of 6-MSA synthesis by 6-MSA. This communication reports experiments indicating such an inhibition not only by 6-MSA, but also by structural analogues of this compound.

Spores of *P. patulum* (NRRL 2159A) were washed from Czapek-Dox agar slants and added to 50-ml. Erlenmeyer flasks containing 20 ml. of Czapek-Dox medium. (Czapek-Dox medium contained 50 g glucose, 2·0 g sodium nitrate, 1·0 g potassium dihydrogen phosphate, 0·5 g potassium chloride, 0·37 g magnesium sulphate (dried powder, 65·6 per cent magnesium sulphate), 0·01 g $FeSO_4 \cdot 7H_2O$, in 1 l. distilled water. Slants contained 1·5 per cent agar in addition.) The inoculum usually contained about 5×10^6 spores per flask.

After an appropriate period of growth at room temperature (25° C), 8 μc. of 1-^{14}C-sodium acetate (50 mc./mmole) was added, and 10-μl. portions of the growth medium were spotted at timed intervals on thin-layer plates[9] coated with 'Adsorbosil'. Carrier 6-MSA was added to the spots, plates were developed in hexane–ether–acetic acid, 80 : 15 : 2, and the 6-MSA spots were detected by blue fluorescence under ultra-violet. The silica gel from each spot was transferred to a scintillator bottle along with 'Cab-o-sil' and toluene scintillator solution (4 g 2,5-diphenyloxazole, 0·05 g 1,4-*bis*-2-(5-phenyloxazolyl)-benzene, 1 l. toluene) and assayed for radioactivity in a Packard 'Tri-Carb' liquid scintillation counter. The method of Lands and Hart[10], in which plates are sprayed with 'Neatan', was helpful in transferring the adsorbent.

It was difficult to obtain a definite criterion for the age and condition of culture flasks which would give the best rate and extent of incorporation since germination time and growth rate varied from one experiment to the next. We generally used cultures in which the synthesis of patulin had begun, when the optical density of a 1:100 dilution of the growth medium reached 0·1 at 276 mμ (ε reported 13,350[11]; found 14,900). Results should be compared only with controls in the same experiment.

The rate of incorporation of 1-^{14}C-acetate into 6-MSA was decreased in the presence of added 6-MSA, as shown in Table 1. The concentration of 6-MSA normally found in the culture medium at this time was between 0·1–0·5 μmoles/ml. as estimated by visual comparison with standards of thin-layer plates after exposure to iodine vapour. Values obtained by extraction of the acidified growth medium with ether, chromatography of the extracts on silicic acid, and measurement of the absorption at 310 mμ[11] also fell within this range. The concentration at which inhibition was observed was thus only slightly greater than that already present, suggesting that the inhibition could be a factor in controlling 6-MSA metabolism.

Table 1. INHIBITION OF 6-MSA SYNTHESIS BY 6-MSA

Cultures were grown for 7 days at room temperature before addition of 1-^{14}C-acetate. 10-μl. samples were assayed at various times as described in the text. Numbers in the table refer to radioactivity incorporated into 6-MSA 30 min after addition of acetate. This time was chosen to best reflect the initial rate of incorporation. Duplicate experiments are given.

Addition of 6-MSA* (μmoles/flask)	^{14}C in 6-MSA (c.p.m./10 μl) Exp. 1	Exp. 2
None	195	195
33	85	45
80	10	10
132	5	5

* *p*H of 6-MSA solution was adjusted to 4·2 in order to prevent changing the *p*H of the cultures.

Structural analogues of 6-MSA also show a similar inhibition. Table 2 describes similar experiments with phenol, benzoic acid, and salicylic acid.

Table 2. INHIBITION OF 6-MSA BY STRUCTURAL ANALOGUES

Conditions were similar to those for Table 1 except for the age of cultures when acetate was added. In experiment No. 3, flasks were grown 8 days; in experiment No. 4, 10 days; and in experiments Nos. 5 and 6, 6 days.

Additions (μmoles/flask)	^{14}C in 6-MSA after 30 min (c.p.m./10 μl) Exp. 3	Exp. 4	Exp. 5	Exp. 6
None	85	85	109	150
Phenol (212)	45	35	—	—
Benzoic acid (164)	35	5	—	—
Salicylic acid (145)	—	—	5	5

It should be emphasized that it is the rate of incorporation of acetate into 6-MSA which is being inhibited, and it is not clear whether a specific step of 6-MSA synthesis or some more general metabolic process is being affected. It would be premature to classify this as a general feed back inhibition until an effect can be demonstrated on an isolated enzyme in the pathway of 6-MSA synthesis. A repression of enzyme synthesis should be ruled out because the inhibition is seen immediately after addition of 6-MSA.

The possibility that 6-MSA and salicylic acid were inhibiting by binding some essential metal ion was tested by investigating the effect of EDTA, which is a considerably stronger metal complexing agent than salicylic acid[12]. EDTA may have a slight inhibitory effect on the rate of incorporation as indicated in Table 3, but the effect is not so great as with 6-MSA and salicylic acid at equivalent

concentrations. Another effect, however, was noticed with EDTA that was not seen with the other compounds investigated. The total incorporation of radioactivity into 6-MSA at longer times after acetate addition was increased by EDTA.

Table 3. EFFECT OF EDTA ON 6-MSA SYNTHESIS

Conditions were similar to those for Tables 1 and 2. Data were obtained in same set of flasks as experiments Nos. 3 and 4, Table 2, and experiments are numbered accordingly. Incorporation into patulin is also shown.

Experiment	Additions (μmole/flask)	^{14}C in 6-MSA Time after acetate addition: (min) 20	60	180	300	^{14}C in patulin 90 h
		c.p.m./10 μl.				c.p.m./10 μl.
3	none	65	130	100	110	150
	EDTA (132)	55	125	165	190	475
4	none	70	105	95	100	200
	EDTA (132)	36	80	170	295	530

Such an effect could be explained by an inhibition of a competing pathway of acetate metabolism making labelled acetate available for a longer period of time. An inhibition of the further metabolism of 6-MSA could also explain the phenomenon; however, incorporation of acetate into patulin, one of the known products of 6-MSA, was also increased by EDTA. Patulin was assayed in a similar manner to 6-MSA by developing the plates in ether–hexane. 75 : 25, and locating spots by a brief exposure to iodine vapour. Normally, radioactivity began to appear in patulin about the time it began to disappear from the 6-MSA fraction (usually 10–30 h after addition of labelled acetate), which would be expected from their presumed precursor-product relationship.

If the biosynthetic pathways of 6-MSA and fatty acids are similar, it should be of interest to determine whether 6-MSA also inhibits fatty acid synthesis.

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Antagonism between Alloxan and Caffeine

IT has been reported[1] that mice with the hereditary obese hyperglycaemic syndrome react paradoxically to alloxan administration. (The obese hyperglycaemic syndrome entails, *inter alia*, extreme adiposity (with weight up to 120 g instead of about 30 g in non-obese littermates), pancreatic islets hyperplasia, moderate hyperphagia, extreme inactivity, a high blood glucose (generally 250–450 mg per 100 c.c.) despite high pancreatic and circulating insulin-like activity, insulin resistance and extreme sensitivity to cold[2]. The adipose tissue of animals with this syndrome shows a number of metabolic idiosyncrasies, in particular, enormously increased capacity for lipogenesis despite decreased glucose uptake[3] and an absence of increase in fat mobilization by epinephrine or FMS[4]. It appears likely that the primary inborn error is the lack of the normally present repressor to glycerokinase in the white adipose tissue[5] with the pancreatic hyperplasia, hyperinsulinism, increased hepatic lipogenesis, etc., secondary. Contrary to what could be expected on the basis of countless experiments by many investigators working with alloxan in many species, administration of a dose of alloxan which is diabetogenic to the non-obese littermates (or of twice that dose) causes a marked decrease in blood glucose lasting many months and a massive regranulation of the otherwise completely degranulated hyperplastic beta cell tissue of the islets of Langerhans[1].

This finding led us to investigate in this syndrome the action of substances related chemically to alloxan (purines, pyrimidines and an alkaloid, that is, uric acid, lactim and lactam forms, adenine, cytosine, guanine, hypoxanthine, uracil, thymine, xanthine and caffeine). Of these, only caffeine has a marked and reproducible effect[6]. The sensitivity of obese hyperglycaemic mice to the toxic effects of caffeine is extreme; while the LD_{50} is 10 mg per animal for lean littermates (500 mg per kg of body-weight), it is only 3·5 mg per animal for the obese hyperglycaemic (80 mg per kg of body-weight). While a 24-h or 48-h fast increases tolerance to alloxan, it increases the toxicity of caffeine. The effect of caffeine on blood glucose-levels of obese hyperglycaemic mice is remarkable. While in non-obese animals after an injection of 5 mg of caffeine (not lethal in any animal) the blood glucose (determined by the Nelson–Somogyi method[7]) shows a transient 60 per cent fall but goes back to normal within 24 h and stays normal thereafter, in obese mice a dose of 2 mg of caffeine (30 per cent lethal) causes the blood glucose-level to rise from 300 mg per 100 c.c. to nearly 500 mg in 6 h, 540 mg in 12 h, and more than 600 mg on the average after 4 days, with the blood glucose staying above 500 mg per 100 c.c. for several months. In fact, so sensitive are the obese hyperglycaemic mice to the action of caffeine that it is enough to replace their drinking water by instant coffee for a week to elevate their blood glucose for 75 days[6].

The mechanism of this effect is as mysterious as that of alloxan. Histological investigations and the use of carbon-14-labelled caffeine have so far given us no clue to the mode of action of this substance. The striking contrast between the actions of these two substances suggested that an examination of the effect of simultaneous injection in obese hyperglycaemic mice and in normal mice would be useful. This preliminary report indicates that caffeine and alloxan are direct antagonists. Among a number of experiments demonstrating this antagonism, the following are typical.

When alloxan was injected intraperitoneally, in saline, with dextrose solution available for 30 h, to groups of six to ten animals, a dose of 5 mg caused the blood glucose of lean mice to rise in two weeks from an average of 115 mg per cent to an average of 205; that of 7·5 mg alloxan from 115 to 470; that of 10 mg was almost uniformly lethal; the blood glucose of obese hyperglycaemic mice was reduced by 5 mg of alloxan from 450 to 180 mg per 100 c.c. in 10 days, then climbed back to 400 mg per 100 c.c. by the end of 2 weeks; after injection of a dose of 7·5 mg, the blood glucose went down on the average from 500 to 350 mg per 100 c.c. in the 2 week period; after injection of a dose of 10 mg, the average blood glucose went down from 420 to 175 mg per 100 c.c. in 2 weeks, having touched a minimum of 120 mg per 100 c.c. after 1 week. Again, as expected from previous experiments, the intraperitoneal administration of a single dose of 2 mg of caffeine to obese mice causes a rise in blood glucose from 350 to 500 mg per 100 c.c. during the course of the next 4 days, the blood glucose reaching 450 mg per 100 c.c. after 2 weeks; the blood glucose of lean mice is essentially unaffected after administration of 5 mg of caffeine.

In non-obese mice, the simultaneous administration of 5 mg of caffeine neutralized the effect on blood glucose of 5 mg of alloxan, with the blood glucose only temporarily rising from 120 to 140 mg per 100 c.c.; again, with 5 mg

of caffeine injected at the same time, the administration of 7·5 mg alloxan held the temporary rise in blood glucose to 25 mg per 100 c.c. or less. When 10 mg of alloxan was given, the simultaneous administration of the same dose of caffeine reduced the mortality from 100 to 30 per cent and again eliminated temporary (or even transient) diabetes. The action of the two substances on the histology of the pancreas (fixation in Bouin's solution with mercuric chloride stain with aldehyde thionin stain) also shows the antagonism observed with blood glucose: granulation of the beta cells of the islets of Langerhans, which is classically eliminated after administration of alloxan, was preserved when caffeine was injected simultaneously. In obese hyperglycaemic mice, a dose of 2 mg of caffeine neutralized (except for a temporary drop) the blood glucose lowering effect of 5 or 7·5 mg alloxan. When 10 mg of alloxan was injected at the same time as 2 mg of caffeine, the drop in blood glucose was about half what it was without caffeine (from 470 to 230 mg per 100 c.c. after 10 days; 285 mg per 100 c.c. after 2 weeks). The action of alloxan in causing the appearance of granulation in the otherwise degranulated beta cells of the islets of obese hyperglycaemic mice was decreased in varying degree by caffeine.

The close relationship between the chemical formulae of caffeine and alloxan (which led to the examination of the effect of caffeine in the first place), and the fact that the antagonism as regards blood glucose and histological appearance of the pancreas is seen in two situations where the actions of the two drugs are entirely reversed, make it likely that both compounds are acting on the same beta cell structures as true chemical antagonists.

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Quantitative Determination of Purine Derivatives in the Presence of Denatured Protein

RECENTLY Currie[1] reported the strong adsorption of purine bases on precipitated protein. In spite of repeated washings with 8 per cent perchloric acid, only 30–40 per cent of the added quantity of xanthine, hypoxanthine and uric acid was recovered. The determination of the bases was carried out enzymatically with xanthine oxidase. As perchloric acid is at present being used as the standard method for deproteinization much of the work concerning the metabolism of purine derivatives would, as a result, be incorrect[2–4].

We have, therefore, deproteinized rabbit kidney and liver tissue with 0·4 N perchloric acid; the tissues were rapidly chilled in liquid air after dissection. Solutions of hypoxanthine, xanthine and adenine were added and the concentrations of these substances were determined by ion exchange. Hypoxanthine and xanthine can be separated by anion exchange ('Dowex 1, *X*4', 200–400 mesh, Cl^-) using a solution of sodium tetraborate (*p*H 8·0; 0·1 N Cl^-) as the elution solvent. In addition, hypoxanthine can be determined alone on a cation exchanger ('Dowex 50*W*, *X*4', 400 mesh, Na^+) using a buffer of ammonium formate (*p*H 3·0; 0·05 N Na^+). With the same resin 1-methyladenine, adenine and 6-aminopurine (ammonium formate buffer *p*H 5·5; 0·35 N Na^+) can be separated[5].

Table 1. DEPROTEINIZATION WITH 0·4 N PERCHLORIC ACID AND WITH THE ADDITION OF DIFFERENT PURINE BASES

Substance	Substance/fresh weight in μmol/g	Tissue	Extractions with 0·4 N perchloric acid	Quantity of substance recovered (per cent)
Hypoxanthine	2·1	kidney	—	98·6
Hypoxanthine	2·1	liver	—	85·7
Hypoxanthine	2·1	liver	2	100·5
Hypoxanthine	0·074–0·15	liver	2	102
Xanthine	0·66	liver	2	101
Adenine	1·6	liver	2	108

We recovered 98 per cent of the added hypoxanthine in kidney tissue without additional extractions. Even smaller amounts (0·1 μmol/g fresh weight) were completely recovered. The adenine contents were not corrected against the control values, which probably resulted in excessive yields. A quantitative determination of the methylated adenine derivatives was dispensed with.

It was not possible to confirm by the foregoing experiments that by deproteinization with perchloric acid a loss of 60–70 per cent of the purine bases occurs due to adsorption on protein. All bases added were recovered quantitatively. Probably the experiments of Currie are explained by the inhibition of xanthine oxidase by traces of potassium perchlorate[6]. Apparently lithium perchlorate has no effect on the enzyme.

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Control of Nicotinamide-adenine Dinucleotide Phosphate Synthesis in the Livers of Rats treated with Ethionine

INJECTION of tryptophan, nicotinic acid or, particularly, nicotinamide leads to high hepatic levels of NAD but very little increase in the hepatic NADP content[1–3]. This suggests that at super-normal levels of NAD factors other than NAD may be rate-limiting in NADP synthesis. NADP synthesis is a single-step reaction requiring only three components: two substrates, NAD and ATP, and one supernatant enzyme, NAD^+-kinase (*E.C.* 2.7.1.23). Using the observation of Shull[4] that ethionine injection causes a drastic fall in the hepatic ATP concentration *in vivo*, Greenbaum, Clark and McLean[5] studied the effect of a lowered ATP concentration on the synthesis of NAD and NADP 5 h after ethionine was injected. They found that neither the enzyme synthesizing NAD (NMN adenylyl transferase, *E.C.* 2.7.7.1) nor the enzyme forming NADP (NAD^+-kinase) was affected at this particular time. However, while NAD fell only slightly, NADP was reduced to about 60 per cent of the control value. It was suggested that ATP was involved in the control of NADP synthesis.

It seemed of interest to determine how the nucleotides varied hour by hour for the first few hours after injections since Shull[4] has shown that the hepatic ATP-level falls

to about 20 per cent of the control value 2–3 h after ethionine injection. Accordingly, female albino rats, of initial body-weight 200 g, were starved overnight and injected intraperitoneally with an aqueous solution of ethionine (500 mg/kg body-weight). They were killed by cervical dislocation at hourly intervals for the first 7 h after the injection and the nicotinamide nucleotides were extracted and assayed by the method of Greenbaum, Clark and McLean[6].

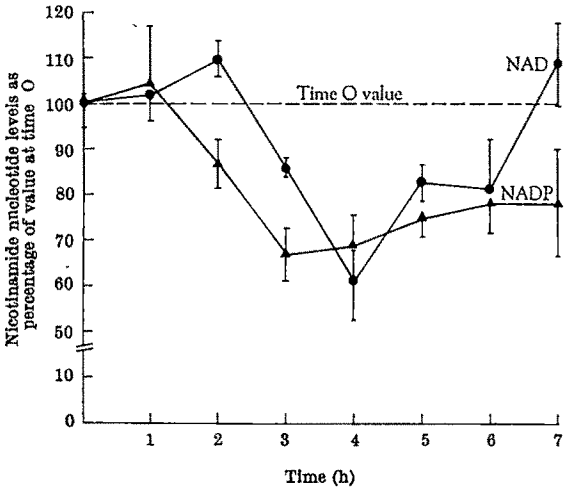


Fig. 1. Time course of the effect of ethionine on the NAD and NADP content of rat liver. The rats were given 100 mg of L-ethionine by intraperitoneal injection. They were killed at hourly intervals starting at 1 h and finishing at 7 h after injection. The oxidized and reduced forms of the nicotinamide nucleotides were extracted in 0·1 N hydrochloric acid and 0·1 N sodium hydroxide, respectively, and all four nucleotides estimated separately by the method of Greenbaum, Clark and McLean (ref. 6). The results are expressed as percentages of time O values calculated from the μg of nucleotide (oxidized plus reduced) per total liver. The vertical bars represent twice the *S.E.M* and each point represents the average of four rats. The values at time O for NAD were 3,561 ± 184 μg/total liver and for NADP, 1,564 ± 34 μg/total liver

The results are shown in Fig. 1 as the combined NAD^+ and NADH and $NADP^+$ and NADPH values expressed as a percentage of the time O values, since it is the total amount of nucleotide with which these experiments are concerned. Also they are calculated on the total liver basis since this is probably the most significant in relation to the whole animal.

The initial small increases in the nicotinamide nucleotides found in the first hour or so seem to be caused by the injection procedure rather than the ethionine since control animals injected with a similar amount of water showed the same trend (unpublished observations). This is contrary to the work of Yasin and Bergel[7], who reported little change (a slight loss if anything) in hepatic NAD after injecting water into rats. The hepatic NAD content starts to decrease after 2 h and reaches a minimum at 4 h, the level being about 60 per cent of the value at time O. After this the NAD content returns to the original values and at 7 h is, in fact, slightly higher than it was at 2 h. The NADP content of the liver starts to fall slightly earlier than the NAD, that is, after about 1 h and it reaches a minimum of about 65 per cent of the time O value at 3 h. Over the next 4 h it increases only slightly, some 10 per cent of the time O value, so that by 7 h NADP has not returned to the original values in the same way as NAD. Thus both NAD and NADP fall but only NAD returns to its normal level within the time span under consideration.

For the purposes of these results NADP synthesis should be considered in terms of the available NAD and ATP since the NAD^+-kinase shows no change 5 h after ethionine injection[5]. At 4 h when both NAD and NADP are at the respective minimal values, the ATP concentration of the liver is about 20 per cent of the normal value[4]. Thus a situation exists in the latter half of the time course when there is competition for the available ATP, and under these conditions NAD synthesis can proceed much faster relatively than NADP synthesis, leading to the normal values of NAD but still depressed values of NADP found at 7 h. It has been suggested that this greater facility for NAD synthesis rather than NADP synthesis in conditions of ATP (adenine moiety) limitation may be due to difference in the K_m for ATP of the enzymes involved in NAD and NADP synthesis and/or to the different intracellular localizations of these enzymes and the available ATP (ref. 5). Several workers[4,8,9] have attributed the effects of ethionine on cellular metabolism to its formation of *S*-adenosyl ethionine by means of the methionine activating enzyme (methionine adenosyl transferase. *E.C.*2.4.2.13). In this way ethionine causes an 'adenine' limitation in the cell. It is interesting, therefore, to note that Cantoni[10] has reported that the methionine activating system is present in the soluble fraction of the cell. Thus it may be suggested that the nuclear 'adenine' limitation is less severe than that of the cytoplasm, leading to the differential effects of NAD and NADP synthesis.

The observation of Dietrich and Yero[11] that, concomitant with the formation of high NAD-levels on nicotinamide injection, the hepatic ATP-level declines, also bears on the foregoing results and in addition on the observations of several workers[1–3] that nicotinamide injection causes high NAD-levels with very little corresponding rise in NADP. In both situations there is a limitation of available ATP leading to a priority system which allows NAD synthesis but not NADP synthesis. It is suggested also that at supernormal NAD-levels ATP will be the rate-limiting factor in NADP synthesis. However, in the first half of the time course a relationship seems to exist between NADP and the other substrate of NADP synthesis, NAD. Both NAD and NADP decrease in parallel and to levels approximately 2/3 of their time O values, although there is a time lag between their falls. This result is interesting in the light of the observations of Clark, Greenbaum and McLean[12] that in precancerous liver and hepatomas a similar relationship between NAD and NADP is apparent at normal and low NAD-levels. Investigations are continuing to establish more clearly the factors involved in the control of nicotinamide nucleotide synthesis and their relationship to overall metabolic control.

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Inhibitory Action of Glucides on Trypsin Activity

GLUCIDES, usually unimportant reagents, can affect the activity of enzymes. Farber and Wynne[1] have reported that some monosaccharides have marked inhibitory effect on the activity of pancreatic proteinase. A similar observation has been made by Whitaker *et al.*[2] with α-amylase. This report is concerned with the inhibitory effect of some mono- and di-saccharides on the activity of trypsin.

Trypsin activity was determined with azocasein substrate, prepared according to Charney and Tomarelli[3]. The trypsin (Merck) was used in 0·1 M phosphate buffer (*p*H 7·5). Glucides were dissolved in the enzyme solution. The reaction mixture, which contained 12·5 mg/ml. azocasein, 1 mg/ml. trypsin, and 1 M (in some cases 0·5 M) glucide, was kept 10 min at 40° C. After precipitating the non-digested proteins with trichloroacetic acid (final concentration 3 per cent), and mixing the filtrate with N sodium hydroxide, the extinction was obtained by means of a Pulfrich photometer with filter *S* 42, using for comparison blanks formed in the same way but without incubation. Trypsin activity was expressed as a percentage of the activity without glucides.

The effect of different concentrations of glucose was first determined (Fig. 1). It may be observed that the inhibitory effect runs parallel with the concentration of glucose. In order to compare the inhibitory effect of different glucides, the following were tested in 1 M concentration: D-arabinose, D-glucose, D-galactose, D-mannose, D-fructose, maltose and saccharose. The two disaccharides were also tested in 0·5 M concentration (Table 1).

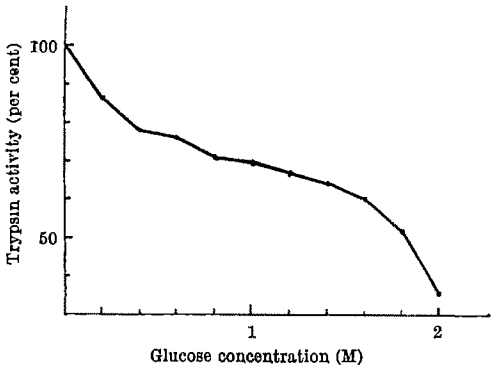


Fig. 1. Change of activity of trypsin with glucose concentration. Incubation time, 10 min; concentration of trypsin 1 mg/ml.; concentration of substrate (azocasein) 12·5 mg/ml. at *p*H 7 5 (in 0 1 M phosphate buffer). Rate of hydrolysis measured photometrically

The strongest inhibitory effect is shown by galactose. In general, except for galactose and arabinose, the inhibitory effect seems to follow the size of the glucidic molecules. These exceptions, as well as the remarkable differences noted in the behaviour of hexoses, suggest, however, that the spatial configuration of the sugars also plays a part in this inhibitory effect. There is no correlation between the inhibitory effect and the solubility of sugars.

The inhibitory effect is principally due to the steric inhibition of the enzymatic reactions, and evidently differs from the well-known substrate inhibition. The glucidic molecules mechanically isolate those of the enzyme and substrate from each other. No doubt, however, the increase of viscosity caused by the sugars also contributes to the decrease of the reaction velocity. The phenomenon is consequently a non-specific retardation rather than a true inhibitory effect, which is non-competitive in type, and which should be called 'retardative inhibition' in order to distinguish it from the other types of inhibition. A similar mechanism plays a part in the protective effect of glucides against the heat inactivation of trypsin[4].

Table 1. INHIBITORY EFFECT OF DIFFERENT GLUCIDES ON TRYPSIN ACTIVITY

Glucide	Concentration of glucide (M)	Trypsin activity %*
D-Arabinose	1	70·8
D-Glucose	1	70·0
D-Galactose	1	54·4
D-Mannose	1	84·3
D-Fructose	1	64·2
Maltose	1	58·8
Maltose	0 5	80·9
Saccharose	1	54·8
Saccharose	0·5	77·1

* Trypsin activity as percentage of activity in the absence of glucides.

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Tumour-promoting and Tumour-inhibiting Fractions from Tumours

RELATIVELY simple preparatory procedures reported from our laboratories[1] have been extended to obtain tumour-promoting fractions (MF_1 and MF_2) and a tumour-inhibiting fraction from lyophilized ascites sarcoma 37 (carried in *BT* mice) and *dbrB* adenocarcinoma (carried in *DBA*/1 mice).

The lyophilized tumour tissue was ground with 4 per cent sodium taurocholate, centrifuged, and the residue treated again with sodium taurocholate. Supernate and residue were combined, shaken, taken to *p*H 7·4 with sodium hydroxide, acidified to *p*H 6·6 with acetic acid, and centrifuged: from this treatment we obtained residue 1 and supernate 1. Residue 1 was washed with sodium taurocholate, extracted twice with acetone, centrifuged, and the final residue, dried *in vacuo*, was fraction MF_1. Supernate 1 was passed through a Seitz filter, the filtrate was brought to *p*H 4·3 with acetic acid, and the precipitate which formed (residue 2) was separated by centrifuging to give supernate 2. Residue 2 was extracted with acetone–water (1 : 1), centrifuged and re-extracted with acetone. The final residue, separated by centrifuging and dried *in vacuo*, was fraction MF_2.

Supernate 2, after Seitz filtration, was adjusted to *p*H 7 with sodium hydroxide and lyophilized. It was then extracted with benzene–ethanol (2 : 1), centrifuged and re-extracted with benzene–alcohol, and the insoluble portion was dried *in vacuo*. It was treated with absolute alcohol, and the soluble supernate separated by centrifuging and evaporated nearly to dryness on a water bath. Addition of ether produced a flocculent white precipitate which was the inhibiting fraction: this was separated by drying *in vacuo*.

Assays of MF_1 and MF_2 fractions from Sarcoma 37 and *dbrB* adenocarcinoma were made in *BT* and *DBA*/1 mice, respectively. The inhibitory fraction was investigated only against the *dbrB* tumour. 0·1 ml. of sarcoma 37 as a 1 : 5 dilution of ascites fluid from a donor was injected subcutaneously into the abdominal region of *BT* mice. The *dbrB* tumour was prepared as a 1 : 1,500 dilution of Type *B* suspension previously described[2] and 0·05 ml. was injected subcutaneously in the abdominal region of *DBA*/1 mice. Five days after tumour inoculation the appropriate fraction was injected intravenously into the tail vein. Fractions MF_1 and MF_2 were homogenized in distilled water (11 mg per ml.) and 0·05 ml. was administered. The inhibiting fraction was given in a 0·1-ml. dosage containing 33 mg per ml. Groups of ten to twelve mice were used for each experiment and control group (which only received the tumour material). Measurements were made of tumour size every 2 days, beginning on the fifth or sixth day after challenge. All control animals

developed tumours. At 10–11 days' post-challenge the sarcoma 37 tumour in mice which received MF_1 and MF_2 were 84–115 per cent and 20–30 per cent larger, respectively, than the tumours in the control mice. At similar periods the *dbrB* tumour was 51–53 per cent larger in mice which received MF_1 and 75–122 per cent larger in those which received MF_2. Greater differences were usually observed at earlier readings as the control tumours tended to catch up with the experimental ones as the post-challenge time increased. With the inhibiting material 50 per cent of the *DBA*/1 mice failed to develop palpable tumours and tumour growth was slow in the remaining mice. As the period of observation increased, the tumours which grew in the treated mice tended to overtake the control tumours so that at 27 days' post-challenge they were only 12 per cent smaller than those in the untreated mice, a figure of doubtful significance.

The techniques used for preparation and analysis of RNA and DNA generally followed the procedure of Kleinschmidt and Manthey[3]. Quantitative estimations were based on the procedure of Vischer and Chargaff[4]. RNA components were present in the MF_1 and MF_2 fractions and were concentrated in the latter. For MF_1 the mole per cent ratio $\frac{\text{adenine/uridylic acid}}{\text{guanine/cytidylic acid}}$ was 0·84. For MF_2 the ratio was 0·93. Hydrolysis studies of DNA components were less successful. The $\frac{A/T}{G/C}$ ratio for DNA of MF_1 was considerably less than one, and DNA from MF_2 gave poor chromatograms. Purines were present in small quantities and the pyrimidines gave diffuse light blue spots under ultra-violet which could not be satisfactorily resolved for quantitative determinations. Qualitatively similar results were obtained with a spontaneous mammary adenocarcinoma from *C3H* mice, but higher concentrations of purines were found in sarcoma 37. These pyrimidine abnormalities were not encountered in MF_2 fractions from mouse spleen or kidney or in sodium deoxyribonucleate subjected to the same hydrolytic procedure.

The protein moiety from MF_1 of *dbrB* adenocarcinoma contained aspartic acid, glutamic acid, serine, alanine, valine, phenylalanine, isoleucine, proline, threonine, histidine, lysine, and arginine. Phenylalanine and threonine were lacking in MF_2 which contained taurine. Nitrogen contents of MF_1 and MF_2 were 14·6 and 12·9 per cent respectively. The corresponding phosphorus values were 0·11 and 0·09 per cent. MF_1 and MF_2 from sarcoma 37 and *dbrB* adenocarcinoma gave an ultra-violet absorption maximum at 264 mμ and minimum at 242 mμ. Infra-red spectra of both fractions from all tumours (sarcoma 37, *dbrB*, and *C3H*) gave the expected protein absorption at 3μ, 6·1μ, and 6·5μ. Qualitative analysis revealed *d*-ribose and a deoxysugar.

The tumour-inhibiting fraction contained phosphorus and chlorine but was negative for nitrogen, sulphur, sugars, and protein. Infra-red spectra showed peaks at 3·1, 6·45, and 7·2μ; a slight shoulder at 7·5μ; and peaks at 9·82 and 10·8μ. In limited experiments, heating during final drying seemed to destroy the effectiveness of the tumour-inhibiting fraction.

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PHYSIOLOGY

Chemical Transmission at the Insect Excitatory Neuromuscular Synapse

GLUTAMIC acid mimics the transmitter at the crustacean excitatory neuromuscular synapse[1] and has been tentatively identified with the chemical mediator at the insect peripheral excitatory synapse[2,3]. Similarities in the pharmacology of insect and crustacean excitatory neuromuscular synapses are, perhaps, to be expected since the pharmacological properties of their peripheral inhibitory synapses are remarkably similar[2,4]. In an effort to determine the site of action of glutamic acid on the insect neuromuscular system we have examined the effects of this substance on the electrical and mechanical properties of a locust neuromuscular preparation.

Most pharmacological and electrophysiological investigations of insect neuromuscular systems have, in the past, been made on *in situ* preparations of the larger orthopteran muscles. Because these large, complex preparations are not very suitable for pharmacological investigations we decided to use the locust metathoracic retractor unguis muscle, since this is a small muscle and can be isolated from the insect and kept alive for many hours if perfused with saline[2]. The muscle, which is about 1 cm long and 300μ in diameter and rarely contains more than 20 fibres, was dissected from the locust leg and fixed with a portion of its innervating nerve in a small 'Perspex' chamber and perfused continuously with saline or saline-containing drug. The muscle was stimulated indirectly through platinum electrodes placed on the innervating nerve. Measurements of the electrical properties and recordings of the electrical activity of the muscle fibres were made using conventional intracellular microelectrode techniques. Contractions of the muscle were recorded by attaching the apodeme of the retractor unguis muscle to a strain gauge with terylene thread.

A single phasic contraction of the retractor unguis muscle occurred when the preparation was perfused with glutamic acid (sodium salt) concentrations greater than 10^{-7} w/v (Fig. 1*b*). Maximal glutamate contractions were obtained with concentrations of about 10^{-5} w/v. In the presence of these relatively high concentrations of glutamic acid the neurally-evoked contractions elicited by maximal stimulation of the motor innervation were always reduced in magnitude and sometimes disappeared completely. At the same time, changes in the electrical properties of the muscle fibres were recorded. For example, the membrane potential and effective resistance were initially reduced when the preparation was perfused with concentrations of glutamic acid greater than 10^{-8} w/v (Figs. 2 and 3). Desensitization of the muscle glutamate receptors during prolonged exposure to high concentrations of glutamic acid led, however, to a slow return of the membrane potential and effective resistance to their resting control values (Fig. 3). Concomitant recovery of the neurally-evoked mechanical responses did not occur (Fig. 1*b*) yet the glutamate-insensitive muscle fibres contracted in response to direct electrical stimulation. The decline in magnitude of the neurally-evoked contractions is therefore indicative of transmission failure at the excitatory neuromuscular synapse and not of some effect of glutamic acid on either the electrically excitable membrane or contractile component of the muscle fibre. Transmission failure could be explained by assuming that glutamic acid is the excitatory transmitter. In the presence of high concentrations of this substance the glutamate receptors on the excitatory post-synaptic membranes would be desensitized and transmission blocked. Glutamic acid has, in fact, been identified in the perfusate obtained from the cockroach limb during stimulation of the nerves innervating the limb muscles[3].

The electrical properties of the muscle fibres were not affected by low concentrations of glutamic acid (that is,

concentrations less than 10^{-8} w/v). However, potentiation of the neurally-evoked mechanical responses (Fig. 1*a*) and an increase in the frequency of the miniature excitatory post-synaptic potentials[5,6] occurred during applications of 10^{-12} w/v to 10^{-9} w/v glutamic acid. Since there was apparently no concomitant change in the amplitude of the miniature discharge it is unlikely that potentiation of the neurally-evoked contractions was due to sensitization of the excitatory post-synaptic membrane. Possibly, glutamic acid facilitates the release of excitatory transmitter from the pre-synaptic terminals. With high concentrations of glutamic acid this process would be masked by the changes at the post-synaptic membrane. If glutamic acid is the excitatory transmitter then its pre-synaptic action could be the basis of neuromuscular facilitation at the insect peripheral excitatory synapse.

The cholinesterase found at the end plates on vertebrate striated muscles is thought to limit the action of the excitatory chemical mediator, acetylcholine, on the post-synaptic membrane. Enzymatic breakdown of the chemical mediator at the insect neuromuscular synapse has not yet been demonstrated. It is perhaps significant, however, that when the locust retractor unguis preparation was perfused with saline containing relatively low concentrations of glutamic decarboxylase (10^{-5} w/v of *E. coli* extract; activity 0·6 u./mg), an enzyme which converts glutamic acid to γ-aminobutyric acid (GABA), the mechanical responses elicited by nerve stimulation were reduced in magnitude (Fig. 1*c*). On the other hand, the contractions were potentiated when the muscle was perfused with relatively low concentrations of glutamic decarboxylase inhibitor, phenylhydrazine hydrochloride (Fig. 1*d*). Repetitive contractions of the muscle fibres to single shocks applied to the innervating nerve occur with concentrations of enzyme inhibitor greater than 10^{-5} w/v. Neither glutamic decarboxylase nor phenylhydrazine appears to have any effect on the electrical properties of the insect muscle fibre.

These observations suggest that glutamic acid could be the excitatory transmitter and that a glutamic acid–glutamic decarboxylase system could be the basis for

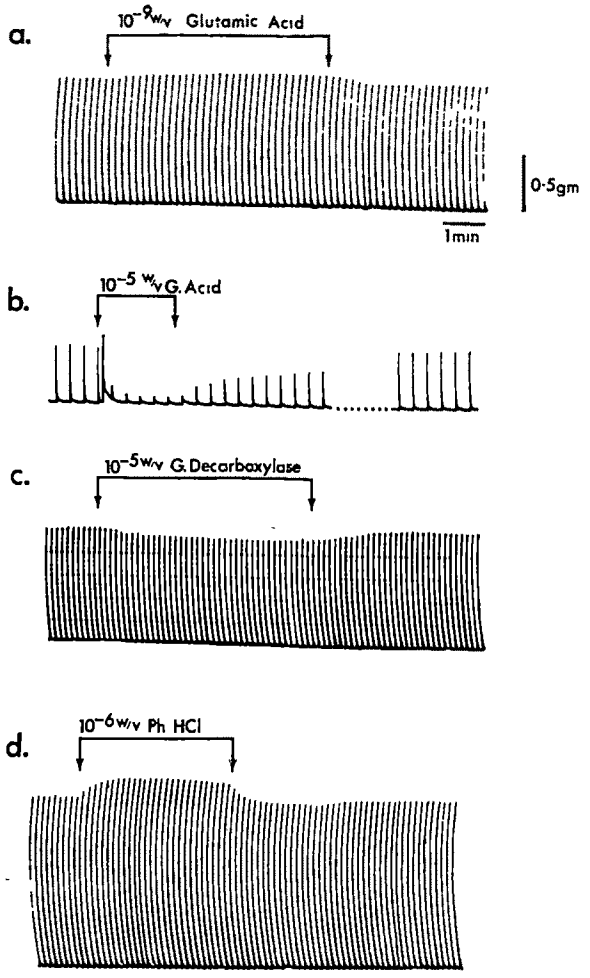


Fig 1. *a*, Potentiation of neurally-evoked contractions of retractor unguis muscle by a low concentration of glutamic acid (10^{-9} w/v). *b*, Maintained depression of neurally-evoked contractions in a relatively high concentration of glutamic acid (10^{-5} w/v). A glutamate-evoked phasic contraction of the muscle occurred immediately following application of the drug. *c*, Depression of neurally-evoked contractions by the enzyme, glutamic decarboxylase (10^{-5} w/v). *d*, Effect of the glutamic decarboxylase inhibitor, phenylhydrazine hydrochloride (10^{-6} w/v), on the neurally-evoked contractions. The mechanical responses are initially potentiated but after a short period the response height started to fall. The decline in magnitude of the contractions could be due to desensitization of the excitatory receptors following accumulation of transmitter at the post-synaptic membrane

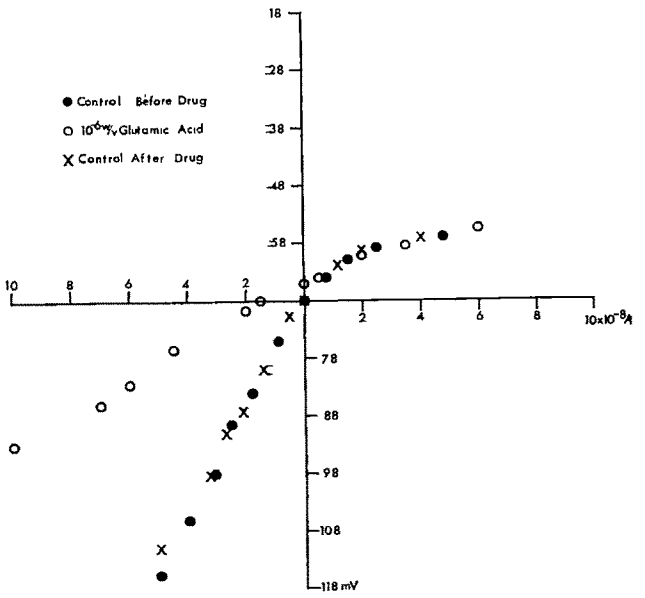


Fig. 2. Current-voltage relation of a single fibre (resting potential = 68 mV) of a locust retractor unguis muscle before, during and after treatment with a relatively high concentration of glutamic acid (10^{-6} w/v). The effective resistance of the muscle fibre fell by almost 70 per cent during application of the drug

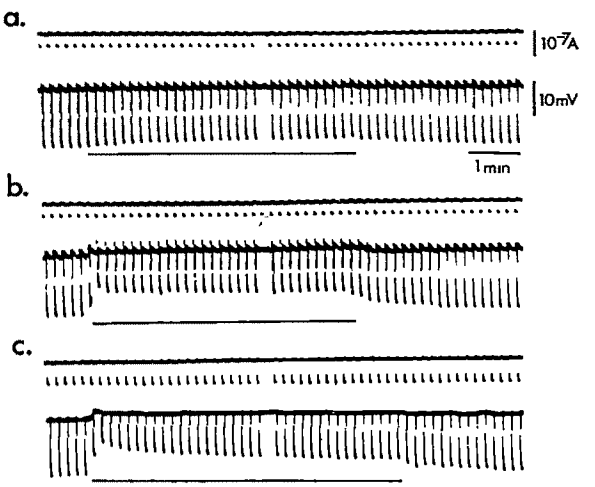


Fig. 3 Hyperpolarizing potentials (lower traces) to constant inward current pulses (upper traces) recorded from three different fibres of a single retractor unguis muscle (membrane potentials (*a*) 75 mV, (*b*) 72 mV, (*c*) 70 mV) Reduction in magnitude of hyperpolarizing potentials during application of (*a*) 5×10^{-8} w/v, (*b*) 5×10^{-7} w/v, and (*c*) 5×10^{-7} w/v glutamic acid indicates changes in effective resistance of muscle fibres. The muscle was perfused with drug during the periods indicated below the lower trace of each record. Note, especially in (*c*), initial fall in membrane resistance followed by slow recovery towards resting control condition

transmission at the insect excitatory neuromuscular synapse. One of the products of the enzymatic destruction of glutamic acid by glutamic decarboxylase, GABA, is known to mimic the transmitter at the insect inhibitory neuromuscular synapse[2,3]. If neuromuscular facilitation at the inhibitory synapse is due to the action of GABA on the terminals of the inhibitory axon then the appearance of GABA as an end product of excitatory neuromuscular transmission could have far-reaching implications.

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Effect of Microwave Radiation on Birds

MUCH has been written over the past years on the effect of microwave radiation on body tissues and animals, but most of the experiments reported have been concerned with the production of heat and its associated behavioural and physiological effects[1-4]. There are very few references to 'athermal' effects such as muscular disturbances[5] and disorientation—phenomena which have not attracted the scientific attention that they merit. In fact, there appears to have evolved a body of opinion dismissing the existence of any effect other than a thermal one on the pretext stated in one report[4] that the photon energy in the frequency band of microwave radiation is insufficient by several orders of magnitude to produce ionization. Apparently no consideration has been given to the effect of induced electrical currents on the activity of the nervous system of an animal. Animal tissues absorb microwave radiation diffusely and since such tissues contain membrane interfaces which are semi-conducting, polarization can occur. If the microwave radiation is in pulsed form, the induced electrical currents will be modulated at the frequency of pulse repetition. It has been demonstrated experimentally that nerve conduction characteristics undergo a profound change when the nerve is subjected to a.c. excitation[6]. Therefore, it is to be expected that microwave radiation would have an effect on the nervous system of an animal depending on the depth of penetration.

Experiments have been conducted on chickens (Old English Games) fourteen weeks old at power levels in the range 10–30 mW/cm^2, at a frequency of 16,000 Mc/s and a pulse repetition rate of 8,000 pulses per sec. A horn antenna was arranged so that it could be mounted above, below or at the side of the cage. With the antenna mounted vertically above the cage it was observed, a few seconds after the onset of radiation, that sustained extensor activity of a wing and leg occurred—a reaction possibly due to the penetration of induced electrical activity to the spinal cord. Shielding first the head of the chicken, and then the body leaving the head exposed produced no significant change in the manifested extensor activity. Radiation from the antenna when mounted below the floor of the cage and pointing vertically upward produced no signs of muscular disturbance, although each chicken did register a startled reaction at the onset of radiation.

These experiments were repeated using pigeons and seagulls (ringbill) with similar but less dramatic results. The pigeons generally registered distress and unsteadiness of gait but not as pronounced extensor reactions. However, it was observed after the experiments that the wings of the exposed birds did not return to their normal fully retracted position for a period of an hour or more.

The seagulls registered considerable distress and unsteadiness of gait but shrugged off the muscular disturbance by repeatedly flapping their wings. There was no observable change in the retracted position of their wings.

Tests were made on the absorption characteristics of the feathers of each species, but the findings were negative.

It is suggested that the low field intensity and short exposure times (less than 60 sec) used in these experiments preclude the production of heat as the causal factor in the manifestation of extensor activity. Further experiments are planned to determine the phenomenological relationship between wave-length, pulse repetition rate and field intensity.

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Action of Mecholyl on the Respiration of Cardiac Muscle *in vitro*

UNTIL now relatively little experimental evidence has been acquired about the detailed regulative function of acetylcholine (ACh) on the metabolism of cardiac muscle. Earlier investigations using the heart–lung preparation showed the oxygen-sparing effect of ACh (ref. 1). ACh seems to increase the energetic efficiency and to preserve the energy sources of the heart muscle tissue[2]. This problem, however, has not been completely solved even by analysis of the direct action of ACh on the cardiac muscle fibre. There are only a few reports on the sparing effect of ACh *in vitro*. An increase of adenosine triphosphate (ATP) and glycogen content in cardiac muscle treated with ACh has been reported[3]. Changes in the oxygen uptake of slices treated with ACh have also been shown by some workers[4]. Recently, the application of ACh or eserine *in vivo* has been shown to decrease the fatty acid utilization of minced myocardial tissue[5].

To obtain further information about this problem, we have studied the effect on the respiration of the isolated rat auricle and tissue slices from both ventricles of mecholyl(acetyl-beta-methylcholine chloride) (MCh) and eserine salicylate *in vitro*. MCh was used instead of ACh because of its small sensitivity to the inactivating action of cholinesterases. The drugs were added to the tissue *in vitro*; the final concentration shown in the Tables refers to their salts. Metabolic activity was measured as oxygen uptake by the direct Warburg technique.

In the first series the action of eserine alone and in combination with MCh has been studied. The rate of respiration of the tissue was stimulated by sodium succinate in the atmosphere of pure oxygen. The results obtained are shown in Table 1. Only slices of left ventricle tissue showed any marked depression of metabolic activity during the second hour of incubation. The final effective concentration of the combined eserine–MCh action cannot, however, be considered a physiological one. It seems of interest that the oxygen uptake of the other myocardial parts remained largely unaffected. Eserine alone (even at concentrations as high as 200 μg/ml.) produced no effects.

In the second series the control respiration rate of the cardiac tissue specimens was diminished by omitting the

Table 1. OXYGEN UPTAKE OF CARDIAC TISSUE *in vitro* AFTER ESERINE AND ESERINE+MECHOLYL (MCh) AT THE HIGHER RATE OF RESPIRATION

Tissue	μl Oxygen/100 mg wet wt /90 min Eserine	Eserine+MCh
Isolated auricles	421·6±21·1 (9)	457 4±19 1 (6)
Slices: left ventricle	354·8± 9·5 (8)	283 0± 7·3 (5)
right ventricle	338 9±19·8 (8)	322 6±26·8 (5)

Medium buffered Krebs-Ringer solution with 0 2 per cent glucose and 20 mM sodium succinate. Eserine salicylate 200 μg ml., MCh=200 μg mecholyl chloride/ml. Gas phase: 100 per cent oxygen, $t=37°$ C. Values are the means ±*S.E.*, figures in parentheses are numbers of experimental animals. Inhibition after mecholyl in left ventricular slices is significant.

Table 2. OXYGEN UPTAKE OF CARDIAC TISSUE *in vitro* AFTER ESERINE +MECHOLYL (MCh) AT THE LOWER RATE OF RESPIRATION

Tissue	μl. Oxygen/100 mg wet wt./90 min Control	Eserine+MCh
Isolated auricles	116 3±4·9 (8)	117·4±4 7 (8)
Slices: left ventricle	70·7±2·8 (7)	72·2±6·2 (8)
right ventricle	72·5±4·3 (7)	73·7±6·4 (9)

Experimental conditions the same as those in Table 1 with exception of the gas phase ($Po_2=0$ 21 atm.) and after omission of succinate. No inhibition after eserine+mecholyl was found.

sodium succinate and using air as the gaseous phase. Under these circumstances, even the aforementioned high combined dose of both cholinotropic drugs did not affect the oxidative metabolism of the three different specimens of myocardial tissue (Table 2).

These findings in sliced ventricular tissue and isolated auricles at the two different levels of respiration used do not support the suggested action of MCh on cardiac energetic metabolism at the cellular level. It seems clear that the sensitivity of the cardiac tissue *in vitro* to MCh and eserine as tested by the Warburg method is surprisingly small. In this connexion, a certain analogy with the behaviour of brain slices treated with ACh *in vitro* may be mentioned. Although there is no doubt about the physiological importance of ACh in the central nervous system, experiments with slices or homogenates of the brain cortex using cholinotropic agents failed to show any effect on the oxidative metabolism of the tissue[6,7].

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Variations in the Plasmalogen Content of Mammalian Heart Muscle

MAMMALIAN heart muscle contains about one-quarter of its phospholipid, on a molar basis, in the plasmalogen form. Unlike brain, where the plasmalogen is almost entirely in the ethanolamine form (glycerol 2-acyl-1-alkenyl-3-phosphorylethanolamine)[1], heart muscle contains both the ethanolamine and choline plasmalogen.

It is also known that the ratio of ethanolamine to choline plasmalogen in the heart muscle of some young mammals is different from that of the adult. In the cardiac muscle of new-born dogs[2], for example, the choline : ethanolamine plasmalogen ratio is low whereas in the adult heart the choline plasmalogen level is very much higher than that of the ethanolamine analogue.

This variation in the ratio of choline to ethanolamine plasmalogen does not, however, appear to be a function only of age. There is a considerable variation within the adults of different species as can be seen in Table 1. Adult rats, mice and sheep, and to a lesser extent ferrets, have a high ethanolamine : choline ratio in their plasmalogens. The hearts of cats, guinea-pigs and ox, however, have a much higher choline : ethanolamine ratio, approaching the 'adult' situation described by Hack and Helmy[2]. Apart from rat, mouse and sheep where the

Table 1 PLASMALOGEN CONTENT OF THE CARDIAC MUSCLE IN SEVERAL MAMMALS

Species	Plasmalogen as mole % total phospholipid	Plasmalogen μmoles/g heart Choline	Ethanolamine	Molar ratio choline : ethanolamine
Rat	13	0 4	3 0	1 : 7 5
Mouse	13	0 8	3·5	1 . 4·4
Sheep[6]	18	1·6	3·7	1 · 2 3
Ferret	25	2·4	4·7	1 2·0
Cat	27	2·9	3 2	1 1 1
Ox	26	2 5	2 3	1 0 9
Guinea-pig	34	3 6	3 3	1 : 0 5

In the experiments, the ethanolamine and choline plasmalogens were determined by the method of Dawson, Hemington and Davenport (ref. 3) using the modification of Ansell and Spanner (ref 4). Total plasmalogen was also determined by the method of Gottfried and Rapport (ref 5).

percentage of phospholipid in the plasmalogen form is low (13–18 per cent), the values lie between 25 per cent and 34 per cent. It would seem that it is the lack of choline plasmalogen rather than an increase in ethanolamine plasmalogen that accounts for the low choline : ethanolamine ratio (Table 1). Until the role of the plasmalogen in heart muscle is known, however, it is difficult to know if the variation in the choline : ethanolamine plasmalogen ratio is of any physiological importance.

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Laser Lesions : Changes in Retinal Excitability

THE electroretinogram and optic tract potentials evoked by photic stimulation exhibit complex changes following the production of a chorioretinal lesion by a pulsed laser beam. Retinal function is not only permanently modified by the lesion, but its production is accompanied by temporary and possibly widespread changes in retino-optic nerve excitability.

Experiments were carried out on cats, weighing between 1·5 and 2·5 kg, anaesthetized by the intravenous injection of pentobarbitone-sodium (30 mg/kg). The right eye was enucleated and the pupil of the left eye fully dilated by the intramuscular injection of atropine sulphate (0·6 mg) and the local application of a mydriatic (phenylephrine hydrochloride). Electroretinograms were recorded with a chlorided silver electrode resting on the rim of the cornea in the upper and outer quadrant, and optic tract potentials were recorded from co-axial electrodes inserted stereotaxically. The photic stimulus was generated by a Mazda '*FA*10' flash tube placed in front of the eye, control responses and responses immediately following the lesion being recorded without interruption. Each lesion was produced in the nasal part of the retina by a ruby rod laser of nominal output 0·5 J and pulse duration 0·5 msec; the lesions varied in diameter from 0·4 mm to 2·2 mm.

In the first series of experiments, using nine cats, the electroretinogram and optic tract potential from the nasal part of the retina were recorded every 2 sec. The *A* and *B* waves of the electroretinogram were depressed immediately following the lesion (Fig. 1): although the *A* wave regained almost complotely its control amplitude, the *B* wave, following an initial recovery, progressively declined over the following 50 min. The optic tract potential was also reduced in amplitude immediately following the lesion and the reduction varied from 18 per cent of the average control amplitude to a complete disappearance (Fig. 1). In most experiments this potential made a partial recovery and in one experiment the control amplitude was regained.

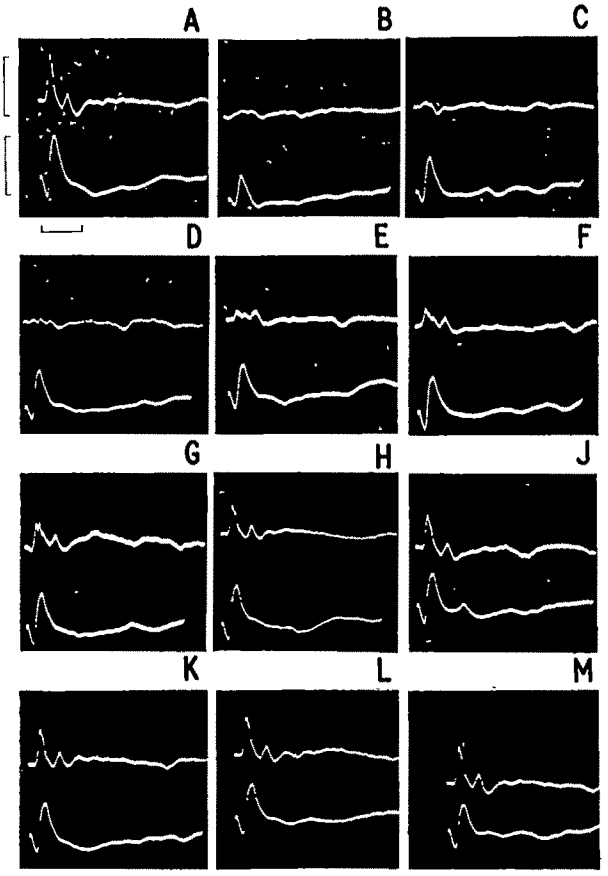


Fig. 1. Effect of a nasal chorioretinal lesion on both the electroretinogram (lower trace) and the nasal optic tract potential (upper trace): *A*, Control records. *B*, *C*, *D*, Responses approximately 2, 6 and 12 sec after the lesion. *E*–*M*, Responses at the 1, 3, 5, 10, 15, 20, 30, 40 min interval after the lesion. The immediate effect is a complete disappearance of the optic tract potential and a fall in the amplitude of the *A* and *B* waves of the electroretinogram. The optic tract potential exhibits a partial recovery but the electroretinogram shows a depression of the *B* wave after an almost complete recovery. The *A* wave of the electroretinogram maintains the recovery observed within the first min. Calibrations: 300 μV and 100 msec

In the second series of experiments, electrodes were placed in both optic tracts and a lesion produced in the nasal part of the retina. In seven of the nine experiments the temporal optic tract potential showed no change during the 40-sec period after the production of the lesion, but in the remaining two experiments the potential showed a decrease which recovered within this period. As in the previous experiments the potential recorded from the nasal optic tract immediately following the lesion was decreased in amplitude.

In these experiments the relation between the electrode and the fibres originating from the part of the retina in which the lesion was made was not known, but it is reasonable to assume that the changes in optic tract potentials are dependent on the spatial relationship between their retinal connexions and the lesion. We consider that changes in potentials recorded from the optic tract of the damaged part of the retina, and the transient fall in amplitude sometimes observed in potentials recorded from the optic tract of the undamaged part of the retina, suggest not only localized permanent damage but also a temporary disorganization of retino-optic mechanisms. The depression in the electroretinogram immediately following the lesion is additional evidence for a change in retinal excitability which may be widespread.

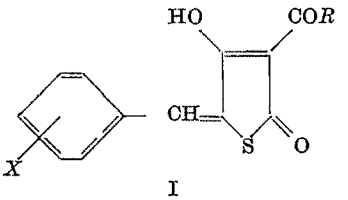
We thank Air Vice-Marshal W. K. Stewart for his interest.

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PHARMACOLOGY

A New Type of Cytotoxic Immunosuppressive Agent: 3-Acetyl-5-(4-fluorobenzylidene)4-hydroxy-2-oxo-2:5-dihydrothiophen

DURING the past 3 years we have been investigating the biological properties of a series of thiophens of the following general formula (I):



I

in which *R* is an alkyl or alkoxy group and the substituent *X* is an electron-withdrawing group such as halogen or nitro. These investigations resulted from the observation that the compound I.C.I. 45,514 (I, *R* = ethoxy, *X* = H) prevented the development of secondary inflamed lesions in rats injected into the foot-pad with a fine suspension of dead tubercle bacilli in liquid paraffin. The use of this syndrome for evaluating chemotherapeutic agents has already been described[1,2].

This communication describes the properties of one of the more active compounds in the series, I.C.I. 47,776 (I, *R* = methyl, *X* = 4-fluoro), which may be prepared by condensation of 4-fluorobenzaldehyde with 3-acetyl-4-hydroxy-2-oxo-2 : 5-dihydrothiophen under either acidic or basic conditions[3]. It melts at 183° C and is soluble in most organic solvents and as its salts in water. It may exist in tautomeric forms other than that indicated. Recently we have found that aqueous suspensions of I.C.I. 47,776 slowly isomerize at room temperature, and special precautions are necessary to ensure that full-strength material is used in small animal experiments. These include grinding under dry conditions and storing aqueous suspensions at 0° C. The isomer is relatively non-toxic and inactive. Compounds in which *R* is alkoxy rather than alkyl do not isomerize.

In chemotherapeutic tests, I.C.I. 47,776 (5–10 mg/kg/day orally) prevented the development, or reduced the severity of secondary inflamed lesions in rats injected with adjuvant into the foot-pad. Similar doses also prevented the development of allergic thyroiditis and allergic encephalomyelitis in the rat, but in a variety of standard anti-inflammatory tests the compound was inactive.

I.C.I. 47,776 proved to be a potent inhibitor of protein and nucleic acid synthesis in suspensions of rat lymph node cells in Eagle's medium (85–95 per cent inhibition at 15 μM). Similar effects were observed with rat liver cells and slices, mouse *L* cells, Landschutz ascites cells and guinea-pig lymph node cells. That inhibition is probably due to interference with generation of high-energy phosphate esters necessary for macromolecular synthesis was demonstrated by inhibition of nicotinamide adenine dinucleotide linked oxidations in isolated mitochondria (95 per cent inhibition at 5–10 μM). This is in contrast with the actions of most cytotoxic drugs which interfere directly with nucleic acid metabolism. Concentrations of I.C.I. 47,776 similar to those which inhibited oxidations in isolated mitochondria also increased the activity of the Mg^{++} dependent adenosine triphosphatase in mitochondria but strongly inhibited the adenosine triphosphatase activated by 2,4-dinitrophenol.

The foregoing results led us to suppose that I.C.I. 47,776 might act *in vivo* as an immunosuppressive agent. This view was confirmed by suppression of the primary antibody response in rats to bovine serum albumin or sheep red cells at a dose of 5 mg/kg/day orally. Moreover, in rats injected with adjuvant into the foot-pad the

development of large pyroninophilic cells and plasma cells in the draining lymph nodes was prevented. Such cells are considered to be involved in antibody synthesis.

Preliminary toxicity investigations with I.C.I. 47,776 in rats and mice have demonstrated haematological and histological changes characteristic of the effects of cytotoxic agents and ionizing radiation. The degree of the effect is closely related to the dose given and parallels the immunosuppressive action already discussed. Because I.C.I. 47,776 has a different mode of action from the cytotoxic agents which are at present available it may prove of interest to study its activity in the treatment of certain malignancies and diseases which involve the participation of an immune response.

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An Anti-viral Drug (M and B 7714) and Bilirubin Conjugation

M AND B 7714 (4-bromo-3-methylisothiazole-5-carboxaldehyde thiosemicarbazone) is a thiosemicarbazone recently introduced for the treatment and prevention of smallpox[1]. The mode of action of M and B 7714 is similar to methisazone but it is slightly less active against rabbit pox[2]. In clinical trials a number of patients have developed an unconjugated hyperbilirubinaemia, and chronic administration of the drug to animals produces an unconjugated hyperbilirubinaemia[3]. I have examined the effect of M and B 7714 on conjugation and biliary excretion to determine the possible site of action of the drug causing jaundice.

The rate of bilirubin glucuronide formation was determined in rat liver slices and rabbit liver homogenates by the method of Lathe and Walker[4]. *o*-Aminophenol conjugation was determined in rat liver slices by the method of Levvy and Storey[5]; in rabbit liver homogenates by the method of Stevenson and Dutton[6]. M and B 7714 was dissolved in ethanol and the ethanol evaporated; the final concentrations were 0·01, 0·1, 1·0 and 10 mM. The effect of M and B 7714 on the maximum hepatic clearance of bromsulphthalein and indocyanine green was determined in male Wistar rats. The maximum hepatic clearance of bilirubin[7] was determined together with the amount in the liver[8] after intraperitoneal injection of the drug.

The addition of M and B 7714 did not lower the rates of conjugation of bilirubin and *o*-aminophenol by rat liver slices. The transferase (UDP glucuronate glucuronyl transferase (acceptor unspecific) *EC*. 2.4.1.17) stage of conjugation was examined in rabbit liver homogenates incubated with ample added uridine diphosphate glucuronic acid. M and B 7714 did not alter the rates of conjugation of these substances by liver (Table 1).

10 mg/100 g, 20 mg/100 g and 30 mg/100 g M and B 7714 suspended in propylene glycol were injected intraperitoneally 1 h before the infusion of bilirubin, indocyanine green and bromsulphthalein. 30 mg/100 g M and B 7714 reduced the maximum hepatic clearance of all three substances (Table 2). When bilirubin was infused, the uptake of bilirubin was not reduced but the percentage of conjugated bilirubin in the liver was decreased.

Table 1. EFFECT OF M AND B 7714 ON CONJUGATION

	Rate of conjugation (mμmoles/mg wet wt/h)			
	Rat liver slices		Rabbit liver homogenate	
Concentration (mM)	Bilirubin	*o*-Aminophenol	Bilirubin	*o*-Aminophenol
0	0·062	0·36	0·40	3·95
0·01	0·048	0·35	0·60	5·0
0·1	0·049	0·42	0·49	4·46
1·0	0·058	0·42	0·43	3·95
10·0	0·055	0·37	0·42	3·95
No. of experiments	(3)	(3)	(4)	(2)

Table 2. EFFECT OF M AND B 7714 ON MAXIMUM HEPATIC CLEARANCE

	Maximum hepatic clearance (μg/100g body-wt./min)			
Dose	Bilirubin	Percentage conjugated in liver	Brom-sulphthalein	Indocyanine green
Control	74(14)	75	73(8)	4·7(16)
10 mg/100 g	73(2)	80	—	3·5(1)
20 mg/100 g	61(4)	67	—	6·1(1)
30 mg/100 g	46(4)	52	25(3)	2·1(5)

No. of experiments in parentheses.

The effect of M and B 7714 on conjugation and biliary excretion was investigated to try and determine the site of action of the drug causing an unconjugated hyperbilirubinaemia. An unconjugated hyperbilirubinaemia can be due to defective uptake of bilirubin or defective conjugation. It is unlikely that defective uptake occurs because bilirubin is present in the liver in slice and infusion experiments in amounts comparable to control experiments.

The effect of M and B 7714 on the *in vitro* conjugation experiments is unusual; it may be that there is no effect because of the extreme insolubility of the compound. In the *in vivo* experiments the excretion of bilirubin and dyes and the amount of conjugated pigment in the liver are reduced; it is possible that the substance is converted *in vivo* to a hepatotoxic material which then inhibits glucuronyl transferase and dye excretion. No such transformation occurs *in vitro*.

I thank Valerie Price for her technical assistance, Prof. N. H. Martin for advice and encouragement, the British Empire Cancer Campaign, May and Baker Ltd., and the Board of Governors of St. George's Hospital for financial assistance.

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Toxicity of Dimethyl Sulphoxide to the Goldfish, *Carassius auratus*

INVESTIGATIONS of the comparative toxicities of organic pesticides to fish have to some extent been hampered by the insolubility of these compounds in water. The most widely used solvent in such tests is acetone, but this compound has important disadvantages. Although it is relatively non-toxic to fish (in this laboratory goldfish have tolerated 18 p.p.t. for at least one week) acetone in solution has a high oxygen demand which may lead to oxygen depletion in the test media[1,2], and if the compound under evaluation, or the solvent vehicle, is volatile it is clearly undesirable to rely on artificial oxygenation.

The low mammalian toxicity of dimethyl sulphoxide (DMSO), together with its favourable volatility, miscible nature and solvent properties[3–5], suggested that this compound might offer certain advantages as an alternative solvent to acetone. Accordingly some preliminary toxicity tests with the goldfish were undertaken which are reported here.

The tests were conducted in Huntingdon tap water of hardness 32 deg., which has been shown to be non-toxic to

fish, at a temperature of 15° C. Fish were exposed in all glass aquaria of 15 l. capacity, each of which contained a maximum of five goldfish 2·0–2·5 in. long. Under these conditions, the 48 h median tolerance limits, or LC_{50} values, were found to be 24 p.p.t. for acetone and 43 p.p.t. for DMSO. At 32 p.p.t. DMSO five goldfish survived for ten days without exhibiting signs of respiratory stress or symptoms of toxic reaction. In a similar concentration of acetone the median period of survival was about 90 min.

Jacob *et al.*[6] have shown that, in a solution containing 15 per cent DMSO, certain compounds are transported across the dog urinary bladder mucosa at a more rapid rate than when they are dissolved in an equivalent strength acetone solution. At levels of 6 and 18 ppt DMSO in water containing 0·05 p.p.m. DDT (70–74 per cent *p.p'.* isomer. A gift from Cocker Chemical Co. Ltd., Oswaldtwistle), the median period of survival of goldfish has not been found to differ significantly from that in tests where the DMSO was replaced by an equivalent amount of acetone. In all tests the value lay between 15 and 24 h. This would tend to indicate that at these comparatively low concentrations DMSO is unlikely to affect unduly the rate of absorption of organic pesticides.

These results suggest that DMSO might be a more suitable solvent for organic pesticides being tested on fish than the more commonly used acetone. Possession of a lower order of toxicity means that quantities adequate to ensure more efficient dispersal of the pesticide in the test water can be used.

The main disadvantage of DMSO resides in its dipolar aprotic nature so that the possibility exists that certain compounds dissolved in it may undergo chemical change. It has already been noted, for example, that solutions of two organophosphorous compounds in DMSO undergo colour changes after 24 h[4].

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HAEMATOLOGY

Haemoglobin Polymorphism in the Orang Utan and an Animal with Four Major Haemoglobins

IN examining haemolysates from ten orang utans from the Yerkes Regional Primate Center collection we found four different haemoglobin phenotypes including one in which three major haemoglobin zones were present. The samples, kindly supplied by Prof. G. Bourne and Dr. J. Moor-Jankowski, were first analysed on starch gels using a *tris*-ethylenediamine tetraacetic acid (EDTA) buffer system[1] and were later investigated by hybridization[2,3] of the component haemoglobins with various other haemoglobins.

Four animals had a single major component migrating slightly anodally to human Hb-A at *p*H 8·6; we refer to this as Hb-A^{OR} (Fig. 4*b*). A minor component similar to human Hb-A_2 in concentration but more anodal in mobility was also present in this phenotype. Two animals had a single major haemoglobin more cathodal than Hb-A^{OR} (Fig. 1*d*) and similar in mobility to the minor component already mentioned. We refer to this major component as Hb-B^{OR}. These animals also had a minor component but it was slower than Hb-B^{OR}. Three other animals had both Hb-A^{OR} and Hb-B^{OR} in similar concentrations (Fig. 1*c*) and a rather weak minor component of the slower type.

The last animal (No. 8) had three major haemoglobin zones and a weak minor component of the slower type (Fig. 1*a*). The middle zone (M) was the same as Hb-A^{OR} in mobility and the slow zone the same as Hb-B^{OR}. The relative strengths of the three zones eluted from a maize starch block were fast = 26·0 per cent, middle = 47·5 per cent and slow = 26·5 per cent, which suggests that the middle zone might contain two haemoglobins.

Human cases with more than two major haemoglobins have occasionally been reported[1]. They are explicable on the basis that both normal and abnormal α-chains and also normal and abnormal β-chains are synthesized. Combination of these chains *in vivo* yields four kinds of haemoglobin, Hb-A ($\alpha_2^A\beta_2^A$), and α-chain variant ($\alpha_2^X\beta_2^A$), a β-chain variant ($\alpha_2^A\beta_2^Y$) and the doubly abnormal type ($\alpha_2^X\beta_2^Y$). If the mobility of the last-named is the same as Hb-A only three zones are separated in electrophoresis.

If the phenotype of orang No. 8 was produced in this way the middle zone should contain both Hb-A^{OR} together with a haemoglobin with variant α- and β-chains. Treatment of this zone by the hybridization procedure should yield the fast and slow variants and this was found to be so (Fig. 2*a*, *b*). Similarly the isolated fast and slow zones when mixed and hybridized should yield the middle zone, which in fact they do (Fig. 2*c*, *d*).

The slow zone of orang No. 8 is indistinguishable electrophoretically from the component, Hb-B^{OR}, of five other animals. It is a reasonable assumption that the slow haemoglobin in all these animals is the same, although this could only be established by structural investigations.

It remains to determine whether Hb-B^{OR} differs from Hb-A^{OR} in the α- or β-chain. Hb-A^{OR}, unlike human Hb-A, forms no separable hybrids with Hb-canine[4], but two hybrids, respectively slower and faster than the

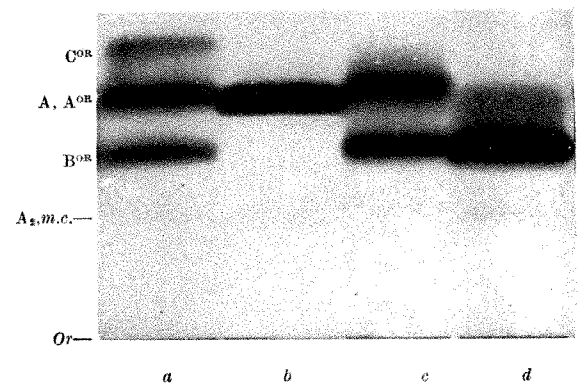


Fig. 1. *Or*, Origin; +, indicates anode. Some haemoglobin phenotypes of the orang. *o*-Tolidine/peroxide stain. (*a*), No. 8: three major Hb zones and slow minor component (*m.c.*); (*b*), human Hb-A and Hb-A_2; (*c*), Hb-A^{OR}, Hb-B^{OR} and slow minor component; (*d*), Hb-B^{OR} and slow minor component

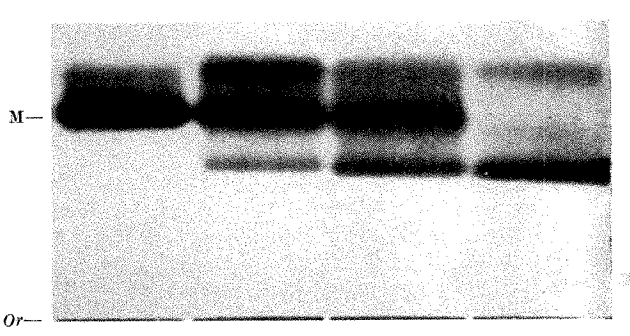


Fig. 2. Recombination of major zones from orang No. 8. *o*-Tolidine/peroxide stain. (*a*), Middle component (M); (*b*), middle component after hybridization treatment; (*c*), fast and slow components after hybridization treatment; (*d*), fast and slow components

parent species, are seen when it is recombined with human Hb-A (Fig. 3*b*). The more anodal of these hybrids was shown to have the same mobility as the single hybrid zone formed when Hb-A^{OR} is recombined with human Hb-H (β_4) and is therefore $\alpha_2^{A or}\beta_2^A$ (Fig. 3*a*). No separable hybrids were obtained when Hb-B^{OR} from two different animals and also the slow zone from No. 8 were recombined with Hb-A, suggesting that the α-chains of Hb-B^{OR} is less negatively charged than that of Hb-A^{OR} so that the hybrid $\alpha_2^{B or}\beta_2^A$ has the same mobility as Hb-A. Hb-B^{OR} also yielded no separable hybrids with human Hb-C, a slow β-chain variant, but gave two hybrid zones respectively faster and slower than the parent haemoglobins with Hb-canine. These results are consistent with Hb-B^{OR} having a variant α-chain. The fast zone from No. 8, which we will call Hb-C^{OR}, forms no separable hybrids with Hb-A or Hb-canine but yields two hybrid zones ($\alpha_2^A\beta_2^{Cor}$ and $\alpha_2^{A or}\beta_2^C$) lying between the parent haemoglobins, with Hb-C. These findings are consistent with Hb-C^{OR} having a variant β-chain.

Orang No. 8 and also those animals carrying Hb-B^{OR} have a minor component slower than that in animals with Hb-A^{OR} alone. This is explicable if the minor component of the orang like human Hb-A_2 is composed of an α-chain combined with another chain comparable with the δ-chain of man, though not necessarily the same in composition. Animals with Hb-B^{OR} would then be expected to form *in vivo* a minor component containing the variant α-chain, as happens with certain human α-chain variants such as Hb-G_α^5. In No. 8 and in heterozygotes also synthesizing normal α-chains the faster type of minor component would also be present but masked by Hb-B^{OR}.

The minor component was isolated from an animal with only Hb-A^{OR} as a major component by elution from a carboxymethyl cellulose column followed by electrophoresis on a maize starch block[5]. Equal volumes of this purified material (2 per cent) and human Hb-H (8 per cent) were then mixed and dialysed at *p*H 4·7 for 24 h.

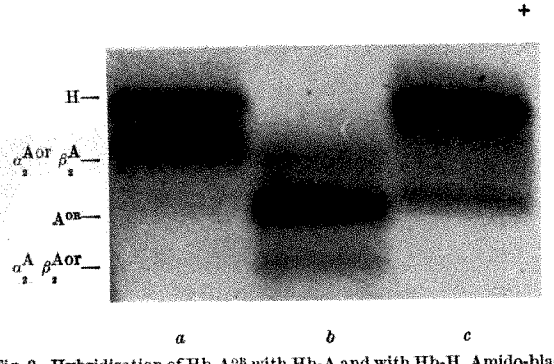


Fig. 3. Hybridization of Hb-A^{OR} with Hb-A and with Hb-H. Amido-black stain. (*a*), Hb-A^{OR} and Hb-H after hybridization; (*b*), Hb-A^{OR} and Hb-A after hybridization; (*c*), Hb-A^{OR} and Hb-H

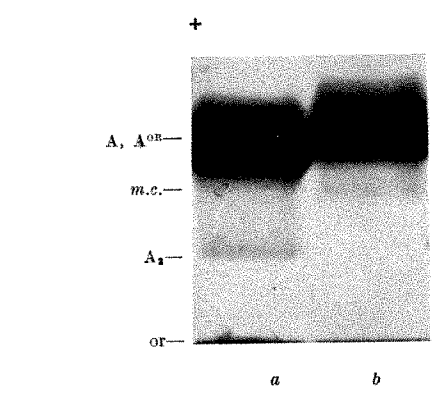


Fig. 4. (*a*), Hb-A and Hb-A_2; (*b*), Hb-A^{OR} and fast minor component (*m.c.*). *o*-Tolidine/peroxide stain

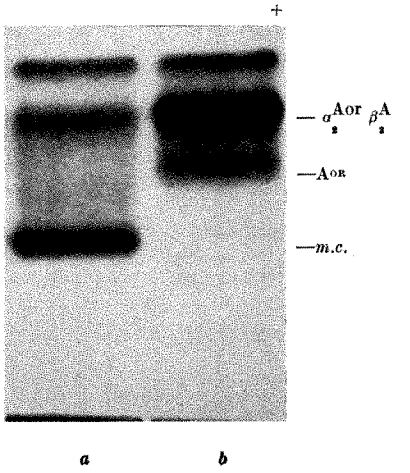


Fig. 5. (*a*), Minor component (fast type) after hybridization with Hb-H; (*b*), Hb-A^{OR} after hybridization with Hb-H. *o*-Tolidine/peroxide stain

After redialysis to *p*H 6·9 this material was compared on starch-gel with $Hb\text{-}A_{OR}$ mixed with Hb-H and treated in the same way (Fig. 5). The minor component with Hb-H yielded a hybrid zone of the same mobility as that formed by Hb-A^{OR} with Hb-H, suggesting that its α-chains are the same as those of Hb-A^{OR}.

It has been reported[6,7] that a minor component occurs in the chimpanzee and gorilla. We have found that a minor component very similar to human Hb-A_2 in mobility and concentration was present in every one of forty-two chimpanzees from the Yerkes collection and in one gorilla from this source. On the other hand, we have not found a minor component in specimens from several species of *Papio, Macaca mulatta M. cyclopis, M. speciosa, M. sinica, M.* (*Cynopithecus*) *nigra*[8], *Erythrocebus patas* and *Cercopithecus aethiops*. In *M. irus*, however, a minor component occurred in some 20 per cent of more than 200 animals. In contrast to human Hb-A_2 and, it appears, to the minor component of the orang, it contained a variant chain combined with a normal β-chain[9].

The high incidence of haemoglobin variants in this small sample of orangs is remarkable. The haemoglobin phenotypes of two other orangs (one from Borneo and one from Sumatra) have been reported[7] and each was considered to have two major components; however, comparison of the illustrations with our own material suggests that one had Hb-A^{OR} and a minor component and the other Hb-B^{OR} alone.

Unfortunately, we have very little information about the histories of the animals on which we have worked, but they were obtained at various times from dealers[10] and it seems unlikely that they are closely related animals from a restricted locality. Though it would be rash to regard them as a representative sample of the species, our results suggest that the variant Hb-B^{OR} may be very common in the orang. The incidence of cases like No. 8 will depend on the frequencies of the α- and β-chain variants involved, provided that such phenotypes are not subject to strong selection, and we may infer that another variant, Hb-C^{OR}, is also quite frequent unless we prefer to regard its occurrence in our small sample as a rare event.

Very little is known of the natural history of the orang[11], but its distribution is restricted to Borneo and Sumatra and it is probably the most numerically reduced of the great apes. Its decline has doubtless been accelerated by human interference in recent times. It is said to occur singly or in small groups but since these are believed to be inconstant in composition the breeding units are probably larger. Nevertheless, in view of the diminished numbers of the species as a whole, the mating populations may well be small enough for loss of variability by genetic drift to be appreciable and it is therefore interesting to find evidence that haemoglobin polymorphism persists. We

have also reported[9] polymorphism of haemoglobins and several other blood proteins in another asiatic primate of restricted distribution, namely *Macaca* (*Cynopithecus*) *nigra*, which is confined to the northern peninsula of Celebes.

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The Quantitative Relationship of the Rh-like (LW) and D Antigens of Human Erythrocytes

PREVIOUS investigations have shown that the injection of human D-positive and negative, rhesus and other monkey red cells into guinea-pigs produces an Rh-like (LW) antibody which reacts more strongly with D-positive than D-negative red cells. The Rh-like (LW) antigen is absent from Rh_{null} (– – –/– – –) red cells and also from the blood of several unusual persons with apparently normal Rh antigens. Some of the latter individuals have produced LW antibodies which resembled those induced in guinea pigs by immunization with human and monkey red cells.

Several investigators[1,2] have reported that D positive red cells from a variety of genotypes, including the –D–/–D– cell with its exceptionally strong D antigen, give essentially the same titration score with human and guinea-pig anti-LW sera. Regis and Yunis[3], however, have recently reported that the LW reactivity of human erythrocytes with guinea-pig and anti-human red cell sera appears to decrease in the following order: cDE/cDE > cDe/cde > CDe/CDe > cde/cde. Gibbs and Rosenfield[4] also observed with hand titrations of a guinea-pig anti-rhesus reagent that the order of reactivity of the LW antigens of eight siblings of a family (Pr) appears to be the same as that of their D antigens: CDe/cde > CDe/CDe > cD^uE/cde > cD^uE/CDe. In view of the importance of these observations to the elucidation of the relationship of LW to the Rh system, it was of interest to confirm these findings with quantitative procedures.

The results of our preliminary investigations to determine whether there is a relationship between the LW and D activities of human erythrocytes are shown in Table 1. The bloods of the mother and a sibling of the Caucasian Rh_{null} propositus (Mrs. L. M.) were included in this study. Levine *et al.*[1] have reported that the Rh antigens of these individuals are abnormally weak but their LW antigens give normal indirect Coombs titration scores with human anti-LW serum. The 50 per cent endpoint titres listed in the table were obtained by the log-probit assay method of Wilkie and Becker[5] using precisely standardized suspensions of test cells. Antiserum-cell mixtures were equilibrated by the aggregation method (without centrifugation) with an incubation period of 3 h at room temperature for the guinea-pig sera and at 37° C for human sera. With all cells tested, the slopes of the anti-LW assay curves were parallel. The titres of the anti-LW sera correlated with the anti-D titres for all Rh genotypes tested. The human anti-LW serum gave the most distinct differentiation (16·7-fold difference between cDE/cDE and CDe/Cde cells); the guinea-pig anti-baboon serum proved the least effective, giving only a three-fold difference between the strongest and weakest D cells.

Table 1. 50 PER CENT ENDPOINT TITRES* OF ANTI-LW SERA WITH HUMAN RED CELLS OF VARIOUS GENOTPYES

	Guinea-pig sera†		Human sera	
	Anti-rhesus RBC (1:)	Anti-baboon RBC (1:)	Anti-LW (1:)	Anti-D (1:)
cDE/cDE	10·4	370	67	2,000
cDE/cde	5·9	250	N.T.	975
CDe/cDE	5·9	255	45	1,055
CDe/cde(FC)	5·0	245	16	333
CDe/cde(JB)	N.T.	N.T.	12	202
CDe/cde(II-10)	4·1	178	9·1	109
CDe/cde(III-2)†	3·8	185	8·3	111
CDe/Cde	1·5	128	4·0	12
cde/cde	negative	9	negative	negative

* 50 per cent endpoint titre = $1/HD_{50}$.
† Guinea-pig sera were absorbed with normal cde/cde cells.
‡ Individuals II-10 and III-2 are the mother and sibling of the Rh_{null} propositus, Mrs. E. M.

The quantitative relationship between the amount of D antigen on the cell and LW activity, indicated by the data, provides strong evidence that the specificities of LW antibodies are complementary to some portion of the structural configuration of D determinants. This is consistent with the observation that blocking D sites with incomplete anti-D does not reduce the LW reactions of the cell[6]. The parallelism of anti-LW assay curves found with cells of Rh genotypes which give assay curves of highly diversified slopes with anti-D sera[7] also suggests that LW and D antibodies are not attaching to identical sites on the D structure.

The observation that the mother and sibling of the Rh_{null} propositus have weak LW reactions comparable to their weak D reactions does not agree with the findings of Levine *et al.*[1] that their LW antigens are as strongly reactive as those of normal CDe/cde control cells. This is an important observation since quantitative studies[8] have confirmed that the D expression of these two family members have been suppressed, presumably by the same genetic mechanism which has caused the Rh_{null} condition of the propositus. The fact that the LW reactivities of these individuals are diminished concomitant with the suppression of their D reactivities is strong supportive evidence that LW antibodies are reacting with the D determinants of red cells.

In view of the above findings and the fact that Rh_{null} bloods, which are devoid of all detectable Rh substances, also lack LW antigens, the possibility that LW determinants may be associated with a portion of the Rh substance common to all normal Rh molecules should be re-examined. This theoretical possibility, suggested by Murray and Clark[9] as early as 1952, is attractive, since it provides reasonable explanations of all findings. The occurrence of LW negative individuals with normal Rh antigens, for example, can be explained by the absence of the LW determinants of the Rh molecule analogous to the absence of determinants from the Rh substances of –D–/–D–, cD–/cD– and C^wC–/C^wD– genotypes. The multitude of variants of Rh antigens already documented makes it most probable that some genetic alteration(s) exist which affect chemical groupings on Rh substance that are not directly involved in the C, D, E, c and e specificities of the molecule. The argument could be raised in opposition that the absence or modification of LW determinants on Rh molecules would not account for the LW negative phenotypes of D positive individuals if LW antibodies react with D determinants. The simplest explanation is that the chemical groupings on the large D structure which confer LW complementarity are absent in these individuals or their linkage to the D structure differs and the spatial orientation and geometry differ in consequence. The postulation that LW determinants are associated with Rh substance implies that LW is a product of Rh genes. However, by analogy with the presence of the products of independent Lewis genes

on A and B substances, these determinants could be the expression of independent LW genes.

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Variation of Red Cell Acid Phosphatase in Two Species of Kangaroos

INHERITED variation has been recently demonstrated in the transferrin of an Australian marsupial, *Macropus rufus* (=*Megaleia rufa*)[1]. So far no other variation has been shown in the blood of this or other marsupials. The present communication reports the investigation of red cell acid phosphatase in two species of Australian marsupials, *M. rufus* and *M. giganteus*, the former commonly known as the red kangaroo and the latter as the grey kangaroo.

The animals used in the present study were kept in captivity at the C.S.I.R.O. Division of Wildlife Research, Canberra. Blood was collected by puncture of the caudal vein. The method for determining acid phosphatase patterns was that described by Hopkinson, Spencer and Harris[2] with minor modifications. The haemolysates were prepared by macerating clotted blood, washing the resultant cells three times with physiological saline and lysing them with an equal volume of distilled water. The haemolysates were stored at $-15°$ C until use. Horizontal starch-gel electrophoresis using thick filter paper inserts (Whatman No. 17) was carried out with a potential gradient of 5·5 – 6·0 V/cm for 16–17 h at $+5°$ C and at *p*H 6·0 (gel buffer: 0·005 M succinic acid and 0·0092 M *tris*, bridge buffer: 0·41 M citric acid/sodium hydroxide). The gels were prepared with an 8·5 per cent starch concentration rather than with the 9·7 per cent recommended by the manufacturers (Connaught Laboratory hydrolysed starch, lot 209–1). After electrophoresis the gels were sliced horizontally and placed in flat glass dishes with covers. The cut surfaces were then covered with a solution of 0·005 M phenolphthalein diphosphate in 0·05 M citrate buffer at *p*H 6·0 and incubated for 2–3 h at 37° C. After incubation the reagent was decanted and about 3 ml. of concentrated ammonium hydroxide poured over each gel. Red zones appeared in areas where phenolphthalein had been liberated from phenolphthalein diphosphate by acid phosphatase.

Twenty-six out of fifty-four red kangaroos showed a pattern which is tentatively called the 'K' type (Fig. 1, 1). This consisted of two components, and the faster was slightly smaller than the slower component. Both were oval in shape. Eighteen other animals showed the 'K' pattern but with an additional small zone of acid phosphatase which was found to be moving ahead of the faster component (Fig. 1, 2). This zone is probably due to some change in the protein during storage because it was not demonstrated in haemolysates of freshly collected blood samples from three of the animals that had previously shown it. Eight of the remaining ten red kangaroos showed a pattern different from the 'K' type. In this pattern two components migrating at the same rate as the components found in 'K' type were present but the faster was smaller and less intensely stained than the slower moving component (Fig. 1, 3). However, this changed pattern could not be reproduced in the haemolysates of freshly collected blood samples from two of the ten animals but instead a normal pattern of the 'K' type was observed. This seems to suggest that the alteration in pattern is probably due to changes during storage. No phosphatase activity was detected in the red cells of the remaining two kangaroos.

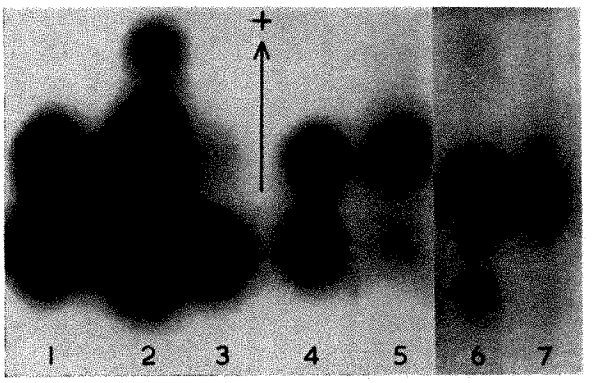


Fig. 1. Red cell acid phosphatase types found in red and grey kangaroos and some of the patterns found in man. The types are from left to right: 1, 'K'; 2, altered 'K'; 3, altered 'K'; 4, 'G'; 5, altered 'G'; 6, human B; 7, human BA

Eighteen grey kangaroos were examined and twelve showed a different pattern which is tentatively called the 'G' type (Fig. 1, 4). The two components in this type were of the same staining intensity and migration rate as the components of the 'K' type. However, the components were usually of equal size and were round in shape. Two grey kangaroos showed the typical 'K' type pattern and four showed an atypical 'G' type. In this atypical pattern there were two components with mobilities similar to the 'G' type components but the slower was less intensely stained than the faster moving component (Fig. 1, 5). This is also probably due to changes during storage and was not observed in two animals from which fresh samples were obtained.

The present work has shown difference in the pattern of red cell acid phosphatase in the two species of Macropods investigated. No evidence of polymorphism was found in the red species but the pattern was usually different from that of the grey species. Two distinct patterns were found in the grey animals and they are believed to represent a true polymorphism. Unfortunately, it was not possible to test this hypothesis because an insufficient number of animals with known pedigrees was examined.

The nature of the changes in electrophoretic mobility resulting from storage in a number of samples is being investigated. It is of interest that in the red species the fast component was presumably unstable and yielded a third area with enzymatic activity. In the grey species the slow component appeared to have deteriorated during storage.

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Fibrinogen as a Co-factor in the Reaction of Platelets with Kaolin

WE have shown[1] that the clotting time of platelet-rich plasma (PRP) is progressively shortened by incubation at 37° C with a suspension of kaolin, and that this reflects a progressive increase in availability of platelet factor 3 (PF3) as well as activation of the plasma clotting system. Since maximal acceleration of clotting by kaolin was only achieved when platelets and plasma were activated together, it seemed likely that a plasma component was required for the reaction of platelets with kaolin. In order to determine whether a plasma coagulation factor was concerned in this reaction, investigations were carried out on the PRP of patients with a series of congenital coagulation defects. Equal volumes (0·1 ml.) of PRP and kaolin (5 mg per ml. of buffered saline, *p*H 7·3) were incubated at 37° C for 20 min and then recalcified with 0·1 ml. of 0·025 M calcium chloride, 0·1 ml. of a partially purified preparation of the deficient factor (or of buffered saline) being added in each case immediately before or after incubation with the kaolin. In the cases of factor-XII and factor-XI deficiency, activation product (prepared by the method of Nossel[2]) was used in place of the respective purified factors. Factors V and VIII were prepared by ammonium sulphate precipitation from barium sulphate-adsorbed platelet-poor oxalated normal plasma and factors VII, IX and X by adsorption of normal serum on to barium sulphate and subsequent elution into 5 per cent trisodium citrate. The fibrinogen reagent used (kindly provided by the Blood Products Laboratory of the Lister Institute) was prepared from fresh plasma by the method of Kekwick and Mackay[3] and was 66 per cent clottable; it also contained plasminogen and some factor VIII and anti-von Willebrand activity.

The results of this series of experiments are shown in Table 1, from which it can be seen that in every case, except those of fibrinogen deficiency, there was no significant difference between the degree of correction of the clotting time of PRP, whether the deficient factor was added before or after incubation with kaolin. It thus appears that the activation of PF3 by kaolin does not depend on prior activation of factors XII and XI, and is independent of all the clotting factors tested except fibrinogen. Horowitz[4], using a Stypven time system, has also found that factors V, VIII, X, XI and XII are not required for PF3 activation by celite.

In order to determine the quantitative relationship between fibrinogen and PF3 activation, PRP from the patient with a plasma fibrinogen concentration of 15 mg per 100 ml. was incubated for 20 min with an equal volume of kaolin suspension in the presence of a series of dilutions of fibrinogen; a mixture of equal parts of fibrinogen (310 mg per 100 ml.) and 0·025 M calcium chloride was then added, so that the clotting time of the mixture was not significantly influenced by the final fibrinogen concentration, but only by that present during incubation with kaolin. The results (Fig. 1) show that PF3 availability is significantly reduced only when the fibrinogen concentration falls below about 30 mg per 100 ml. This is in close agreement with the fibrinogen concentration of 20 mg per 100 ml. which Caen *et al.*[6], in investigations on the same patient, found necessary for spontaneous platelet aggregation and adhesion to glass.

Table 1. KAOLIN CLOTTING TIME OF DEFICIENT PLATELET-RICH PLASMA

Deficient factor	(Per cent)	Clotting time (sec) Deficient factor added before incubation with kaolin	Deficient factor added after incubation with kaolin	Deficient factor not added
XII	(<1)	39	36	110
XI	(<1)	38	39	97
X	(10)	39	37	65
IX	(2)	32	32	70
VIII	(<1)	39	44	107
VII	(<1)	36	33	29
V	(<1)	41	47	120
Fibrinogen (1 mg/100 ml.*)		30	62	>600
Fibrinogen (15 mg/100 ml.†)		30	77	166

* Reference 5.
† Reference 6.

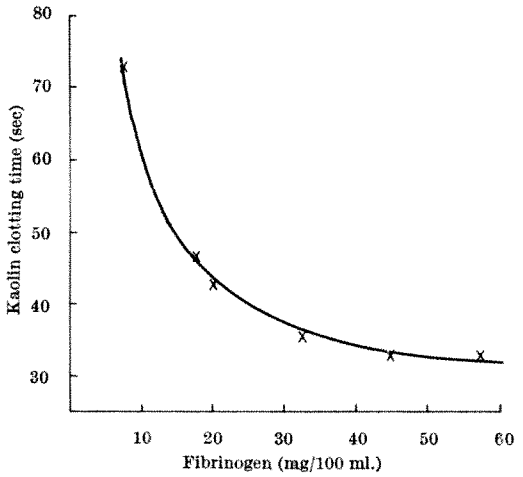


Fig. 1. Relationship between kaolin clotting time of platelet-rich plasma and fibrinogen concentration during incubation with kaolin

Evidence has also been presented[7–9] that fibrinogen is an essential co-factor for the aggregation of platelets by adenosine diphosphate (ADP). We have found that the activation of PF3 by incubation of PRP with kaolin is always associated with the formation of mixed aggregates of platelets and kaolin particles, and that both are inhibited by ADP antagonists and by EDTA, and fail to occur in thrombasthenia[1,10,11]. Platelet/kaolin aggregation also failed to occur during the investigations on afibrinogenaemic PRP reported here, unless fibrinogen was added. These findings thus suggest that PF3 availability in this system results from the adhesion of platelets to kaolin and from their aggregation, and that these reactions are dependent on the action of ADP, which here, as in other systems which have been investigated, requires fibrinogen as a co-factor.

These experiments do not, of course, resolve the question whether other plasma components besides the coagulation factors are also required for this reaction. Similar investigations on patients with von Willebrand's disease have given inconclusive results, chiefly for the reason that the kaolin clotting time of their PRP has usually differed too slightly from that of normal controls to allow the effect of plasma fractions to be properly evaluated.

We are grateful to the various physicians who have allowed us to study their patients, and particularly to Drs. J. P. Caen and E. Gugler, who also put their laboratories at our disposal. This work was supported by a grant from the Medical Research Council.

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IMMUNOLOGY

Synthesis of Antibody *in vitro* by Lymph Node Fragments using Absorbable Gelatin Sponges

It has been conclusively demonstrated that fragments of lymph nodes obtained from previously immunized rabbits can be secondarily stimulated *in vitro* with the specific antigen[1,2]. Richards *et al.*[3] and Richardson[4] have shown that the antibody released by fragments *in vitro* is actually synthesized *de novo*. In the main, glass wool pads have been used to support the fragments in the culture. Unfortunately, the use of glass wool pads impairs histological investigation of the fragments during antibody synthesis. The glass wool cannot be sectioned on a microtome so that any fragment studied must be removed from the pad, resulting in loss of the periphery of the fragment which clings tightly to the glass wool. The cells which migrate from the tissue into the interstices of the pad are also not available for histological study. An ideal supporting medium for combined histological and antibody studies should be capable of being sectioned so that the cells can be observed in their relationships to each other and to the supporting medium. In order to pursue this type of investigation, we decided to investigate the possible use of absorbable gelatin sponges, as a replacement for the glass wool.

Young, adult New Zealand rabbits were immunized with rat serum albumin (RSA) (prepared by ammonium sulphate fractionation of whole rat serum, pure by the criterion of immunoelectrophoresis) and bovine serum albumin (BSA) (obtained from Pentex Incorporated, Kankakee, Illinois). The rabbits were given three intravenous injections of antigen (20 mg per injection) and three foot pad injections, bilaterally (10 mg per foot pad per injection) over a period of 10 days. All injections were made in saline. After a period of 3–4 months, during which time the serum antibody titre decreased to a low level, the rabbits were killed by intravenous administration of pentothal (50 mg per kg body weight) and the popliteal lymph nodes were excised within a period of 5 min. The nodes were trimmed of excess fat and cut into fragments of about 1–2 mm^3 in sterile Hanks's solution (obtained from Difco Laboratories, Detroit, Michigan). Six to eight fragments were placed into each Leighton tube and either a glass wool pad or absorbable gelatin sponge ('Gelfoam', Upjohn Co. of Canada, Montreal) was then overlaid. The fragments were then incubated, at room temperature for 2 h, with 0·5 mg of the antigen in 1 ml. Eagle's medium (HeLa, ten times, obtained from Difco Laboratories) containing fresh normal rabbit serum (20 per cent), glutamine (0·04 per cent), sodium bicarbonate (0·135 per cent), streptomycin (50 μg per ml.) and penicillin (100 u./ml.)[1,2]. The fragments in the control tubes were incubated with the modified Eagle's medium only. The fragments were then washed three times with aliquots of the medium and incubated with 1 ml. of medium at 37° C. The medium was changed every 3 days and tested for the presence of antibody by the tanned cell haemagglutination technique[5].

Table 2. Synthesis of Antibody to Bovine Serum Albumin *in vitro* using Glass Wool Pads and Absorbable Gelatin Sponges

Table No.	BSA incubated with fragments	Type of support used	Antibody titres* of supernatants in days of culture 6	9	12	15	18
1		Glass	320	640	320	320	40
2	–	wool	20	0	0	0	0
3			160	320	160	160	40
4		Glass	40	640	640	640	160
5	+	wool	40	320	1,280	640	80
6			40	320	640	320	160
7		Gelatin	0	0	0	0	0
8	–	sponge	0	0	0	0	0
9			0	0	0	0	0
10		Gelatin	0	0	0	20	0
11	+	sponge	0	80	320	320	40
12			0	80	160	80	80

* Titres less than 10 are consicered to be negative.

As can be seen from Tables 1 and 2, the gelatin sponges functioned at least as well as glass wool pads in the facilitation of the immune response *in vitro*. In point of fact, the sponges appeared to act in a more discriminate fashion in the presence of BSA (Table 2) since the control tubes with gelatin sponges contained no detectable antibody whereas the control tubes with glass wool pads invariably contained antibody, but in much lower titre than the experimental tubes.

In summary, it has been demonstrated that absorbable gelatin sponges can replace glass wool pads in the maintenance of antibody-forming lymph node fragments *in vitro* without any adverse effect in so far as the peak titre of antibody formation is concerned or with respect to the temporal aspects of the *in vitro* antibody formation curve. The utilization of gelatin sponges should facilitate histological examination of the fragments since they can easily be fixed and sectioned, properties which are not possessed by glass wool.

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Cytotoxic Effects of Antisera against Dye–Protein Complexes

It has been shown in rabbits that antibodies are formed following immunization with dye–protein complexes. These antibodies react with stained cells and reveal a cytotoxic activity in the presence of active complement[1,2]. Sulphorhodamine *B* was coupled to bovine albumin according to Uehleke[3]. Rabbits were immunized with this dye–protein complex and an antiserum (DPC-antiserum)

Table 1. Synthesis of Antibody to Rat Serum Albumin *in vitro* using Glass Wool Pads and Absorbable Gelatin Sponges

Tube No.	RSA incubated with fragments	Support used	Antibody titres* of supernatants in days of culture 6	9	12	15	18	21	24	27	30	33
1		Glass wool	1,280	1,280	1,280	640	160	160	0			
2	–		1,280	1,280	640	320	40	20	0			
3			640	640	160	80	20	0	0			
4		Glass wool	5,000	10,000	5,000	5,000	1,280	640	320	160	40	0
5	+		5,000	10,000	5,000	2,560	640	320	160	160	20	0
6			2,500	5,000	2,560	1,280	320	160	20	0	0	0
7		Gelatin sponge	80	80	80	40	40	40	0			
8	–		80	80	80	40	40	20	0			
9			40	80	80	40	20	0	0			
10		Gelatin sponge	320	5,000	10,000	5,000	5,000	5,000	5,000	640		0
11	+		320	5,000	10,000	5,000	5,000	5,000	2,560	640		0
12			0	5,000	5,000	1,280	1,280			Contaminated		

* Antibody titres less than 10 are considered to be negative.

was obtained which gave a positive reaction in Landsteiner's inhibition test. Cytotoxic activity was assayed in different systems.

(1) Sulphorhodamine *B*-stained cells of Ehrlich's ascites tumour (stained EAC) of the mouse; addition of DPC-antiserum to stained EAC produces cytotoxic changes[1,2]. A similar effect is obtained by addition of specific antisera against EAC[1]. Normal rabbit's serum does not reveal such activity.

(2) Solid Ehrlich's tumours stained intravitally with sulphorhodamine *B*; characteristic necroses occur after intratumoral injection of DPC-antiserum. These necroses differ significantly from spontaneous necroses[1,2]. In this assay normal rabbit's serum is inactive.

(3) Cytotoxic effects of DPC-antisera on stained HeLa cells in tissue culture; HeLa cells were cultivated in bottle cultures (medium: Gey's solution with addition of 10 per cent cord serum, 0·25 per cent lactalbumin, 'neomycin' and penicillin) and transplanted in bottles with mounted cover-slides. Staining of cells was performed by adding 1 ml. of sulphorhodamine *B* (1 : 5,000) to 9 ml. of culture medium. 24 h later, the cultures were washed with Gey's solution. In the assay, 1 ml. Ringer's solution (zero-control), 0·5 ml. DPC-antiserum and 0·5 ml. active complement or 0·5 ml. normal rabbit serum and 0·5 ml. active complement, respectively, were added to 9 ml. of the culture medium. The changes of the cultures were regularly controlled. At different intervals cover-slides were withdrawn, fixed in alcohol and stained with iron-haematoxylin. Fig. 1 shows HeLa cells stained with sulphorhodamine *B* after addition of normal rabbit serum. Fig. 2 shows the same cells after addition of DPC-antiserum. A comparable cytotoxic effect is produced by addition of specific anti-HeLa cell serum prepared according to McAllister *et al.*[5].

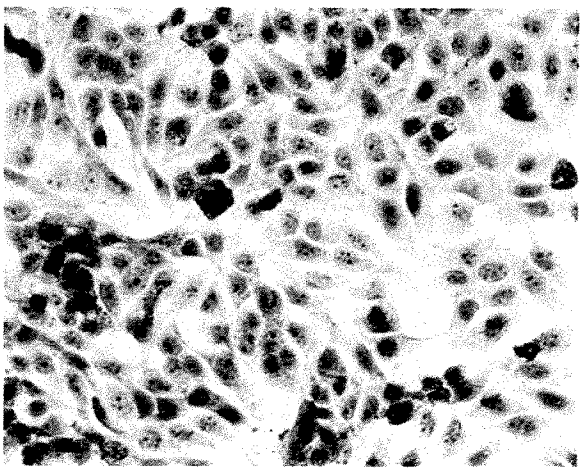


Fig. 1. Effect of normal rabbit's serum and active complement on sulphorhodamine *B*-stained HeLa cells in tissue culture (4 h)

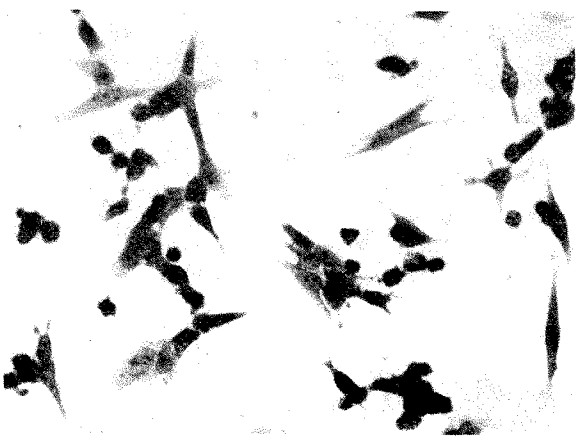


Fig. 2. Effect of DPC-antiserum and active complement on sulphorhodamine *B*-stained HeLa cells in tissue culture (4 h after addition)

The results demonstrate the possibility of producing a cytotoxic effect with a non-cytotoxic serum and a non-toxic dye as a hapten. It is necessary, however, that the cells have bound the dye and that the serum contains antibodies against this component.

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HISTOCHEMISTRY

Histochemical Observations on the Lipids of Sound Human Dentine

EVIDENCE for the presence of lipids in sound human dentine has indicated that they are present in the dentinal tubules[1], though, following an unmasking treatment with hot pyridine, they also have been observed in the matrix, at the junction of dentine and predentine[2]. This communication reports on the use of other unmasking techniques, combined with various histochemical stains, to investigate further the lipid components of this tissue.

Undecalcified ground sections of dentine, approximately 7μ thick, were prepared from fresh and fixed non-carious human molar and pre-molar teeth. Fixation was in 10 per cent neutral formol saline, or 7 per cent ethyl alcohol. Sections were stained, without previous treatment, or following one of a series of progressively intense unmasking techniques. These consisted of 0·02 N hydrochloric acid alone, or followed by water at 20° C, 60° C or 100° C, or alternatively 0·02 per cent acetic or trichloracetic acid at 60° C or 100° C, each for wide ranges of time. Following etching in 0·02 N hydrochloric acid at 20° C for 1–2 min, sections were also treated with 1 per cent sodium ricinoleate, 0·1 per cent trypsin, or chymotrypsin, 1 mg/ml. collagenase, hyaluronidase or phospholipase *D*, each in the appropriate buffer, at 37° C for 2–4 h, then washed in running water at 20° C for 24 h (ref. 3). Following these unmasking procedures the sections were stained by one of the following methods: acetone-Sudan black *B*[3], acetone-acetylated Sudan black *B*[3], osmium tetroxide vapour[4], Nile blue sulphate[5], phosphomolybdic acid[6], or Luxol fast blue[7]. Some sections, stained with Sudan black *B*, were extracted with many different solvents at 20° C and 60° C, and also in 3 : 1 methanol : chloroform, in a Soxhlet extractor, for periods up to a hundred days, then restained. Similar stained sections were also decalcified and mounted in a watery medium, or alternatively dehydrated and mounted in balsam. Contact microradiographs were obtained of some sections prior to staining, to relate the development of calcification to the staining results, and comparable sections were incinerated in air at 600° C.

In untreated sections, only the contents of the dentinal tubules and any uncalcified peritubules matrix around them; stained by these methods, however, progressively more intense unmasking, from brief etching to boiling acetic acid (8 min), revealed a changing picture. The peritubular dentine matrix (that is, any matrix arranged

around, as distinct from between, the tubules), adjacent to the predentine, was the first to stain heavily, and as the intensity of the unmasking was increased, so the peritubular matrix in the more peripheral dentine was stained. However, a zone adjacent to the enamel failed to stain under all circumstances. With more prolonged and intense unmasking, the zone adjacent to the predentine, which had stained in the first instance, failed now to do so Thus, including the predentine, four zones became discernible, numbered 1 to 4, from the pulp. Zone 3 was reduced in size in radicular dentine, so that in the apical third it was absent, and in the middle third it was seen, in transverse sections, as two crescents, labial and lingual. The junction between zones 2 and 3 was seen to coincide with the calcification of the peritubular matrix, which then became hypercalcified, as seen on the microradiographs. The very intimate relationship between the organic and inorganic fractions of the dentine matrix was illustrated in incinerated sections where carbon (identified by electron-spin resonance) was retained in the hypercalcified peritubular matrix of zone 3, and mirrored in part the appearance of a stained section.

The extraction of the lipids from the dentine matrix was extremely difficult, 75–100 days' extraction being necessary to prevent subsequent restaining. The soap and enzymes employed failed to unmask the lipids under these conditions, and complete decalcification of a dentine section resulted in subsequent diffuse and faint staining. When an undecalcified section was stained satisfactorily with Sudan black *B*, then decalcified and mounted in a watery medium, apart from some shrinkage little change was observed. However, after passing such a section through alcohol and xylene, and mounting in balsam, all the stain was removed other than traces around the tubules.

The positive results obtained with the stains employed indicate the presence of choline-containing phospholipids (probably lecithins) in peritubular dentine matrix. These are in such intimate relationship with both the organic and inorganic fractions that some disorganization of the tissue is necessary to unmask these elements. However, more intense treatment such as complete decalcification results in their loss. For these reasons dentine sections have been stained as thin hard sections, the surfaces of which have been disorganized to some degree, while the centre acts as a reservoir for the components.

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RADIOBIOLOGY

Are Iodotyrosines Normal Constituents of Plasma?

The question of whether or not the iodotyrosines exist normally in the circulation has been the subject of considerable experiment and discussion during the past seven years, beginning with a report by Werner and Block[1] that they detected iodine in the iodotyrosine areas of chromatograms of serum from human subjects. Some subsequent investigations have provided supporting evidence while others have not[2–6]. Experimental evidence has not led to a satisfactory answer to the basic question because the concentrations of circulating iodine-containing substances are so low and a variety of artefacts may arise as a result of manipulations during the analysis[7]. In addition, not all assumptions inherent in present methods have been subject to direct substantiation. The extent of breakdown of iodotyrosines prior to analysis is unknown; added tracers may not behave as the native substances due to differences in protein binding; and these substances may not be principally contained in the plasma, which has been the only fraction of blood investigated by most research workers. Consequently, the conclusion accepted at present is only conditional: if mono- and di-iodotyrosine are normally present, they are probably protein bound and do not occur in significant amounts as free substances in normal plasma samples[8].

Thermal neutron activation analysis[9,10] or mass analysis[11] of iodine-129 increases the sensitivity of iodine analysis by a factor of at least a thousand over other methods. Replacing the natural iodine of the body with this isotope allows applications of this increased sensitivity of detection to the quantitation of iodine in biological samples[12]. It appeared feasible to study extrathyroidal iodine metabolism with the same analytical power previously used in the investigations of intrathyroidal iodine metabolism because this increase in sensitivity approximates the ratio of thyroidal to extrathyroidal iodine concentration. Our first step was to attempt to measure the circulating iodotyrosines.

Rats were maintained for 3–6 months on a diet which supplied daily 5–7 μg of iodine, 86 per cent of which was iodine-129. Samples were obtained by pooling the plasma of six such rats. 5 or 10 ml. portions were taken for analysis. Iodine-131-labelled carriers of mono- and di-iodotyrosine, triiodothyronine and thyroxine (hereafter referred to, respectively, as MIT, DIT, T-3 and T-4) were added to the portions. The iodoamino-acids were then extracted into hot *n*-butanol, concentrated, and chromatographed in 2-butanol–3 per cent ammonia (3 : 1) by the procedure used by Block *et al.*[13]. The separated iodoamino-acids were identified by autoradiography. Each spot was removed from the chromatograph and rechromatographed in a second solvent—*n*-butanol–dioxane–3 N ammonium hydroxide (4 : 1 : 4)—to assure the purity of the specific iodo compounds. The spots from these paper chromatograms which contained the doubly purified substances were placed in quartz vials, sealed under vacuum, and irradiated for 16 h in a thermal neutron flux of approximately 10^{13} n/cm²/sec. The vials were then broken and dropped with their contents into a few millilitres of 0·5 per cent sodium hydroxide containing 20 mg of carrier iodide. The radiation damage to the chromatographic paper made it soluble in the dilute sodium hydroxide. The iodine was separated by the distillation procedure of Bowen[14] and any residual activity due to radiobromine removed by extraction with hydroxylamine according to the procedure of Cosgrove *et al.*[15]. The iodide thus purified was precipitated as silver iodide and counted in a 3 in. × 3 in. thallium-activated sodium iodide crystal. The γ-ray spectrum and the half-life of each sample were determined to assure that radiochemical purity had been achieved. After the iodine-130 activity had decayed, the iodine-131 activity was compared with the activity of the original carrier chromatograms. This allowed for the calculation of an overall yield of the procedure for each sample. Standards of iodine-129-labelled DIT were analysed at the same time.

Control experiments consisted of adding labelled iodide, or one of the labelled iodoamino-acids, together with non-labelled carriers of the other iodoamino-acids and processing the plasma by the foregoing procedure. Some control experiments were done without the addition of the non-labelled carriers. The distribution of label on these was determined. We found no evidence of exchange reactions or of coupling of the iodotyrosines to form thyronines or of breakdown of the thyronines into iodotyrosines. Some deiodination occurred during chromatography, but this was corrected for in the yield calculation. The

alteration of the tracer activity due to the neutron exposure was less than 1 per cent.

The results are summarized in Table 1. MIT and DIT iodine was found in all samples analysed. The amount ranged from 0·006 to 0·01 μg per 100 c.c. of pooled rat plasma samples. This was equal to between 1 and 2 per cent of the value obtained for thyroxine iodine.

Table 1. IODOAMINO-ACID COMPOSITION OF RAT PLASMA DETERMINED BY NEUTRON ACTIVATION ANALYSIS OF IODINE-129 IN PAPER CHROMATOGRAMS OF THE *n*-BUTANOL EXTRACT OF POOLED PLASMA FROM RATS IN ISOTOPIC EQUILIBRIUM WITH THIS ISOTOPE

Sample	Micrograms iodine per 100 c.c. plasma Pooled sample 1 (6 rats)	 Pooled sample 2 (6 rats)
MIT	0 008	0·006
DIT	0 007	0·010
T-3	0·177	0 065
T-4	0 916	0·999
Total	1·108	1·080

Since both iodine-129 and iodine-131 are fission products, we had to assume that the iodine-131 carriers might be contaminated with iodine-129 as this would result in a significant blank. Due to the great differences in half-lives of iodine-129 and iodine-131, the relative amount of iodine-129 would increase as the carrier solutions aged. 5 ml. portions of plasma from individual rats were processed after the carrier solutions had aged 28 days. Blank samples for the activation analysis procedure were prepared by adding iodine-13-labelled carrier to the areas just above and below the MIT and DIT spots on the final paper chromatograms. Because of the age of the carriers and the decreased volume of plasma (5 ml. compared with 10 ml.), the blank would contain a relatively greater amount of iodine-129 than in the experimental runs. The results are summarized in Table 2. In all three control experiments, the iodine-129 content of the iodotyrosine spot was greater than either of the two adjacent control areas.

Table 2

Sample	Micrograms iodine per 100 c.c. plasma
Rat 1, 5 c.c. plasma	
Blank (above)	0·001
MIT	0·004
Blank (below)	0·001
Rat 2, 5 c.c. plasma	
Blank (above)	0 003
MIT	0 008
Blank (below)	0·001
Rat 3, 5 c.c. plasma	
Blank (above)	0·001
DIT	0·006
Blank (below)	0·001

The iodine-131 used contained a trace of iodine-129 which resulted in a non-zero blank. By adding the iodine-131 labelled carrier to the areas just above and below the MIT and DIT spots on the final paper chromatograms and analysing for iodine-129, it was shown that MIT and DIT iodine was greater than the blank.

We have concluded that the iodotyrosines are normally present in the circulation of the rat. Because blank values were not subtracted from the original values, the results reported may contain as much as a 10 per cent positive error. Substitution of iodine-125 which is not fission produced as the tracer and other alterations of the procedure are in progress which should allow even more precise quantitation of these substances. Some experimental evidence has been obtained which suggests that the iodotyrosines concentrations in plasma may decrease with time after the samples have been removed from the animals. Also we have found, by iodine-125 isotopic equilibrium studies, that a significant portion of the circulating iodine is contained in the non-plasma portion of the blood.

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Inhibition of Oxygen Consumption of Liver Homogenates from Mice injected with Radioprotectors in the Presence of Pyruvate

FOLLOWING the working hypothesis put forward by Bacq and Alexander[1] according to which the 'chemical shock' induced by the injection of radioprotectors, at radioprotective dose, must have some importance in the mechanisms of action of these substances, we have measured the oxygen consumption of liver homogenates of mice injected with a dose of radioprotector when pyruvate was offered as substrate.

Preliminary experiments by one of us[2] have shown that after intravenous injection of cystamine (2 hydrochloric acid, 170 mg/kg), the largest decrease of oxygen uptake was observed with livers from mice killed 20 min after the injection (Table 1). There is no question about the nature of the metabolic upset: the decreased pyruvate utilization with accumulation of ketoacids points to an inhibition at the first metabolic step.

The following facts confirm and extend these findings. (*a*) The inhibition is already evident in the animal 10 min after an intraperitoneal injection of cystamine (Table 1). (*b*) The intravenous injection of mercaptoethanol (156 mg/kg) does not result in inhibition of respiration in the same conditions when the liver is taken 20 min after injection. Mercaptoethanol has no radioprotective action[3]. (*c*) This inhibition of oxygen consumption in the presence of pyruvate is not a specific action of sulphur-containing radioprotectors since the same phenomenon has been observed with livers of mice killed 20 min after

Table 1. OXYGEN CONSUMPTION (μl./h/10 mg DRY WEIGHT) BY SWISS MOUSE LIVER HOMOGENATE IN PRESENCE OF 50 μMOLES OF PYRUVATE
Effect of cystamine (2 hydrochloric acid, 170 mg/kg)

Controls	Time between intravenous injection and killing				Controls	Time between intraperitoneal injection and killing	
	3 min	10 min	20 min	30 min		3 min	10 min
21·4 ± 2·24	11·3 ± 2·33 0·02 > *P* > 0·01	15·2 ± 1·5 0·05 > *P* > 0·02	13·7 ± 1·4 *P* < 0·01	13·8 ± 2 01 0·02 > *P* > 0·01	20·66 ± 1·44	19·6 ± 1·60	13·7 ± 3·6 0·02 > *P* > 0·01

The main cavity of each Warburg vessel contained 2 8 ml. of a 1 : 10 (w/v) liver homogenate in the following medium: sodium chloride, 130 mM; potassium chloride, 12·8 mM; magnesium sulphate, 0 65 mM; phosphate buffer *p*H 7·4, 5 mM; the side-arm of each vessel contained either 0·2 ml. (50 μmoles) of a pyruvate solution or 0·2 ml. of Ringer's solution. The oxygen consumption due to pyruvate was equated to the difference between the oxygen uptake in the presence and absence of added substrate.

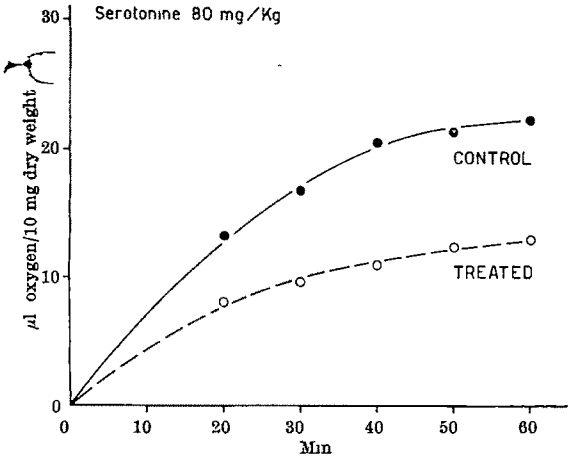


Fig. 1. Oxygen consumption of Swiss mouse liver homogenates in presence of pyruvate; same experimental conditions as in Table 1. The animals were killed 20 min after they had been injected intravenously with serotonin

injections of serotonin (80 mg/kg, Fig. 1). The radioprotective action of this amine is well known.

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BIOLOGY

Some Experiments on the Nutrition of Plants growing on Coal Mine Waste Heaps

COLONIZATION of abandoned pit heaps in Britain begins after a few years with the appearance of scattered grasses and herbs which gradually spread until eventually they form a more or less complete cover. At some stage during the development of this grass/herb community, shrubs and trees begin to colonize the heaps and these in turn spread until after a period of from thirty to a hundred years closed woodland may be formed[1].

Ecologists who have examined this process have tended to concentrate attention on the initial stages of colonization and to stress the importance of the lack of surface stability and drought as the environmental factors which control colonization (see, for example, Rees[2] and Brierley[3]). While there is no doubt that these factors are important, especially in the early stages, there is little published information concerning the role of other factors, such as nutrition, or of the factors which control the nature and productivity of the vegetation once the surface has been stabilized and other physical factors have been ameliorated. This report describes some experiments which were designed to reveal whether mineral nutrition plays any part in pit-heap ecology.

There is some indirect evidence from other sources which suggests the importance of nutrition. It has been recognized for a long time by those concerned with the reclamation of industrial wastelands that pit heaps are very infertile[4] and that it is almost impossible to establish a plant cover on heaps without adding fertilizers. Also, it has been observed that pit-heap vegetation may tend to be calcicole or calcifuge, depending on the acidity of the particular heap. This suggests that nutrition may be important because it has been shown for other situations[5-7] that calcicole and to some extent calcifuge[7] behaviour depends on nutritional factors.

Our first experiment involved a laboratory assessment of the nutritional status of a large number of contrasting pit-heap soils. In the experiment a number of typical pit-heap species were grown from seed on the different soils in a growth cabinet at 17° C. The soils were treated with different nutrient solutions and after 5 weeks the plants were harvested, dried and weighed. Every pit-heap soil we examined, including freshly exposed and weathered, burnt and unburnt shales with *p*H values of from 3·2 to 7·8, was severely deficient in phosphorus and nitrogen. However, the response to phosphorus or nitrogen depended on the particular species and the acidity of the soil. Some typical results are given in Figs. 1 and 2. *Deschampsia flexuosa*, which is only found on heaps with acid soils, responded to added phosphate when it was grown on the acid soil (Fig. 1). When the soil was limed to a *p*H of about 6 it responded even more to added nutrients, possibly because of an effect of the *p*H on nutrient

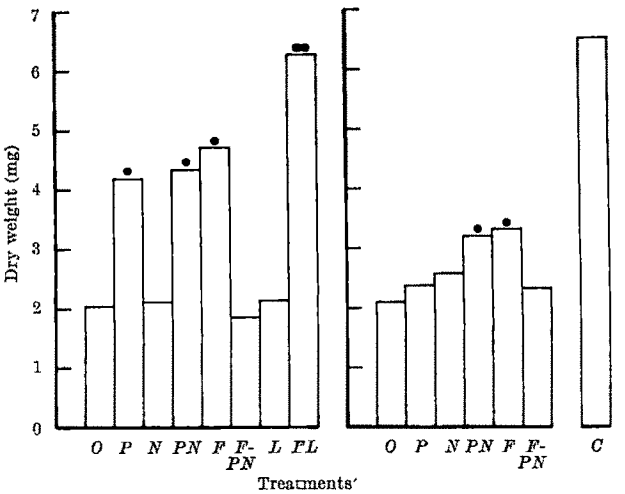


Fig. 1. Dry weight of *Deschampsia flexuosa* produced in 5 weeks on two pit-heap soils of different *p*H with various nutrient treatments. Left, unburnt shale, *p*H 3·2. Right, burnt shale, *p*H 7·4. Nutrient treatments based on Hewitt (ref. 8) applied weekly as follows, treatment *O*, watered with distilled water, *P* watered with solution containing 9 p.p.m. phosphorus as 'AnalaR' $NaH_2PO_4.2H_2O$, *N* solution containing 140 p.p.m. nitrogen as 'AnalaR' potassium nitrate; *PN*, solution containing both *P* and *N* as above; *F*, Hewitt (ref. 8) solution containing all major and micro-elements, *F-PN*, Hewitt solution minus *P* and *N*, *L*, treated with lime to a *p*H of about 6; *F+L*, Hewitt solution and limed; *C*, growth of *Deschampsia* on a control soil. Yields marked ● significantly (1 per cent level) different from unmarked yields. Yields marked ●● significantly (1 per cent level) different from those marked ●

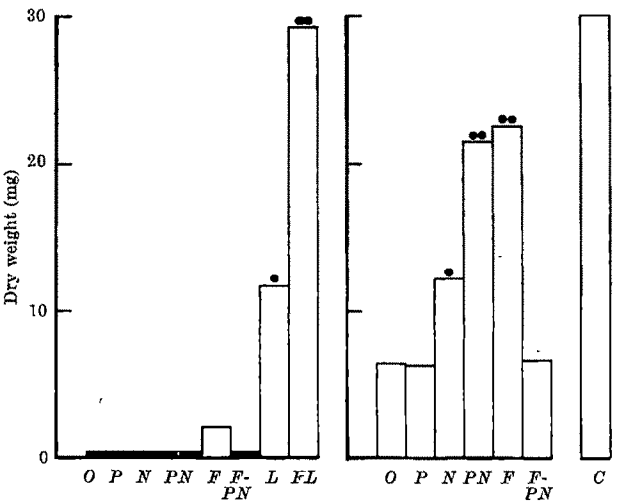


Fig. 2. Dry weight of *Plantago lanceolata* produced in 5 weeks on the same soils as in Fig. 1. Treatments as for Fig 1, except that *C* = growth of *Plantago* on a control soil

availability or to alleviation of a metal toxicity. The latter possibility is suggested because toxic levels of aluminium, manganese and iron are known to occur in many acid soils[5,9]. On the soil with a high *p*H (7·4) *Deschampsia* again responded to nutrients but the effect was much smaller and less clear cut. The performance of *Plantago lanceolata*, a species which is found only on less acid heaps and heaps with high *p*H, on the same two soils as were used in the *Deschampsia* experiment provided an interesting comparison (Fig. 2). It was impossible to keep the seedlings of *Plantago* alive on the acid soil unless it was limed. When it was limed, then there was a pronounced response to nutrients. *Plantago* responded to added nitrate on the soil with the high *p*H (7·4) but showed an even greater response when phosphate was supplied with the nitrate. The two species showed markedly contrasting behaviour on the two soils. We have some evidence that the poorer performance of *Plantago* compared with that of *Deschampsia* on the acid soil and the necessity for a prior liming of the soil before significant growth occurred may have been due to greater susceptibility of *Plantago* to a metal toxicity. If this proves to be the case, the situation is clearly very similar to that described by Rorison[5] for the growth of calcifuges and calcicoles on an acid soil. As yet we can advance no reasons for the difference in performance of the two species on the soil with the high *p*H.

Field experiments have been concerned with the effects of nutrition on seedling establishment and on the growth and productivity of established vegetation.

In the experiments on seedling establishment, batches of seeds of various species were sown in May in 20 cm² plots at the same sites previously assayed in the pot experiments. Plots were given different nutrients applied as 'AnalaR' chemicals in powder form and the numbers and appearance of seedlings were recorded at intervals over the next 7 months. Table 1 gives some records for *Plantago lanceolata* at three sites. It is clear that at the time of writing the nutrient treatments have had no effect on establishment but have had an enormous effect on the growth of the seedlings (Fig. 3).

Finally, the results of one of the field experiments on the effects of nutrients on established vegetation are given in Table 2. An area with a fairly uniform and well-established sward of *Agrostis tenuis* was chosen and 1 m² plots were marked out on it in a randomized block design. Three nutrient treatments were applied as 'AnalaR' chemicals in powder form in June, then 4 months later the vegetation of each of the plots was cropped. Cropping consisted of removing shoots and leaves just above ground-level with a pair of shears. The harvested vegetation was sorted into living and dead material before being dried and weighed. There was a massive increase

Table 1 PERCENTAGE SURVIVAL OF SEEDLINGS OF *Plantago lanceolata* AT THREE FIELD SITES

Site	*p*H	Control*	*N, P, K*, Mg added†	*N, P, K*, Mg added and limed‡
Cramlington I	4·6	0	0	2
,, II	5 6	15	13	13
,, III	6 5	13	15	—
				No liming treatment

* Control. No nutrients or lime added
† Nitrogen, phosphorus, potassium and magnesium added as in Willis (ref. 11).
‡ As †, but limed (3 g/plot).

Table 2. EFFECTS OF NUTRIENTS ON A SWARD OF *Agrostis tenuis* GROWING ON A PIT-HEAP SOIL

Treatment*	Dry weight per m² (g) Living material	Dead material
0	4·61	52·14
L	11·15	48 56
F	64·96†	67·24
L+F	153·97†	18·25‡

* Treatments designated as in Table 1.
† Yields significantly (1 per cent level) different from those in the same column.
‡ Yield significantly (1 per cent level) lower than yields in the same column.



Fig. 3. Silhouettes of *Plantago lanceolata* plants taken from plots after seven months growth in a field experiment. Left, no nutrient treatment. Right, plot treated with nitrogen, phosphorus, potassium and magnesium, and limed at sowing time

in the growth and productivity of the vegetation to which nutrients had been added. In addition, the combination of liming and nutrients resulted in a considerable reduction in the amount of dead material present in the plots. This might have been due to an effect on the senescence of leaves (both phosphorus and nitrogen deficiency accelerate senescence[10]) and to a faster decay rate of dead material.

In conclusion, the foregoing experiments suggest that the vegetation of pit heaps is greatly influenced by nutritional factors. Because physical factors, such as surface stability, have previously been shown to be important, a considerable ecological problem is posed, namely, the estimation of where, when and to what extent different factors affect the vegetation of a particular pit heap.

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Pesticide Residues in the Irish Environment

IN recent years there has been some public concern regarding the extent to which pesticide residues are found in wild-life. The existence of environmental contamination by pesticides in Britain has been recognized by the Advisory Committee on Pesticides and Other Toxic Chemicals[1]. The Crop Husbandry and Crop Protection Department of the Agricultural Institute, as part of its programme, has been keeping this problem under review. Accordingly, an investigation of the occurrence and distribution of residues of organic mercury and the organic chlorine insecticides, lindane (γ-BHC), aldrin, dieldrin and *pp'*-DDT, in the Irish environment was undertaken. Because of the maritime nature of the country and its position on the western edge of Europe, the findings should be of particular interest.

Samples for chemical analysis were received from the Veterinary Research Laboratory and the Fisheries Division of the Department of Agriculture and Fisheries, the

Veterinary College of Ireland, and members of the public. With the exception of the birds' eggs, all the specimens submitted were from species either found dead or moribund, some in circumstances suggestive of possible pesticide poisoning.

The specimens were analysed for organic chlorine residues using the extraction and 'clean-up' method of de Faubert Maunder *et al.*[2], followed by the gas-liquid chromatographic procedure detailed by Dimick and Hartmann[3]. Organic mercury residues were determined by the method described by Gage[4]. The lowest levels detectable were 0·002 p.p.m. aldrin, 0·01 p.p.m. lindane, dieldrin and *pp'*-DDT, and 0·015 p.p.m. organic mercury.

The results of the analyses, which were carried out in 1964 and 1965, are summarized in Tables 1 and 2.

The organs and tissues of one foxhound, eleven pheasants, one kestrel, two thrushes, three pigeons, one bullfinch, four salmon and three trout were examined (Table 1). Organic chlorine residues, principally dieldrin, were found in twenty-one out of the twenty-six specimens analysed. In addition, two pheasants and the three pigeons contained organic mercury residues. Death in these cases could be attributed to pesticide poisoning. The high concentration of dieldrin (13·89 p.p.m.) in the breast muscle of one pheasant probably caused the death of this bird. In the pathological examinations which were carried out by the Veterinary Research Laboratory, Abbotstown, County Dublin, enteritis was observed in all six fatalities. This is in accord with the possibility of death occurring from organic chlorine poisoning[5].

It was later established that river pollution was responsible for the three trout mortalities and it was suggested that the four salmon fatalities were due to a cold water form of columnaris disease caused by a myxobacterium *Cytophaga* (s. *Chondrocuccus*) *columnaris*[6], but this has not been confirmed.

Thirty-one birds' eggs collected from three sites in 1965 were analysed (Table 2). The mallard, guillemot, fulmar, razorbill and kittiwake eggs were all obtained from the Great Saltee Island, County Wexford, which lies at the junction of St. George's Channel and the Atlantic. The presence of organic chlorine residues in guillemot, kittiwake and shag (*Phalacrocorax aristotelis*) eggs collected from this site in 1964 has already been reported[7]. The levels of the residues present in the 1965 eggs did not prevent development of the embryos, as most of the eggs were in various stages of incubation at the time of analysis. The residues in the mallard eggs were considerably lower than those in the guillemot, fulmar, razorbill, and kittiwake eggs, which were nearly all of the same order. These sea birds, which range more widely than the mallard, possibly fed on molluscs and fish in waters already contaminated with organic chlorine residues. Moore and Tatton[7] have suggested that the eggs of sea birds might be useful indicator organisms for detecting changes in environmental contamination by persistent pesticides.

The results show that low levels of organic chlorine residues are present in a wide variety of biological material. The possibility that these residues are due to industrial contamination by Irish sources seems very remote. Relatively few fatalities may be definitely attributed to the organic chlorine insecticides. The work is being continued.

I thank Mr. C. B. Adams and the Veterinary Research Laboratory, Abbotstown, County Dublin, for the pathological examinations of the bird fatalities, Dr. A. E. J. Went, of the Fisheries Division of the Department of Agriculture and Fisheries, for the salmon samples, Mr. D. Cabot, University College, Galway, for the mallard and sea birds' eggs, other donors for specimens, Miss C. Kenna, Miss J. Oliver and Mr. J. O'Shea for technical

Table 1. ORGANIC MERCURY AND ORGANIC CHLORINE RESIDUES IN MAMMALIAN, AVIAN AND FISH TISSUES
(All results expressed in p.p.m. on a wet weight basis)

Species	No. of specimens analysed			Range of organic mercury	Range of lindane	Range of aldrin	Range of dieldrin	Range of *pp'*-DDT
	Total	With organic mercury residues	With organic chlorine residues					
Foxhound (*Canis familiaris*)	1	0	1	ND	0·37–0·72	0·06–0·08	0·21–0·61	0·10–0·30
Pheasant (*Phasianus colchicus*)	11	2	9	2·53–5·15	0·025–0·58	0·01–0·20	0·03–13·89	0·03–0·58
Kestrel (*Falco tinnunculus*)	1	0	0	ND	ND	ND	ND	ND
Thrush (*Turdus* spp.)	2	0	0	ND	ND	ND	ND	ND
Pigeon (*Columba* spp.)	3	3	3	0·015–5·33	0·025–0·67	0·02–0·04	0·065–6·16	0·06–0·19
Bullfinch (*Pyrrhula pyrrhula*)	1	0	1	ND	0·22–0·28	0·18–0·26	0·70–1·89	0·34–0·54
Salmon (*Salmo salar*)	4	0	4	ND	0·01–0·15	0·01–0·21	0·02–0·285	0·03–0·71
Trout (*Salmo trutta*)	3	0	3	ND	0·05–0·30	0·04–0·43	0·11–0·62	ND

ND, none detected.

Table 2. ORGANIC MERCURY AND ORGANIC CHLORINE RESIDUES IN BIRDS' EGGS
(All results expressed in p.p.m. by weight with respect to total egg contents)

Species	No. of eggs analysed			Range of organic mercury	Range of lindane	Range of aldrin	Range of dieldrin	Range of *pp'*-DDT
	Total	With organic mercury residues	With organic chlorine residues					
Pheasant (*Phasianus colchicus*)	2*	0	1	ND†	0·03	0·004	0·23	0·065
Hen-harrier (*Circus cyaneus*)	2*	0	1	ND	0·03	0·01	0·33	0·12
Mallard (*Anas platyrhyncha*)	6*	0	5	ND	0·01–0·015	0·002–0·01	0·06–0·125	0·01–0·04
Guillemot (*Uria aalge*)	6*	1	5	0·57	0·005–0·04	0·003–0·06	0·25–0·44	0·07–0·46
Fulmar (*Fulmarus glacialis*)	4*	0	3	ND	0·03–0·04	0·01	0·33–0·44	0·41–0·54
Razorbill (*Alca torda*)	6*	0	5	ND	0·01–0·045	0·01–0·09	0·30–0·43	0·46–0·72
Kittiwake (*Rissa tridactyla*)	5*	1	4	0·61	0·02–0·04	0·01–0·02	0·29–0·38	0·32–0·55

* Includes one egg analysed only for organic mercury residues. †ND, none detected.

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Occurrence of Mesoplastra in a Cryptodiran Turtle, *Lepidochelys olivacea*

MESOPLASTRAL bones, a characteristic feature of most amphichelid turtles, are to-day restricted to members of the Pleurodiran family Pelomedusidae. Of the three genera of this family, two (*Podocnemis* and *Pelomedusa*) have the mesoplastra reduced to small, widely spaced bones separating the hyoplastra and hypoplastra at the outer part of their common suture. Only in *Pelusios* are they retained in their pristine contiguous condition, a retention perhaps connected with the necessity of the hyoplastra having a straight posterior margin to allow flexure of the hinged plastron to occur. The mesoplastra of *Pelomedusa* may be missing as an occasional variant[1], but there appears to be no record of mesoplastra having been recorded in a cryptodire; indeed, a fossil plastron with mesoplastra would definitely not be classified as cryptodiran at all. It is therefore of interest to record the finding of a plastron of the Pacific ridley (*Lepidochelys olivacea*) in which the hyo- and hypoplastra appear to be separated by a pair of well-defined contiguous mesoplastra (Fig. 1). The plastron was found separate from the remainder of the animal on Shell Beach, North-West District, British Guiana. (The discovery of *Lepidochelys* in British Guiana is documented elsewhere[2,3].) The specimen was not collected, but a colour slide was taken of it, close inspection of which leaves no doubt that the breaks are true sutures and not sulci or post-mortem fractures.

It is possibly significant that the specimen involved should be a *Lepidochelys*, as the Cheloniidae are closer to the Amphichelydia than any other turtle family, judging by such features as the retention of infra-marginals and other small plastral laminae (though the non-retractile neck and roofed-over skull are probably secondary reversions). Moreover, the ridley is the most primitive member of the family, having the most highly ossified shell, and showing a curious reversion to a (supposedly primitive) multi-scutate condition of the carapace; though in this connexion it must be remembered that the typical testudinate pattern of five centrals and four laterals was established even among Jurassic amphichelids. The ridley also lacks the specialization of large size shown by other members of the Cheloniidae.

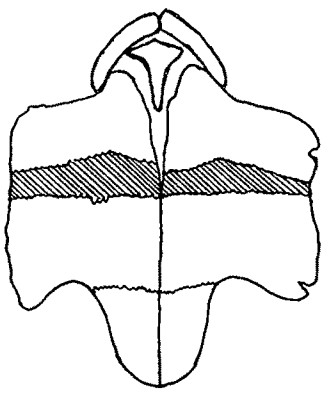


Fig. 1. Plastron of *Lepidochelys olivacea* with mesoplastra

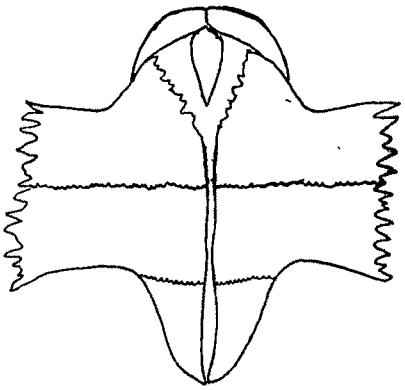


Fig. 2. Normal plastron of *Lepidochelys olivacea*

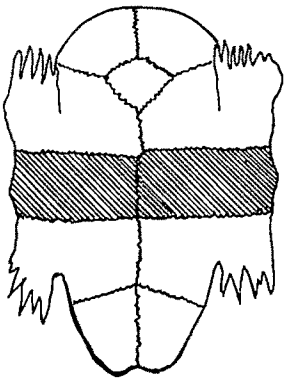


Fig. 3. Plastron of a Jurassic amphichelid (*Pleurosternum* sp.) from the Purbeck Limestone of Swanage, Dorset (Oxford University Museum No 13797)

In view of the symmetry of these aberrant 'mesoplastra', their occurrence in the primitive *Lepidochelys* and the close similarity between them and the mesoplastra of amphichelids, one is tempted to suggest that they have a true homology with the mesoplastra of primitive turtles. However, considering the very long time since a *Lepidochelys* ancestor had mesoplastra, it is perhaps more likely that the present specimen merely shows a casual division of embryonic ossification centres. Neither hypothesis can be tested, of course, even if the specimen were in hand.

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Collection of the Liquid Fraction of Bee Venom

THE liquid fraction of honey bee venom (*Apis mellifera* L.) can be collected by a modification of the device described by Benton, Morse and Stewart[1] and Benton[2].

This device consists of a flat surface over which steel wires are stretched at $\frac{1}{4}$-in. (6·36-mm) intervals. The wires are alternately grounded and charged. The bees, on being shocked, sting down through a sheet of nylon parchment taffeta; the venom is left on the underside of

the nylon when the sting is removed by the bee. The venom is allowed to dry and is then scraped free with a sharp blade. The venom so collected is slightly contaminated with pollen grains and dust.

To collect the liquid fraction, two sheets of thin polyethylene ('Handiwrap', Dow Chemical Co.) (0·0254-mm thick) are stretched over the collection board and under the nylon parchment taffeta; the sting of the honey bee cannot easily penetrate thicker sheets of polyethylene. The bees, when shocked, sting down through the taffeta and the two sheets of polyethylene. The venom is deposited as small droplets on the underside of the last sheet of plastic. The first sheet of polyethylene, covered with contaminants, is discarded. It is possible to collect about 2 g of venom from twenty colonies of bees in about 2 h with the original apparatus; the modified apparatus described here collects only about half that amount.

Some venom is lost between the sheets of plastic and in the nylon, but the material that is obtained is free of contaminants. To obtain the venom as a liquid, one must remove the last sheet of plastic rapidly, extract it with physiological saline solution or de-ionized water and quick-freeze the solution. Pure, dried venom can be obtained by drying the solution under aseptic conditions.

The thin sheet of polyethylene does not retain the stingers and the bees do not appear to suffer any adverse effects in the process of venom collection.

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Pregnancy Failure in Laboratory Mice after Multiple Short-term Exposure to Strange Males

An olfactorily induced pregnancy failure in recently inseminated laboratory mice has been shown to result from enforced cohabitation with a strange or alien male, following which the female would return to oestrus[1]. The females were exposed for a period of 12, 24, 48, or 72 h, with maximum pregnancy failure (approximately 80 per cent) occurring at the two longer periods, and about half as many failures with 12 h exposure[2,3]. In the aforementioned experiments, the female was continually subjected to the presence of the strange male. The purpose of the present investigation was to determine if multiple, short-term exposures of the newly inseminated female to strange males would reduce the pregnancy rate.

Our experiments were conducted with virgin albino females, 50–60 days of age, weighing approximately 25 g, and showing a perforate vaginal orifice. Opaque plastic window boxes 22 in. by 5·25 in. by 5·5 in. high with wire mesh tops were used throughout as cages. Females were paired individually with a stud male (albino) of proved fertility and examined each morning for the presence of a vaginal plug. The male was removed from the female's cage when she showed a vaginal plug. The female remained in her home cage for 24 h, after which she was subjected to one of four treatments, as follows: The female was (*a*) left undisturbed in her cage for 7 days (control); (*b*) placed for 7 days in a cage containing a resident wild male house mouse; (*c*) transferred to a cage containing a resident wild male house mouse for three 15 min periods per day for 4 days; (*d*) transferred to an empty cage for periods identical to (*c*). Each treatment consisted of fifty animals. Females were killed on the seventh day in order to determine whether or not they were pregnant. All treatments were tested for significance by χ^2 analysis. Wild males were used as strange males, in part to prove their capacity to block pregnancies and in part to provide as wide a spectrum of odours as possible, thus assuring maximum pregnancy failure.

For the fifty females in treatment *a* there were forty-five pregnancies (90 per cent), while those in treatment *b* had only twenty-two pregnancies (44 per cent). This is a significant reduction in pregnancy rate ($P < 0.001$). For the fifty females subjected to wild males for short periods (treatment *c*) there were twenty-five pregnancies (50 per cent). This pregnancy rate is also a significant reduction from the control ($P < 0.001$) but is not significantly different from the constant exposure to the wild males (treatment *b*). Of the fifty females exposed to empty cages (treatment *d*) there were forty pregnancies (80 per cent), which was a significant increase in pregnancy rate from the continuous or periodic exposure to the wild males ($P < 0.001$) but was not significantly different from the control (treatment *a*).

Our data indicate that multiple short-term exposure of a recently inseminated female to a wild male will cause essentially the same degree of pregnancy failure as the continuous exposure to a wild male. The 15 min exposure may be considered a longer period of contact than might occur under natural conditions; however, we believe that the short exposure approximates more closely the degree of contact in the environment than continuous exposure. Since the exposure periods were 4–12 h apart, we suggest that in the natural habitat the periodic contact of a recently inseminated female with several male mice or trails containing their odours would be adequate to induce some pregnancy failure, and thus would contribute toward decreased recruitment in the population. Recent investigations[4,5] indicate (*a*) in two high-density natural populations, a general failure of adult females to become pregnant as well as an increase in the percentage of parous, non-pregnant females in the older age classes at the time of and immediately following the peak in the population; and (*b*) an increase in the reproductive rate when females from confined populations were allowed to disperse into uninhabited areas.

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MICROBIOLOGY

Some Observations on Staphylococcal Pigmentation

Various substances added to nutrient agar media are known to enhance colonial pigmentation of *Staphylococcus aureus*. These include sterile cow's milk[1,2], fermentable carbohydrate[3], sodium chloride[4], glycerophosphoric acid[5] and glycerol monoacetate[6]. Not all these are equally effective, however, the most intense pigmentation being developed on milk agar and on glycerol monoacetate agar. Glycerol monoacetate agar has the additional advantage that strains of *Staph. aureus* growing

on it can be readily differentiated into a number of colour varieties—yellow, orange, buff and white—after incubation for 48 h. In our hands, milk agar has given irregular results. Thus, although the medium has consistently induced strongly pigmented growth, some batches of it have shown little or no differentiation between the colour types, even after prolonged incubation, while other batches have developed striking colour differentiation after incubation for only 24 h. These inconsistencies led us to examine different components of cow's milk in order to determine the nature of its pigment-stimulating fraction.

In an earlier study of the pigment-stimulating properties of milk, Johnston[7] found that neither casein nor lactose appreciably increased pigmentation, but he obtained good pigmentation on media containing calcium. Our investigations with skimmed milk, and with some of the substances present in the water phase of milk, including lactose, calcium, casein and citrate, have yielded disappointing results. A wide range of concentrations of these substances, both singly and in various combinations, was examined in heart infusion agar (Difco), but in no case was colonial pigmentation of staphylococci much stronger than on the nutrient base without these additives. Skimmed milk agar supported moderate orange pigmentation of some strains, but the colour was never very intense and colour differentiation was absent.

Since cream agar[8], which consists of the 'top off the bottle' of unhomogenized cow's milk, added to the nutrient agar base to give a final concentration of 10 per cent, gave consistently intense colonial pigmentation with obvious colour differentiation after incubation for 48 h, we next tested 'pure' cream fat as a pigment-stimulating substance. Cream fat was extracted from ordinary commercial cream either by ether extraction or by heating at 60° C after preliminary separation by centrifugation, and butter-fat was separated from commercial butter by heating at 60° C. These fats, which were emulsified into the hot molten nutrient agar base with a Silverson mixer, gave very intense colonial pigmentation and overt colour differentiation at 48 h.

It was now clear that the fat components of whole milk are the factors responsible for enhanced colonial pigmentation of *Staph. aureus* growing on milk agar, an observation that is in accord with the pigment-stimulating effect of glycerol monoacetate.

The two components of cream and butter-fat—glycerol and fatty acids—were next examined. Although Willis and Turner[6] reported that glycerol does not significantly enhance staphylococcal pigmentation, the alcohol was re-examined, but again with negative results. The fatty acids of cream and butter were obtained (1) by saponification of the fat with alcoholic sodium hydroxide, acid precipitation, and washing, and (2) by digestion of the fat with excess of aqueous pancreatic lipase (Calbiochem), separation by heating to 60° C, and washing. These mixtures of fatty acids were neutralized with 1 N sodium hydroxide, sterilized and incorporated into the nutrient agar base. On these cream- and butter-soap media, staphylococcal pigmentation was more intense than we have seen on any other medium, and excellent colour differentiation was obtained after incubation for only 24 h.

Of the various fatty acids present in cream and butter-fat, we obtained commercial preparations of those listed in Table 1, and tested them at a range of concentrations in the nutrient agar base. The results shown in Table 1 are those obtained after incubation for 48 h, at the optimum concentration for each of the fatty acids tested.

Human fat[6] incorporated into the nutrient base by emulsification causes colonial pigmentation and colour differentiation which is at least as good as that obtained with cream and butter fat, but cod liver oil is not quite so effective.

These observations, which will be reported in detail elsewhere, indicate that staphylococcal pigmentation is greatly favoured by the presence of a variety of fatty acids. Mevalonate, which is an intermediate in the carotenoid pathway, itself enhances pigmentation slightly, but is much less active than the fatty acids. Carbohydrate metabolism appears to contribute very little towards colonial pigmentation.

Table 1. PIGMENT-STIMULATING EFFECT OF SOME FATS AND FATTY ACIDS ON STRAINS OF *Staphylococcus aureus*

Fats and fatty acids incorporated into heart infusion agar (Difco)		Pigment production and colour differentiation after 48 h at 37° C: Yellow	Orange	White
Acetic	—Na salt	±	++	++
,,	—glycerol ester	+++	+++	++++
Butyric	—Na salt	±	±	±
,,	—glycerol ester	±	+	±
Caproic	—methyl ester	+	++	++++
Caprylic	—Na salt	+	++	++++
Capric	—methyl ester	−	++	++++
Lauric	—Na salt	NG	NG	NG
,,	—glycerol ester	−	−	−
Myristic	—Na salt	NG	NG	NG
Palmitic	—Na salt	±	++	++++
Stearic	—Na salt	++	++	++++
,,	—glycerol ester	++	+++	++++
Oleic	—Na salt	±	++	++++
,,	—K salt	±	++	++++
,,	—glycerol ester	+++	+++	++++
Cod liver oil		±	++	++++
Human fat		+++	+++	++++
,, ,,	—Na salt	+++	+++	++++
Cream fat		+++	+++	++++
,, ,,	—Na salt	++++	++++	++++
Butter fat		+++	+++	+++
,, ,,	—Na salt	++++	++++	++++
Commercial sodium soap (beef and mutton tallow)		+++	+++	++++
'Tween 60'		±	++	+++
'Tween 80'		±	++	+++
'TEM 4T'		+++	+++	++++

NG, No growth; −, no pigment; ±, poor pigment; +, ++, +++, ++++, increasing intensity of pigment.

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Effect of Bacitracin on *in vitro* Phagocytosis

PREVIOUS investigations of the influence of antimicrobial compounds on phagocytosis indicated that polypeptide antibiotics like bacitracin and polymyxin *B* retarded ingestion of polystyrene latex (PSL) particles or *Staphylococcus aureus* by guinea-pig polymorphonuclear leucocytes (PMN)[1]. Although the levels of antibiotic necessary to produce this inhibition exceeded the therapeutic level for the guinea-pig, it was of interest to discern the mode of action of this peptide on the ingestion process and possibly gain further insight as to the basic mechanism of phagocytosis. This communication embodies some of the evidence for the chelating effect of the peptide and the essential participation of metal ions in phagocytosis.

The acquisition and preparation of PMN together with the determination of phagocytic activity in the presence of bacitracin were performed as previously described[1]. The washed polymorphonuclear leucocytes were allowed to ingest particles in Krebs–Ringer solution with and without $MgSO_4 \cdot 7H_2O$ and calcium chloride. All media were prepared with de-ionized, glass distilled water. Baci-

tracin was added as indicated. The phagocytic index was expressed as:

$$P.I. = \frac{\text{Number of PMN with} > 10 \text{ particles/cell}}{500 \text{ PMN counted}}$$

Phagocytosis of PSL and *Staph. aureus* by PMN was significantly reduced in the absence of Mg^{++} and Ca^{++} (Fig. 1). The addition of bacitracin or EDTA to a medium containing these ions likewise decreased the number of particles ingested. It can be seen in Fig. 1 that phagocytosis was almost fully restored if Mg^{++} or Ca^{++} was provided after a 30-min period of incubation in a medium which was initially devoid of these ions. It was also noted that the chelator EDTA exerted appreciably less effect on ingestion of PSL than staphylococci.

While serum, used at a concentration of 30 per cent, exerted little effect on the ingestion of PSL by PMN, it stimulated the engulfment of staphylococci by 20 per cent or more. The inhibition by bacitracin or EDTA was observed in the absence of serum. Since PSL and bacteria exhibited different properties relative to their adsorption in the presence of serum, it was omitted from experiments which tested the reversal of bacitracin inhibition by metal ions.

An indication of the chelating effect of bacitracin became evident when phagocytic ingestion was observed to decrease in Krebs buffer solution; a medium which contains metal ion levels very near those in serum. When phagocytosis was conducted in Krebs medium, the *P.I.* decreased in proportion to the concentration of bacitracin. Maximal retardation of ingestion occurred when the concentration of bacitracin was approximately 1·4 mM (Fig. 2) assuming a molecular weight of 1,422 (ref. 2).

The addition of Mg^{++} or Ca^{++} reversed the inhibition by bacitracin, the latter being more effective up to the concentration found in serum. Further increase in Ca^{++} resulted in an appreciable decrease in phagocytosis of PSL while increasing Mg^{++} appeared to potentiate the ingestion (Fig. 2). The bottom abscissa in the figure represents values obtained in the presence of 1·4 mM bacitracin. The antibiotic appeared to bind or promote the binding of several moles of Mg^{++} per mole of peptide since at the point of complete restoration of ingestive capability the ratio of Mg^{++} to bacitracin was 14 : 1.

The notion that bacitracin prevents phagocytosis by competition for metal ions and thus thwarts adsorption is suggested in the following experiment: A suspension of PMN in Krebs buffer was permitted to adsorb PSL at 0° C in the absence of bacitracin for 15 min. The excess (non-adsorbed) PSL was then washed off and the polymorphonuclear leucocytes were examined microscopically to assess the adsorption. Afterwards, bacitracin was added and the polymorphonuclear leucocytes were incubated for 30 min at 37° C. A significant degree of ingestion was observed (Table 1). If the polymorphonuclear leucocytes were allowed to incubate for 15 min at 0° C in the presence of bacitracin prior to the 'confrontation' with PSL, the usual retardation of ingestion occurred.

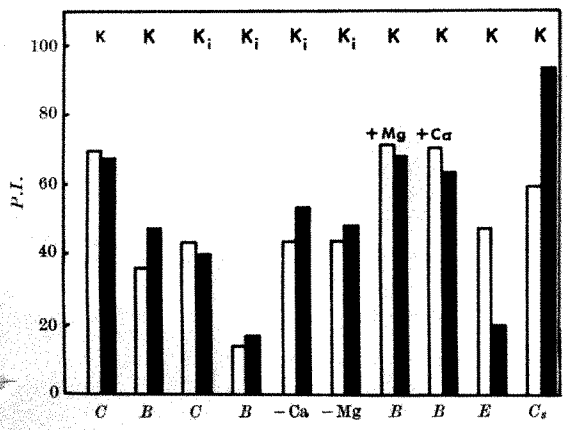


Fig. 1. Phagocytosis of PSL and *Staphylococcus aureus* with and without added metal ions. Each test mixture contained particles and PMN as in Table 1; Krebs buffer (*K*) or Krebs buffer minus Ca^{++} and Mg^{++} (K_i), 67 mM, *p*H 7·4; and $MgSO_4 \cdot 7H_2O$, 15 mM; calcium chloride, 3·0 mM; EDTA, 5·0 mM (*E*) and bacitracin, 1·4 mM (*B*) where indicated. *C*, Control with no bacitracin; C_s, serum added to 30 per cent

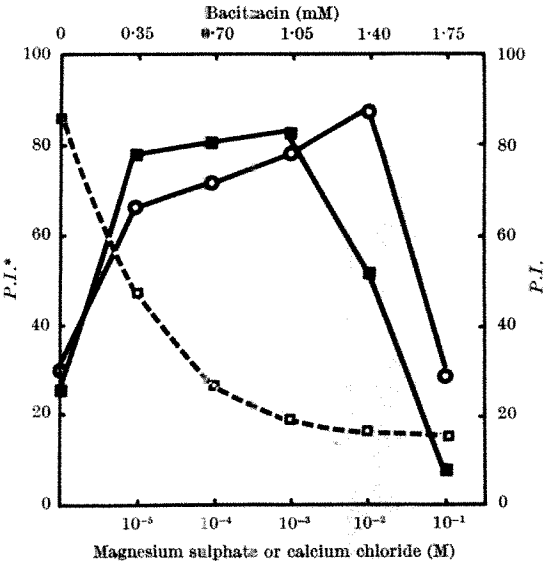


Fig. 2. The reversal of bacitracin inhibition by metal ion. The phagocytic index (*P.I.**) on the left ordinate is expressed as a percentage of the control value, that is the phagocytosis which occurred without bacitracin in complete medium was taken as 100 per cent. The values are a function of those on the bottom abscissa. The *P.I.* on the right ordinate is expressed as in the text and the values are a function of those on the top abscissa. —■—■—, magnesium sulphate; —○—○—, calcium chloride; – – □ – – □ – –, bacitracin

Table 1. ADSORPTION OF PSL PRIOR TO, DURING AND AFTER TREATMENT OF PMN WITH BACITRACIN

Phagocytic system		*P.I.*	*P*
(1) PMN + PSL	37° C, 30 min	67·80 ± 10·70	—
(2) PMN + PSL + BAC	37° C, 30 min	28·15 ± 3·20	< 0·001
(3) PMN + PSL	(0°C, 15 min)		
+ BAC	37° C, 30 min	38·10 ± 3·84	< 0·001
(4) PMN + PSL	0° C, 15 min	8·60 ± 2·12	< 0·001
(5) PMN + PSL	(0°C, 15 min)		
	37° C, 30 min	61·70 ± 3·26	> 0·100
(6) PMN + PSL + BAC	0° C, 15 min	0·10 ± 0·14	< 0·001

Each phagocytic system contained: Krebs buffer, *p*H 7·4, 67 mM; PMN, $2·0 \times 10^7$; PSL, $5·0 \times 10^8$; bacitracin, 1·4 mM where indicated and saline, 0·87 per cent to a final volume of 2·0 ml. The pre-incubation period for adsorption of PSL at 0° C is indicated in parentheses. The *P.I.* and standard error are given in each case. *P* has the usual connotation of significance and refers to the difference between cells ingesting particles after adsorption in the absence and presence of bacitracin. PMN, Polymorphonuclear leucocytes; PSL, polystyrene latex; BAC, bacitracin.

It thus appears that phagocytes which have successfully adsorbed particles are committed to ingest them. The failure of bacitracin to retard ingestion after adsorption suggests that its mode of action is primarily on adsorption and not ingestion. The innocuous effect of the peptide on certain metabolic reactions of PMN (ref. 1) would lend further support to this concept.

Some adsorption or ingestion was noted in the absence of Mg^{++} and Ca^{++} with added bacitracin. The phagocytic system may not be totally deplete with respect to Mg^{++} and Ca^{++} since the PMN could easily have retained tightly bound Mg^{++} or Ca^{++} throughout the preparative procedure. In short, phagocytosis in a given population of PMN cannot be completely terminated unless an actual repulsive barrier is established. Experiments which make it possible to assess properly the interplay of repulsive and attractive forces which occur on the surface of the PMN during phagocytosis constitute a formidable problem in cell biology. Also, while a bridge involving Ca^{++} may be prevalent in one case, the preference for Mg^{++} may be

manifest in another. Such might be the reason for the difference in restorative character of Mg^{++} and Ca^{++} as presented here.

The notion that phagocytosis can be explained in terms of the simple colloidal stability theory of Verwey and Overbeck[3] has been discounted[4] because of a lack of correlation in the zeta-potential of either cells or test particles. A mechanism of phagocytosis involving cross-linking of serum proteins between cells and particles has been proposed[5]. The role of Ca^{++} in adsorption of protein on to the cell surface has been suggested in investigations on the flocculation of bacteria by colloids[6]. In a similar way serum components which bind Ca^{++} very strongly at or near their isoelectric point have been postulated[7].

In view of the evidence presented here, the divalent metal ion although not indispensable in phagocytosis seems to play a supportive part in it. Since Ca^{++} can bind peptides and thereby impart a net positive charge, its association with the negatively charged cell might promote the 'physiological adsorption' which is essential to 'physiological ingestion'. At physiological *p*H bacitracin should, to a certain extent, bind Ca^{++} (refs. 2, 8). The link resulting from this union may then be peptide to peptide or cell to peptide. The competition for a binding site on the cell surface might be such that particles, even if protein coated, would not be adsorbed in a manner sufficient to initiate ingestion. The chemical nature of the surfaces involved must be elucidated before the initial phases of the mechanism of phagocytosis can be understood.

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VIROLOGY

Isolation of Parainfluenza-3 Virus from Water Buffaloes in Egypt

ALTHOUGH various workers have reported the isolation of myxovirus parainfluenza-3 from cattle from different parts of the world[1–4], there is no report of similar viruses from water buffaloes. This report describes briefly the isolation of myxovirus parainfluenza-3 from buffalo calves suffering from pneumoenteritis.

The two strains *MP*-3(45) and *MP*-3(83) were isolated in primary calf kidney cell cultures from the nasal and faecal swabs respectively taken from buffalo calves showing clinical signs of pneumoenteritis. On primary isolation in calf kidney cultures, grown in medium consisting of 0·5 per cent lactalbumin hydrolysate in Hanks's balanced salt solution, cytopathic effect was first observed after 5 days as focal rounding of refractile cells. This progressed to form large and small syncytia. The cell sheet came off the glass wall of the tissue culture tubes after 10–12 days' incubation. On subsequent passages in calf kidney cells, cytopathic effect appeared within 36 h. Both strains formed well-defined plaques on buffalo and calf kidney monolayers. On the seventh day after inoculation, plaques measured 1–1·5 mm on calf kidney cells



Fig. 1. After 7 days of incubation plaques resulting from the inoculation of 10^{-5} (bottle No. 1) and 10^{-6} (bottle No. 2) dilutions of *MP*-3 (45) virus

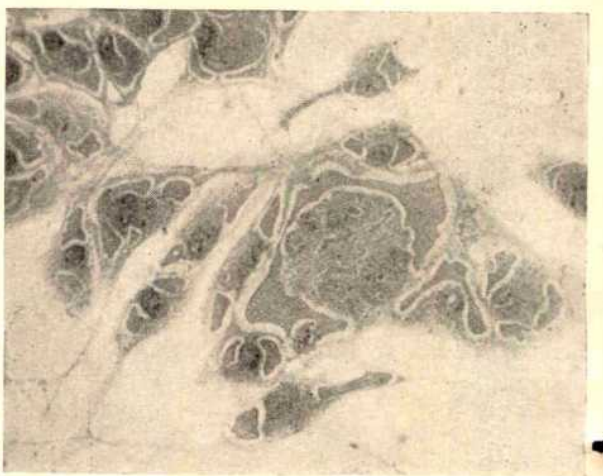


Fig. 2. Cytoplasmic and intranuclear inclusion in water buffalo kidney cells 8 days after inoculation with buffalo strain *MP*-3 (45) of parainfluenza-3 virus. Zenker's haematoxylin and eosin. (× *c.* 334)

(Fig. 1). The titre of the infected fluid was generally $10^{7.8}$ P.F.U. per ml.

These agents produced cytopathic effect in calf, buffalo calf and goat kidney cultures. The viruses also produced distinct intranuclear and intracytoplasmic eosinophilic inclusions in calf, buffalo and camel kidney cultures. Fig. 2 shows inclusions produced by *MP*-3(45) in buffalo kidney cells. Isolate *MP*-3(45) produced giant cells and cytoplasmic inclusions in *MS* cell cultures, and a few cells showed intranuclear inclusions. At 4° C virus *MP*-3(83) agglutinated buffalo, cattle and sheep erythrocytes at high titres (1 in 128) and agglutinated chicken

Table 1. SEROLOGICAL RELATIONSHIP AMONG BOVINE PARAINFLUENZA-3 VIRUS AND BUFFALO STRAINS OF *MP*-3 VIRUS

Virus	Titre of sera* versus indicated virus					
	MP-3 (45)		*MP*-3 (83)		*MP*-3 (Ohio)	
	Neut†	HI‡	Neut†	HI‡	Neut†	HI‡
MP-3 (45)	10,000	8,000	5,000	10,000	5,000	2,000
MP-3 (83)	10,000	2,000	5,000	10,000	2,000	500
MP-3 (*S*) (cattle strain)	5,000	2,000	5,000	5,000	1,000	2,000

* Sera were prepared in rabbits except *MP*-3 (Ohio) which was prepared in calf with no antibodies before infection.
† 100–200 PFU of virus were used in neutralization test.
‡ Against four haemagglutinating units of each virus.

and guinea-pig red cells at low titres (1 in 4), whereas *MP*-45 strain agglutinated sheep, cattle and buffalo red cells at high titre (1 in 128) but did not give haemagglutination with chicken and guinea-pig erythrocytes. This indicates the biological difference between these two strains and also between them and the cattle strains of *MP*-3 virus being studied in our laboratory[5]. Both viruses gave haemadsorption with sheep, cattle and guinea-pig erythrocytes. Exposure of both types to 40 per cent diethyl ether for 30 min at 37° C resulted in complete loss of infectivity as determined by the plaque technique. These agents are also sensitive to 1 per cent trypsin (Difco 1 in 250) and 1 : 500 dilution of sodium deoxycholate. The viruses were neutralized by bovine parainfluenza-3 immune sera kindly supplied by Drs. Hamdy and Bohl of Ohio Agricultural Experimental Station, Wooster, Ohio. As shown in Table 1, cattle and buffalo strains appear to be serologically identical. These viruses seem to possess a unique biological quality regarding their haemagglutination spectrum. Studies are in progress to determine the etiological role of these viruses for the little-studied respiratory diseases of water buffaloes, which are important domestic animals in the Eastern countries.

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PSYCHOLOGY

Spontaneous Alternation and Exploration in Rats

WHEN tested in a T-maze rats tend to alternate their choices spontaneously[1]; that is, when given two successive trials they tend to choose first one arm then the other, rather than the same arm on both trials. They alternate even when no choice has been made on the first trial; thus, if put directly into the goal box at the end of one arm of a previously explored T-maze, and then given a choice between the two maze arms, they will tend to choose the arm leading to the other goal box[2]. In general there is a tendency to go to the part of the environment that has been least recently explored.

A possibly related phenomenon has been found in a T-maze in which one arm leads to a Dashiell maze (a symmetrical network of alleys intersecting at right angles —it will be referred to as a D-maze), while the other ends in a blind alley[3,4]. After a few trials rats show an increasing tendency to choose the arm leading to the D-maze. Like alternation, this may be due to a tendency to go to the part of the environment that has been least recently explored. For if a rat has two areas to explore (such as the D-maze and blind alley), and is given the same time in each, there will tend to be more unexplored, or less recently explored, parts of the larger area; it should therefore choose the path leading to this area more frequently than that leading to the smaller area. Thus rats will prefer the arm leading to the D-maze.

This D-maze experiment has been interpreted rather differently by Berlyne and Slater[5]. They suggest that rats prefer the D-maze because of an aversion for a maze arm in which movement is relatively restricted, as in a blind alley. In support of this interpretation they report an experiment in which rats fail to show any preference for an arm leading to a goal box containing novel stimuli, when the other arm leads to an equally spacious goal box containing familiar stimuli. This implies that the opportunity to explore novel stimuli is not rewarding to rats, and therefore it cannot be the opportunity to explore the D-maze that gives rise to the preference for the arm leading to it. However, while in alternation and the D-maze experiments the environment is more or less stable, in Berlyne and Slater's experiment part of the environment must change from trial to trial; this might produce fear, which would complicate the interpretation of the results. In the following experiment, the tendency to go to the part of the environment that has been least recently explored is pitted against the supposed tendency to avoid places where movement is restricted, using a maze in which the environment is stable.

Twelve male hooded rats were used. The floor plan of the apparatus, consisting of an alley maze of unpainted aluminium, is shown in Fig. 1. On every trial a rat was put into the stem, *S*, and when it had entered one of the arms *A*, *B*, *C* or *D*, a door was closed behind it, either restricting it to a single arm (so that the rat was confined in one of the arms *A*, *B*, *C* or *D*) or restricting it to two arms (so that it was confined either to the alley made up of *A* and *B*, or to *C* and *D*). On one side of the maze a rat was always restricted to a single arm (this side will be referred to as *RS*), while on the other side (*URS*) it was always restricted to two arms. For half the rats *RS* was on the left, for half it was on the right. The rats were hungry (being given food for 1·5 h each day, starting 15 min after the day's testing) and were rewarded in the maze as follows: all rats were rewarded with one 45 mg sugar pellet at the end of each of the two *URS* arms; half the rats were rewarded with one pellet in the *RS* arm chosen, half were rewarded with two pellets in this arm. All rats were left in the two *URS* arms for 30 sec before being picked out for the next trial; half were left in the *RS* arm chosen for 30 sec, half were left in this arm for 15 sec. These different conditions were introduced in order to control against preferences due to differences in reward conditions on the two sides of the maze, or to differences in the time left in the maze. Three trials were given per day for 12 days, with 25 sec between trials.

If rats tend to choose the least recently explored part of the environment, even if it is more restricting, they should prefer *RS*, since they will need to choose *RS* twice in order to explore the whole of it, while they will only need to choose *URS* once. The mean number of choices to *RS* was 21·33 (out of a maximum of 36). This is significantly above chance by Wilcoxon's *T*-test ($T = 4$, one-tailed $P < 0{\cdot}005$). None of the differences between subgroups formed either on the basis of amount of reward on *RS*, or time spent on *RS*, were significant by the Mann–Whitney *U*-test. The result thus confirms the prediction from the hypothesis that rats prefer an arm leading to a D-maze to a blind alley because of a tendency to go to the

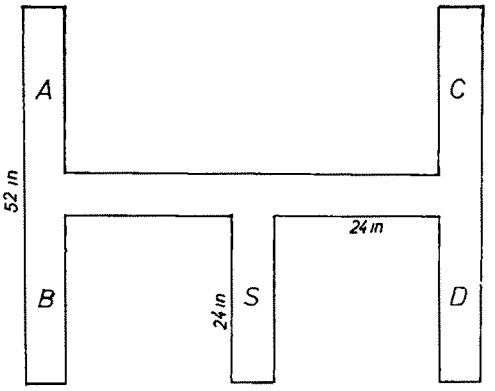


Fig. 1. Ground plan of the maze

least recently explored part of the environment, rather than a tendency to avoid a place where movements are restricted.

In the D-maze experiments, after the rats had settled down to a steady preference, the maze was changed, so that the arm previously leading to the D-maze became a blind alley, and the previous blind alley led to a D-maze. The rats' preferences followed this change, so that they continued to prefer the D-maze. A similar result would be expected in the present experiment if *RS* and *URS* were interchanged. To test this the experiment was continued for a further 12 days, with *RS* and *URS* on the sides of the maze opposite to where they had been for the first 12 days. Contrary to expectation, the preferences did not switch to the new *RS*; the mean number of choices to *RS* was 16·33.

Thus rats prefer an arm leading to a D-maze, and change their preferences when the position of the D-maze is changed; while in the present experiment rats prefer *RS*, part of which is likely to be less recently explored than the rest of the environment, but do not change their preferences when the position of *RS* is changed. The first part of the experiment supports the hypothesis that rats prefer a D-maze because of a tendency, as in alternation, to go to the least recently explored part of the environment. A possible explanation of the failure to change preferences is that when responses are rewarded they become increasingly stereotyped, and rats become insensitive to changes in the environment that do not affect the reward. Thus the preference for *RS* may become fixated and continue even though no longer appropriate to the exploratory tendencies through which it was originally acquired. In the D-maze experiments no primary reward, such as food, was given, so that the preference would not become fixated.

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An Acoustic Factor in Letter Cancellation

CONRAD[1,2] has shown that intrusive errors in the recall of letters presented either auditorily or visually are acoustically similar to the presented items. Thus confusions in recall occurred mainly between *B*, *C*, *P*, *T* and *V* and between *F*, *M*, *N*, *S* and *X*. Subsequent work (for example, Wickelgren[3]) has confirmed and extended these findings.

The following experiment is reported because the results would appear to extend the already important phenomenon of 'acoustic confusion' to an area hitherto considered unrelated to short-term memory.

Twenty naval ratings were presented with foolscap sheets containing a passage of prose reproduced from an article in *Punch*. They were requested to go through the sheets crossing out all the letters '*e*' and were allowed 15 min to complete as much of the material as they could. The emphasis of the instruction lay towards speed rather than accuracy.

The first two paragraphs (329 words) of the completed work were examined to determine which particular *e*'s had remained uncancelled. The results were analysed under three categories: (1) words in which the *e* was pronounced; (2) words in which the *e* was silent; (3) 'the's. The results of this gross analysis are presented in Table 1, from which it is clear that the probability of missing a silent *e* is greater than that for missing a pronounced *e*. This difference is statistically significant ($P < 0.002$, on a two-tailed binomial test). The *e* in 'the' had the greatest probability of being omitted; this being significantly greater than silent *e*'s ($P < 0.024$) and the pronounced *e*'s ($P < 0.002$).

Silent *e*'s are commonly found at the end of words and it was therefore necessary to discover whether it was the predominantly late position of the silent *e* which caused it to be missed rather than the fact that it was silent. Accordingly, a positional analysis was carried out in which the words containing *e*'s were divided into: (1) those in which *e* occurred at the terminal position; (ii) those with *e* in the penultimate position; (iii) those in which *e* occurred in a position other than last or penultimate (the 'early' *e*). Table 2 presents the probabilities of missing an *e* which is silent or pronounced under the three positional categories. It is evident that, after controlling for the position of the *e* in the word, silent *e*'s are still significantly more likely to be omitted than pronounced *e*'s. Position in the word does have an effect, however, in that early *e*'s are less likely to be missed than later ones.

Table 1. PROBABILITIES OF MISSING AN *e* IN 'THE', WHEN PRONOUNCED AND WHEN SILENT

'The'	Pronounced	Silent
0·32	0 04	0·15

Table 2. PROBABILITIES OF MISSING A PRONOUNCED OR SILENT *e* RELATED TO POSITION IN WORD

Position category	Pronunciation category: Pronounced	Pronunciation category: Silent	Significant level (*P*) (pronunciation)
Early in word	0·03	0 06	N.S.
Penultimate	0 07	0·14	0·024
Terminal	0·12	0·18	0·042
	$P = 0.027$	$P = 0.042$	
	N.S.	N.S.	
	Significance level (Position)		

N.S., not significant.

The tentative conclusion to be drawn from these findings is that the acoustic image of a word is scanned as well as the visual stimulus. The results would seem to suggest that the acoustic image is examined for characteristics normally associated with the presence of an *e* in the printed word and that the *e* is more likely to be missed if an acoustic correlate is lacking.

The high proportion of missed *e*'s in 'the' could possibly be due to three factors: (*a*) because the *e* in 'the' is normally pronounced /ə/; (*b*) because the *e* is in the terminal position; (*c*) because 'the' is a highly redundant word. which may be 'taken for granted' and thus not scanned. The first possible explanation (*a*) is probably inadmissible. A comparison was made between words in which the *e* was pronounced /ə/ as in were or inserted with those in which it was pronounced /ɛ/ (as in 'set') or /i/ (as in 'seat') and it was found that /ɛ/ or /i/ were, if anything, the more likely to be missed, but this difference was quite insignificant. (The words used in this comparison were selected such that chance positional factors were controlled.) The second possible explanation (*b*) may be partly true, but cannot be the only explanation, since the *e* in 'the' is much more likely to be missed than terminal *e*'s in other words (see Table 2). The third explanation (*c*) would therefore seem preferable, but further work is needed to establish the case positively.

It is of interest that, since the *e*'s pronounced /ə/ were certainly no more likely to be missed than *e*'s pronounced /ɛ/ or /i/, the nature of the acoustic correlate seemed to be unimportant.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, May 9

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Prof. L. Berry "The Nile in the Sudan".

UNIVERSITY OF LONDON (at the Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, London, W.2), at 5 p.m.—Dr. R. C. Valentine: "The Construction of a Virus" (second of a series of four Almroth Wright Lectures).*

BRITISH SOCIETY FOR THE HISTORY OF SCIENCE (in the Council Room of the Science Museum, Exhibition Road, London, S.W.7), at 5.30 p.m.—Dr. A. I. Sabra. "The Representation of White Light—Hooke *v.* Newton".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "An Engineer's Ideas on Some of the Electromagnetic Fundamentals" opened by Mr G. F. Shotter.

SOCIETY FOR VISITING SCIENTISTS (at the English Speaking Union, Dartmouth House, 37 Charles Street, London, W.1), at 7.30 p.m.—Discussion Meeting on "Teaching Methods in Biology and Medicine". Chairman: Dr. J. R. Ellis. Speakers. Prof. Marc Klein: "The Training of the Doctor in France"; Dr J. Badenoch "The Tutorial System at Oxford".

Tuesday, May 10

SOCIETY OF CHEMICAL INDUSTRY, AGRICULTURE GROUP (at 14 Belgrave Square, London, S.W.1), at 10.30 a.m.—Annual General Meeting, followed by a meeting on "Tropical Crops".

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 2 p.m.—Annual General Meeting. 5.30 p.m.—Mr. A. Schilling: "A New Step in the Appreciation of Lubricants for Modern Automotive Diesel Engines"

UNIVERSITY OF LONDON (at the Middlesex Hospital Medical School London, W.1), at 5 p.m.—Prof Lord Cohen of Birkenhead: "Why Study Medical History?"*

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. S. Jones: "Automatic Control in Railway Systems".

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. G. P. Hutchinson. "Interlocking in Large Electricity Substations—a Fundamental New Approach".

PARLIAMENTARY AND SCIENTIFIC COMMITTEE, GENERAL COMMITTEE (in Committee Room 6, House of Commons, Westminster, London, S.W.1), at 5.30 p.m.—Discussion on "Recent Developments with Regard to Antibiotics and the Control of Infections", initiated by Dr. Frank Hartley and Dr F. L. Rose.

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, Royal School of Mines, London, S.W.7), at 5.30 p.m.—Prof D. W. Fuerstenau: "Mineral-Water Interfaces III. An Interfacial Approach to the Geochemistry and Extraction Metallurgy of Sea Floor Nodules".*

UNIVERSITY OF LONDON (at the Institute of Archaeology, 31–34 Gordon Square, London, W.C.1), at 5.45 p.m.—Mr. D. H. French: "Excavations at Can Hasan, 1961–65".*

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (in the Film Theatre, Shell Mex House, Strand, London, W.C.2), at 6 p.m.—34th Annual General Meeting.

Wednesday, May 11

GEOLOGICAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. R. W. R. Rutland: "A Tectonic Study of Part of the Philippine Fault-Zone", Dr W. W. Bishop and Dr. A. F. Trendall: "Erosion-Surfaces, Tectonics and Volcanic Activity in Uganda".

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5.30 p.m.—Annual General Meeting, followed by Mr. R. S. R. Fitter: "A Year's Work for Conservation".

CHALLENGER SOCIETY (at the Linnean Society, Burlington House, Piccadilly, London, W.1), at 5.45 p.m.—Annual General Meeting, followed by Mr. L. Draper: "The Benefits of Wave Research".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2), at 6 p.m.—Prof D. Gabor, F.R.S. "Holography".

Thursday, May 12

MINERALOGICAL SOCIETY (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 3 p.m.—Meeting on "The Nature and Origin of Ore-forming Fluids".

UNIVERSITY OF LONDON (at Bedford College, Regent's Park, London, N.W.1), at 5.15 p.m.—Prof N. Miliott. "Evolution and the Eye of the Beholder—Victorian Biological Science in Retrospect".*

UNIVERSITY OF LONDON (in the Beveridge Hall, Senate House, London, W.C.1), at 5.15 p.m.—Prof. B. M. Foss. "Education as Art, Science and Technology—a Psychologist's View" (inaugural lecture).*

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, Westminster, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Prevention of Air Entrainment in Vortices", introduced by Mr. G. A. J. Young.

UNIVERSITY OF LONDON (at the Middlesex Hospital Medical School, London, W.1), at 5.30 p.m.—Prof. Fred Lembeck (Tubingen). "Successful Errors in Pharmacology".*

Friday, May 13

INSTITUTION OF ELECTRICAL ENGINEERS, CONTROL AND AUTOMATION DIVISION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion Meeting on "The C.E.I.—Its Implications on Engineering Courses", opened by Prof M. G. Say.

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof Ronald King: "Your Servant—The Electron".

Saturday, May 14

ASSOCIATION OF CLINICAL BIOCHEMISTS, SOUTHERN REGION (at the Middlesex Hospital Medical School, Cleveland Street, London, W.1), at 10.15 a.m.—Meeting on "Some Aspects of Enzymology".

Monday, May 16

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C.2)—Colloquium on "Main Power Distribution in Industry".

UNIVERSITY OF LONDON (at the Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, London, W.2), at 5 p.m.—Prof M. Abercrombie: "The Pathology of Cell Movement" (third of a series of four Almroth Wright Lectures).*

UNIVERSITY OF LONDON (in the Botany Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Prof. David H. Brown (Washington University, St. Louis): "Enzymic Reactions in Glycogen Synthesis".*

UNIVERSITY OF LONDON (at Bedford College, Regent's Park, London, N.W.1), at 5.30 p.m.—Prof. A. Tarski: "What are Logical Notions?"*

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER and an ASSISTANT LECTURER (with a special interest in either pure mathematics or statistics) IN MATHEMATICS—The Registrar, University of Strathclyde, George Street, Glasgow, C.1 (May 12).

LECTURER (with qualifications in educational psychology and/or sociology) IN EDUCATION—The Registrar, Bradford Institute of Technology, Bradford, quoting Ref. 131Y/E (May 12)

ASSISTANT LECTURER or LECTURER (preferably with experience in some branch of phycology) IN THE DEPARTMENT OF BOTANY—The Registrar, The University, Liverpool, 3, quoting Ref. CV/32 (May 14).

DIRECTOR OF THE AUDIO-VISUAL AIDS AND PROGRAMMED LEARNING UNIT—The Registrar, The University, Liverpool, quoting Ref. CV/11/N (May 14).

LECTURERS/ASSISTANT LECTURERS (4) (with a good honours degree, appropriate research or teaching experience, and preferably with an interest in one or more of the following fields Vertebrate zoology, embryology, physiology, histology or marine ecology) IN THE ZOOLOGY SECTION OF THE DEPARTMENT OF BIOLOGICAL SCIENCES—The Registrar, Portsmouth College of Technology, Hampshire Terrace, Portsmouth, Hampshire (May 14).

ASSISTANT EXPERIMENTAL OFFICER (with a pass degree, H.N.C., F.I.M.L.T. or similar qualification, previous laboratory experience, and preferably a working knowledge of microbiology) for work concerned with genetical aspects of animal diseases and to supervise the running of a mouse colony—The Secretary, A.R.C. Animal Breeding Research Organization, King's Buildings, West Mains Road, Edinburgh, 9 (May 16).

LECTURER (with a good honours degree in engineering and preferably an interest and experience in thermodynamics) IN MECHANICAL ENGINEERING—The Registrar, The University, Newcastle upon Tyne, 2 (May 16).

LECTURER (with a good honours degree in physics, academic or industrial research experience, and preferably with research interests in one of the following fields: acoustics, nuclear physics; magnetism or biophysics) IN THE DEPARTMENT OF PURE AND APPLIED PHYSICS—The Secretary, Royal College of Advanced Technology, Salford, 5 (May 16).

MAP CURATOR (preferably geography graduate with an interest in maps and bibliographic work) IN THE DEPARTMENT OF GEOGRAPHY—The Registrar, The University, Leeds, 2 (May 16).

LECTURER IN HUMAN PHARMACOLOGY IN THE DEPARTMENTS OF THERAPEUTICS AND PHARMACOLOGY—The Secretary, University of Edinburgh, South Bridge, Edinburgh (May 18).

LECTURER or SENIOR LECTURER IN HISTOLOGY OR NEUROANATOMY IN THE DEPARTMENT OF ANATOMY, Faculty of Medicine—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (May 19).

ASSISTANT IN PSYCHOLOGY for work in social psychology—The Secretary to the University Court, The University, Glasgow (May 20).

ASSISTANT LECTURER or LECTURER (with research experience in the fields of structural and metamorphic geology) IN THE DEPARTMENT OF GEOLOGY—The Secretary, University College, Gower Street, London, W.C.1 (May 20).

ASSISTANT LECTURER (preferably with an interest in quantitative genetics or population biology) IN THE DEPARTMENT OF AGRICULTURAL BOTANY—The Registrar, University College of North Wales, Bangor, North Wales (May 20).

ASSISTANT LECTURER (preferably with research experience in fields other than soils or dairying) IN THE SECTION OF FOOD AND AGRICULTURAL BACTERIOLOGY (situated in the Department of Agriculture)—The Registrar, The University, Leeds, 2 (May 20).

EXPERIMENTAL OFFICER (with H.N.C. or equivalent qualifications and preferably experience in gas chromatography and scintillation counting) IN PHARMACEUTICAL CHEMISTRY to work under the Professor of Pharmaceutical Chemistry—The Registrar, Bradford Institute of Technology, Bradford (May 20).

I.B.M. FELLOW (with a good honours degree or Ph.D in some branch of science or engineering and at least one year's experience in programming a digital computer) IN SIMULATION STUDIES IN THE DEPARTMENT OF SYSTEMS ENGINEERING—The Secretary, University of Lancaster, Bailrigg House, Lancaster (May 20).

ASSISTANT LECTURER IN PHILOSOPHY—The Registrar, Magee University College, Londonderry, Northern Ireland (May 21).

LECTURER IN EDUCATION in the field of the psychology of education—The Registrar, The University, Nottingham (May 21).

DEMONSTRATOR/SENIOR DEMONSTRATOR IN THE DEPARTMENT OF ZOOLOGY—The Registrar, The University, Newcastle upon Tyne, 2 (May 23).

TEMPORARY LECTURER or ASSISTANT LECTURER IN GEOCHEMISTRY IN THE DEPARTMENT OF EARTH SCIENCES—The Registrar, The University, Leeds, 2 (May 23).

LECTURER (graduate in biochemistry with postgraduate qualifications) IN THE DEPARTMENT OF BIOCHEMISTRY, University of Ibadan, Nigeria—The Secretary, Senate Committee on Higher Education Overseas, University of London, Senate House, London, W.C.1 (May 24)

COMPUTING ASSISTANT (with a good honours degree in mathematics or in a pure or applied science, together with some experience of large-scale digital

computing machines) IN THE ELECTRONIC COMPUTING LABORATORY—The Registrar, The University, Leeds, 2 (May 27).

LECTURERS (preferably with experience in theoretical physics, experimental nuclear physics, solid state physics or astrophysics) IN PHYSICS—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (May 27).

LECTURER IN THE DEPARTMENT OF MEDICAL PHYSICS to take charge of and pursue research in clinical physics work embracing radioisotopes and scanning, radiotherapy and radiodiagnosis—The Secretary, The University, Aberdeen (May 28).

SENIOR LECTURER or LECTURER IN THE DEPARTMENT OF PHYSICS—The Registrar, The University, Keele, Staffs (May 28).

ASSISTANT LECTURER (Probationary) or LECTURER (man or woman graduate, with special interests in stratigraphy, including palaeontology and/or sedimentary geology) IN GEOLOGY—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (May 30).

LECTURER/SENIOR LECTURER and a LECTURER IN THE DEPARTMENT OF MATHEMATICAL STATISTICS, University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, May 30).

LECTURERS/SENIOR LECTURERS (2) IN THE DEPARTMENT OF PHYSIOLOGY, University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, May 30).

ASSISTANT LECTURER (with special qualifications in some field of experimental botany) IN THE DEPARTMENT OF BIOLOGY—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (May 31).

LECTURERS IN THE DEPARTMENT OF ELECTRICAL ENGINEERING—The Registrar, The University, Newcastle upon Tyne, 2 (May 31).

SENIOR LECTURER/LECTURER IN GEOGRAPHY at the University of New England, Armidale, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, May 31).

SENIOR LECTURER or LECTURER (with a Ph.D. degree or equivalent) IN PHYSICAL CHEMISTRY at the University College of Townsville, University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, June 3).

PROFESSOR OF PURE MATHEMATICS at the University of New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, June 30).

READER/SENIOR LECTURER/LECTURER IN THE DEPARTMENT OF HISTORY AND PHILOSOPHY OF SCIENCE, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, June 30).

LECTURER or SENIOR LECTURER (with a degree in veterinary science registrable in the State of Victoria, preferably with postgraduate experience in teaching and small animal medicine) IN VETERINARY MEDICINE (SMALL ANIMALS) IN THE DEPARTMENT OF VETERINARY CLINICAL SCIENCES, University of Melbourne, Australia—Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, July 1).

CHAIR OF ELECTRICAL ENGINEERING—The Academic Registrar, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

CHAIR OF ELECTRONICS—The Academic Registrar, Loughborough University of Technology, Loughborough, Leicestershire, quoting Ref. 12/AF.

KEEPER (preferably graduate with cartographical qualifications and administrative experience) OF THE MAP ROOM—The Director, Royal Geographical Society, 1 Kensington Gore, London, S.W.7.

LECTURER or ASSISTANT LECTURER (pure mathematician) IN THE DEPARTMENT OF MATHEMATICS—The Secretary, University College, Gower Street, London, W.C.1.

POSTDOCTORAL RESEARCH ASSISTANT IN THE DEPARTMENT OF CHEMISTRY for work under the direction of Dr. K. J. Morgan, on the determination of optical dispersion by spectroscopic methods—The Secretary, University of Lancaster, Bailrigg House, Lancaster.

READER (with at least five years teaching experience in light current and an adequate record of university and/or industrial research) IN ELECTRONICS AND TELECOMMUNICATIONS WITHIN THE DEPARTMENT OF ELECTRICAL ENGINEERING, University of Khartoum—The Secretary, Inter-University Council for Higher Education, 33 Bedford Place, London, W.C.1.

RESEARCH STUDENTS, RESEARCH FELLOWS and RESEARCH ASSISTANTS IN THE DEPARTMENT OF CHEMISTRY, for research into two broad fields, namely, electrochemistry and gas kinetics—Prof. Graham Hills, Department of Chemistry, The University, Southampton.

SENIOR RESEARCH ASSISTANT (with a degree or H.N.C. in electrical engineering or applied physics, or an equivalent qualification, and experience in electronics) IN THE MULLARD RADIO ASTRONOMY OBSERVATORY—The Secretary, Department of Physics, Cavendish Laboratory, The University, Free School Lane, Cambridge.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

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Educational Productions, Ltd. Filmstrip No. C6620: Some Common British Seaweeds. (Botany Series.) 34 frames (colour)+notes. (East Ardsley, Wakefield: Educational Productions Ltd., 1965.) 30s. [23

British Broadcasting Corporation. The Measurement of Audiences. By Robert Silvey. (BBC Lunch-time Lectures, Fourth Series—4.) Pp. 15. Local Radio in the Public Interest: the BBC's Plan Pp. 15. (London: British Broadcasting Corporation, 1966.) [83

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Department of Education and Science. Lubrication (Tribology)—Education and Research: a Report on the Present Position and Industry's Needs. Pp. viii+80. (London: H.M. Stationery Office, 1966.) 6s. 6d. net. [253

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The Physics Exhibition Handbook 1966. (50th Exhibition of Scientific Instruments and Apparatus, Alexandra Palace, London, 28th–31st March, 1966.) Pp. xix+229+71. (London: The Institute of Physics and The Physical Society, 1966.) [253

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Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Mechanical Engineering, 1964–65. Pp. 65. Annual Report of the Wool Research Laboratories, 1964–65. Pp. 58. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1965.) [43

Studia Forestalia Suecica. Nr. 31: The Identification of the Mycelia of Wood-Decay Fungi by their Oxidation Reactions with Phenolic Compounds. By A. Kaärik. Pp. 80. 15 kr. No. 32: Ökologische und Physiologische Studien uber die Larchenminiermotte, *Coleophora laricella* HBN. Von Hubertus E. Eidmann. Pp. 226. 18 kr. Nr. 33: Cone Crop Fluctuations in Scots Pine and Norway Pine. (An Investigation based on the cone counts carried out by the National Forest Survey in the years 1954–1962 and on the reports submitted by Forest Service Rangers on the cone setting in the years 1909–1961.) By Stig Hagner. Pp. 21. 4 kr. (Stockholm: Svenska Skogsvårdsföreningen, Box 16316, 1965.) [43

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A MEASURE OF REFORM

BY now time will have ceased to stand still at Oxford. The Franks Commission has at last reported, and the assimilation of its recommendations has begun. Other institutions will be closely concerned. Universities elsewhere, for example, will be affected if Oxford eventually adopts the new proposals on the admission of undergraduates. So will be the schools and their pupils. Then the British Government will be deeply interested in what the Commission has to say. Is Oxford a needlessly expensive way of teaching undergraduates? Or is it an especially good way of doing the job? Or is it both things at once?

The first thing to be said is that the Franks Report* is a most valuable public document. Its scope is enormous and it touches every aspect of university life, public and private. A reader can learn what kinds of schools send students to Oxford, and what proportion of the teaching staff takes dinner in hall at night. For months to come, no doubt, Oxford senior common rooms will be occupied in trying to tell which colleges are which in those anonymous tables which show how individual colleges pay, and otherwise reward, their Teaching Fellows. Then, running through the splendid language of the Report, there is the constant theme that Oxford is not an independent and autonomous university but a part of the educational system of the United Kingdom. Such qualities as the University can claim are but measures of its obligations. From this principle spring important egalitarian arguments —the view that the machinery for admitting students must be fair and seen to be fair, and that the richer colleges should be taxed to help the poorer ones. As an analysis of the present state of affairs, the Franks Report is entirely worthy of the great university it describes.

But will it accomplish what the critics say must be done? This, unfortunately, is not certain. In many ways the Report bears the marks of being an internal document—about Oxford by Oxford and, some will say, for Oxford. At several points the Commission has clearly been prevented by its familiarity with the existing state of affairs from contemplating seriously some extreme but effective remedies for acknowledged defects. In this spirit, for example, it has settled for a compromise on the appointment (by election) of the Vice-Chancellor. He is to be a full-time man at last, and is to be paid a salary; however, the reasons given for deciding to elect a new Vice-Chancellor every four years are so unconvincing that the accompanying proposals to strengthen the office of the Registrar are an inadequate assurance that in future it may sometimes be possible to tell who speaks for Oxford.

This is not to decry the whole Report. Most of the recommendations on the internal administration of the University are wise, and will make the university more effective as well as more efficient. If the proposals on the relationship between the University and the colleges are adopted, for example, a great many long-standing absurdities will be swept away. It is particularly sensible that all academic appointments should jointly involve the university and a college. The proposals on the finances of the colleges are courageous; the notion that essentially autonomous charitable institutions should regard their endowments as held in trust for the university as a whole will do much to change the character of Oxford for the better.

It is therefore all the more surprising that the Report should evade some crucial issues. The proposals on admissions, for example, are an awkward and probably unworkable compromise. It is understandable that the university should wish to attract more of the bright young people who do not at present spend an extra year in the sixth-form of a secondary school, preparing themselves for the competition. It makes very little sense, however, to say that there should be an alternative examination for those who are relatively unprepared. For if the new examination is fair, why should the majority of entrants to Oxbridge be required to spend an extra year at school on needlessly specialized study? And if selection is possible at all after a mere four terms of sixth-form work at school, why should not Oxford take pot luck with the system of university admissions that seems to provide most other universities with the undergraduates they need? Those passages in the Report of the Commission which urge the development of an entirely new system of admitting students to all British universities appear occasionally to suggest that Oxford has sought refuge in the delay there would certainly be in the design and development of an entirely novel system for entry to all British universities. The Commission seems to have lacked a sense of how the competitiveness of the Oxbridge entrance examinations distorts the balance of the curriculum in the schools. It also appears to have been surprisingly uninformed of the studies of the relationship between the universities and the schools now being carried out by the Schools Council and by the Committee of Vice-Chancellors.

The recommendations on the growth of Oxford are also disappointing. The Commission appears to have been needlessly dazzled by the social anthropology of the college system. The calculation is that a college with more than 500 members ceases to be viable. This leads to the conclusion that the student body (postgraduate and undergraduate) in 1980 or soon after may be 13,000, which is an increase of nearly a third more than the present size. It is also true that the growth now foreseeable will occur where it is most needed—there will be more postgraduates and more women. Yet Oxford will remain predominantly a university for the humanities. It is hard to see how some of the exciting developments now planned by scientists at Oxford can be successfully accommodated within the limits proposed by the Franks Commission. Fifteen years from now, Oxford could well be sorry that the Commission did not increase its estimate of science students by two thousand or so.

Picking holes in the Franks Report is going to be easy enough, but this does not imply that its publication will have no effect. On the contrary, it is bound to mark a turning point in the long development of Oxford, and the undertone of the Report makes it plain that the members of the Commission are aware of how eagerly their work is being awaited. After the criticisms of the Robbins Report, Oxford was a despondent place; now, three years later, the mood is very different. The University has

* See also page 667 of this issue of *Nature*.

come to recognize how its great virtues are not inseparably bound up with the out-dated practices which frequently invite criticism. The Franks proposals on postgraduates, for example, will be welcomed with enthusiasm in most colleges. So will be the administrative reforms intended to make the University more cohesive. In one sense the Franks Commission has already attained one of its principal objectives, for the public discussions which accompanied the process of collecting evidence has awakened the University to the opportunities of change. Even at All Souls, there will be a measure of delight.

CLOSING THE MOHOLE

THE future of the American project to drill a hole through the mantle of the Earth now seems in doubt. A week ago the Committee on Science and Astronautics of the U.S. House of Representatives jibbed at approving the new estimate of 28 million dollars for the cost of completing the Mohole. It remains to be seen whether the corresponding Senate committee will take the same view, but the prospects are not encouraging. For even if the Senate should consider that present costs are justifiable, the difference between the House and the Senate would somehow have to be reconciled before work could go ahead. Nobody will be surprised that those most closely associated with the Mohole project have been cast down by this latest twist in the long and tortuous history of this project.

Two separate questions arise. The wisdom of the House Committee's decision is, for example, open to question, chiefly because the Mohole project is now so far advanced. A technique for drilling deep holes in the ocean floor has been developed, and orders for the drilling barges have been placed. By cancelling now, the U.S. Congress will save only a proportion of the total cost of the Mohole. It is capricious, to say the least of it, for the politicians to pull hard on the purse-strings at this late stage. It would have been much more to the point if they had taken a hard sceptical look at the project four years ago, for there was then good reason for believing that the drilling programme was being pushed ahead too quickly, and with too little preliminary study. A more deliberate programme might have been easier to contain within the bounds of a public budget, and might have been more rewarding as well. But Congress cannot put the clock back to the beginning by a crude cancellation.

The second issue is even more alarming. Hitherto, Congress has not exercised to any important extent its constitutional right to arbitrate on the fine details of scientific programmes financed by public money. It is true that plans for building—and siting—big particle accelerators have usually been examined in detail by Congressional committees, and the National Institutes of Health have occasionally been showered with more money than they could usefully spend, but the great public agencies have usually been allowed to manage their own affairs within the framework of a budget agreed by Congress. Though the details of the programmes of the National Science Foundation have been examined by Congress in the process of accounting for the spending of public money, Congress has usually trusted the judgment of its scientific public servants on the disposition of these funds. On the face of it, the Mohole decision looks like a departure from this practice. It could be a dangerous precedent.

HELPING WISELY

International Aid

By I. M. D. Little and J. M. Clifford. Pp. 360. (London: George Allen and Unwin, Ltd., 1965.) Cloth 45*s*. net; Paper 25*s*. net.

INTERNATIONAL aid follows no general pattern; it is a tangled skein of threads beginning with the motives and objectives of the donor countries, often entangled with international organizations, and ending with a group of largely unco-ordinated projects in each of the recipient countries. In *International Aid*, Mr. Little and Mrs. Clifford have performed a valuable service in setting out and analysing the efforts of the more developed countries to help those less fortunate. They have had a difficult task, but have carried it out with considerable success. At times it may seem that they have tried too hard to equate quite different forms of aid, but the exercise has value in that it brings out very clearly the complexity of the problem.

In the early chapters, dealing with the history and quantity of aid, the authors describe its many forms; thus it may be a gift of money with or without any strings, or the supply of materials, equipment or services, or again it may be a loan at any interest rate from nil to the full commercial level and with time of repayment differing equally widely. Further, aid may be given on a bilateral basis or through one of the international agencies or organizations, to which many nations contribute, sometimes freely, at others with restrictions in the form of blocked currencies. The tables given in the book are valuable in that they bring together the available facts, but they should only be read in the light of the qualifications well set out in the text.

One point is clearly brought out, namely that British aid has been far from generous compared with that of some other countries. Moreover, it has not followed any set policy or shown the degree of understanding of the problems involved which might have been expected of the largest ex-colonial power. The limited total of aid has arisen from Britain's own financial difficulties, but the funds available might have been used more effectively if the administration at home and in the recipient countries had been better. Both in the Government departments in Britain and on the staffs of the High Commissions and the Embassies there have been few professional advisers either in the economic or the technical aspects of the work. The few were grossly overloaded so that they were compelled to dash from place to place, with no opportunities to make the intensive studies required for the best allocation of aid. The staffs of the High Commissions and Embassies were more concerned with political questions, as befitted their training, so that the immediate advantage of agreeing quickly to a local demand has been allowed to outweigh the long-term gain which would have resulted from a careful detailed study of the needs of a country. The politician and diplomat have had an unnecessary fear of being branded as 'neo-colonialist'. They have not appreciated that a newly independent country, while violently resenting any political interference, would welcome economic and technical help provided this did not involve long-term contracts of employment and left the final decision to the country concerned, even though the technical advice might point to only one conclusion. This willingness to accept technical help is well illustrated in the recently published report of the Agricultural Research Council of Central Africa, where, in spite of all political differences, Rhodesia, Zambia and Malawi join with the United Kingdom and the United States in financing the work of an international staff.

One of the greatest needs of the less developed countries is for technical assistance. The staffing of a new and highly technical project is often completely beyond the means of a developing country. Help is required to get the project started and in the training of the men who will ultimately

run it. All too often funds which have been made available are left unused for long periods because the necessary staff cannot be found. In desperation men who are not really suited for the work may be recruited with resulting disappointment in the recipient countries.

Here, too, Britain has failed to grasp the opportunities offered. In agriculture and other branches of food production, there were men in the colonies with special training and valuable experience. With independence these men had, in most cases, to choose between short-term contracts or leaving with a generous compensation, which directly or indirectly came out of aid the colonies received. Many left and retired or took up other work unrelated to agriculture in the developing countries. If they could have been recruited into a corps of specialists with permanent career prospects they would have been invaluable in the fight to increase food production. The developing countries would have been saved the cost of compensation and so, to that extent, have required less aid. The loan of these men would have provided most valuable help at a relatively low cost. It is only recently that efforts have been made to meet this need by adding supernumerary posts to the universities, research institutes and the advisory services. Unfortunately, very many of the potential recruits have been lost.

The book includes a most interesting chapter on the principles of aid giving. We are all inclined to be smug about the aid we provide, convincing ourselves that our intentions are altruistic. Yet much of the aid given has, for its objective, either commercial or political advantage. This may be direct or it may arise from the economic development of the country receiving the aid.

Another chapter of special interest is that dealing with the co-ordination of aid administration. Anyone who has been concerned with this work must be conscious of the lack of co-ordination among the international agencies and between the individual donor countries. There is an equal lack of co-ordination between the different ministries in the recipient countries. The authors discuss the many aspects of this complex problem, bringing into focus the doubts and fears which are shared by all those concerned with giving and receiving aid.

This book is one to buy and read and then to retain for reference. It is not easy reading but the effort is well worth while. It should be compulsory reading for all politicians, for civil servants in the appropriate ministries including the Treasury and for our representatives in the less developed countries. W. K. SLATER

BIOPOIESIS

Current Aspects of Exobiology

Edited by G. Mamikunian and M. H. Briggs. Pp. xi+420. (London and New York: Pergamon Press, Ltd., 1965.) 120*s*.

CURRENT Aspects of Exobiology consists primarily of papers delivered at a meeting on exobiology held at the Jet Propulsion Laboratory during February 26–28, 1963. Four papers presented at this meeting were not available for publication and the editors have replaced them with other reviews. The contents of the volume are best described by the titles of the various chapters: "Investigation of Organo-Chemical Evolution"; "Organic Remains in Meteorites"; "Micro-organisms of Middle Precambrian Age from the Animikie Series, Ontario, Canada"; "The Survival Capabilities and the Performance of Earth Organisms in Simulated Extraterrestrial Environments"; "The Possibility of a Primordial Lunar Life"; "Possibilities of Life on Mars"; "Life-Detection Experiments"; "Biological Contamination of the Planets"; "The Radio Search for Intelligent Extraterrestrial Life"; "Trends and Problems in Exobiology".

References are included in each chapter, and at the end of the volume is a lengthy list dealing with "considerations of the existence of life on other planets and for the universe and in origin of life" plus some material on the "physical and chemical nature of the planets" considered relevant.

The field of exobiology is a very exciting area of contemporary research, dealing as it does with what is probably the most important and intriguing problem to mankind—the origin of life and its possible existence on extraterrestrial bodies. Regrettably the present volume does not do justice to the field and will prove of only limited usefulness to the scientific community. The long time delay between preparation of the material and publication of the book results in much of the material being outdated and in many important recent developments being omitted. Moreover, the editing has been somewhat loose with an unfortunate lack of balance and unevenness of treatment. Thus one of the most intensively studied areas, the nature of organic matter and organized elements in carbonaceous chondrites, has only sixteen pages devoted to it, whereas one of the two longest chapters, sixty-four pages in length, deals with a highly controversial theory that oceans existed on the Moon for extensive periods of time and that a lunar life originated therein. While Chapter 2 is devoted solely to carbonaceous chondrites, an extensive discussion of the same topic is contained in Chapter 5. Similarly, a portion of the material discussed in "Possibilities of Life on Mars" is again repeated in "Life-Detection Experiments". Several chapters are marked by a subjective approach which is apparently motivated by the desire that life has originated externally and independently of Earth. This is quite notable in Chapters 2, 5 and 6, and detracts significantly from these discussions. Finally, the merits of the extensive bibliography must be questioned since there is no indication of the extent of the literature search, either in terms of journals and books or in terms of years. Presumably it is intended to appeal to the scientist working in the field, but because of these shortcomings he could not rely on the competence of the list and would perforce carry out his own detailed literature search.

Despite these critical comments, the volume will be a useful addition to a serious exobiology library because of the presence of excellent articles on certain areas, notably organo-chemical evolution (Part 1 of Chapter 1); pre-Cambrian micro-organisms (Chapter 3); life-detection experiments (Chapter 7); biological contamination of the planets (Chapter 8); and the search for intelligent extraterrestrial life (Chapter 9). However, the spotty nature of the coverage and the high price will discourage most scientists and many libraries from adding it to their collections. D. G. REA

MOLECULES AND EVOLUTION

Internal Factors in Evolution

By Lancelot Law Whyte. Pp. xxiii+81. (London: Tavistock Publications Ltd., 1965.) 18*s*.

INTERNAL Factors in Evolution is a timely book. There has been an undercurrent of thought in the minds of scores, perhaps hundreds, of biologists, including many geneticists, over the past 25 years, suggesting that the picture drawn by modern genetical studies is insufficiently adequate and comprehensive. Such doubts have crystallized around the problem of the randomness of mutation, the directiveness imposed by the persistence of cultural preferential trends over many generations and latterly by the rise of molecular biology with its evidence for highly complex organization at a subsidiary level. Thus J. B. S. Haldane (1959), towards the end of a life devoted mainly to research in genetics, was himself forced to the conclusion that "it is almost certain that facts will

be discovered which will show that the theory of natural selection is not fully adequate to account for evolution".

The essence of Whyte's book is the conclusion that the conditions of biological organization restrict, to a finite discrete spectrum, the possible avenues of evolutionary change from a given starting point. The nature of life limits its variation and is one factor directing phylogeny. Thus the mutations of which the consequences reach the Darwinian test have already been sifted by an internal selection process. This internal selection restricts the directions of evolutionary change by internal organizational factors. The author says "there may be no mutations which can be fully ascribed to chance", and he quotes Bachelard (1953) that there is implicit in quantum mechanics a structuring tendency for complex systems to form more complex forms of ordering. He then suggests, though obviously with some doubts, that this could perhaps be the basis of internal selection. Thus, the attempt is made to complement the Darwinian principle of external adaptive selection by a new type of selection acting directly on changes inside the organism at the molecular and chromosomal levels. Under this principle, random mutations are subjected to a selection process dependent on their compatibility with the internal organization and processes of the organism, before the adult faces environmental competition. It is his view that biological order, co-ordination and organization have equal importance with random statistical competition and that this concept is, in a radical philosophical sense, complementary to Darwin's principle and has, therefore, significant implications for the psychological and social sciences as well as for biological sciences. Medawar (1960) is quoted as having suggested that there is in systems of polymers a 'repetitiousness' or tendency towards elaboration, so that ever more complex sets of genetical instructions are offered for trial and that this may provide a basis for advancing complexity; and I think the general response for molecular biologists to Whyte's views will be favourable.

The book is brief, trenchant and profound. The fact that it may not contain any ideas which are absolutely new is no weakness; rather it is in this that much of its strength lies. Whyte is to be congratulated on having brought together and unified in discussion a number of ideas that have been somewhat on the fringe of biological thought in recent years. All who are interested in the fundamental ideas of unity, ordering, complexity, adaptiveness and directiveness—and that is to say almost all biologists—should read this book. Once they have read it they are unlikely, whatever their response, to forget it. They may disagree on how good the evidence is for some of the conclusions and on how far ideas put forward have been adequately covered by earlier writers. They can hardly argue that the problems raised are other than topical and fundamental.

W. H. THORPE

ADSORPTION FROM SOLUTION

Adsorption from Solutions of Non-Electrolytes

By J. J. Kipling. Pp. xii+328. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1965.) 73*s*.

THE understanding of adsorption from solution requires not only a knowledge of the dispositions of, and interactions between, the solution molecules in a surface phase, but also a knowledge of the corresponding situation in the bulk of the solution. Solution and liquid state theory are not, however, in a well-developed state, at least as compared to that for gases, and if only for this reason we may expect adsorption from solution to be a difficult field of investigation. The understanding of adsorption on to solid surfaces from solution requires in addition an intimate knowledge of the solid surface and its interactions with the liquid phase components. The complexities of such systems are obviously immense and it is scarcely surprising that adsorption from solution on to solids has the appearance of being a rather neglected subject. *Adsorption from Solutions of Non-Electrolytes* by J. J. Kipling, is the first book of its kind and reflects accurately the present state of the subject.

This book is almost entirely concerned with adsorption on to solids. There are sections on liquid–vapour and liquid–liquid interfaces, but these are included more for purposes of comparison than for their intrinsic interest. Apart from this limitation, the book is very comprehensive and is based on the extensive experience and work of the author in this field. The early chapters describe the methods of investigating adsorption while the later ones are devoted to specific systems, and the results that are now available to us. The latter are presented systematically. Five chapters deal successively with adsorption from completely miscible liquid mixtures, from partially miscible liquids, of gases, solids and polymers from solution and of adsorption from multicomponent systems. Among the remaining chapters are descriptions of the kinetics of adsorption and the thermodynamics of adsorption. The book contains a considerable amount of data and probably all the important references. The material is well presented and very readable.

Although it will be primarily of interest to research workers in surface chemistry, there are many sections which can readily be used for undergraduate instruction. In my opinion, this is an excellent and much-needed book and is to be wholeheartedly recommended. The only disappointing feature is the price.

D. A. HAYDON

ELECTRONS AND HOLES AT THE SURFACE

Semiconductor Surfaces

By A. Many, Y. Goldstein and N. B. Grover. Pp. xvi+496. (Amsterdam: North-Holland Publishing Company, 1965.) 110*s*.

THE advent of the transistor after the Second World War did more than revolutionize the electronics industry. It set solid-state physics on its feet. It provided, in the study of germanium and silicon, the first really critical test of the electronic theory of solids, one of the triumphs of quantum theory. It stimulated research into many aspects of the solid state. And it presented, in the *p–n* junction, the first really simple example of an electrified double layer.

The great success of *p–n* junction theory has rested largely on two simplifying features: the very low concentrations of the charged particles, and the fact that the electrons and holes move in the same medium on both sides of the junction. In much of the recent research on silicon and germanium surfaces these two advantages have been retained. Electrons and holes in the semiconductor remain, as a rule, the only mobile charge carriers. The bending of the bands at the surface, even in extreme cases of inversion or accumulation, is rarely so great as to introduce degeneracy. The amount of bending, expressed by the surface potential or the electric field normal to the surface, can be controlled at will by using an external electrode (the field effect) or by chemical means. Passage of current parallel to the surface is used to determine excess densities of electrons and holes, and to measure surface mobilities, which are lower than in the bulk. These and other techniques have been elaborated and applied with considerable success to silicon and germanium surfaces, and it is fair to say that we have by now reliable and detailed models both for clean surfaces and for real (oxidized) surfaces, at least up to and including the fast states on the inside of the oxide. The slow states on the outside present a more difficult problem.

Semiconductor Surfaces is the first text-book to deal comprehensively with this new and thriving branch of solid-state physics, and should not be confused with a number of conference symposia bearing similar titles. It will certainly be in great demand. The authors, already well known for their original research in the field, have produced a well-integrated and readable account of the present state of the art, both theoretical and experimental. After a masterly résumé of the theory of bulk semiconductors, which many readers will value for its own sake, there is a mainly experimental chapter on surface preparation and its effect on the lattice structure. Then there are two theoretical chapters: in the first the authors describe the space-charge region in some detail in terms of solutions to the Poisson–Boltzmann equation, using their own improved system of normalized co-ordinates, and displaying graphically the more important results; the second gives the quantum theory of surface states and the associated statistics. Chapters 6 and 7 describe experimental methods, with main emphasis on the field effect, though surface recombination and various emission phenomena are dealt with at some length. The next chapter gives the theory of electron and hole transport at the surface, with experimental results. A final long chapter contains a critical account of our present knowledge of silicon and germanium surfaces.

The presentation is excellent. There are remarkably few errors or misprints. Ample use is made of diagrams and graphs. The text is well documented, the final chapter alone containing more than 400 references. If the book has a fault it is to be found perhaps in its supreme air of competence and confidence. One is left with the feeling that here is a subject thoroughly explored, put in order and offered up to technology on a plate: of course there is much to be done, but the conceptual framework is now established. Is it? What are its limitations? How accurate is the Poisson–Boltzmann approximation? Surely discreteness of charge is important in a narrow surface channel. We are left wondering. Image forces are barely mentioned, and are assumed to exist only outside the surface. No distinction is drawn between elemental and ionic semiconductors. The more enquiring reader will need to look beyond this book for a view of the future. But most of the students, engineers, and experimental physicists to whom it is addressed will find it a mine of information and instruction, an invaluable introduction to the literature, and a real pleasure to read. J. I. CARASSO

GOOD ENOUGH TO EAT

Principles of Sensory Evaluation of Food

By Maynard A. Amerine, Rose Marie Pangborn and Edward B. Roessler. Pp. x+602. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 156*s*.

A POLISH proverb claims that fish, to taste right, should swim three times—in water, in butter and in wine. The early efforts of the basic scientists in the food industry were directed at improving the preparation, preservation, and distribution of safe and nutritious food. Our memories of certain foodstuffs eaten during the Second World War suggest that, although these might have been safe and nutritious, they certainly did not taste right nor were they particularly appetizing in appearance or smell. This neglect of the sensory appeal of foods is happily becoming a thing of the past. Indeed, in 1957 the University of California considered the subject of sufficient importance to warrant the setting-up of a course in the analysis of foods by sensory methods. *Principles of Sensory Evaluation of Food* grew out of this course, and is the first of a series of monographs on food science and technology to be published by the Academic Press. The authors hope that it will be useful to food technologists in industry and also to others engaged on research into the problem of sensory evaluation of foods.

The scope of the book is well illustrated by the chapter headings: "The Sense of Taste"; "Olfaction"; "Visual, Auditory, Tactile, and Other Senses"; and "Factors Influencing Sensory Measurements". There are further chapters on panel testing, difference and directional difference tests, quantity–quality evaluation, consumer studies, statistical procedures (including design of experiments), and physical and chemical tests. An attempt has clearly been made to collect every possible piece of information which might be useful, more than one thousand five hundred references being quoted. As a result, the book seems at first sight to be an exhaustive and critically useful review of the literature. This it certainly is, but this is by no means its only achievement, for there are many suggestions for further lines of research, and the discursive passages are crisply provocative of new ideas and new ways of looking at established findings.

Of particular interest is the weight given to the psychological aspects of perception, both objectively and subjectively. The relation between stimuli and perception is well covered, and includes a valuable discussion of the uses and disadvantages of the Weber fraction in the evaluation of differences. It is interesting to find that in spite of many attempts to separate and define the modalities of taste, nothing better has been achieved than the familiar classification into sweet, sour, salty and bitter. Nor is there as yet any clear-cut evidence of the physiological nature of the taste stimulus. With regard to smell, systems of classification are of little value because of the extraordinary sensitivity of the nose and because the response to the stimulus is so subjective. The authors suggest that a classification based on the size, shape and electronic status of the molecule involved merits further investigation, as does the theoretical proposition that weak physical binding of the stimulant molecule to the receptor site is a necessary part of the mechanism of stimulation.

Apart from taste and smell, there are many other components of perception of the sensations from food in the mouth. The basic modalities of pain, cold, warmth and touch, together with vibration sense, discrimination and localization may all play a part, as, of course, does auditory reception of bone-conducted vibratory stimuli from the teeth when eating crisp or crunchy foods. In this connexion the authors rightly point out that this type of stimulus requires much more investigation, suggesting that a start might be made by using subjects afflicted with various forms of deafness. It is, of course, well known that extraneous noise may alter discrimination, and the attention of the authors is directed to the work of Prof. H. J. Eysenck on the "stimulus hunger" of extroverts and the "stimulus avoidance" of introverts[1]. (It is perhaps unfair to speculate, not that the authors do, that certain breakfast cereals rely on sound volume to drown any deficiencies in flavour, or that the noisier types are mainly eaten by extroverts.)

One very minor criticism of the book is that almost nothing is mentioned concerning the effects of dentures on perception of food sensation. With the world population of old people increasing, it would be thought that the masticatory status, in terms of deficient natural dentitions or full dentures, and its relationship to food selection or rejection, would be of importance to food technology. There are many accounts of investigations on these lines in the dental literature which could have been included.

The authors believe that scientific investigations of the sensory analysis of foods must be based on a thorough knowledge of sensory physiology and an understanding of the psychology of perception. This book gives ample evidence that they can justify their belief. Although they modestly say that it is directed primarily at food technologists, there is no doubt of its value to many others. There is much to interest, inform and even surprise workers

in many other fields, both in the basic sciences, including the behavioural and the clinical. The binding, printing, paper and general layout of the book are unusually good, and it is structurally tough enough to stand up to the rather traumatic life of the frequently handled laboratory reference book. Although its price in Britain may seem high, the quality and quantity of the information contained in it should make cost a very secondary consideration.

D. C. BERRY

[1] Eysenck, H. J., *Experiments with Drugs* (Pergamon Press, Ltd., Oxford, 1963).

INSECT MYCOSES

Pilzkrankheiten bei Insekten

Anwendung zur Biologischen Schadlingsbekämpfung und Grundlagen der Insektenmykologie. Von Dr. Erwin Müller-Kogler. Pp. xvi+444. (Berlin und Hamburg: Verlag Paul Parey, 1965.) 98 D.M.

THE study of entomogenous fungi, for long one of the better known by-ways of mycology, has during recent years been attracting increasing attention because of deepening interest in the biological control of harmful insects. One of the earliest investigations on entomogeneous fungi is a landmark in the history of microbiology. In the 1830's Agostino Bassi, an Italian lawyer turned farmer, after prolonged and ingenious experimentation, established the concept of pathogenicity by proving that the muscardine disease which then ravaged the silkworm industry was caused by the fungus later named in his honour *Beauveria bassiana*. Interest in *B. bassiana*, which is pathogenic for many kinds of insects, has persisted, and there are more references to it in *Pilzkrankheiten bei Insekten* than to any other fungus. Index entries to *B. bassiana* occupy four of the seven columns of the general index devoted to the genus *Beauveria*, which is only surpassed by the combined eight columns of entries for *Entomophthora* and *Empusa*, two genera which, as the author notes, taxononomic mycologists now consider should be united.

If the first book on entomogenous fungi in general, that is to say, on fungal parasites, symbionts, and saprophytes of insects, has yet to be written, *Pilzkrankheiten bei Insekten* is nevertheless most welcome. It breaks new ground in applied entomogenous mycology by bringing together a comprehensive account of attempts to control insect pests, both experimentally and in the field, by the use of fungi and of the underlying theoretical considerations.

The stage is set in Part 1 (pp. 1–62) in which efforts to control insect and mite infestations of crop plants and forest trees during the period 1937–63 are concisely summarized. Some seventy examples of attempts to control diverse pests by the use of more than twenty different species of fungi are assembled, and in addition there are a dozen instances of the use of *B. bassiana* combined with DDT or other insecticides which frequently gave better control than that by either component.

Part 2 (pp. 63–146) includes sections on fungi, the diagnosis of insect mycoses, and the isolation and culture of the fungi involved, but only clues to other works are given for their detailed identification. Consideration is also given to virulence and techniques for the bulk production of spores, and methods for their application are considered in some detail.

To control outbreaks of fungal disease is sometimes difficult but to induce infection, particularly epidemics, under field conditions and at will is even more difficult. This is reflected in Part 3 (pp. 147–347), in which aspects of pathogenesis such as the infection process and the subsequent progress of the pathogen and its effects on the host, host resistance, and the factors in the external environment—particularly those which constitute 'weather'—are dealt with, and for certain of these environmental factors the data are conveniently tabulated. Another table lists fungal hyperparasites recorded on entomogenous fungi and finally there is a brief consideration of epizootics.

The whole work is well documented and there is a 40-page bibliography.

G. C. AINSWORTH

HOMAGE TO LITTLEWOOD

Papers presented to J. E. Littlewood on his 80th Birthday Edited by J. D. Weston. (Proceedings of the London Mathematical Society, Third Series, Vol. 14A.) Pp. v+314. (Oxford: Clarendon Press; London: Oxford University Press, 1965.) 80*s*. net.

PROBABLY no account of the most famous collaboration in the whole field of mathematical discovery will ever be written: the writings of Hardy contain only the most cursory of asides, and Littlewood's fascinating essay on his own mathematical education ends with the tantalizing words: "I soon began my 35-year collaboration with Hardy". Burkill (*Mathematical Gazette*, 38, 47) gives, on the authority of Harald Bohr, the axioms on which the partnership rested, including that which asserted that if one wrote a letter to the other, the recipient was under no obligation to reply to it, or even to read it. Speculation is bound to be ill-informed and possibly impertinent; but on looking again at Hardy's early papers, published before the joint work began, it is possible to feel that, sparkling as they are, they are not quite "in the Hobbs class", to use Hardy's own phrase for supreme excellence, whereas those of the partnership, which began about 1913 with papers on the summability of series and Tauberian theorems, proceeding to the great sequence of memoirs on problems in '*partitio numerorum*', undoubtedly are. Wiener's dichotomy, classing Hardy as the man of clarity and original ideas, Littlewood as the man of power and indomitable persistence, is an oversimplification, in so far as it suggests that each had to supply something the other lacked. The partnership was *sui generis*, and defies dissection; but because this volume is in homage to Littlewood, we may fairly quote Hardy's remark, that he knew of "no one else who could command such a combination of insight, technique and power".

Twenty-six research papers, by friends and colleagues, including many former pupils, ranging over wide domains of mathematics, form an impressive tribute to a great mathematician and a great mathematical teacher. There were no histrionics in Littlewood's Cambridge lectures; the solution of difficult problems appeared a simple matter, until a close investigation in the hope of determining just how the miracle was worked suggested that the lecturer had brought his own rock-climbing skills into the lecture-room, and had exhibited there, too, the almost instinctive ability to detect the precise hand- or foot-hold from which the optimum leverage could be derived. Comment on the process was not withheld; and novices, perplexed by the sets of zero measure which cropped up as exceptions in many real-variable theorems, could be grateful for the emphasis on the informal principles, that every measurable set is nearly a sum of intervals, every integrable function is nearly continuous, and every convergent sequence of functions is nearly uniformly convergent. When these lectures were published in book form, the informal comment, the motivations of the arguments, the care to isolate and emphasize the crux of a proof, contributed substantially to guiding the reader through an intricate domain, in which the content and style prompted Rado to say that reading the book "induces an exalted state of mind closely akin to that experienced when listening to a great symphony or string quartet".

Papers presented to J. E. Littlewood on his 80th Birthday, printed in the best Clarendon Press style, makes a worthy present from the London Mathematical Society to not the least distinguished of its many famous presidents.

T. A. A. BROADBENT

OXFORD AND THE FRANKS REPORT

THE University of Oxford will now know the worst The Report of the Commission of Inquiry under Lord Franks has now been published (May 12, 1966, Clarendon Press). Externally, the chief consequences will be new policies on the admission of undergraduates, on the balance between undergraduates and graduates and between the arts and the sciences. For the rest, the report recommends important changes in the administration of the university including a proposal to centralize the machinery for making decisions without losing the appearance, and some of the reality, of democracy; the taxation of richer colleges for the benefit of the poorer, and the re-organization of academic life in such a way that the separation between the colleges and the university is less pronounced than it has been in recent centuries. It is also proposed that All Souls should have three years "to set its house in order", and in particular to decide how to spend its excess investment revenue. Apart from being a vehicle for specific proposals for change, the Franks Report is also a fascinating and frequently an eloquent exercise in the sociology of a university which is, for better and worse, without compare.

The Franks Commission was created by Oxford in February 1964, after the criticisms of Oxford which appeared in the Robbins Report. The terms of reference were wide, and the Commission was required to assess "the part which Oxford plays now and should play in the future in the system of higher education in the United Kingdom, having regard to its position as both a national and an international university". Among the questions specifically referred to were the administration of the University and the arrangements for the appointment of a Vice-chancellor, the relationship between the University and outside bodies including those from which public funds are obtainable, the relationship between the autonomous university and the substantially autonomous colleges, and matters of finance, staffing, curriculum, selection, examination and postgraduate teaching. The Commission collected evidence throughout 1964, and its work included special surveys, based on questionnaires, of matters such as the academic needs of undergraduates and the work-load of the academic staff. The members were Lord Franks, Sir Lindor Brown, Mrs. J. Floud, Sir Robert Hall, Miss M. G. Ord, Mr. M. Shock and Mr. J. Steven Watson.

The Commission readily accepts a good many of the criticisms made of Oxford in the Robbins Report and elsewhere. Thus it accepts that "an effective administrative system which combines democracy with decision is the first need of Oxford. Unless this is secured, other reforms cannot be undertaken with real hope of success". It accepts "that the mists through which Oxford permits itself to be seen can obscure much more than its statistics". The result, the Commission says, is the prevalence of misconceptions which "arise from Oxford's failure to take the trouble to explain itself". At the outset, the Commission also notes the criticisms that "Oxford is a privileged place", that its academic efficiency is low, that the university as a whole pays too little attention to postgraduate teaching and that the college system, which emphasizes the importance of undergraduate work, exacerbates the neglect of research.

Admissions to Oxford

The Franks Commission says that the problem of selecting undergraduates has been complicated since the end of the Second World War by the great increase in the number of young people seeking entrance to universities and by the wish of schools other than independent and direct grant schools (which are partly dependent on public funds but independently governed) to prepare students for universities. The existing procedure for selecting candidates, based on an entrance examination administered by the colleges, is said to be valuable in itself but to be attended by undesirable consequences. For one thing, the entrance examination supposes that candidates will have spent more than two years in the sixth-form of a British school and will have been provided with knowledge extending well beyond the Advanced-level examination taken by all senior school leavers.

Though the Commission does not accept that Oxford is biased against pupils from maintained schools, and though it can point with satisfaction to the steady increase in the proportion of those admitted to Oxford from maintained schools (19 per cent of male entries in 1938 and 40 per cent in 1965), the Commission does accept that "Oxford is not yet receiving applications from candidates coming from these schools on the scale that the number of prospective university entrants would warrant". The result, says the Report, is that Oxford is impoverished in the quality of its students and their diversity, and that it is exposed to the "suspicion of privilege and social injustice". This difficulty is likely to be increased by the growth of comprehensive schools. "The low application rate from the maintained schools is evidence that Oxford is lacking efficiency as it fishes for promising talent in the national pool: from this point of view the present entrance examination is neither academically successful nor socially just."

What is to be done? The Franks Commission argues that it will be impracticable and undesirable for Oxford to maintain a selection system independent of that used by other British universities, especially as its proportion of all university entrants in Britain declines from the present 7 per cent. But A-level examinations are not a good basis for selection, because they are not designed for university entrance, because university selectors do not play a part in their design and because A-level marks "are not necessarily a good guide to promise in university work". In the long run, the Commission holds, there will have to be a nation-wide system for selecting undergraduates for entry to British universities, but it also says it would not help if Oxford were to throw in its lot with the other universities in the immediate future, and were to rely on the machinery of the Universities Central Council on Admissions (UCCA), because that would "increase the already undesirable pressure which university requirements at A-level puts on the work of the schools". Thus it is that the Franks Commission recommends "that the long-term solution lies in the adoption of a new common admissions system by all universities, and we recommend that Oxford should work towards the initiation of plans for the reform of the admissions system on a national scale. What is wanted is a completely fresh look at the problem of university selection. We think that Oxford is well placed to take an initiative in this matter".

Recognizing that all this cannot come about overnight, the Franks Commission has proposed an interim revision of the Oxford entrance procedure, principally to meet the fear that the university is not sufficiently accessible to pupils who do not spend a third year in the sixth form at their secondary schools. Briefly, it is proposed that there should be two parallel entrance examinations, one for candidates who have spent more than two years in the sixth form, and another for those who offer themselves a year earlier in the school chronology, and who are sufficiently mature (17·5 years in the December of the academic

year in which entry is sought). The Franks Commission reckons that some 10 per cent of the present intake of undergraduates at Oxford would at present qualify under such a procedure, and it suggests that the university as a whole should aim at selecting some 20 per cent of its intake by means of an examination taken before A-level. (The Commission sets its face against a fixed quota.)

Closed scholarships should be abolished, and open scholarships and exhibitions reduced in number so that no more than 10 per cent of the undergraduate population of any college hold scholarships, and in such a way that the value of all awards to undergraduates should be worth £50 a year, and should be dependent on evidence of satisfactory work year by year.

Modest Growth

If the Commission has its way, the undergraduate population will grow modestly in the years immediately ahead, and the postgraduate population will grow much more quickly. Table 1 summarizes recent experience of the growth of the student population and the predictions of the Franks Commission.

Table 1

	1928–29	1948–49	1965–66	1980–85 (est.)
Undergraduates				
Arts, etc.	3,402	4,679	5,080	5,000–5,500
Science	714	1,480	2,518	3,250–3,750
Graduates				
Arts, etc.	309	717	1,235	1,750–2,250
Science	48	354	991	2,000–2,500
All students	4,559*	7,294*	9,824	13,000 (est.)

* A few students who cannot be classified as undergraduates or graduates are omitted.

Predictions about the future size of the postgraduate population are based partly on the recognition of a growing need of postgraduate teaching and partly on the arguments of faculties at Oxford which have provided the Commission with estimates of their own futures. Thus the Commission recommends a growth of the postgraduate population from 23 per cent at present to at least 30 per cent.

The comparatively small increase in the total size of the university is held, in the Report, to be necessary if the collegiate structure of the university, and the advantages thereof, are to be retained. A college, the argument goes, should not be so big that "no one person can hope to know all the members", which is said to be roughly equivalent to the rule of thumb that no college should have more than 500 members, students and senior members. The Franks Commission also holds that the university would lose its cohesion if the number of colleges were to grow without restraint.

The Commission is far less specific about the directions in which such expansion as it recommends should take place. It directs attention to the plans for expansion which already exist in the fields of science and technology, but points out that changes in the numbers of students following courses of different kinds have come about as a result of the policies of individual colleges on the admission of undergraduates. Obviously the Franks Commission is anxious that there should in future be a better co-ordination of these separate tendencies, apparently through the Council of the Colleges, the formation of which is intended to serve the whole cause of co-ordinating college policy.

Conditions of Work

The Oxford tutorial system is the most surprising of the many aspects of academic life at Oxford that the Franks Commission would like to see changed. Sensibly, however, the Report points out that no system of tutorials can at present cover the whole of an undergraduate syllabus without an excessive expenditure of time and effort. It also accepts a great deal of the argument from university teachers to the effect that present concern for the tutorial system entails too great a burden on the academic staff, and recommends that no student should be expected to write more than one tutorial essay a week during term (though he may attend more than one tutorial in the company of others).

The proposals on the conditions of service for academic staff may do much—if they are eventually accepted by the university—to simplify the movement of academics out of and into Oxford. The complications of the present system under which teachers may be appointed either by colleges or by the university, though sometimes by both, are to be removed by a common system of appointment under which university and colleges share responsibility. The arrangements proposed would create the category of "Fellow-lecturer", appointed jointly by the university and by one of the colleges, and required to teach for "up to fourteen hours" a week. Some Fellow-lecturers would owe their first allegiance to the university, and others to a particular college, but none would be entirely in one camp. Stipends would vary a little—Franks thinks not much—according to the college. University readers would increasingly be freed from undergraduate teaching to spend time on postgraduate instruction and supervision, and should have salaries of up to £3,250 a year, with an extra £400 for those retaining the teaching duties in colleges. The Franks Commission would also create more teaching posts at the professorial level, and though there is no suggestion of abandoning the system of a fixed salary (of £4,100) for all professors, it is planned that a professor who is also head of a department can retain his special responsibility allowance without having to do the administration that would normally be involved.

A measure of the extent to which Oxford teachers are said to be overburdened with teaching at present is the calculation of the Commission that the university would have to (and should) recruit a further 182 teachers to bring the size of its teaching staff to the level used as a national standard by the University Grants Commission. Further regularities should flow from the set of detailed proposals that would ensure that college Fellows are equitably and fully charged for the benefits in kind which attachment to a college may sometimes bring.

For the rest, the Report of the Commission makes several recommendations about the improvement of the co-ordination of teaching programmes, for postgraduates and undergraduates alike. Examinations are to be co-ordinated, and continuity preserved from one year to another; arrangements made to see that all university posts should carry with them the automatic right to an M.A. degree and the right that stems from it to take part in the government of the university; the Queen will be asked to forgo her prerogative to appoint the Regius professors at Oxford (with the exception of the canons of Christ Church); and all colleges will be expected to obtain university consent before making senior research appointments.

The Report is scornful of All Souls and of the successive attempts of that college, spread out over the years since the Royal Commission on Oxford in 1922, to spend the whole of its investment income now amounting to close on £250,000 a year. The report suggests that All Souls should aim to spend at least £100,000 on academic activities of its own, and that the college should present a plan to this end to the University within three years.

On general administration and finance, the Report suggests the creation of networks of interlocking committees for general and academic purposes. Within them, the Vice-Chancellor would become more powerful than at present, though it is not proposed to appoint permanent Vice-Chancellors at Oxford but merely to extend the term of office from two years to four. Arrangements are to be made for taxing the richer colleges to support the poorer, which will make it easier for the latter to take on their full share of postgraduate students.

THE ORIGIN OF CONTINENTS

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THE origin of continental nuclei has long been a puzzle. Theories advanced so far have generally failed to explain the first step in continent growth, or have been subject to serious objections. It is the purpose of this article to examine the possible role of the impact of large meteorites or asteroids in the production of continental nuclei.

Unfortunately, the geological evolution of the Earth's surface has had an obliterating effect on the original composition and structure of the continents to such an extent that further terrestrial investigations have small chance of arriving at an unambiguous answer to the question of continental origin. Paradoxically, clues to the origin and early history of the surface features of the Earth may be found on the Moon and planets, rather than on the Earth, because some of these bodies appear to have had a much less active geological history. As a result, relatively primitive surface features are preserved for study and analysis.

In the case of both the Moon and Mars, it is generally concluded from the appearance of their heavily cratered surfaces that they have been subjected to bombardment by large meteoroids during their geological history[1,2]. Likewise, it would appear a reasonable hypothesis that the Earth has also been subjected to meteoroid bombardment in the past, and that very large bodies struck the Earth early in its geological history.

The largest crater on the Moon listed by Baldwin[1] has a diameter of 285 km. However, if we accept the hypothesis of formation of some of the mare basins by impact, the maximum lunar impact crater diameter[1] is probably as large as 650 km. Based on a lunar analogy, one might expect several impact craters of at least 500 km diameter to have been formed on Earth. By applying Baldwin's[1] equation (7–1*A*), the depth of such a crater should be about 20 km. Baldwin admits that his equation gives excessive depths for large craters so that the actual depth should be somewhat smaller. Based on the measured depth of smaller lunar craters, a depth of 10 km is probably a conservative estimate for the depth of a 500 km impact crater. Baldwin's[1] equation (7–11*A*) gives the depth of the zone of brecciation for such a crater as about 75 km. The plasticity of the Earth's mantle at that depth makes it impossible to speak of "brecciation" in the usual sense. However, local stresses may be temporarily sustained at that depth, as shown by the existence of deep-focus earthquakes. Thus, short-term effects might be expected to a depth of more than 50 km in the mantle.

Even without knowing the precise effects, there is little doubt that the formation of a 500-km crater would be a major geological event. Numerous authors have considered the geological implications of such an event. Donn *et al.*[3] have, for example, called on the impact of continent-size bodies of sialic composition to form the original continents. Two major difficulties inherent in this concept are the lack of any known sialic meteorites, and the high probability that the energy of impact would result in a wide dissemination of sialic material, rather than its concentration at the point of impact[4].

Gilvarry[5], on the other hand, called on meteoroid impact to explain the production of ocean basins. The major difficulties with this model are that the morphology of most of the ocean basins is not consistent with impact, and that the origin and growth of continents is not adequately explained.

We agree with Donn *et al.*[3] that the impact of large meteorites or asteroids may have caused continent formation, but would rather think in terms of the localized addition of energy to the system, rather than in terms of the addition of actual sialic material.

The formation of a 10 km deep crater would result in a decrease in pressure of approximately 2,500 atm.[6]. The effects of fracturing down to a depth of more than 50 km would strongly decrease the pressure locally at greater depths. It is impossible to speculate in detail on the sequence of events which followed the impact, but it is

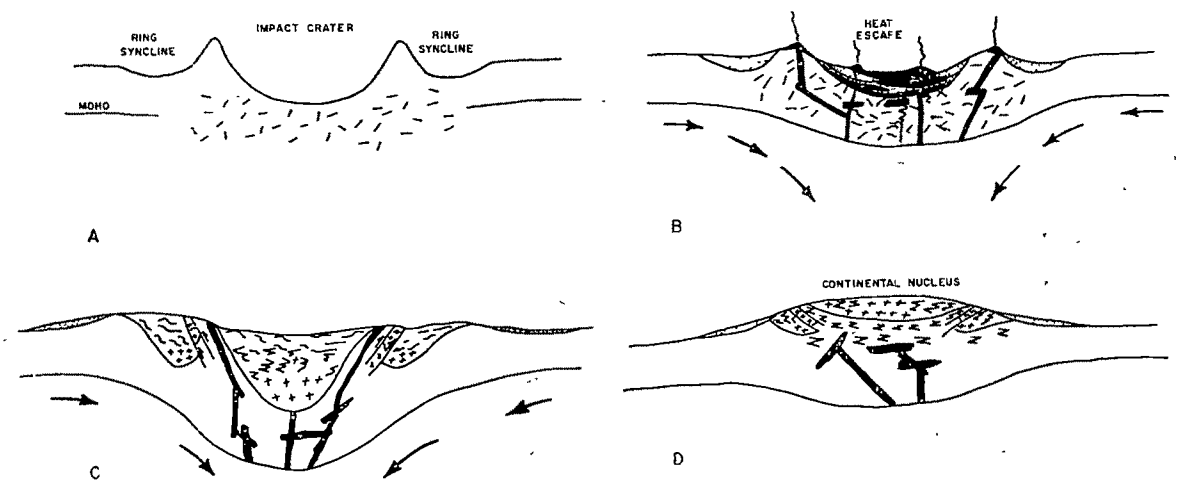


Fig. 1. Proposed mechanism of formation of the continental nucleus. *A*, Immediately after the impact. *B*, Shortly after a new Mohorovičić discontinuity may be formed, deeper than before. Heat released at the surface causes cooling and downflow in the mantle. *C*, Sinking and compression caused by the downflow. *D*, When the heat loss is terminated, downflow stops and the continental nucleus rises. Downflow may restart at the borders, resulting in geosyncline formation and continental growth

reasonable to assume that considerable volcanic action would result from the combination of sudden release of pressure and crustal fracturing[7]. Erosion of crater rim material and volcanism should then jointly fill the crater basin and circumferential syncline, which are typical features of large impacts, with low-density sediments and volcanics (see Fig. 1). As a result of this density differential, the entire complex would be isostatically uplifted to form a continental nucleus. It is of interest to note, in this regard, that the apparent age of the lunar maria (3·6 billion years[8]) is close to that of the oldest continental rocks[3].

Once the continental nucleus has been formed as suggested here, subsequent continental growth could occur in a number of ways. The temperature at a depth of 10 km early in the geological history of the Earth is not known, but 200° C is probably a very conservative estimate, considering the present-day extrapolated temperature at this depth of about 300° C. Before the formation of the continents, convection currents in the mantle were also probably distributed in some simple geometric pattern[9]. The impact would have caused the exposure at the surface of material originally at a temperature of at least 200° C. Even without volcanism, such a structure would be a powerful geothermal sink, conceivably able to modify the convection currents. The most logical modification would be the downflow of the cell immediately under the crater, with all the geosynclinal properties of such a phenomenon[9]. When sediments were sufficiently thick to result in a thermal blanket, the downflow would shift to the edge of the newly formed "protocontinent". From here on, the process would continue as described by Vening Meinesz[9]. Fig. 1 shows diagrammatically such a process.

Kennedy's theory[10], in which the Mohorovičić discontinuity is held to be a phase change, also explains the process by which a geosyncline would sink and accumulate a great quantity of sediments, provided that a basin is present in the first place. Under this theory, the first effect of sediments deposited in the basin and ring syncline of the continental nucleus would be to increase the pressure under them, without any significant increase in temperature. This would cause the Mohorovičić discontinuity, which must have initially migrated downward in response to the reduced pressure attendant on crater formation, to move upward toward the surface. The resulting increase in density would cause the area to sink. As more sediments were added to the sinking trough, their low thermal conductivity would finally result in an increase in temperature, which would cause a downward migration of the Mohorovičić discontinuity. The resulting decrease in density would result in a general uplift of the area. The uplift would create a topographic gradient at the edge of the lifted area, where sediments would be again deposited, thus perpetuating the process.

Whatever the precise history of continental growth, it would appear that the localized additions of energy to the Earth's crust, which would result from the impact of large meteorites or asteroids, could produce continental nuclei through the formation of impact structures. Convection currents and/or the migration of a phase change boundary would assist in the formation of a nucleus, as well as in subsequent continental growth. Other mechanisms could, however, function just as well in continent formation, given the initial localized addition of energy resulting from an impact.

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DDT RESIDUES IN ADELIE PENGUINS AND A CRABEATER SEAL FROM ANTARCTICA

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IN the 20 or more years of use of DDT for insect control in the United States, traces of this pesticide or its breakdown products have become ubiquitous. Even fish in remote streams and those that spend their lives on the high seas have been shown to contain residues[1]. It has been speculated that DDT residues are universally distributed[2].

An opportunity to examine the pesticide content of animals living in the most remote part of the globe came during a visit to the Antarctic. This article reports on the pesticide content of Adelie penguins and a crabeater seal collected at Ross Island, and relates this content to the biology of the animals and to the possible distribution routes of the contaminants.

Samples of liver and fat were collected (by W. J. L. S.) during the third week of February 1964 from six adult male Adelie penguins, *Pygoscelis adeliae*, and one young male crabeater seal, *Lobodon carcinophagus*, at Cape Crozier, Ross Island (77° 27′ S., 169° 14′ E.). The two species were selected because they rarely range beyond the limits of the pack ice and can therefore be considered true residents of the Antarctic region. They both feed on crustacea, as do most of the whalebone whales. The rookery where the penguins were collected is the largest (about 300,000 breeding birds) of the most southerly breeding places for the Adelie, and its location in the Ross Sea is as remote from pesticide-contaminated portions of the world as could be hoped for.

The penguins analysed (Table 1) were selected to represent extremes of physiological condition[3]. Parents actively feeding their chicks are not accumulating body fat (our specimens weigh 4·0 and 4·5 kg, and their blubber is 2 mm thick), but at the end of the season when the chicks are grown the Adelies gorge with food at sea and rapidly

Table 1. QUANTITIES OF DDT AND ITS METABOLITES FOUND IN SIX ADULT MALE ADELIE PENGUINS AND A YOUNG MALE CRABEATER SEAL COLLECTED FROM CAPE CROZIER, ANTARCTICA, FEBRUARY 16–20, 1964

Specimen No	Species and status	Physiological condition	Weight of bird (kg)	Blubber thickness (mm)*	Tissue†	Wet weight (g)	Analysis Parts per billion DDE	DDD	DDT	Total
6169	Adelie	Parent‡	4·5	2	Liver	29 43	20	0	0	20
6151	Adelie	Parent§	4·0	2	Liver	24 20	28	0	8	36
6160	Adelie	Pre-moult	6 0	12	Liver	63 79	15	0	1	16
					Fat	10·78	45	0	0	45
6161	Adelie	Pre-moult	6·3	12	Liver	54·70	10	2	13	25
					Fat	51·43	19	0	5	24
6162	Adelie	Pre-moult	6·5	10	Liver	47 65	14	0	10	24
					Fat	15·87	63	6	18	87
6163	Adelie	Post-moult	2·9	2	Liver	17·69	83	16	16	115
					Fat	17·20	83	0	69	152
6164	Crabeater	—	—	—	Liver	93·75	7	2	4	13
					Fat	176·12	17	7	15	39

* Indicates state of nourishment. Measurement of fat layer beneath feathers over pectoralis major muscle 3 cm in front and below the axilla.
† Fat was from the lower abdomen in all the penguins and from beneath the skin (blubber in the seal).
‡ Parent arrived from sea with a full stomach of shrimp and a small quantity of fish.
§On the beach waiting to depart for sea. Stomach was empty.

gain weight and fat deposits (6·0–6·5 kg and blubber 10 or 12 mm thick) prior to the moult. The birds come to land or stay in the pack ice to moult and fast for about 3 weeks, losing 45 per cent of their body-weight[4]. Our post-moult bird weighed 2·9 kg.

A sample of fat was also taken from an emperor penguin, *Aptenodytes forsteri*, which had been collected by Dr. Edward Wilson in July 1911 during the British Antarctic Expedition's Winter Journey and left, partly exposed to wind and snow, for the past 52 years on the floor of a stone igloo[5] a few miles from the Cape Crozier emperor rookery.

The greatest care was taken to avoid contamination from clothing and hands when the Adelie or seal specimens were collected. Dissecting instruments and the glass containers used for transport were thoroughly cleaned, then rinsed with acetone. Samples were individually wrapped in aluminium foil, placed in one glass jar per animal and sealed with aluminium foil lining the cap. The specimens were frozen within 2 h of collection and remained so until chemical analysis.

No special precautions against contamination were taken with the emperor penguin material as it was not collected with subsequent pesticide analysis in mind. A flipper with attached frozen axillary fat was removed from the 52-year-old carcass by knife and bare hands and stored in a plastic bag. Like the other specimens, the emperor fat remained frozen until analysed.

Residue analyses were made at the Patuxent Wildlife Research Centre (by C. M. M. and W. L. R.) by electron-capture gas chromatography, with confirmatory identification by thin-layer chromatography. The samples were prepared by grinding, then drying with sodium sulphate. They were then extracted for 7 h in 'Soxhlet' apparatus with petroleum ether, then 7 h with isopropanol–hexane (1: 1); extracts were carried separately thereafter. Acetonitrile–hexane (1: 1) partitioning was used to clean up the petroleum ether extracts; hexane partitioning for the isopropanol–hexane extraction; elution was through a 'Florisil' column (3: 1 hexane–benzene). Gas chromatography was carried out with a 'Jarrell-Ash' gas chromatograph fitted with an electron capture detector; column, 5 per cent *SE*-30 on 80/90 mesh 'Anakrom *ABS*' in 0·25 in. × 44 in. glass column, nitrogen pressure 30 lb./in.^{2}, range 10^{-9} amp; column temperature 150° C, injector temperature 195° C. Thin-layer chromatography was also carried out as a confirmatory measure on eluates from all samples combined. Readings were made on 200 × 200 mm plates coated with aluminium oxide *G* (0·25 mm thick) and activated at 130° C for 0·5 h. Some fat remained, and the combined sample was spotted on four plates in 10 μl. aliquots. 10 μl. of the sample was also placed on standards to compensate for the distortion of R_F values. The plates were developed with hexane, but only the standards were sprayed with Mitchell's silver nitrate solution and exposed to ultra-violet light. That portion of the plates corresponding to DDE, DDD, and DDT was removed with a vacuum zone collector, extracted and re-read by gas chromatography. The total of 11·8 μg of DDE from individual samples agreed closely with the amount (12·4 μg) found after combining the samples. The eluate was again concentrated, then developed, sprayed. and read by thin-layer chromatography. DDE and DDT were observed, but the amount of DDD was insufficient.

Separate portions of the fat and liver of the same individuals were also analysed at the Wisconsin Alumni Research Foundation. The samples in this case were prepared by low-temperature drying and grinding with sodium sulphate. Extraction was for 8 h in 'Soxhlet' with ethyl-ether and 'Skelly *B*'. Acetonitrile partitioning was used for cleaning up, followed by elution through 'Florisil' column with ethyl-ether and 'Skelly *B*' (5 : 95). Gas chromatography was carried out using a 'Barber-Colman' 5,000 series gas chromatograph with an electron-capture detector; the column consisted of acid- and base-washed and silenized 'Chromport *W*' coated with 5 per cent Dow Corning 200 (12,500 centistokes) in 0·25 in. × 48 in. column, nitrogen flow 75 c.c./min, column temperature 210° C, injector temperature 240° C, detector temperature 230° C.

All samples from the six Adelie penguins and the crabeater seal contained minute traces of DDT and its metabolites ranging from 13 to 115 parts per billion in liver and 24 to 152 parts per billion* in fat (Table 1). No DDT products were detected in the emperor penguin fat, which came from a bird killed over 30 years before the world-wide use of DDT, nor were residues of other chlorinated hydrocarbons found in any of the specimens. Analyses by the Wisconsin Alumni Research Foundation also showed the presence of DDE and DDT in comparably minute traces ranging from 11 to 64 parts per billion in liver and in fat. These results are remarkably close, considering the minute quantities involved. Moreover, the portions tested by the two laboratories were different samples from the same individuals, not sub-samples of a single-blended preparation.

The Adelie (No. 6163) that had undergone considerable post-moult weight loss still contained residues that were high in concentration (though not in total quantity) by comparison with others in the series. This indicates that the extensive metabolism of fatty tissue that occurs during moult does not result in the complete elimination of the pesticide residue from the fat or liver. This is what would be expected on the basis of experimental studies with rats in which animals subjected to enforced weight loss through food deprivation showed increased DDT concentration in the fat that remained, thus indicating that the rate of loss of fat exceeded the rate of loss of pesticide[6].

* U.S. billion; 1×10^9.

On the basis of present knowledge, the quantities of DDT in the penguins and seals would be judged an unlikely hazard to them. DDT residues in living animals collected in the United States independently of known treatment programmes vary in amount from quantities similar to those in our Antarctic specimens to amounts thousands of times greater[7]. One investigation of DDT residues in human body fat in the United States showed approximately 7 p.p.m.[8].

With so little available information on the distribution of DDT in Antarctic wildlife, we can only speculate on its mode of transport to the area and its incorporation in the ecosystem. Several possibilities can be considered.

Local contamination from man is unlikely. DDT is not used in Antarctica, though it is possible that a certain amount is carried on fur and woollen clothing. Evidence against direct contamination from clothing during collection comes from the fact that the emperor penguin fat was negative despite few precautions during collection. Moreover, with one exception, the positive specimens showed a higher proportion of residues in the fat than in the liver, as would be expected under conditions of low chronic exposure. Contamination from faulty handling would have been more likely to have given equal quantities in fat and liver.

Waste disposal at the United States and New Zealand stations at Hut Point, Ross Island, about 50 miles from Cape Crozier, is another possible source of some DDT contamination of the local marine life. A total of more than 1,000 men stay at these stations in summer and 200 in winter. Packing material, food waste, old clothing, and frozen human excreta are dumped on the sea ice and liberated to the sea during a brief period in late summer when the ice breaks up.

A conservative approximation of the quantities of DDT discarded in food waste can be made on the basis of the normal content of DDT residues in the United States diet. This amount has been estimated to be 0·184 mg/man/day[9]. If food waste is assumed to equal half the total food consumed, then feeding a group of 1,000 men for 100 days would provide 9 g of DDT residues. A portion of ingested DDT will also be eliminated in the faeces, but the amounts eliminated at such low levels of intake have not yet been estimated. However, there would be rapid dilution in the water of McMurdo Sound of any traces in the waste from such a small human population. It is, perhaps, also significant that no other chlorinated hydrocarbons were detected in the penguins and seal, yet they would be liberated in minute quantities, like DDT, in food waste.

Another possibility is that the DDT residues reached the South Polar regions by air currents. DDT recovery from the air has been reported in the United States by the U.S. Public Health Service[10], and in several unpublished investigations. There is accumulating evidence to indicate that bacteria, fungal spores, strontium-90, and even land arthropods[11] have been transported to Antarctica by air. The dilution of the air would be so great, however, that we consider it unlikely that this is the principal source of contamination of the fauna. Two samples of ice were examined by the Southeast Water Laboratory, Public Health Service, Athens, Georgia[12]. One sample represented glacial ice from Byrd Station (80° S., 119° W.) deposited prior to 1940 and the other, glacial ice deposited more recently. Neither of these samples contained traces of any pesticide. DDT would have been detected if it had been present in concentrations of as little as 0·05 part per billion.

It is also possible that DDT might have been carried into Antarctic waters by ocean currents. The penguins and seals feed in the Antarctic surface current, which, moving northwards, sinks below the warmer sub-Antarctic water at the Antarctic Convergence. Although there will be mixing on the Convergence, it would be difficult for contaminants to spread directly southwards against the Antarctic surface current. Contaminated water would be more likely to gain access to the Antarctic by way of the warm deep current which, passing upwards between the Antarctic intermediate current and the Antarctic bottom water, is responsible for the upwelling of nutrients in the Antarctic region. This warm deep current originates chiefly in the north Atlantic Ocean. It has been estimated, however, that it would take many years for particles to reach the Antarctic from the north Atlantic where it is assumed the pollution is highest. But, as Deacon[13] points out, not all the warm deep current originates from the north Atlantic. Much of it must be formed from the Indian and Pacific Oceans and also from mixing between northward and southward movements in the sub-Antarctic region. Thus, it would be possible for pollution to reach the Antarctic surface water by way of the warm deep current, though it would be expected to take longer to come from the northern than from the southern hemisphere.

Concentration in the food chains would provide the next step in the build-up of pesticide residues. The Adelie and crabeater occupy the third trophic layer of a typically simple Antarctic food chain. Adelies from Cape Crozier feed their young partly on small fish, but mostly on *Euphausia crystallorophias*[14], the dominant shrimp in the neighbourhood of the Ross Ice Shelf and the Victoria Land coast. Farther off the coast, *crystallorophias* is replaced by the most abundant of all the euphausids, *E. superba*, a circumpolar species that occurs predominantly in the east to west Antarctic surface current, but which overlaps with *E. frigida* in the west-wind drift almost to the Antarctic Convergence. In fact, Mackintosh[15] has pointed out that the ten species of southern *Euphausia* overlap (as do other components of the marine fauna) in their distribution in characteristic zones to the sub-tropical Convergence. In addition to the overlap in horizontal distribution, which shows remarkably little seasonal change, there are seasonal and diurnal vertical migrations of these and other plankton which lead them away from the Antarctic surface water into the warm deep current and therefore into the most likely source of contamination. For this reason, the Adelies and crabeaters are more likely to pick up DDT and concentrate it in their tissues from traces in *E. superba*, or even *E. frigida*, when they are feeding in the pack ice away from land.

Finally, there is the possibility of direct contamination of Antarctic waters, and subsequent concentration in the food chains, from the breakdown of the vast hordes of marine organisms, including whales and birds, which move freely across the Antarctic Convergence and into temperate zones. Whales do most of their feeding south of the Convergence, but they move north in the austral winter. Of the bird species, the Arctic tern, *Sterna paradisea*, the giant petrel, *Macronectes giganteus*, the sooty shearwater, *Puffinus griseus*, the Wilson's petrel, *Oceanites oceanicus*, and other species have extensive migrations from the Antarctic and sub-Antarctic to the harbours and coastal waters of most countries of the southern hemisphere, or to the North Atlantic and Pacific Oceans[16]. For example, the Wilson's petrel, reputed to be one of the most abundant birds on earth, breeds around the Antarctic and on its islands, then moves during the austral winter into the Indian, Pacific and Atlantic Oceans. It can be seen in great numbers scavenging off the eastern coast of North America between April and September. A few months later it will be back in Antarctica feeding on *Euphausia superba*. These are but a few examples to emphasize two important points. There are enormous changes in biomass in the surface waters of the South Polar regions according to the seasons, and the southern oceans are linked directly and rapidly with different parts of the world by these and other migrating scavengers and carnivores.

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GUESSING AS A FORM OF INTELLIGENT BEHAVIOUR

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WE have recently been reminded by Hammerton[1] that guessing is not always random. This is scarcely contestable. What is, perhaps, of more interest is Hammerton's contention that the type of correction for guessing often made in intelligence tests of the multi-choice variety is inadequate. He offers evidence in support of the thesis that guesses have a better than random probability of being right and that therefore "It can pay to guess". The implication seems to be that guessing in this context is a mild form of delinquency and is not sufficiently penalized by applying the usual correction formula. Two points arise and need clarification. The first has to do with item uniqueness, while the second involves more fundamental considerations.

To illustrate the argument, and to provide data, Hammerton uses a modified version of Shipley's vocabulary test. With this kind of test it can hardly be denied that on balance it pays to guess when in doubt. Two sorts of reasons account for this. First, the subject may have good grounds for rejecting some of the possibilities, either because he knows their meanings and is sure that they are not synonymous with the word specified, or because they are the wrong part of speech, or for some similar reason. If only one of the distractors is of this form, then a subject's chance of hitting the right answer is not $1/n$ but $1/(n-1)$, and so on. Secondly, as is widely recognized, there is a continuum between ignorance and certainty which may favour guessing in certain circumstances. In vocabulary tests this influence will favour guessing unless the distractors have been specially chosen to mislead the unwary. However, the broad assertion that this influence must favour guessing overlooks the factor of item uniqueness. If we choose items carefully, we can easily demonstrate that guessing raises or lowers scores, or leaves them unaffected.

The following multi-choice item is a good example:

CONDIGN means the same as
BLAME, MEET, PEPPERY, PRAISEWORTHY, SLANDER.

Unless the subject actually knows the right answer he is more likely to choose one of the distractors. The main point of the argument is that it is possible to produce items of this kind and to engineer the distribution of guessing probabilities in any way that takes the fancy. It is a matter of interest to examine why this should be so.

In this particular example we have engineered the situation by:

(*a*) Using a word that is in any case little known.

(*b*) Using a target word that has a far more common meaning than the alternative meaning that corresponds to CONDIGN.

(*c*) Choosing distractors that are plausible. For example, CONDIGN has about it a similar ring to CONDEMN, and so may easily be thought akin to any word involving value judgments.

(*d*) Confusing the subject still further by not giving clues as to whether he should be looking for a verb, adjective or noun. Only PEPPERY and PRAISEWORTHY are clearly adjectives; BLAME and SLANDER can operate as nouns or verbs; while MEET in its common form is a verb, although it is as an adjective that it provides a synonym for CONDIGN.

An experiment has been carried out following the procedure of Hammerton. A vocabulary test was presented to subjects who were also asked to give, with each response, an assessment of their own feelings of confidence or doubt. The subjects—forty in number—were all adults with a good educational background. The test was a modified version of the vocabulary part of Shipley's self-administering test[2]. The even-numbered items were used with the addition of an extra distractor to each item, so that a subject had to choose the correct word from among five words. The CONDIGN item was added to the end. The null hypothesis was that each word on the CONDIGN item would be chosen an equal number of times, and that any observed differences would merely be chance variations. The prediction was that those who 'guess'—those who express some 'doubt' or are 'very doubtful'—on this item would tend to choose one of the distractors rather than the target alternative; in other words, less than 20 per cent of the subjects would choose MEET.

All the subjects were doubtful about the CONDIGN item, and the following results were obtained.

NUMBER OF SUBJECTS CHOOSING TARGET WORD AND DISTRACTORS

Meet	Distractors
1	39

A one-tailed binomial test[3] indicates $P < 0.001$, so that the prediction is confirmed.

* Seconded from University College, London.

So, if we were to apply the usual guessing correction to a test composed of items of this type, the standard correction for guessing would be too punitive. A subject who knew none of the answers, but who valiantly guessed, would be likely to get rather less than 20 per cent right, and would therefore end up with a negative score. And serve him right, some may say. But does it? Here we come to the second main point—one of principle that leads on to far more interesting issues.

When as test constructors we somewhat arbitrarily designate one of the alternatives as 'correct', we risk making the disconcerting discovery that we are less intelligent than some of the people we presume to test. With a dictionary in one hand and a fertile imagination controlling the movements of the pen in the other, test constructors must feel pretty confident in their ability to spot the winner. Subsequent item analysis can be a chastening experience. A typical example came to light in the course of constructing a vocabulary scale that would discriminate reliably among university students. The test constructor (R. T. G.) thought it would be a good idea to include TURGID as an item, on the grounds that some people might easily confuse it with TURBID. TURGID means BOMBASTIC according to the dictionary. However, it would seem that most intelligent people remain unaware of this, and item analysis revealed that it took a relatively low-grade intellect to choose BOMBASTIC as a synonym, MUDDY being much preferred by those scoring high on the test as a whole. Nowadays, TURGID is most often associated with the phrases of TURGID prose or TURGID style. In this context it easily slips into use as a synonym for CONGESTED or OBSCURE, and hence MUDDY. If English is defined as the language used by contemporary intelligent and educated English-speaking people, then the writers of dictionaries would appear to be out of touch with a number of developments in current usage.

The lover of Shakespeare's language may deplore the fact that DISINTERESTED is often misused; particularly since IMPARTIAL has so few synonyms. Nevertheless, we must come to terms with the idiosyncrasies of a vital language that has somehow managed to transform SANCTION into its own antonym. In the interests of public safety, perhaps less regret need be felt when INFLAMMABLE, which is currently in the process of being transformed, is eventually superseded by the Americanized FLAMMABLE. The pronunciation of BANAL provides a related case in point, and that of QUESTIONNAIRE another just as interesting in its own way.

It should be pointed out that the orthodox methods of item analysis do not always reveal weak items of this kind. As a rule, each item is called right or wrong, and all the wrong responses are lumped together for the purpose of computing a bi-serial r or whatever other statistic is used to correlate the item response with either an internal or an external criterion. This method can easily conceal the fact that among the responses designated wrong by the constructor there can lurk one alternative (or even more) that would show up a good deal better than the officially correct response if the more valid distractor were to be designated right and the computation re-run. By lumping together all the so-called wrong responses, the highly intelligent subjects choosing the most valid response(s) are swamped by the subjects who select the really hopeless distractors. Only by sorting responses to each item into as many groups as there are possible responses is it possible to discover which response is, in fact, the most valid. Using this technique, the alternatives can be ordered in terms of validity and the fact that the difference between right and wrong is one of degree can be brought out. In an item analysis of this kind performed on the Cattell III test (Buros[4], p. 440), a disturbingly high proportion of the items were found to be either non-discriminating or wrongly keyed.

Granted, then, that right and wrong are to be considered operationally, rather than as *ex cathedra* pronouncements on the part of the test constructor, we come back to the intelligent subject faced with choosing from among a number of alternatives the one most likely to be scored right. He may, of course, be perfectly sure of the answer and, as is well known, there is usually a significant positive correlation between subjective confidence levels and the probability of being correct. However, the intelligent may be less than certain. In these circumstances we may ask:

(*a*) Is he entitled to guess?

(*b*) Should he be penalized for wrong guesses, and, if so, to what extent?

(*c*) Is it more intelligent to guess than to pass over the item?

The first question will bear little discussion. All answers are in a sense guesses; even if the subject is certain he is right, there is no guarantee that his confidence is justified. If we attempt to make an ethical issue of this—that guessing is in some sense dishonourable—we shall find ourselves forced into a peculiar moral posture. "When in doubt, do nothing" is the injunction, which is a strange request to make of a subject, and may fairly be interpreted as "Have no doubts".

The two remaining questions are inter-related and come closer to the heart of the matter. It has been suggested by Hammerton[1] that guessing in these circumstances is analogous to what goes on in signal detection experiments. This is a perfectly tenable point of view, particularly if the concept of 'gambling' between 'Type I' and 'Type II' errors as used by Swets *et al.*[5] is emphasized. A 'Type I' error occurs when we reject the null hypothesis although it happens to be true; a 'Type II' error occurs when we accept the null hypothesis although it happens to be false. Scientists, for the most part, adopt the convention that it is preferable to accept a high risk of 'Type II' errors, rather than vice versa. There are, however, exceptions to this general rule which are far more germane to real life situations.

For the present purposes, however, it may be more profitable to go to game theory for a more powerful theoretical framework within which to analyse this type of guessing behaviour. In their book, *A Study of Thinking*, Bruner, Goodnow and Austin[6] show how the concept of a pay-off matrix, stemming from the work of von Neumann and Morgenstern[7], can be used to analyse problem-solving behaviour in psychological terms. With intelligence tests we are dealing with problem-solving behaviour of a special kind, and what may not have been fully appreciated until now is that there is a pay-off matrix associated with guessing in this context, even though the matrix is rarely defined clearly or made explicit by the test constructor. This, however, is scarcely the fault of the subject, who has to do the best he can in a relatively unstructured situation.

If we state explicitly how a test is to be scored, the subject can then make rational decisions about his guessing strategies. We can say, where there are five alternative answers to each item, that the subject will be credited with one point for every item correct, and debited a quarter of a point for every item wrong. Using this standard form of correction for guessing, the subject knows that on balance it should pay to guess. If his guesses really are random, then he will, in the long run, neither gain nor lose. If, as is generally the case, subjective confidence and probability of being right are correlated, then on balance he should come out on the right side, which is perfectly reasonable. The ability to make good guesses is the same as the ability to pick the right answer, the difference being purely one of degree. Any subject who in these circumstances refuses to guess (or who refuses to choose, short of subjective certainty) either lacks the intelligence to draw the appropriate conclusion regarding the pay-off function for himself or—if he can draw the conclusion but cannot bring himself to act on it—may be said to be acting irrationally.

An alternative form of explicit instruction would be to tell a subject that he will not be penalized in any way for guessing. Again the intelligent subject will be sure not to leave any item unanswered.

But what of the more common form of instruction that leaves the question of guessing vaguely undetermined? Here the subject has to construct a pay-off matrix for himself by divining what the probable scoring procedure is, and play his game accordingly. This form of instruction will favour the adventurous, even reckless, individual who enjoys risk-taking and believes in making the most of poorly structured situations. The more restrained, pedantic type of person will find himself at a disadvantage and will, almost inevitably, be penalized for his timidity. And this, it may be argued, is what life is like. The person who refuses to take unnecessary risks, no matter how favourable the pay-off function, is left behind in any race—for money, power, prestige or the more human satisfactions associated with the needs for companionship, warmth and affection. In a city, the person who waits until the street is entirely clear of traffic before crossing the road will certainly minimize the risk of being struck down by a vehicle, but he will cross very few roads.

So it would seem that most intelligence tests as presented are, in this matter of guessing, more like personality tests. Furthermore, a case can be made for the paradox that they are more effective as intelligence tests precisely because they do possess this characteristic of being a kind of personality test. This is not a new idea. It is expounded by Wechsler[8] in his theory of non-intellective intelligence, and in which he attacks the widespread assumption that personality and intellectual factors belong to different domains. Wechsler shows that there are personality components integral to mental functioning. In effect, he argues that the level at which an individual functions as an intelligent creature depends, among other things, on his emotional economy. The person who behaves stupidly because of his emotional difficulties is still behaving stupidly. Whether or not he can overcome these difficulties and function at a higher level is a question comparable with that of the extent to which he can overcome an early environmental deficit of an intellectual kind.

This brings the argument back to the two questions with which we began. We can answer the second, more or less adequately, by saying that it is usually more intelligent to guess than pass over an item, but that everything will depend on the pay-off function. It would be simple enough to devise a pay-off function that would penalize subjects so heavily for wrong answers that it might pay to refuse to answer all the items. Existing tests do not, however, go to such extremes, and it is doubtful whether any intelligence test is made more effective as a discriminator by the use even of the standard correction for guessing. Indeed, there are grounds for arguing that test instructions should be more explicit about the penalties for guessing, so that the intelligent subject would be given an opportunity to derive the pay-off function and use his rational powers to overcome whatever personality factors might otherwise inhibit his performance. Without this information he is left in the invidious position of having both to allow for his own personality variables, and to divine the emotional attitude of the test constructor towards guessing.

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OBITUARIES

Prof. L. R. Wager

PROF. L. R. WAGER, professor of geology and Fellow of University College, Oxford, from 1950, died suddenly in London on November 20, 1965, at the age of sixty-one.

Lawrence Rickard Wager was born at Batley, Yorkshire, on February 5, 1904, and educated at Leeds Grammar School. He proceeded to Pembroke College, Cambridge, where he graduated in 1926 with first-class honours in geology in the Natural Sciences Tripos. The award of a Goldsmiths' Company Studentship then enabled him to work at Cambridge for three years under Dr. Alfred Harker, chiefly on the mineralization and metasomatism of the Whin Sill.

This early interest in petrology was to continue for the rest of his life and to earn him a world-wide reputation as a leading authority on the petrogenesis, mineralogy and geochemistry of igneous rocks. Wager was primarily a field geologist, however, in that he advocated and applied the principle that an interpreter of the Earth's history should see the evidence for himself, and in this he was not the sort of man to content himself with a limited objective. During his remarkable life he scaled the higher reaches of Everest, explored the Greenland Ice-cap, mapped 35,000 km^2 of rocks in one Arctic year, produced the most important account of an intrusive complex known in petrological literature, and established and directed, at Oxford, a particularly advanced geochemical research group dealing chiefly with radiometric age and isotope studies on rocks and minerals. The hallmark of a first-rate geologist is the ability to trace and find his evidence on any part of the Earth's surface, to investigate the material using advanced laboratory techniques, and then to co-ordinate the minute details and translate them often in terms of the vastness of the Earth's framework and its time-span. Wager showed more than just the ability to do this, for he was outstanding in each field.

In 1929 he was appointed to a lectureship at the University of Reading, and the period between then and the outbreak of war saw him on several expeditions as both geologist and climber. At Cambridge he had been president of the Mountaineering Club, and in 1930 he was chosen by Gino Watkins to go to East Greenland as a member of his British Arctic Air Route Expedition. The three hazardous exploratory journeys in which Wager participated included a scientific reconnaissance of 200 miles of the treacherous Angmagssalik–Kangerdlugssuaq coast, a 250-mile return sledge journey to relieve the inland Ice-cap weather station, and a 360-mile return journey to climb the highest point then attained in the Arctic (Mount Forel). In 1932 he continued his geological reconnaissance investigations with the Scoresby Sound Committee's Second East Greenland Expedition, and went there again in 1934 with Dr. Charcot in the *Pourquoi Pas?* In 1935, with Augustine Courtauld and others of the British East Greenland Expedition, he climbed the highest mountains in the Arctic (the Watkins Mountains). With two Eskimo families he then wintered in the remote Kangerdlugssuaq region, together with his brother as botanist (Dr. H. G. Wager) and both their wives, and, in the company of W. A. Deer, he conducted a year's

geological survey of about 35,000 km² of Tertiary volcanic country (Southern Knud Rasmussens Land) and a remarkably detailed study of the associated plutonic complexes. He returned to the region in 1953 as joint leader of the East Greenland Geological Expedition, and at the time of his death had obtained financial support for a large-scale second geological expedition, which he was to have led jointly with Prof. W. A. Deer (Cambridge) in 1966 and 1967.

Wager's skill on rock and ice was already well known when, in 1933, he joined Hugh Ruttledge's Everest expedition. On May 30 of that year, hampered by the beginning of frost-bite and with the oxygen supply from the air reduced to one third of normal, he and Wyn Harris reached 28,100 ft. in an assault on the summit. "Knowing these men as I do, they would not turn aside from a climb within the limits of the possible", wrote Ruttledge, but they were still capable of bringing back an ice-axe dropped by Mallory in 1924 and, in Wager's case, specimens from the limestone cliff that had barred their way to the summit. Wager wrote papers on the structural and geomorphological features of the Everest region, and on the fossilized organisms that lay in rocks uplifted nearly six miles above the seas in which they had once lived.

In 1939, after a number of papers by Wager on the Tertiary volcanic rocks of the region, he and W. A. Deer published a memoir on the petrology of the Skaergaard intrusion of East Greenland (*Medd. om Grønland*). Few treatises have had such an impact on the science of petrology, and even to-day it comprises one of the standard references on gabbroic layered intrusions. This large body of Tertiary igneous rock, exposed in the mountains and fjords of Kangerdlugssuaq, was shown by the authors to have formed by the slow cooling and crystal fractionation of about 500 km³ of basalt magma in a subsurface, funnel-shaped magma chamber. They proposed that as the crystals of a varied mineral assemblage formed, they were transported by magmatic convection currents to the floor of the chamber and deposited as successive layers. The near-perfect arrangement of the layers, exposed over about 7,500 ft. of thickness, provided the investigators with successive crops of minerals in equilibrium with the contemporary liquids over a relatively wide temperature range (starting at about 1,150° C), and Wager and Deer provided a searching account of the textural, mineralogical and chemical variations, as well as a hypothesis, widely accepted to-day, on the mechanisms of formation of layered intrusions.

In 1944, Wager went to Durham as professor of geology after war service as an officer in the Royal Air Force. He had been engaged on aerial photograph interpretation, advising troops on Arctic warfare, and sailing in Arctic seas with the Murmansk convoys, and was mentioned in dospatches.

At Durham and, since 1950, at Oxford, he continued his profitable researches into the petrology of the Skaergaard intrusion. Joined by colleagues and a host of postgraduate students, the researches fell chiefly into three categories: mineralogical studies on the silicate, oxide and sulphide minerals; geochemical studies on major and trace elements and on radioactive isotope ratios in the rocks and minerals; and calculations aimed at an understanding of the crystallization history of the intrusion. He was also concerned with investigations into the petrology of other layered intrusions in East Greenland and South Africa, of the Hebridean volcanic centres of Skye and Rhum, and of Recent volcanic centres in the West Indies. The results of his extensive work on layered intrusions have formed the basis of a book almost completed at the time of his death, and due to be published this year (Wager and Brown, *Layered Igneous Rocks*). At Oxford, Wager was responsible for building up the facilities for petrographic, mineralogical and geochemical teaching and research which were to make it one of the better equipped centres in Britain. In addition, his period there saw an appreciable increase in the size of the whole department, including a new wing in 1964, and in the number of staff and students pursuing studies in most branches of geology.

Elected to a Fellowship of the Royal Society in 1946, he was also a Fellow of the Geological Society of London (vice-president, 1951–53), of the Royal Geographical Society, and of the Mineralogical Society of America; a member of the Mineralogical Society (president, 1960–63); and one-time president of Section C of the British Association for the Advancement of Science (1958). He served on several university, national, and international committees, and was a founder editor of *Geochimica et Cosmochimica Acta* and *Journal of Petrology*. He was awarded the Arctic Medal (1933); the Mungo Park Medal (1936); the Lyell Fund (1935), the Bigsby Medal (1945), and the Lyell Medal (1962) of the Geological Society of London; and the Spendiarov Prize at the eighteenth International Geological Congress in 1948.

Wager was an inspiring teacher and a born leader, his integrity and seriousness of purpose instilling in all who worked with him a deep sense of loyalty. He set high standards and was unstinting in his appreciation of those who tried to meet them, for he had a liberal mind and a generous heart. Staff and students could always depend on his valuable advice and would find him ready to spend any spare time he might have on discussing their technical or personal problems, however trivial. He had a rather retiring personality, was reluctant to talk of his exploits, and rarely displayed his deeper emotions. Yet his authority, drawn from a splendid association of courage, intellect, and gentle modesty, could never fail to impress, even in a casual encounter.

He is survived by his widow, who greatly helped him in his work, two sons and three daughters.

G. MALCOLM BROWN

Prof. W. F. Whittard

PROF. WALTER FREDERICK WHITTARD, professor of geology in the University of Bristol, died on March 2, at the age of 63.

Born in Clapham, London, on October 26, 1902, Whittard was educated at Battersea County Secondary School; he was a founder member of the school Natural History Club, and an early interest in geology was established by the finding of fossils in the Chalk near Purley. After a brief spell at the Chelsea Polytechnic, he proceeded to the Imperial College of Science and Technology to read geology under W. W. Watts. There he also studied zoology, so that Whittard's subsequent major interests in stratigraphy and palaeontology especially were determined early in his undergraduate days.

After obtaining his first degree, Whittard went to Shropshire at the instigation of Watts to study rocks of Llandovery age, and it was during this period that he developed a love for Shropshire and the Welsh Borderland which was to last the whole of his life. Following the award of the Ph.D. degree, Whittard went up to Sidney Sussex College, Cambridge. There he moved on to the study of Ordovician rocks, still in Shropshire, a critical area for a better understanding of the history of the Ordovician period.

In 1929, Whittard seized a chance opportunity to join the Cambridge East Greenland Expedition; he went as chief geologist, and carried out much useful work. This experience gave Whittard an enthusiasm for the Arctic regions which was later inherited by some of his students.

In 1931 he was appointed assistant lecturer in geology at the Imperial College of Science and Technology. As lecturer, Whittard remained there until 1937, when he was elected to the Chaning Wills chair of geology in the University of Bristol, lately vacated by Trueman. He

took over a department organized on traditional lines which accorded with his specialisms; despite this, with characteristic forward planning, Whittard began to adapt the department to the changes beginning to affect geology. This work was halted by the Second World War, but with the end of hostilities he resumed the modernization. It was during the difficult immediate post-war years that Whittard served the University well as dean of the Faculty of Science; both then and later he brought into play a sane and balanced judgment in the many University committees on which he served.

Whittard was elected a Fellow of the Royal Society in 1957. From 1952 to 1954 he had been president of the Bristol Naturalists' Society, and from 1961 to 1962 had served as vice-president of the Palaeontological Association. He was awarded the D.Sc. degree of the University of London, and in 1964 he became a Fellow of the Imperial College of Science and Technology. The last honour he was to receive was in 1965 when the Geological Society awarded him the Murchison Medal, and this brought considerable pleasure.

As a geologist, Whittard showed a wide diversity of interests which is borne out by the variety of his published works. Many papers dealt with the geology of Shropshire in which he had been actively engaged for nearly forty years. Whittard had accumulated an immense amount of detailed information on the geology of south Shropshire, and his field-maps were models of execution; it is a tragedy that he did not live to publish the geological map he had planned. Whittard wrote several papers on the geology of East Greenland, and on the Bristol district; he was joint editor of *Bristol and its Adjoining Counties* (1955).

In later years, after the completion of the Shropshire field-work, Whittard entered a fresh field of research. Excited by W. B. R. King's work on the geology of the floor of the English Channel, and with the co-operation of the director and staff of the Marine Biological Station, Plymouth, he threw himself with his usual energy into a systematic programme of coring and geophysical exploration of the Western Approaches. This work quickly produced important results, including the nature of marine sediments of Miocene and Pleistocene age, previously known nowhere in Britain. Through this research, the seventeenth Colston Symposium was devoted to "Submarine Geology and Geophysics", and the resulting publication was edited jointly by Whittard and his colleague, R. Bradshaw.

Whittard will be remembered above all for his painstaking work in palaeontology. He contributed to our knowledge of no less than five animal phyla, including the Amphibia, and he was an acknowledged expert on trilobites. For some years he had been engaged on the Palaeontographical Society's monograph, *Ordovician Trilobites of the Shelve Inlier, Western Shropshire*; at the time of his death the major part of this work had been published. It is a model of meticulously careful work, excellently illustrated, and the geological world is the poorer for the non-completion of a work in which Whittard's unique experience was of paramount importance.

Whittard was a man of quiet charm who enjoyed a happy home life. He found joy in his garden and especially in his collection of rock plants and alpines. At his home at Westbury-on-Trym he relaxed with music, reading and book-binding. He was keenly interested in the welfare of his students, and found gratification in having taken part in the training of so many young geologists.

Prof. Whittard is mourned by his wife and son. He will be missed acutely by his many friends and by geologists everywhere. His death is a grievous loss especially to British geology, and to the University of Bristol which he had served so loyally and well for almost thirty years.

F. WOLVERSON COPE

NEWS AND VIEWS

Royal Society of London

AT a meeting of the Royal Society on April 21, the following were elected foreign members of the Society: PROF. J. BRACHET, professor of animal genetics and biochemistry, Université Libre de Bruxelles, Brussels; PROF. H. K. HARTLINE, Rockefeller Institute, New York; PROF. L. E. F. NÉEL, professor of experimental physics, Université de Grenoble, Grenoble; PROF. A. WEIL, Institute for Advanced Study, Princeton, New Jersey.

U.S. National Academy of Sciences

THE following have been elected foreign associates of the National Academy of Sciences: PROF. H. ALFVÉN, professor of theoretical electrodynamics and mathematics, Royal Institute of Technology, Stockholm; PROF. P. M. S. BLACKETT, president, The Royal Society, London; SIR JOHN ECCLES, professor of physiology, Australian National University, Canberra; PROF. M. EIGEN, Max Planck Institute of Theoretical Chemistry, Gottingen; PROF. E. KATCHALSKI, Department of Biophysics, Weizmann Institute of Science, Rehovoth; PROF. K. LORENZ, director, Max Planck Institute for Behavioural Physiology, Bavaria; PROF. J. PIAGET, professor of psychology, University of Geneva; PROF. B. SANDER, professor emeritus of mineralogy and petrography, University of Innsbruck; PROF. P. SWINGS, director, Institute of Astrophysics, University of Liège; PROF. H. TAMIYA, Institute of Applied Microbiology, University of Tokyo.

Earth Sciences in the University of Leeds : Prof. E. Irving

DR. E. IRVING, senior scientific officer in the Division of Geomagnetism at the Dominion Observatory, Ottawa, has been appointed to a new chair of geophysics in the Department of Earth Sciences in the University of Leeds. He will take up the appointment in July. Dr. Irving was educated at Colne Grammar School, served in the Army from 1945 until 1948 and graduated at Cambridge in 1951. His research work, begun in Cambridge and continued after 1954 in the Australian National University, where he held a research fellowship and later a senior fellowship, has been largely devoted to the study of the magnetic properties of rocks and their application to problems in the earth sciences, in particular those relating to the theory of continental drift, and to such other problems as past climates and the distribution of oil. His recent book on palaeomagnetism is a comprehensive survey of this subject. At Leeds, where his work will fit well with that of the recently established geochronological unit under Dr. Dodson, and with the Research Institute of African Geology, he will develop research on geomagnetism and other geophysical methods which can help to solve some of the fundamental problems of the Earth.

Reactor Centrum Nederland

THE fifth summary of activities of Reactor Centrum Nederland, covering the period July 1, 1964–June 30,

1965, describes the development of a pressurized water reactor suitable for ship propulsion—the so-called NERO project. Part of this, carried out in association with Euratom, has been completed (Pp. 15. The Hague: Reactor Centrum Nederland, 1965). Work at Petten is concerned with the reactor core, consisting of sintered uranium oxide fuel in zircalloy tubes. The fuel contains discrete particles of burnable neutron poison, for which UB_4 and Dy_2O_3 are being considered. For testing, both the low-flux and the high-flux reactors have been used, and the effect of the introduction of poisoned fuel rods on the power distribution has been measured in the critical facility KRITO, using a two-zone core of 3·1 per cent and 3·8 per cent enrichment. The principal problem of the NERO design in the field of materials is the compatibility of the neutron poison particle with the fuel matrix, both during the fabrication of the fuel and during its life in the reactor.

The three reactors at Petten each play a part in the reactor development work and KRITO is used exclusively for that work. The 200-MW high-flux reactor is owned by Euratom as part of that organization's research establishment adjoining the RCN site but is operated by RCN. Two new loops and one new beamhole experiment have been installed. The experiment consists of a triple-axis spectrometer for the investigation of crystal lattice dynamics by the inelastic scattering of neutrons. Two other beamholes are in use for crystal structure studies by means of neutron diffraction. New information on the magnetic ordering in cobalt (II) oxide has been obtained. The 10-kW low-flux reactor has been made more versatile by the addition of a trolley in the large sub-critical facility which occupies one side of the reactor.

Rubbish in Estuaries

A STUDY by the U.K.A.E.A. Isotope Research Division has illustrated the difficulties of selecting areas for the deposition of spoil and sewage. The initial studies, carried out in 1961 and 1964 in the Firth of Forth, show that the Oxcars deep region, which has been used as an area for the deposition of spoil dredged from Rosyth dockyard, is totally unsuitable for the purpose. Far from being swept out to sea, the silt moves upstream, and is ultimately deposited near the region from which it was originally dredged.

The technique used in the experiments utilized radioactive tracers. Glass was prepared to the same density and size distribution as the silt, and scandium-46 was incorporated to enable the movement of the silt to be detected by scintillation counter. A hopper containing 750 tons of silt from Rosyth dockyard was inoculated thoroughly with tracer material and deposited in the normal way in Oxcars deep, immediately after slack high water. Within 24 hours, traces of silt were detected above the Forth Bridge, while below Oxcars deep no trace could be found. This pattern was maintained throughout the nine weeks in which measurements were made, despite the fact that deposition of the spoil was made at a state of the tide least likely to carry material upstream. Deposition of spoil is normally carried out at all states of the tide.

A subsequent study in 1965 of an area superficially less attractive for the dumping of silt has given different results. Although this area is shallower than the Oxcars deep, it is in an ebb channel, and the silt is swept out into the estuary.

Water in the United States

THE drought that has persisted in the eastern United States for the past few years, causing New York City to be classified as a 'disaster area', has emphasized that water supply can be as much a problem in industrial areas as in the farming areas of India and Africa. However, the problem for the United States should, with proper measures, be soluble. In a pamphlet for the United States Geological Survey, entitled *Has the United States Enough Water?*, A. M. Piper concludes that the United States can be assured of a sufficient water supply for essential needs within the near future if proper measures are taken soon. These include research before any decisions are made concerning management of water supplies; a realization that there is no one solution for all situations; compromise among potentially competitive uses; and large-scale investment for new water-management works.

Nearly all the fresh water comes from precipitation; rainfall in the United States varies from less than 4 in. per annum in the Pacific South-west to more than 85 in. in the Pacific North-west. This range of rainfall is complicated by an equally wide variation in potential 'evapotranspiration'—water loss from open-water surface and vegetable transpiration—ranging from 18 in. per annum in the Rocky Mountains to 60 in. in Death Valley. Where evapotranspiration exceeds precipitation, the land is arid; where precipitation is greater, water overflows into streams.

Where local needs are greater than the minimum stream-flow (which varies with the seasons), the variations must be suppressed, usually by means of reservoirs. At present the cost of controlling the water in a stream basin continuously has not been acceptable. However, Mr. Piper thinks that the acceptable cost will increase substantially and that the limitation on water storage will be determined by engineering complexities, other potential land uses, or other objectives of stream management.

In 1960, 270,000 million gallons of water were withdrawn each day from rivers in the United States, while 68,000 million gallons were actually consumed, 86 per cent in irrigation. At the present rate of increase, the total use of water will double in 25–30 years. When this increase is extrapolated forwards for each of sixteen regions in the United States, it appears that potential supply exceeds the likely commitment in only three regions; in another seven regions water requirements will be realized if virtually complete regulation of the stream-flow is feasible. In the remaining nine regions it is expected that even with complete regulation of stream-flow, social and industrial developments will be handicapped.

Lens Design at the N.B.S.

THE United States National Bureau of Standards has developed a procedure for the determination of lenses from design data alone, in response to a request from the Air Force Reconnaissance Laboratory at Wright Field and as an alternative to the expensive and time-consuming method of fabricating prototypes and then testing them. The system of optical design analysis is based on the use of spot diagrams, which are analogues of star image tests. In Monograph 93 of the National Bureau of Standards, O. R. Stavroudis and L. E. Sutton present an outline of the methods used to calculate, produce and interpret spot diagrams (United States Department of Commerce. *Spot Diagrams for the Prediction of Lens Performance from Design Data.* Pp. iii + 96. Washington, D.C.: Government Printing Office, 1965. 75 cents).

A spot diagram is a plot of points representing the intersections of rays from a given object point with a focal plane of a lens, and is calculated from the design specifications of the lens by the use of a fast digital computer. Within the framework of geometrical optics, the spot diagram can be interpreted in the same way as a star image produced by a real lens; it is the greatly magnified picture of the image of a point source as it would appear on the film plane of a camera using the lens. Hawkins and Linfoot in 1945 were the first to publish a report on spot diagrams when they described the performance of several designs of Schmidt cameras, and Herzberger, in 1947, followed with a report on the method of calculating the spot diagrams.

Since the introduction of fast digital computers, interest in spot diagrams has considerably increased. The computational procedures used to obtain the diagrams provide additional information about the lens, including data for estimating the resolving power, and information on vignetting, distortion and chromatic aberration.

The monograph emphasizes, however, that the spot diagram as a representation of the image of a point object is subject to certain limitations. Each ray is represented as contributing an equal amount to the intensity distribution of the image whereas, in reality, there is a considerable variation in the flux density associated with rays which travel along different paths through the lens. Moreover, each surface is entered at different angles of incidence, which means that different proportions of energy are transmitted. The intensity distribution in the dense portion of a spot diagram is likely to be underestimated, since the superimposition of one point of a spot diagram on another leads to only a single point. The diffraction effects due to the wave nature of light are not taken into account in the computation.

International Conference of Engineering Societies

At a meeting in Paris during April 13–15 representatives from the engineering federations—the Engineering Societies of Western Europe and the United States of America (E.U.S.E.C.), the Commonwealth Engineering Conference (C.E.C.), the Fédération Européenne d'Associations Nationales D'Ingenieurs (F.E.A.N.I.), and Union Panamericana de Asociaciones de Ingenieros (U.P.A.D.I.)—together with participants from Czechoslovakia, Hungary and the U.S.S.R., agreed on the need for a world organization of engineers. A proposed constitution for an International Conference of Engineering Societies was drawn up and will be submitted to the four organizations as soon as possible.

Eleanor Roosevelt International Cancer Fellowships

The International Union against Cancer, with the funds provided by the American Cancer Society, is to award fellowships for research on cancer. They will be awarded to persons on the staff of universities, teaching hospitals, research laboratories or similar institutions, and will be made to those investigating either the experimental or the clinical aspects of cancer. The fellowships will usually be awarded for one year, and applications must be received before September 1, for the period beginning March 1, 1967. Application forms and further information can be obtained from International Union against Cancer, P.O. Box 400, CH—1211, Geneva 2.

Gordon Research Conferences 1966

The Gordon Research Conferences, which are designed to stimulate research in universities, research foundations and industrial laboratories, will be held this year at five locations in New Hampshire and one in Washington during June 13–September 2. Sixty-six five-day conferences will be held, covering a wide range of topics in the physical and biological sciences. The purpose of the conferences is to discuss the most recent developments, analyse their significance, and provoke suggestions for future research. Further information and forms of application can be obtained from Dr. W. George Parks, Colby Junior College, New London, New Hampshire. Residence and registration charges for each conference will amount to 115 dollars.

Growth of University of Sussex

Lord Fulton, the Vice-Chancellor of the University of Sussex, who is retiring this summer, stresses in the sixth annual report of the University the rapid expansion in the 5 years since its foundation in 1961:

	1961–2	1962–3	1963–4	1964–5	1965–6
Academic faculty	9	63	117	197	
Students	55	442	898	1,504	2,090
Applications for admission		3,080	4,765	5,960	10,382

University News: Birmingham

Dr. P. J. Garner has been appointed to the newly established chair of chemical engineering. Dr. R. C. Curran has been appointed to the chair of pathology. The title of honorary professor in the Department of Electronic and Electrical Engineering has been conferred on Dr. E. V. D. Glazier, head of the Physics and Electronic Department at the Royal Radar Establishment. The title of honorary professor in the Department of Physics has been conferred on Dr. D. H. Parkinson, head of the Physics Group at the Royal Radar Establishment.

London

Dr. T. I. Shaw, principal scientific officer at the Plymouth Laboratory of the Marine Biological Association of the United Kingdom, has been appointed to the second chair of zoology, tenable at Queen Mary College. The title of reader in experimental haematology has been conferred on Dr. G. I. C. Ingram in respect of his post at St. Thomas's Hospital Medical School.

Dr. D. E. Barton, reader in statistics in University College, has been appointed to the chair of statistics tenable at Queen Mary College. The title of professor of virology has been conferred on Dr. L. H. Collier in respect of his post at the Lister Institute of Preventive Medicine.

Southampton

Dr. A. Carrington, assistant director of research in organic and inorganic chemistry, University of Cambridge, has been appointed to the fourth chair of chemistry as from October 1, 1967.

Appointments

Prof. R. L. Bisplinghoff, deputy head of the Department of Aeronautics and Astronautics in the Massachusetts Institute of Technology, has been appointed head of the Department as from July 1. He succeeds Prof. C. S. Draper, who will continue as a senior member of the faculty of the Department and as director of the Instrumentation Laboratory, which he founded more than 25 years ago.

Mr. G. C. Gaut, research director of the Plessey Co., Ltd., and Mr. A. Ross, chairman of the United Dominions Trust and a director of Eagle Star Insurance and of the Australia and New Zealand Bank, have been appointed part-time members of the National Research Development Corporation.

Mr. R. Eddison has been appointed managing director of Science in General Management, Ltd. (SIGMA), as from May 2. He succeeds Mr. Stafford Beer, who is joining the International Publishing Corporation, Ltd., as development director.

Announcements

Lord Hinton of Bankside has been elected the eighty-first president of the Institution of Mechanical Engineers, in succession to Mr. H. N. G. Allen.

A symposium on "Prostaglandins" will be held in Stockholm during June 6—8. Further information can be obtained from Dr. B. Samuelsson, Department of Chemistry, Karolinska Institutet, Stockholm 60.

The annual delegate conference of the Institution of Professional Civil Servants will be held in Hastings during May 23–25. Further information can be obtained from the General Secretary, Institution of Professional Civil Servants, Northumberland Street, London, W.C.2.

A symposium on "High Temperature Reactors and the *Dragon* Project", organized by the British Nuclear Energy

Society, will be held in London during May 23–24. Further information can be obtained from the Secretary, British Nuclear Energy Society, 1–7 Great George Street, Westminster, London, S.W.1.

AN open day will be held at the Agricultural Research Council Weed Research Organization on May 27. Further information can be obtained from B. A. Wright, A.R.C. Weed Research Organization, Begbroke Hill, Kidlington, Oxford.

A SYMPOSIUM on "Echinoderm Biology" will be held at the Zoological Society of London during May 25–26. Further information can be obtained from Prof. N. Millott, Zoology Department, Bedford College, Regent's Park, London, N.W.1.

AN ordinary meeting of the Scottish Section of the Society for Analytical Chemistry on "Thin-layer Chromatography" will be held in Paisley on May 31. Further information can be obtained from the Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

ERRATUM. In the article entitled "Thromboplastin-induced Hypercoagulability and its Prevention", by Dr. S. Nordstrom *et al.*, which appeared in the May 8, 1965, issue of *Nature*, the second sentence of paragraph one, p. 633, lines 5–8, should read: " 'Trasylol' has proved to be an antithromboplastin *in vitro*[3] and, more specifically, has been shown to block the reaction in which factors VIII, IX, X and contact factors take part[4]".

THE NIGHT SKY IN JUNE

All times are in Universal Time

MOON

New Moon	18d 20h
Full Moon	3d 08h

CONJUNCTIONS WITH THE MOON

Venus	15d 23h, 1° S.
Mars	—
Jupiter	—
Saturn	11d 21h, 2° N.

PLANETS

Times of rising (*R*) and setting (*S*) during the month

Name	*R*/*S*	Beginning	Middle	End	Mag.	D_φ (10^6 miles)	Zodiacal position
Mercury	*S*	Unfavourable	22h 00m	21h 45m	−0·3	103	Gemini
Venus	*R*	2h 25m	2h 00m	1h 50m	−3·5	112	Aries
Mars	—	Unfavourable for observation			—	232	Taurus
Jupiter	*S*	22h 10m	21h 20m	Unfavourable	−1·4	573	Gemini
Saturn	*R*	1h 35m	0h 40m	23h 40m	+1·3	896	Pisces

D_φ is the distance of planet from the Earth on the 15th of the month

OCCULTATIONS OF STARS BRIGHTER THAN MAGNITUDE +6 AT GREENWICH

Star	*R*/*D*	Time	Mag.
λ Vir	*D*	27d 22h 18·4m	+4·6

(*D*, disappearance; *R*, reappearance)

OTHER PHENOMENA

21d 21h, summer solstice

CORRESPONDENCE

Ships and String and Sealing-wax

SIR,—I have read your leader of April 9, 1966, with surprise, for I did not realize well-informed men could see things so differently.

You state: "prices are often uncompetitive and delivery dates too long". This paradox resolves itself. Prices are unremunerative, not uncompetitive, because in an attempt to keep labour forces from disintegrating, contracts have been accepted at too low a price. Further, for political reasons, foreign buyers have been subsidized by H.M. Government, berths are filled and booked for a long time and new deliveries can only be promised for distant dates. Rather than admit this, a shipbuilder required to tender may put forward a discouraging price. The position is aggravated by H.M. Government policy to coax new industries to shipbuilding areas where they compete for skilled labour, particularly in finishing trades. There is also the habit—of old standing—of making delivery promises for components which the maker has but a faint hope to honour.

You state: "Relationships between shipbuilders and the suppliers on whom they rely, particularly the engine makers, are too confused and complacent". However, so far as engines are concerned, the trouble is that half our shipbuilders, and probably all the major ones, build their own and that consequently production is fragmented instead of being concentrated into efficient units. There were, until recently, about nineteen British makers of marine steam turbines. Geddes implies there ought to be only one. I do not know the figures in regard to Diesels. Geddes says there ought to be four only.

The expenditure on research and development, I agree with you, is and has been far too low, but you cannot really relate it to total turnover, that is, the price of ships' hulls. Far too little was spent on steam installations, I agree, and what little was spent was frittered away by moving in all directions simultaneously, instead of being directed to improving economy of use by raising cycle efficiency and trustworthiness.

It is true that the heavy, slow Diesel "came into fashion" because it seemed, on paper, to be economically superior, but cycle efficiency of steam installations has improved to match Diesel efficiency, and steam turbine availability—days per year—has proved itself superior to that of Diesels. The fashion, therefore, will change again. Faster, medium-speed Diesels, I believe, will not be the solution. Not only is the efficiency inherently lower because of the unfavourable ratio of cylinder surface to volume, but also the incidence of failure increases with the number of components.

You speak approvingly of the recent powerful enhancement of the industry's concern for research and its availability to carry it out effectively. Presumably you are thinking of the re-organization of the British Ship Research Association in 1962. This, however, divorced design from 'research and development', and neither was subjected to the discipline arising from intimate connexion with producing, marketing and operating products. I do not know of any large-scale successful commercial undertaking working under such conditions. I therefore look forward to the implementation of the Geddes recommendations.

O. P. T. KANTOROWICZ

86 Saltwell Road South,
Low Fell,
Gateshead 9.

AN EXACT SOLUTION FOR DETERMINING MULTIPLE HIT FREQUENCIES IN FULLY CORRELATED FIRE

By E. V. GADECKI

Technical Operations, Incorporated, Burlington, Massachusetts

RECENTLY, Terry[1] outlined a mathematical construct for determining the probability of exactly m hits with n weapons when the distribution of shots is circular normal and centred at a point h units from the target origin. Terry proposes that the final integral for obtaining the unconditional probability that exactly m weapons will hit the target when n weapons are released can be evaluated numerically by employing the Laguerre–Gauss quadrature formula. In this article, an exact solution for this integral is provided in terms of a series.

Using the same notation as Terry, we shall proceed in a similar manner. The ballistic dispersion and aiming error are characterized by standard deviations σ_B and σ_A, respectively. The probability that a single weapon drawn at random strikes the target is given in polar coordinates as:

$$p=\int_0^R\int_0^{2\pi}\frac{1}{2\pi\sigma_B^2}r\exp\left[-\frac{1}{2\sigma_B^2}(r^2+h^2-2h\,r\,\cos\theta)\right]\mathrm{d}\theta\,\mathrm{d}r \qquad (1)$$

Rearranging terms in equation (1), we have:

$$p=\frac{1}{\sigma_B^2}\exp\left[-\frac{h^2}{2\sigma_B^2}\right]\int_0^R r\exp\left[-\frac{r^2}{2\sigma_B^2}\right]\left[\int_0^{2\pi}\frac{1}{2\pi}\exp\left[\frac{rh\cos\theta}{\sigma_B^2}\right]\mathrm{d}\theta\right]\mathrm{d}r \qquad (2)$$

The integral within the brackets can be expressed as a modified Bessel function:

$$\int_0^{2\pi}\frac{1}{2\pi}\exp\left[\frac{rh\cos\theta}{\sigma_B^2}\right]\mathrm{d}\theta=J_0\left[\frac{irh}{\sigma_B^2}\right]=I_0\left[\frac{rh}{\sigma_B^2}\right]$$

Accordingly, equation (2) can be rewritten as

$$p=\frac{1}{\sigma_B^2}\exp\left[-\frac{h^2}{2\sigma_B^2}\right]\int_0^R r\exp\left[-\frac{r^2}{2\sigma_B^2}\right]I_0\left[\frac{rh}{\sigma_B^2}\right]\mathrm{d}r \qquad (3)$$

Expanding $I_0\left[\frac{rh}{\sigma_B^2}\right]$ in its series form, we have:

$$I_0\left[\frac{rh}{\sigma_B^2}\right]=\sum_{k=0}^{\infty}\frac{1}{k!k!}\left(\frac{rh}{2\sigma_B^2}\right)^{2k}$$

Therefore,

$$p=\frac{1}{\sigma_B^2}\exp\left[-\frac{h^2}{2\sigma_B^2}\right]\int_0^R r\exp\left[-\frac{r^2}{2\sigma_B^2}\right]\sum_{k=0}^{\infty}\frac{1}{k!k!}\left(\frac{rh}{2\sigma_B^2}\right)^{2k}\mathrm{d}r$$

Since the series is obviously convergent after a finite number of terms, we can expand the series and integrate term by term. Using integration by parts and finite induction the following expression is arrived at:

$$p=\exp\left[-\frac{h^2}{2\sigma_B^2}\right]\sum_{i=0}^{\infty}\frac{\left(\frac{h^2}{2\sigma_B^2}\right)^i}{i!}\left[1-\exp\left[-\frac{R^2}{2\sigma_B^2}\right]\sum_{j=0}^{i}\frac{\left(\frac{R^2}{2\sigma_B^2}\right)^j}{j!}\right] \qquad (4)$$

Now the probability that m of n rounds will hit the target with aiming error, σ_A, is given by:

$$P_{[n,m]}=\int_0^{\infty}\frac{h}{\sigma_A^2}\exp\left[-\frac{h^2}{2\sigma_A^2}\right]\binom{n}{m}p^m(1-p)^{n-m}\,\mathrm{d}h \qquad (5)$$

If $m=n$, we have:

$$P_{[n,n]}=\int_0^{\infty}\frac{h}{\sigma_A^2}\exp\left[-\frac{h^2}{2\sigma_A^2}\right]p^n\,\mathrm{d}h \qquad (6)$$

or substituting equation (4) into equation (6), we have:

$$P_{[n,n]}=\int_0^{\infty}\frac{h}{\sigma_A^2}\exp\left[-\frac{h^2}{2\sigma_A^2}\right]\left\{\exp\left[-\frac{h^2}{2\sigma_B^2}\right]\sum_{i=0}^{\infty}\frac{\left(\frac{h^2}{2\sigma_B^2}\right)^i}{i!}\left[1-\exp\left[-\frac{R^2}{2\sigma_B^2}\right]\sum_{j=0}^{i}\frac{\left(\frac{R^2}{2\sigma_B^2}\right)^j}{j!}\right]\right\}^n\mathrm{d}h \qquad (7)$$

Now, since R and σ_B are constants in the expression:

$$1-\exp\left[-\frac{R^2}{2\sigma_B^2}\right]\sum_{j=0}^{i}\frac{\left(\frac{R^2}{2\sigma_B^2}\right)^j}{j!}$$

and only i is the variable, let us use G_i to represent this expression, that is:

$$G_i=1-\exp\left[-\frac{R^2}{2\sigma_B^2}\right]\sum_{j=0}^{i}\frac{\left(\frac{R^2}{2\sigma_B^2}\right)^j}{j!} \qquad (8)$$

Now using equation (8) in equation (7), we have:

$$P_{[n,n]}=\int_0^{\infty}\frac{h}{\sigma_A^2}\exp\left[-\frac{h^2}{2\sigma_A^2}\right]\left\{\exp\left[-\frac{h^2}{2\sigma_B^2}\right]\sum_{i=0}^{\infty}\frac{\left(\frac{h^2}{2\sigma_B^2}\right)^i}{i!}G_i\right\}^n\mathrm{d}h$$

$$=\int_0^{\infty}\frac{h}{\sigma_A^2}\exp\left[-\frac{h^2}{2\sigma_A^2}\right]\exp\left[-\frac{h^2n}{2\sigma_B^2}\right]\left\{\sum_{i=0}^{\infty}\frac{\left(\frac{h^2}{2\sigma_B^2}\right)^i}{i!}G_i\right\}^n\mathrm{d}h$$

$$=\int_0^{\infty}\frac{h}{\sigma_A^2}\exp\left[-h^2\left(\frac{\sigma_B^2+\sigma_A^2n}{2\sigma_A^2\sigma_B^2}\right)\right]\left\{\sum_{i=0}^{\infty}\frac{\left(\frac{h^2}{2\sigma_B^2}\right)^i}{i!}G_i\right\}^n\mathrm{d}h \qquad (9)$$

Let

$$v=h^2\left(\frac{\sigma_B^2+\sigma_A^2n}{2\sigma_A^2\sigma_B^2}\right)$$

Therefore:

$$\mathrm{d}v=h\left(\frac{\sigma_B^2+\sigma_A^2n}{\sigma_A^2\sigma_B^2}\right)\mathrm{d}h$$

$$\frac{\sigma_B^2\,\mathrm{d}v}{\sigma_B^2+\sigma_A^2n}=\frac{h}{\sigma_A^2}\,\mathrm{d}h$$

$$\frac{h^2}{2\sigma_B^2}=\frac{\sigma_A^2\,v}{\sigma_B^2+\sigma_A^2n}$$

Equation (9) becomes:

$$P_{[n,n]}=\frac{\sigma_B^2}{\sigma_B^2+\sigma_A^2 n}\int_0^\infty \exp(-v)\left\{\sum_{i=0}^{\infty}\frac{\left(\frac{\sigma_A^2 v}{\sigma_B^2+\sigma_A^2 n}\right)^i}{i!}G_i\right\}^n [dv$$

Let

$$C_n=\frac{\sigma_A^2}{\sigma_B^2+\sigma_A^2 n};\quad K_n=\frac{\sigma_B^2}{\sigma_B^2+\sigma_A^2 n}$$

Accordingly,

$$P_{[n,n]}=K_n\int_0^\infty \exp(-v)\left\{\sum_{i=0}^{\infty}\frac{(C_n v)^i}{i!}G_i\right\}^n dv \qquad (10)$$

Now the following two formulae are needed:

(I) $$\sum_{i=0}^{\infty}\frac{a_i x^i}{i!}\sum_{j=0}^{\infty}\frac{b_j x^j}{j!}=\sum_{k=0}^{\infty}\sum_{L=0}^{k}\binom{k}{L}a_L b_{k-L}\frac{x^k}{k!}$$

(II) $$\int_0^\infty e^{-x}\sum_{i=0}^{\infty}a_i\frac{(bx)^i}{i!}dx=\sum_{i=0}^{\infty}a_i b^i$$

Therefore, we have:

$$P_{[0,0]}=K_0\int_0^\infty \exp(-v)\,dv=K_0(1)=1$$

$$P_{[1,1]}=K_1\int_0^\infty \exp(-v)\sum_{i=u}^{\infty}\frac{(C_1 v)^i}{i!}G_i\,dv$$

Using formula (II):

$$=K_1\sum_{i=0}^{\infty}G_i C_1^i$$

$$P_{[2,2]}=K_2\int_0^\infty \exp(-v)\left\{\sum_{i=0}^{\infty}\frac{(C_2 v)^i}{i!}G_i\right\}^2 dv$$

Using formula (I):

$$=K_2\int_0^\infty \exp(-v)\left\{\sum_{i=0}^{\infty}\sum_{j=0}^{i}\binom{i}{j}G_j G_{i-j}\frac{(C_2 v)^i}{i!}\right\}dv$$

Using formula (II):

$$=K_2\sum_{i=0}^{\infty}\sum_{j=0}^{i}\binom{i}{j}G_j G_{i-j}C_2^i$$

Now set:

$$G_i^{(2)}=\sum_{j=0}^{i}\binom{i}{j}G_j G_{i-j}$$

And more generally:

$$G_j^{(t)}=\sum_{j=0}^{i}\binom{i}{j}G_j^{(t-1)}G_{i-j}\text{ where }t\geqslant 2$$

Therefore,

$$P_{[2,2]}=K_2\sum_{i=0}^{\infty}G_i^{(2)}C_2^i$$

Continuing this process and using finite induction, we find that:

$$P_{[n,n]}=K_n\sum_{i=0}^{\infty}G_i^{(n)}C_n^i\text{ for }n\geqslant 2 \qquad (11)$$

Now,

$$P_{[n,m]}=\binom{n}{m}\sum_{i=0}^{n-m}\binom{n-m}{i}(-1)^i P_{[m+i,m+i]} \qquad (12)$$

Equation (12) is derived by expanding equation (5) into a sum of integrals.

As a further simplification $P_{[1,1]}$ can be shown to be equal to:

$$1-\exp\left[-\frac{R^2}{2(\sigma_B^2+\sigma_B^2)}\right]$$

by expanding the series and terms within and then changing the order of addition.

As a summary of the results, then:

$$P_{[n,m]}=\binom{n}{m}\sum_{i=0}^{n-m}\binom{n-m}{i}(-1)^i P_{[m+i,m+i]}$$

where

$$P_{[0,0]}=1$$

$$P_{[1,1]}=1-\exp\left[-\frac{R^2}{2(\sigma_B^2+\sigma_A^2)}\right]$$

$$P_{[n,n]}=K_n\sum_{i=0}^{\infty}G_i^{(n)}C_n^i\text{ for }n\geqslant 2$$

where

$$K_n=\frac{\sigma_B^2}{\sigma_B^2+\sigma_A^2 n};\quad C_n=\frac{\sigma_A^2}{\sigma_B^2+\sigma_A^2 n}$$

$$G_i^{(n)}=\sum_{j=0}^{i}\binom{i}{j}G_j^{n-1}G_{i-j}\text{ for }n\geqslant 2$$

and

$$G_i=1-\exp\left(-\frac{R^2}{2\sigma_B^2}\right)\sum_{j=0}^{i}\frac{\left(\frac{R^2}{2\sigma_B^2}\right)^j}{j!}$$

I have written computer programmes to evaluate $P_{[n,m]}$ by this exact method, as well as by using the Laguerre–Gauss Quadrature Method with 2 abscissae and with 5 abscissae and, also by using the Gaussian Quadrature Method, with 14 abscissae while making the interval of integration finite. For comparison, I used the same input data into these four programmes. The radius, aiming error, and ballistic dispersion were all chosen to be 10 ft. and the number of rounds was 5. The results of the four programmes are given in Table 1.

Table 1

	Gauss (14 abscissae)	Laguerre–Gauss (2 abscissae)	Laguerre–Gauss (5 abscissae)	Exact
$P_{[s,0]}$	0·34446243	0·34454247	0·34455280	0·34455280
$P_{[s,1]}$	0·33457011	0·33539236	0·33538066	0·33538066
$P_{[s,2]}$	0·21411442	0·21302425	0·21301963	0·21301963
$P_{[s,3]}$	0·08546478	0·08554481	0·08554819	0·08554819
$P_{[s,4]}$	0·01942290	0·01955569	0·01956195	0·01956195
$P_{[s,5]}$	0·00193162	0·00193640	0·00193676	0·00193676

It was expected that the accuracy of the results should be better as Table 1 is read from left to right. The results tend to bear this out especially with the amazing correlation between the Laguerre–Gauss Quadrature Method with five abscissae and the exact method.

The comparison was inconclusive as to which method was faster. The programmes all had the same recorded time, which was also the minimum time the operators of the computer would record. It is my opinion that with an increase in the number of rounds the exact method would be shown to be faster.

[1] Terry, E. R., *Nature*, **205**, 663 (1965).

DAMAGE PROBABILITIES IN FULLY CORRELATED FIRE

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I HAVE recently developed a construct for determining the probability of exactly m hits with n weapons when the distribution of shots is circular normal and is centred at a point h units from the origin of a circular target[1]. The construct could be used for determining the probability of exactly m damaging hits if the conditional damage function is assumed to be the circular 'cookie-cutter' function. For this damage function, the probability of damage with a single weapon is unity within some area about the weapon strike or burst point and is zero outside this area. More precisely, if the conditional damage probability to the target is $D(x, y)$ and the joint probability density function that describes the density of shots in the target plane is $f(x, y)$, then:

$$D(x, y) = \begin{cases} 1 & x^2+y^2 \leq R^2 \\ 0 & x^2+y^2 > R^2 \end{cases}$$

For this function, the probability of damage is:

$$p = \iint_{x^2+y^2 \leq R^2} f(x, y)\, dx dy$$

In this article, I shall develop a construct for determining the probability of multiple damaging hits for the same conditions outlined in my earlier work, except that the conditional damage function is assumed to be the circular Gaussian damage function first introduced by von Neumann[2]. For many situations, this is a more exact expression of the conditional damage function than the cookie-cutter function. Unlike the cookie-cutter function, as the radial distance from the strike or burst point increases, the damage probability gradually decreases from unity at the strike or burst point to zero. More precisely, for this situation we may write:

$$D(x, y) = \exp\left[-\frac{x^2+y^2}{a^2}\right] = \exp\left[-\left(\frac{r}{a}\right)^2\right]$$

where a is the equivalent damage radius. We note that:

$$\int_{-\infty}^{\infty}\int D(x, y)\, dx dy = \Pi\, a^2$$

As in my earlier investigation, I shall assume that the pattern of weapons is released on the target with a bivariate circular normal aiming error characterized by a standard deviation σ_A, and each weapon comprising the burst pattern is distributed about the mean point of impact or centroid of the pattern with a bivariate circular normal ballistic dispersion characterized by a standard deviation σ_B. I shall further assume that the ballistic dispersion for each of the weapons and the aiming error are mutually independent. Although a conditional damage probability is associated with each weapon comprising the burst pattern, no cumulative damage effect is assumed; that is, the probability of target damage is independent of the number of weapons striking the target.

Accordingly, the probability that a single weapon drawn at random will damage the target is given by:

$$p = \frac{1}{2\pi\sigma_B^2}\int_{-\infty}^{\infty}\int \exp\left\{-\frac{1}{2\sigma_B^2}\left[(x-h)^2+y^2\right]\right\} \exp\left[-\frac{x^2+y^2}{a^2}\right] dx dy$$

$$= \frac{1}{2\pi\sigma_B^2}\int_{\theta=0}^{2\pi}\int_{r=0}^{\infty} \exp\left[-\frac{1}{2\sigma_B^2}(r^2+h^2-2hr\cos\theta)\right] \exp\left[-\left(\frac{r}{a}\right)^2\right] r\, dr\, d\theta$$

$$= \frac{1}{2\pi\sigma_B^2}\int_{\theta=0}^{2\pi}\int_{r=0}^{\infty} \exp\left[-\frac{1}{2a^2\sigma_B^2}(a^2r^2+a^2h^2-2a^2hr\cos\theta+2\sigma_B^2r^2)\right] r\, dr\, d\theta \quad (1)$$

If we rearrange equation (1) thus:

$$p = \left\{\frac{1}{\sigma_B^2}\exp\left(-\frac{h^2}{2\sigma_B^2}\right)\int_{r=0}^{\infty} r\exp\left[-\left(\frac{a^2r^2+2\sigma_B^2r^2}{2a^2\sigma_B^2}\right)\right]\right\} \left[\frac{1}{2\pi}\int_{\theta=0}^{2\pi}\exp\left(\frac{hr\cos\theta}{\sigma_B^2}\right)d\theta\right] dr \quad (2)$$

we note that:

$$\frac{1}{2\pi}\int^{2\pi}\exp\left(\frac{hr\cos\theta}{\sigma_B^2}\right) = J_0\left(\frac{ihr}{\sigma_B^2}\right) = I_0\left(\frac{hr}{\sigma_B^2}\right)$$

which is the zero-ordered Bessel function of pure imaginary argument. Expanding $I_0(hr/\sigma_B^2)$ in its series form gives:

$$I_0\left(\frac{hr}{\sigma_B^2}\right) = \sum_{i=0}^{\infty}\frac{1}{(i!)^2}\left(\frac{hr}{2\sigma_B^2}\right)^{2i}$$

If we set:

$$u \equiv \frac{a^2r^2+2\sigma_B^2r^2}{2a^2\sigma_B^2}$$

and

$$du = \frac{a^2+2\sigma_B^2}{a^2\sigma_B^2}\, r\, dr$$

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equation (2) can be put in the form:

$$p = \exp\left(-\frac{h^2}{2\sigma_B^2}\right)\left(\frac{a^2}{a^2+2\sigma_B^2}\right)\int_{u=0}^{\infty} \exp(-u)\, I_0\left(\frac{ha}{\sigma_B}\sqrt{\frac{2u}{a^2+2\sigma_B^2}}\right) du$$

$$= \exp\left(-\frac{h^2}{2\sigma_B^2}\right)\left(\frac{a^2}{a^2+2\sigma_B^2}\right)\sum_{i=0}^{\infty}\frac{1}{(2^i i!)^2} \int_{u=0}^{\infty}\left(\frac{ha}{\sigma_B}\sqrt{\frac{2u}{a^2+2\sigma_B^2}}\right)^{2i}\exp(-u)\, du \quad (3)$$

Noting that:

$$\int_0^{\infty}\frac{(Ku)^i e^{-u}}{i!}\, du = K^i$$

and putting equation (3) in the form:

$$p = \exp\left(-\frac{h^2}{2\sigma_B^2}\right)\left(\frac{a^2}{a^2+2\sigma_B^2}\right)\sum_{i=0}^{\infty}\frac{1}{2^{2i}i!} \int_{u=0}^{\infty}\frac{1}{i!}\left[\frac{2h^2a^2u}{\sigma_B^2(a^2+2\sigma_B^2)}\right]^i \exp(-u)du \quad (4)$$

$$p = \exp\left(-\frac{h^2}{2\sigma_B^2}\right)\left(\frac{a^2}{a^2+2\sigma_B^2}\right)\sum_{i=0}^{\infty}\frac{\left[\frac{h^2a^2}{2\sigma_B^2(a^2+2\sigma_B^2)}\right]^i}{i!}$$

we obtain

$$= \exp\left(-\frac{h^2}{2\sigma_B^2}\right)\left(\frac{a^2}{a^2+2\sigma_B^2}\right)\exp\left[\frac{h^2a^2}{2\sigma_B^2(a^2+2\sigma_B^2)}\right]$$

$$= \frac{a^2}{a^2+2\sigma_B^2}\exp\left[\frac{h^2a^2}{2\sigma_B^2(a^2+2\sigma_B^2)} - \frac{h^2}{2\sigma_B^2}\right]$$

$$= \frac{a^2}{a^2+2\sigma_B^2}\exp\left[-\frac{h^2}{a^2+2\sigma_B^2}\right] \quad (5)$$

Since the ballistic dispersions of all the weapons comprising the burst are independent of one another, the conditional probability of damaging the target with exactly m of n weapons is given by:

$$Q_{[n,m]} = \binom{n}{m} p^m (1-p)^{n-m} \quad (6)$$

and the probability density function for determining whether the mean point of impact is within an annulus of width dh at a distance h units in any direction from the centre of the target is given by:

$$dp_h = \frac{1}{\sigma_A^2}\exp\left(-\frac{h^2}{2\sigma_A^2}\right) h\, dh \quad (7)$$

Accordingly, the probability of exactly m of n weapons damaging the target is:

$$P_{[n,m]} = \frac{1}{\sigma_A^2}\int_{h=0}^{\infty}\exp\left(-\frac{h^2}{2\sigma_A^2}\right)\binom{n}{m}p^m(1-p)^{n-m}\, h\, dh \quad (8)$$

where p is given by equation (5).

Rearranging equation (8):

$$P_{[n,m]} = \frac{1}{\sigma_A^2}\binom{n}{m}\int_{h=0}^{\infty} h\exp\left(-\frac{h^2}{2\sigma_A^2}\right)p^m(1-p)^{n-m}\, dh \quad (9)$$

we can readily obtain a solution by expanding the binomial and integrating term by term. If n equals m, then the probability of exactly n of n weapons damaging the target is:

$$P_{[n,n]} = \frac{1}{\sigma_A^2}\int_{h=0}^{\infty} h\exp\left(-\frac{h^2}{2\sigma_A^2}\right)p^n\, dh$$

$$= \frac{1}{\sigma_A^2}\int_{h=0}^{\infty} h\exp\left(-\frac{h^2}{2\sigma_A^2}\right)\left[\frac{a^2}{a^2+2\sigma_B^2}\exp\left(-\frac{h^2}{a^2+2\sigma_B^2}\right)\right]^n dh \quad (10)$$

Setting

$$u \equiv \frac{h^2}{2\sigma_A^2}$$

$$du = \frac{h}{\sigma_A^2}\, dh$$

$$K \equiv \frac{2\sigma_A^2}{a^2+2\sigma_B^2}$$

and

$$W \equiv \frac{a^2}{a^2+2\sigma_B^2}$$

we can put equation (10) in the form:

$$P_{[n,n]} = \int_{u=0}^{\infty}\exp(-u)\left[W\exp(-Ku)\right]^n du$$

$$= W^n\int_{u=0}^{\infty}\exp(-u)\left[\exp(-Ku)\right]^n du$$

$$= W^n\int_{u=0}^{\infty}\exp(-u)\exp(-nKu)\, du$$

$$= W^n\int_{u=0}^{\infty}\exp\left[-u(nK+1)\right]du = \frac{W^n}{nK+1}$$

$$= \frac{a^{2n}}{(2n\sigma_A^2 + 2\sigma_B^2 + a^2)\,(a^2 + 2\sigma_B^2)^{n-1}} \qquad (11)$$

Accordingly, we then have:

$$P_{[0,0]} = 1$$

$$P_{[1,1]} = \frac{W}{K+1} = \frac{a^2}{2\sigma_A^2 + 2\sigma_B^2 + a^2}$$

$$P_{[2,2]} = \frac{W^2}{2K+1} = \frac{a^4}{(4\sigma_A^2 + 2\sigma_B^2 + a^2)\,(a^2 + 2\sigma_B^2)}$$

$$P_{[3,3]} = \frac{W^3}{3K+1} = \frac{a^6}{(6\sigma_A^2 + 2\sigma_B^2 + a^2)\,(a^2 + 2\sigma_B^2)^2}$$

$$\vdots$$

$$P_{[n+1,n+1]} = \frac{W^{n+1}}{(nK+K+1)} = \frac{a^{2(n+1)}}{(2(n+1)\sigma_A^2 + 2\sigma_B^2 + a^2)\,(a^2 + 2\sigma_B^2)^n}$$

The probability of exactly m of n damaging hits on the target for any m of n is:

$$P_{[n,m]} = \binom{n}{m} \sum_{i=0}^{n-m} (-1)^i \binom{n-m}{i} P_{[m+i,m+i]}$$

$$= \binom{n}{m} \sum_{i=0}^{n-m} (-1)^i \binom{n-m}{i} \frac{W^{m+i}}{mK + iK + 1} \qquad (12)$$

where from above:

$$W = \frac{a^2}{a^2 + 2\sigma_B^2} \qquad (13)$$

and

$$K = \frac{2\sigma_A^2}{a^2 + 2\sigma_B^2} \qquad (14)$$

Comparing the results given here with those of my earlier investigation, we see that the circular Gaussian damage function not only provides a more realistic description of the conditional damage function for certain situations, but also facilitates obtaining the integrals and implifying the resulting expressions[3]. From the point of view of target damage assessments, however, since a considerable number of variables must be considered, it is evident that a conditional damage function that accurately describes the effects of these variables will be far more complex than either the cookie-cutter or Gaussian damage functions.

For some weapon terminal effects, the conditional damage function appears to lie somewhere between these two functions. A sequence of functions of this type has been proposed by Dobbie[4]. The first member of the sequence is the Gaussian damage function and the limiting member is the 'cookie-cutter' damage function. It has the property that the probability integrals for most situations can be computed in finite form in terms of known functions. The second member of the sequence yields probabilities that lie approximately half-way between the corresponding probabilities for the Gaussian and 'cookie-cutter' damage functions. This property can be used to approximate the difficult cases arising out of using the 'cookie-cutter' function. Since the second member of the sequence is generally a better description of the conditional damage function than the 'cookie-cutter' function, it would appear to be more feasible to use the probabilities provided by this second member of the sequence.

Our assumption apropos of cumulative damage effects is questionable, since we would normally expect more damage from several weapons striking the target than from a single weapon. The problem here is not one of mathematics but of a paucity of data on the vulnerability of targets subjected to multiple hits. From the viewpoint of operational effectiveness, however, several questions arise. If the situation is such that a single hit is insufficient to destroy a target, then an attempt should be made to make the ballistic dispersion negligible in comparison to the aiming error. If the resulting pattern is too small, however, either all weapons will strike the target or none. It can be argued of course that if a single weapon strikes a vulnerable component of the target and does not damage it, then a larger calibre weapon or different fusing is required. If, on the other hand, only one hit is sufficient to damage the target, then the ballistic dispersion should be increased. As the resulting pattern is enlarged, the probability of at least one hit approaches a maximum representing the optimum pattern[5].

Addendum. The solution to equation (1) can be obtained more readily by separating the basic integral accordingly:

$$p = \frac{1}{2\pi\sigma_B^2} \int_{-\infty}^{\infty} \exp\left\{-\left[\frac{(x-h)^2}{2\sigma_B^2} + \frac{x^2}{a^2}\right]\right\} dx \int_{-\infty}^{\infty} \exp\left\{-\left[\frac{y^2}{2\sigma_B^2} + \frac{y^2}{a^2}\right]\right\} dy$$

The first integral can be written:

$$\int_{-\infty}^{\infty} \exp\left(-\frac{x^2}{2\sigma_B^2} + \frac{xh}{\sigma_B^2} - \frac{h^2}{2\sigma_B^2} - \frac{x^2}{a^2}\right) dx = \exp\left(-\frac{h^2}{2\sigma_B^2}\right) \int_{-\infty}^{\infty} \exp\left[-\left(\frac{1}{2\sigma_B^2} + \frac{1}{a^2}\right)x^2 + \frac{xh}{\sigma_B^2}\right]$$

We note that

$$\int_{-\infty}^{\infty} \exp\,(-ax^2 + bx)\,dx = \frac{1}{\sqrt{a}} \exp\left(\frac{b^2}{4a}\right) \int_{-\infty}^{\infty} \exp\,(-y^2)\,dy = \sqrt{\frac{\pi}{a}} \exp\left(\frac{b^2}{4a}\right)$$

This together with the corresponding expression for y reduces equation (1) to

$$p = \frac{a^2}{a^2 + 2\sigma_B^2} \exp\left[-\frac{h^2}{a^2 + 2\sigma_B^2}\right]$$

[1] Terry, E. R., *Nature*, 205, 663 (1965).

[2] von Neumann, J., in *Optimum Spacing of Bombs and Shots in the Presence of Systemic Errors, B.R.L.* Report No. 241 (Aberdeen Proving Ground, Maryland: Ballistic Research Laboratories, 1941).

[3] Gadecki, E. V., *Nature*, preceding article.

[4] Dobbie, J. M., *Probability-of-Damage Problems of Frequent Occurrence*, O.E.G. Study No. 626 (Washington, D.C.: Operations Evaluation Group, 1959).

[5] Terry, E. R., *Nature*, 206, 1135 (1965).

EFFECTIVENESS OF PATTERNS OR GROUPS OF WEAPONS RELEASED WITH A SINGLE AIMPOINT IN THE PRESENCE OF ZERO BALLISTIC DISPERSION

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IT has been shown that if a pattern or group of weapons released with a single aimpoint is absolutely 'rigid', each weapon in the pattern will impact at its intended aimpoint and only two parameters—the number of weapons in the pattern and the spacing between weapons —are required to define the pattern[1]. Such a condition would arise if the patterns were released in the absence of ballistic dispersion. Admittedly, dispersionless patterns never exist in reality. Our interest in investigating these patterns was solely to provide one of several checks on the logic of the previously reported computer programmes[1-4]. However, our equations can be used satisfactorily as approximations when the ballistic dispersion is small.

When a pattern of weapons is released in a salvo in which there is zero spacing between weapons, if the conditional damage function is the circular 'cookie-cutter' function[2], the presence of zero ballistic dispersion will result in the probability of no less than a specified number of damaging hits being independent of the number of weapons in the pattern and equal to the probability of damaging the target with a single weapon drawn at random. The probability of exactly m of n damaging hits on a circular target of radius R when the conditional damage function is the circular 'cookie-cutter' function and the distribution of shots is circular normal and centred at a point h units from the target origin can be determined from:

$$P_{[n,m]}=\frac{1}{\sigma_A^2}\int_{h=0}^{\infty}\exp\left(-\frac{h^2}{2\sigma_A^2}\right)p^m(1-p)^{n-m}\,h\,dh\ (0\leqslant p\leqslant 1) \quad (1)$$

where:

$$p=\frac{1}{\sigma_B^2}\exp\left(-\frac{h^2}{2\sigma_B^2}\right)\int_{r=0}^{R}\exp\left(-\frac{r^2}{2\sigma_B^2}\right)I_0\left(\frac{rh}{\sigma_B^2}\right)r\,dr$$

$$=\exp\left(-\frac{h^2}{2\sigma_B^2}\right)\sum_{i=0}^{\infty}\frac{\left(\frac{h}{2\sigma_B^2}\right)^i}{i!}\left[1-\exp\left(-\frac{R^2}{2\sigma_B^2}\right)\sum_{j=0}^{i}\frac{\left(\frac{R^2}{2\sigma_B^2}\right)^j}{j!}\right] \quad (2)$$

$I_0(x)$ is the zero-ordered Bessel function of pure imaginary argument, and σ_A and σ_B are the aiming error standard deviation and ballistic dispersion, respectively[3,4]. When $\sigma_B=0$, we have

$$p=\begin{cases}1, & \text{when } h<R\\ 0, & \text{when } h>R\end{cases} \quad (3)$$

Accordingly, rewriting equation (1):

$$P_{[n,m]}=\frac{1}{\sigma_A^2}\int_{h=0}^{\infty}\exp\left(-\frac{h^2}{2\sigma_B^2}\right)p^m(1-p)^{n-m}\,h\,dh$$

$$+\frac{1}{\sigma_A^2}\int_{h=R}^{\infty}\exp\left(-\frac{h^2}{2\sigma_A^2}\right)p^m(1-p)^{n-m}\,h\,dh \quad (4)$$

if we set $m=n$ and replace the variable of integration by a function of a new variable, we obtain:

$$P_{[n,n]}=\int_{u=0}^{R^2/2\sigma_A^2}e^{-u}\,du=1-\exp\left(-\frac{R^2}{2\sigma_A^2}\right)=P_{[1,1]} \quad (5)$$

Similarly, if we set $m=0$, we obtain:

$$P_{[n,0]}=\int_{u=R^2/2\sigma_A^2}^{\infty}e^{-u}\,du=\exp\left(-\frac{R^2}{2\sigma_A^2}\right)$$

$$=P_{[1,0]}=1-P_{[1,1]} \quad (6)$$

so that $P_{[n,0]}=1-P_{[n,n]}$. By inspection of equation (4) we see that for all values of m such that $0<m<n$, $P_{[n,m]}=0$. Accordingly, since the probability of at least m of n damaging hits, say $P_{n,m}$, can be obtained from the following relationship:

$$P_{n,m}=1-P_{[n,0]}-P_{[n,1]}-P_{[n,2]}-\cdots-P_{[n,m-1]}$$

$$=1-\sum_{i=0}^{m-1}P_{[n,i]}=\sum_{i=m}^{n}P_{[n,i]} \quad (7)$$

we may write:

$$P_{n,m}=P_{[n,n]}=1-\exp\left(-\frac{R^2}{2\sigma_A^2}\right),\ (\sigma_B=0) \quad (8)$$

If we assume now that the conditional damage function is the circular Gaussian damage function[2], the probability of damaging the target with exactly m of n weapons is given by:

$$P_{[n,m]}=\binom{n}{m}\sum_{i=0}^{n-m}(-1)^i\binom{n-m}{i}\frac{W^{m+i}}{mK+iK+1},\ (n\geqslant m) \quad (9)$$

where

$$W=\frac{a^2}{a^2+2\sigma_B^2} \quad (10)$$

$$K=\frac{2\sigma_A^2}{a^2+2\sigma_B^2} \quad (11)$$

a is the equivalent damage radius, and the probability of damaging the target with a single weapon drawn at random is given by:

$$p=\frac{a^2}{a^2+2\sigma_B^2}\exp\left(-\frac{h^2}{a^2+2\sigma_B^2}\right) \quad (12)$$

When $\sigma_B=0$, W is unity, $K=2\sigma_A^2/a^2$, and equation (9) reduces to:

$$P_{[n,m]}=\binom{n}{m}\sum_{i=0}^{n-m}(-1)^i\binom{n-m}{i}\frac{a^2}{2\sigma_A^2(m+i)+a^2},$$

$$(n\geqslant m,\ \sigma_B=0) \quad (13)$$

For this damage function the probability of at least m of n damaging hits is not equal to the probability of damaging the target with a single weapon drawn at random (given by equation (12)), since this latter probability, irrespective of the aiming error, always assumes some finite value between zero and unity, that is, $p\neq 0$.

If a pattern is released with a certain non-zero spacing between weapons, then whether or not a weapon in the pattern damages the target will depend on the pattern reference point (whose aimpoint is the aimpoint for the pattern as a whole) and the distance its aimpoint is offset from the pattern reference point. We shall illustrate this with an example drawn from stick (train) bombing[1,5,6]. Consider a vulnerable infinite-line or strip target of width $2W$ so located that the centreline of the target is coincident with the x axis. An aircraft approaches the target on a perpendicular course and releases a linear pattern of n weapons, aiming the centre weapon at the centre of the target with linear spacing Δy_i. To simplify the problem, it is assumed that n is an odd integer and that the linear spacing between weapons is less than the target width. Damage obtains from a hit with the ith weapon in the pattern if it impacts in the target plane at a point y_i such that:

$$p_i = \begin{cases} 1, \text{ if } -W \leqslant y_i \leqslant W \\ 0, \text{ otherwise} \end{cases} \qquad (14)$$

If the aimpoints of the individual weapons in the pattern are defined in relation to the aimpoint of some reference point in the pattern, say $\bar{y}_A$, and the pattern aimpoint is the aimpoint of the reference point (the centre weapon, for our example), then the probability of at least m of n damaging hits with a pattern of n weapons is given by:

$$P_{n,m} = \int_{-\infty}^{\infty} f_A(\eta)\, Q_{n,m}\, d\eta \qquad (15)$$

where

$$f_A(\eta) = \frac{1}{\sqrt{2\pi}\,\sigma_A} \exp\left\{-\frac{1}{2}\left[\left(\frac{\eta-\bar{y}_A}{\sigma_A}\right)^2\right]\right\} d\eta \qquad (16)$$

$$Q_{n,m} = \sum_{j=m}^{n} (-1)^{j-m} \binom{j-1}{m-1} S_j^{(n)}, \quad (m = 1, 2, \ldots, n) \qquad (17)$$

$$S_1^{(n)} = \sum_j p_{ij}, \; S_2^{(n)} = \sum_{j<k} p_{ij}\, p_{ik}, \ldots,$$

$$S_n^{(n)} = p_{i1}\, p_{i2} \cdots p_{in} \qquad (18)$$

$$p_i(\eta) = \frac{1}{\sqrt{2\pi}\,\sigma_B} \int_{-W}^{W} \exp\left\{-\frac{1}{2}\left[\left(\frac{y-\bar{y}_i}{\sigma_B}\right)^2\right]\right\} dy_i$$

$$= \operatorname{erf}\left(\frac{W-\bar{y}_i}{\sigma_B\sqrt{2}}\right) \operatorname{erf}\left(\frac{W+\bar{y}_i}{\sigma_B\sqrt{2}}\right) \qquad (19)$$

$\bar{y}_i$ is the mean point of impact of the ith weapon where:

$$\bar{y}_i = \eta + \Delta y_i \qquad (20)$$

and $d\eta$ and dy_i are the error and target co-ordinate systems similarly oriented with origins at the target centre and the pattern reference point, respectively. Here $f_A(\eta)$ is the aiming error probability density function for determining whether the pattern reference point is placed between η and $\eta + d\eta$. The $Q_{n,m}$ and $p_i(\eta)$ are conditional probabilities of obtaining at least m of n damaging hits with a pattern of n weapons and of obtaining a damaging hit with the ith weapon in the pattern, respectively, given that the aiming error is placed at η.

If $\sigma_B = 0$ and we assume for the moment that all the p_i are equal (that is, that the weapons in the pattern are released with zero spacing), then damaging hits obtain only under the conditions expressed by equation (14). Thus, for this situation the pattern reference point, $\bar{y}_A$, must be placed such that $-W \leqslant y_A \leqslant W$. The probability that the pattern reference point is so placed, which is also the probability that at least m of n weapons damage the target, is given by:

$$P_{n,m} = \frac{1}{\sqrt{2\pi}\sigma_A} \int_{-W}^{W} \exp\left[-\frac{1}{2}\left(\frac{\bar{y}_A}{\sigma_A}\right)^2\right] dy$$

$$= \operatorname{erf}\left(\frac{W}{\sigma_A\sqrt{2}}\right) \qquad (21)$$

Now let us assume that the weapons are released with a specified non-zero spacing. To determine the probability of no less than a specified number of damaging hits, we must first determine the maximum number of hits possible, taking into consideration the target width and pattern length, l. The latter is equal to the product $(n-1)\Delta y_i$. If the pattern reference point (that is, the centre weapon in the pattern) misses the target centre by a distance greater than $(2W+l)/2$, none of the weapons will damage the target. Conversely, if the pattern reference point is placed a distance less than $(2W+l)/2$ from the target centre, damaging hits will obtain. Thus, if the pattern reference point is placed in a central strip of width $2W+l$, at least one weapon damages the target and the probability that the pattern reference point is so placed is:

$$P_{n,1} = \frac{1}{\sqrt{2\pi}\,\sigma_A} \int_{-(2W-l)2}^{(2W+l)/2} \exp\left[-\frac{1}{2}\left(\frac{\bar{y}_A}{\sigma_A}\right)^2\right] dy$$

$$= \operatorname{erf}\left(\frac{2W+l}{\sigma_A\, 2\sqrt{2}}\right) \qquad (22)$$

Similarly, if the pattern reference point is placed in a central strip of width $2W+l-2\Delta y_i$, at least two weapons will damage the target, and the probability that the pattern reference point is so placed is:

$$P_{n,2} = \frac{1}{\sqrt{2\pi}\,\sigma_A} \int_{-(2W-l-2\Delta y_i)/2}^{(2W+l-2\Delta y_i)/2} \exp\left[-\frac{1}{2}\left(\frac{\bar{y}_A}{\sigma_A}\right)^2\right] dy$$

$$= \operatorname{erf}\left(\frac{2W+l-2\Delta y_i}{\sigma_A\, 2\sqrt{2}}\right) \qquad (23)$$

For the general case, we can show in a similar manner that, if the pattern reference point is placed in a central strip of width $2W+l-2\Delta y_i(r-1)$, at least m damaging hits obtain, and the probability that the pattern reference point is so placed is:

$$P_{n,m} = \frac{1}{\sqrt{2\pi}\,\sigma_A} \int_{-[2W+l-2\Delta y_i(m-1)]/2}^{[2W+l-2\Delta y_i(m-1)]/2} \exp\left[-\frac{1}{2}\left(\frac{\bar{y}_A}{\sigma_A}\right)^2\right] dy$$

$$= \operatorname{erf}\left[\frac{2W+l-2\Delta y_i(m-1)}{\sigma_A\, 2\sqrt{2}}\right] \qquad (24)$$

It is our belief that had these findings been generally appreciated in the past, damage probability curves in previously published works on the phenomenology of releasing weapon patterns with a single aimpoint might have been drawn somewhat differently.

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A DIGITAL COMPUTER ANALYSIS OF PALAEOLITHIC FLINT ASSEMBLAGES

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BORDES'S numerical methods of describing and classifying complex assemblages of flint artefacts have provided a new approach to Palaeolithic studies. These methods describe assemblages by the percentage of occurrence of a fixed range of types and classify them on this basis[1]. However, the presentation and analysis of this kind of data are by no means straightforward and it has proved difficult to compare comprehensively more than a few assemblages together, and also to make use of the full range of data in classification. This article describes experiments with one possible aid to the study of these and similar kinds of data.

A multi-dimensional scaling procedure developed by Shepard[2] and Kruskal[3] primarily for use in psychological research has been applied to archaeological material. The method operates on a set of 'units', in this case flint assemblages, any two of which are related by a numerical 'similarity coefficient'. It arranges points, each corresponding to a unit, in a prescribed number of dimensions so that the distances between the points reflect the magnitudes of the corresponding similarities. Configurations of points in one or two dimensions form 'maps' which are convenient to study, and which may reveal structure inherent in the data.

Full details of the procedure are given elsewhere[4]. Briefly, a digital computer is programmed (our programme is written in Algol) to generate a random configuration of points in a prescribed number of dimensions and then iteratively to rearrange the points until a configuration which best fits the data is obtained. For any configuration a measure of goodness of fit may be calculated which we call the 'strain' (Shepard's 'delta'). That configuration with the least strain is the most valuable. Normally, when an optimal two-dimensional configuration has been achieved, it is then collapsed into one dimension and used to initiate a search for the best linear arrangement of the points.

The application of this method to archaeological material has been tested most fully so far on a series of thirty Iron Age brooches which could be described numerically by the presence and absence of various features. In these tests[4], the analysis produced configurations which were consistent with a rough chronology deduced from independent evidence. Its performance also compared favourably with a series of 'intuitive' analyses carried out on the same material by a group of professional archaeologists.

For the experimental analysis of Palaeolithic material to be described here, sixteen assemblages were chosen from a wide range of well-documented sites of the Mousterian culture—the culture regularly associated with Neanderthal remains. A number of stratified assemblages were included since their known chronological ordering would help in assessing the value of the computed results.

The data, all taken from authors who used Bordes's list of categories, were used in two forms. The first was Bordes's 'essential' form which excludes some retouched flakes of dubious significance (for example, some that may have been 'retouched' by natural agencies). These essential data have been modified slightly for the present analysis to exclude Bordes's miscellaneous category 62 ('divers') and to include 'bifaces' as an additional single category within the list of types. The second form of data used was Bordes's 'real' data, which included all retouched flakes.

A similarity coefficient S_{ij} was calculated for each pair (i,j) of assemblages according to the formula:

$$S_{ij} = \sum_{t=1}^{n} (P_{it}\, P_{jt})^{\frac{1}{2}}$$

where the summation is over the n types, and where P_{it} is the percentage of flakes of assemblage i which is of type t. The formula takes the square roots of the percentages to improve the characteristics of the coefficient.

Repeated analyses from different random starting configurations were carried out, and low-strain configurations were produced in two dimensions for both the essential and the real data, but in one dimension for the essential data only. This implies that the real data were not consistent with a linear arrangement of the assemblages. Successive low-strain configurations from the same data varied within only narrow limits. Typical configurations are illustrated in Figs. 1 and 2.

For the essential data, which are perhaps theoretically more reliable, the two-dimensional configuration suggests

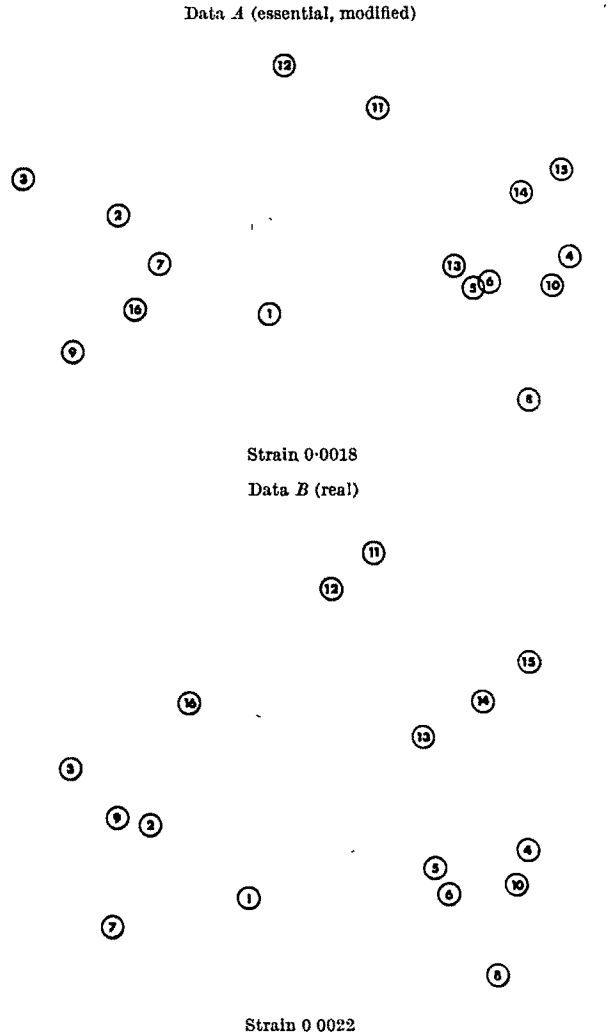


Fig. 1. Computed two-dimensional configurations of sixteen Mousterian assemblages: (1)–(3) Pech d'Azé (Dordogne) levels 4, *B*, 7 (ref. 8), (4)–(7) Chadourne (Dordogne) levels *D*, *C*, *B*, *A* (ref. 9); (8)–(9) Haute-Roche (Charente), lower and middle levels (ref. 10); (10) l'Ermitage (Vienne) (ref. 11); (11) Oissel (Seine-Inf.) (ref. 12), (12) Houppeville (Seine-Inf.), série claire (ref. 12); (13) Rigabe (Var) level *G* (ref. 13); (14) Prince (Grimaldi) foyer *E* (ref. 14); (15) Kokkinopulos (Greece), surface finds (ref. 15); (16) Romani (Barcelona), levels 9/9*a*/9*b* (ref. 16)

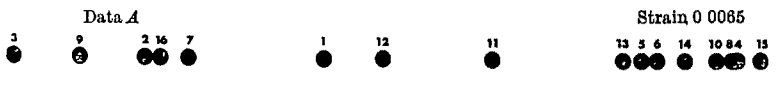


Fig. 2. Computed one-dimensional configuration of sixteen Mousterian assemblages from modified 'essential' data. 'Real' data refused to yield a low strain configuration in one dimension

two principal clusters of points around the ends of a main axis (I to the right, II to the left) with two assemblages, 11 and 12, apart, perhaps suggesting a third focus (III) roughly equidistant from I and II.

The arrangement of the stratified assemblages is consistently with earlier assemblages to the right of later (1–2–3; 4–6/5—7; 8–9). Assemblages 5 and 6, which apparently form an exception, have been shown by Vértes not to differ significantly in their content although they are from different levels in the Abri Chadourne[6]. Assemblages 11 (Oissel at the base of Loess récent I) and 12 (Houppeville at the base of Loess récent II), which may be dated relatively by their geological position, conform to the general pattern.

The same consistent placing of stratified assemblages is found in the one-dimensional configuration.

The two-dimensional configuration obtained from the real data reproduces the main features just described. However, assemblages 13, 14 and 15 are here separated from the main cluster I, and the relative position of some single assemblages within the other main group II varies considerably. These differences between the configurations derived from the essential and the real data were obtained consistently.

It would be unwise to draw general conclusions from the analysis of so few assemblages as this. However, it may be noted that the three main clusters which suggest themselves in the two-dimensional configurations correspond broadly, though not completely, with the main divisions of the Mousterian which Bordes suggests (largely on the evidence of discontinuity in the frequency distribution of racloirs). Group 1, to the right of the diagram, corresponds with Bordes's Charentian, and group III (assemblages 11 and 12) with his typical Mousterian. However, group II includes assemblages variously classified by Bordes as Denticulate Mousterian (7 and 9), and as Mousterian of Acheulian tradition types *A* and *B* (assemblages 1 as type *A*, 2 and 3 as type *B*). It is interesting that for both sets of data the scaling analysis, which is effectively 'polythetic' in Sneath's[6] terms, relates the important assemblage 1 to other assemblages from the Dordogne (to 2, a higher level in the same cave, and to 7) rather than to assemblages from the Seine basin (11 and 12). This is despite a racloir index which would certainly group 1 with 11 and 12 were a monothetic system of classification based on the racloir frequency followed.

The assemblages separated from our group I by the real data, but not by the essential data, are from south-easterly sites and include 15, the patinated series of surface flints from Kokkinopilos, Greece.

The placing of assemblage 1, and the seemingly strong correlation between typological development and chronology which is implied by the positioning of the stratified assemblages perhaps lend support to Mellars's view[7] that typological differences between some of the main Mousterian groups may have more chronological significance than Bordes himself admits.

From our preliminary work we are encouraged to believe that this method of analysis provides a convenient and objective means of presenting archaeological data which it is otherwise difficult to study. It is likely to reveal clustering and trends within the material and so to aid classification and interpretation. In the analysis described here, it seems that the main axis of the two-dimensional configurations may well correspond with time, and in addition definite clusters are indicated which emphasize variation in a second dimension around this main axis. Thus multidimensional scaling provides a kind of improved seriation of the material, avoiding many of the limitations inherent with normal seriation techniques. An optimal one-dimensional configuration produces seriation with relative spacing of points as well as ordering, and is accompanied by a numerical measure of the distortion involved in obtaining a linear representation of the data. An optimal two-dimensional configuration is distorted only to the extent that the data require three or more dimensions for their accurate representation, and again this distortion is measured. Without safeguards of this nature, seriation is liable to assume linearity where none exists.

We plan to use these methods to analyse a more complete series of relevant Palaeolithic assemblages.

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OCCURRENCE OF COMPOSITIONALLY ZONED ALMANDITIC GARNETS IN REGIONALLY METAMORPHOSED ROCKS

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ALMANDITIC garnets have received much attention from metamorphic petrologists for a variety of reasons, including: (1) the use of almandite as an isograd mineral in regional metamorphic sequences; (2) the possible relation of garnet composition to host-rock composition, metamorphic grade, and the partitioning of elements between co-existing phases[1–4]; (3) the use of the internal textures of garnets in investigating phases of garnet growth (that is, metamorphism) in relation to phases of deformation[5–6]; and (4) the use of garnet in

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studying nucleation and growth of metamorphic minerals[7]. As a result of such studies, a reasonable amount of data on the chemical composition of almanditic garnets in regional metamorphic terrains has accumulated, but few investigations have been made of the variation in chemical composition of garnets within a single microscope slide, both from the point of view of estimating the attainment of chemical equilibrium, and also in evaluating the relation of any chemical variation to the internal textural variation. From this and other points of view, we have recently investigated a number of metamorphic minerals from two regional metamorphic terrains using the electron-probe microanalyser in the Department of Mineralogy and Petrology, University of Cambridge, and a brief summary of the results on almanditic garnets is presented here.

The rocks investigated come from two distinct regions, the Glen Esk area in the south-east Grampian Highlands of Scotland (B. H.), and the Sulitjelma region, North Norway (K. J. H.). The rocks studied from the Glen Esk area are pelites, with the typical mineral assemblage: quartz, plagioclase, muscovite, biotite, garnet, staurolite, ±kyanite, ±sillimanite, and come from the staurolite, kyanite and sillimanite zones of Barrow[8]. The garnets in these rocks commonly show straight or slightly sigmoidal inclusion trails (S_i) of fine-grained quartz and iron ore. The material studied from Sulitjelma ranges in grade from the biotite to the kyanite zone, and consists of pelites and calcareous pelites of the following mineral assemblages: (1) quartz, biotite, muscovite, garnet, ±staurolite, ±kyanite, ±plagioclase; (2) quartz, biotite, clinozoisite, ±calcite, ±hornblende, ±garnet, ±plagioclase. The garnets in these rocks show a wide variety of internal textures, including both S-shaped and straight S_i of fine-grained quartz, clinozoisite and iron-ore; and others with this type of S_i in the core, but with a margin of either clear garnet, or garnet with relatively large inclusions. (A 'large inclusion' in this context has a size approximately that of the groundmass crystals surrounding the garnet porphyroblast.)

Garnets with no apparent discontinuity in growth. Garnets within this class are those characterized by having, in addition to the possible presence of a very fine dusting (of graphite ?), inclusions of uniform size, with a straight, *S*-shaped, or random arrangement; or those which have no inclusions at all. In addition, these garnets show no sharply marked changes in inclusion pattern or refractive index when viewed in the optical microscope.

The majority of microprobe determinations have been carried out on garnets of this type, and with only rare exceptions a gradational compositional zoning with respect to FeO, MnO, MgO and CaO has been found to occur within individual garnets. This is illustrated in Figs. 1 and 2, which are based on point counts at selected locations across the garnets. In the oxide percentages shown, 'FeO' refers to total iron calculated as FeO, and all percentages are 'apparent concentrations' (that is, percentages uncorrected for absorption, atomic number, and fluorescence effects). The standards used were pure manganese, olivine (9·50 wt. per cent FeO, 49·08 wt. per cent MgO) (K. J. H.), pure iron and pure magnesium (B. H.), and wollastonite (47·44 wt. per cent CaO): the accelerating voltages used were 20 kV for iron, manganese and calcium, and 15 kV for magnesium; and the take-off angle was 40°. Thus the 'true concentration' of the oxide probably does not exceed the 'apparent concentration' by more than 10 per cent for FeO, MnO and CaO, and by more than 40–50 per cent for MgO; and since the last is generally less than 5 per cent by weight of the garnet the relative proportions of the oxides will be only slightly altered by correction.

Overall the most constant characteristic of the zoning observed is a gradual decrease of MnO and gradual increase of FeO from core to margin of the garnets. Forty-one out of forty-seven garnets in sixteen out of eighteen rocks from Sulitjelma show a decrease of MnO from core to margin (the other cases were unzoned), generally of from 2 to 5 per cent MnO, but not uncommonly of as much as 10 per cent MnO (see Fig. 1). FeO shows only a slightly less consistent variation in the opposite sense (but of similar magnitude), rarely remaining constant, or even decreasing slightly towards the margin. In the Glen Esk area garnets a maximum difference of 5 per cent MnO has been observed, but in the majority of cases MnO and FeO vary by only 1–2 per cent, as in Fig. 2; but despite its small size, the variation was found to occur in seventeen out of eighteen garnets (spread over seven rocks) examined for FeO, and seven out of eight garnets examined for MnO (the exceptional garnet in both cases showing no zoning).

For MgO the changes observed within the garnets from the Glen Esk area are very small and variable, but in the Sulitjelma garnets a general small increase from core to margin has been observed. CaO decreases from core to margin in seven out of eight garnets examined from the Glen Esk area, and in the one shown in Fig. 2 the zoning is exceptionally strong. Garnets investigated from the Sulitjelma region are not consistent with respect to CaO, which may increase, decrease, or remain constant from core to margin. Overall the general scheme of the zoning indicates the cation substitution to be:

$$\underset{\text{Core}}{Mn^{++}\ (+Ca^{++})} \rightleftarrows \underset{\text{Margin}}{Fe^{++}\ (+Mg^{++})}$$

Although the zoning in the garnets appears to be roughly concentric, preliminary investigations show that this is not always the case, either because of obvious fracturing or resorption later in the metamorphic history (Fig. 3), or in other cases possibly because of actual variation of growth rate in different directions during formation.

The possible factors involved in the production of this compositional zoning (which may be termed 'cryptic' since there is no indication of it under the optical microscope) are numerous, and it is difficult, with the information at present available, to determine which are the more significant. The general pattern of the zoning shows a change of chemical composition similar to that observed

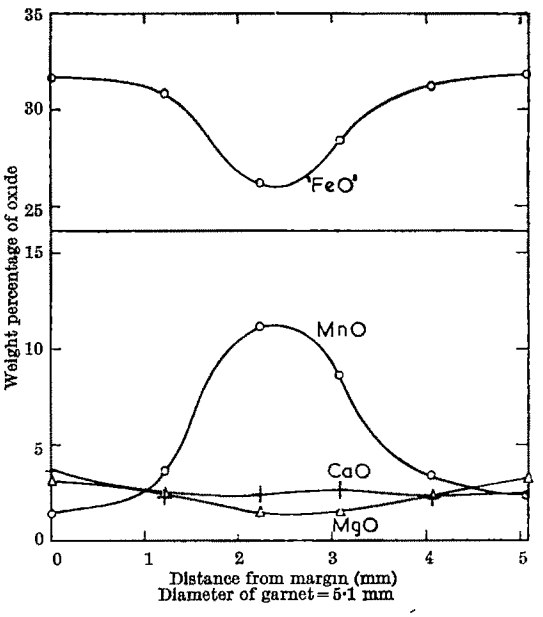


Fig. 1. Cryptic compositional zoning in an almanditic garnet from a garnet–hornblende–mica schist (64 *K* 323) from Sulitjelma, Northern Norway, as revealed by electron-probe analysis. The garnet is euhedral, contains few inclusions (unarranged), and optically appears to be of one phase of growth. The 'weight percentage of oxide' is the 'apparent concentration', that is, the concentration uncorrected for absorption, atomic number, and fluorescence effects (see text)

to occur with respect to grade in almanditic garnets from regional metamorphic sequences, where MnO and CaO decrease and FeO and MgO increase upgrade[4]. It seems possible, therefore, that the observed zoning of individual garnets is largely the result of a similar grade effect, namely, that as the grade increases during growth of the garnets Fe^{++} and Mg^{++} are increasingly stabilized in the lattice[1]. However, excluding metasomatism, other factors might cause similar effects, and one possibility, in connexion with the MnO zoning, arises from the marked affinity for garnet commonly shown by the small amount of MnO in metamorphosed argillaceous rocks[4]. In the rocks in question, practically the whole of the MnO is located within the garnets. It seems quite likely, therefore, that at the beginning of garnet growth (even under constant temperature and pressure conditions, at which both almandite and spessartite are stable), the MnO of the rock will become preferentially concentrated within the garnet; and considering the very small amount of MnO in the rock as a whole it will soon become exhausted in the vicinity of each garnet as the growth of the garnets proceeds. The process might be regarded as a fractionation of much of the rock MnO into the garnet early in the latter's growth, and thus effectively changing the amount of available MnO in the rock.

Other factors which might operate singly, or in combination with one another and those previously noted, in causing the zoning, include possible differences in the diffusion rates of the cations (either at constant or varying temperature), or increase in the available amount of Fe^{++} during metamorphism as a result of reduction of Fe^{+++} by reactions involving graphite[8]. In addition, the effects of chemical reactions involved in the production of other minerals within the rocks must be considered: the variable nature of the CaO zoning in the Sulitjelma rocks (where, in addition to plagioclase, the calcium-bearing minerals, calcite, clinozoisite and hornblende, may be present) as compared with the Glen Esk area rocks (where plagioclase is the only calcium-bearing mineral besides garnet) might be explicable on this basis.

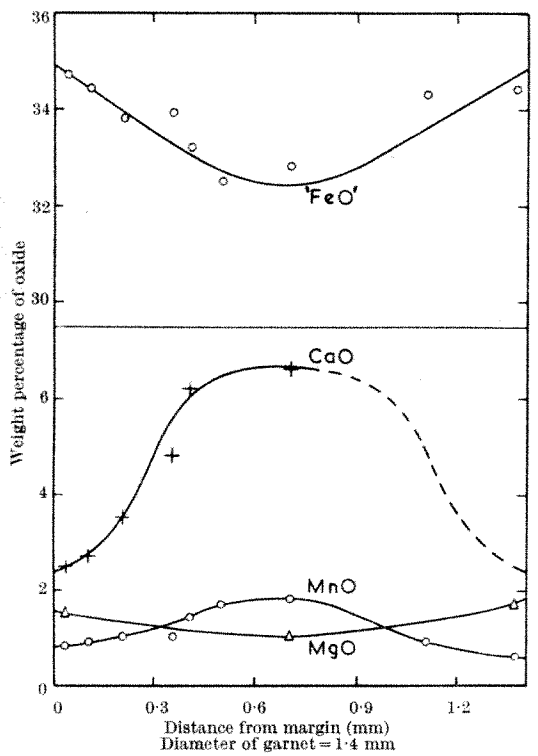


Fig. 2. Cryptic compositional zoning in an almanditic garnet from a garnet–staurolite–kyanite–sillimanite schist from Glen Esk, Scotland (specimen 96417–8, Harker collection, Cambridge), as revealed by electron-probe analysis. The garnet is approximately circular, has very slightly sigmoidal *Si* of fine-grained inclusions, and appears to be of one phase of growth. The 'weight percentage of oxide' is again 'apparent concentration', uncorrected for absorption, atomic number and fluorescence effects

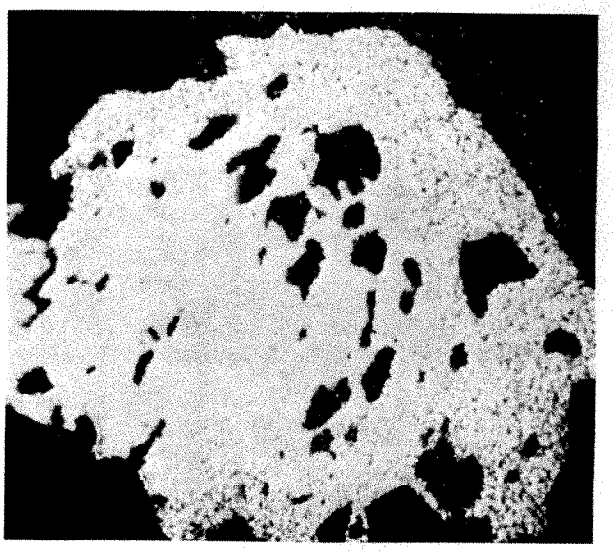


Fig. 3. X-ray scanning photograph taken in manganese K_α radiation of a cryptically zoned almandine garnet from a garnet–hornblende–mica-schist (64 *K* 131) from Sulitjelma, North Norway. The lightest area has the highest MnO content, and it can be seen that this is transgressed by the garnet margin. It is probable that this is due to fragmentation or resorption of the garnet after growth, especially since the lowest MnO content is associated with the (presumably) original euhedral faces. The dark inclusions and surround are quartz, clinozoisite, and biotite, containing only trace amounts of MnO

Garnets with an apparent discontinuity in growth. In the Glen Esk area the only garnets which suggest a possible discontinuity in growth have an interior densely dusted with fine irresolvable inclusions, sharply demarcated from a clear outer zone. Microprobe analysis across these garnets, however, has revealed no sharp change in composition between the clear and clouded parts, and such compositional zoning as exists is only of the gradational type described in the previous section.

Some garnets from the Sulitjelma region show marked evidence of a discontinuity in growth, with cores having straight or S-shaped *Si* of fine inclusions, and rims with large inclusions or none at all. In these cases a sharp change in composition does occur across the junction of the texturally different zones (Fig. 4). In general, FeO is usually greater in the outer zone, while CaO and MgO may either increase or decrease. MnO has never been observed to increase in the outer zone. These marked changes in chemical composition agree with the textural evidence in suggesting two distinct periods of garnet growth.

It may be noted that, except for slight zoning of plagioclase, none of the other minerals (hornblende, biotite, staurolite) investigated in the rocks described shows any compositional zoning.

General deductions. The present work has shown the existence of marked cryptic compositional zoning in virtually all almanditic garnets from different mineral assemblages, in two widely separated regions, and at grades of metamorphism varying from biotite zone to sillimanite zone. It is clear from this that there is a strong possibility that such zoning may occur in similar rocks from other regions, and that the interpretation of bulk chemical analyses of garnets must proceed with caution, both with respect to changes in garnet composition with grade, and also with regard to the partitioning of elements between co-existing phases. There is the possibility that bulk chemical analyses of garnets do not even represent the average chemical composition of the garnet in a rock, because of selective concentration of only one part of the garnet (such as the core) during

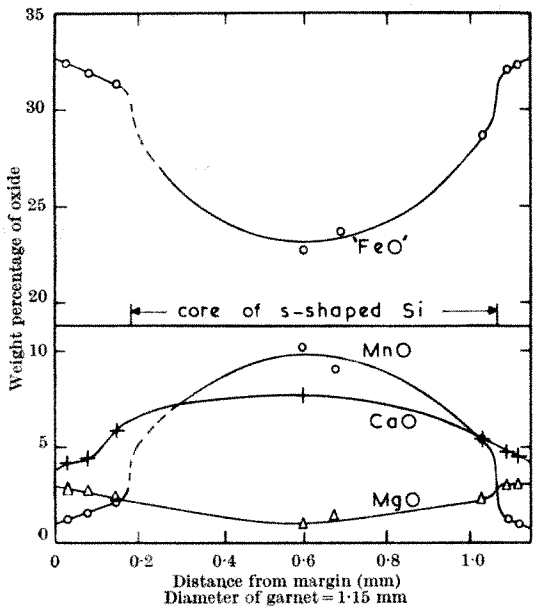


Fig. 4. Discontinuous compositional zoning in an almanditic garnet from a garnet–mica–schist (64 *K* 176) from Sulitjelma, Northern Norway, as revealed by electron-probe analysis. The garnet has a roughly circular core of S-shaped *Si* composed of fine-grained inclusions, and a euhedral margin of irregular thickness containing a few coarse, random inclusions. Thus optically it appears to be of two phases of growth, and this is also indicated by the compositional zoning. 'Weight percentage of oxide' again refers to 'apparent concentration'

separation of the mineral. Thus, one of us (K. J. H.), using magnetic and heavy liquid techniques, separated two pure fractions of garnet of slightly differing density from a garnet–mica–schist, in which there had been only one apparent phase of garnet growth. The distinct differences in chemical composition, especially of MnO, FeO and MgO, may be seen from the analyses of the two density fractions presented in Table 1.

The occurrence and nature of the zoning may, however, be useful in a number of ways. If it may be assumed that the change in composition (particularly of MnO) varies regularly during one phase of garnet growth, the occurrence of the zoning could prove a useful aid in studying such features as the frequency of nucleation, and the relative growth rates both of individual garnet crystals, and of garnets from different rocks, during the course of metamorphism. It may be noted that the data at present available might suggest that within one rock the smaller the garnet the smaller is the amount of zoning; however, further investigation with reference to the elimination of 'cut-effect' is necessary. In addition, the zoning, and variations in it, in different rock types and different areas, might provide some clues as to the chemical reactions occurring in the formation of particular mineral assemblages.

Table 1. ANALYSES OF TWO SEPARATED FRACTIONS OF GARNET FROM A GARNET–MICA–SCHIST (64 *K* 229) FROM SULITJELMA

	A	*B*
SiO_2	37·98	38·14
TiO_2	0·17	0·11
Al_2O_3	21·50	20·95
'FeO'	27·66	28·65
MnO	5·27	3·60
MgO	2·56	3·18
CaO	4·95	5·20
Total	100·09	99·83

The garnet is apparently of one phase of growth (few or no inclusions throughout) and *A* is the slightly denser fraction. It seems probable, from the differences in MnO, 'FeO', and MgO, that *A* is from nearer the core than *B*, but neither *A* nor *B* need necessarily represent the core or margin compositions respectively.

We thank Dr. J. V. P. Long for reading the manuscript and together with Dr. S. J. B. Reed for much useful discussion and invaluable help in using the electron-probe microanalyser, Dr. G. A. Chinner for critically reading the manuscript, and Prof. W. A. Deer for providing the facilities of the Department of Mineralogy and Petrology, Cambridge. The work was carried out during the tenure of research studentships at Cambridge, and University College, London, from the Department of Scientific and Industrial Research.

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DISTRIBUTION OF SUBSTITUTIONAL NITROGEN DONORS IN SYNTHETIC DIAMONDS

By Inst. Lt. M. J. A. SMITH*, R.N., B. R. ANGEL* and Inst. Lt. Cdr. R. G. EMMONS, R.N.
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CONSIDERABLE attention has been devoted to the study of nitrogen impurity in natural diamonds using the technique of electron spin resonance[1–3]. The spectrum obtained is due to the unpaired electron provided by the substitutional nitrogen atom and the main feature is a triplet at $g = 2{\cdot}0024$ caused by interaction with the nitrogen nucleus which has a spin of unity. The distribution of nitrogen is variable and would seem to depend on the method by which the diamond was formed.

In the majority of natural diamonds nitrogen exists substitutionally in the form of non-paramagnetic platelets and such diamonds have recently been classified as Type I*a* (ref. 4).

Type I*b* diamonds contain nitrogen atoms which are not linked in platelet form. The electron spin resonance spectrum is usually narrow (0·3 oersted) and depends on the orientation of the static magnetic field to the diamond lattice. It has hyperfine constants $A = 40{\cdot}8$ oersteds and $B = 29{\cdot}2$ oersteds and typical concentrations lie in the range 10^{14}–10^{17} atoms cm^{-3}. A theory of the interaction for the nitrogen centre has been developed[1,3].

Samsonenko[2] observed electron spin resonance from nitrogen in natural diamonds and obtained two sets of superimposed lines of different widths. Line shape measurements indicated that the broad resonance was probably caused by dipolar interaction between nitrogen centres in regions of elevated concentration. He concluded that pairing of spins takes place and platelets are formed at concentrations of the order of 10^{20} atoms cm^{-3}. Exchange interaction should precede such pairing and evidence for this is provided by the Lorentz form of the central peak.

Huggins and Cannon[5] studied 'RVG'-type ('resinous vitrified grinding'-type) synthetic diamond and obtained the nitrogen spectrum superimposed on a broad resonance due to the transition metal catalyst used during preparation. The nitrogen resonance is considerably broader than is observed with natural diamonds and the three lines overlap considerably. More recent results by Dyer *et al.*[4] using samples of mean diameter 400μ strongly suggest

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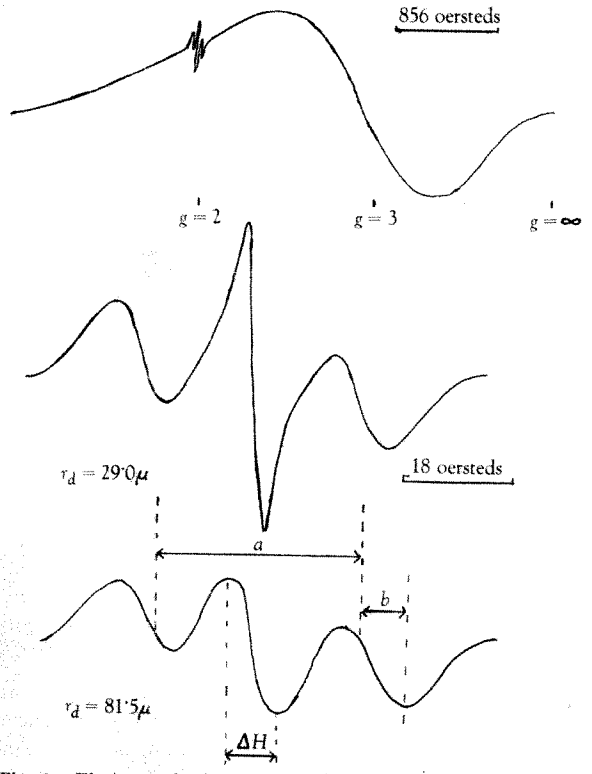


Fig. 1. Electron spin resonance spectrum for 'MBG'-type synthetic diamonds

that the major contribution to the line-width is produced by a spread in the local field due to ferromagnetic inclusions. For the nitrogen concentrations present, the satellite lines are broadened owing to the angular variation of the hyperfine coupling and the random distribution of the diamonds which form the sample. Evidence that nitrogen exists in platelet form is not obtained and synthetic diamond is thought to be wholly Type I*b*. The nitrogen content can be controlled during formation and can have a value up to about 10^{20} atoms cm^{-3}.

In the work presented here the electron spin resonance spectra have been recorded for a range of 'MBG'-type ('metal bonding grinding'-type) synthetic diamonds of different sizes. The diamonds are passed through sieves of known mesh and groups of the same mean diameter are isolated. Each diamond is assumed to be spherical and the diameter of a group is quoted as the mean mesh size of two consecutive sieves. A sample of constant weight, equal to 48 mg, is taken from the appropriate group and consists of between 6,000 and 130,000 single diamonds depending on the group diameter.

A typical spectrum is shown in Fig. 1. For the samples investigated, the concentration of paramagnetic transition metal impurity remains constant at approximately 10^{21} spins cm^{-3}. This is also true for specimens of 'MBS'- ('metal bonding saw') and 'RVG'-type synthetic diamonds which were studied, although *g*-value variations suggest a different catalyst. Ferromagnetic inclusions can clearly be expected.

Table 1 summarizes the results obtained for the nitrogen resonance. The spectrometer was calibrated against diphenyl picryl hydrazyl to obtain the concentration of paramagnetic centres and against methyl methacrylate monomer treated with nitric oxide[6] to define the magnetic field axis. The *g*-value of the central nitrogen resonance is 2·0024 ± 0·0007 and is in agreement with other published results. The splitting between the satellite lines (*a*) is 60 ± 2 oersteds and the width of each satellite (2*b*) is 20 ± 2 oersteds measured as shown in Fig. 1. These three parameters remain constant within the deviations quoted.

Table 1

r_d (μ)	N_{scc} Spins cm^{-3} × 10^{19}	ΔH Oersteds	$\left[\frac{N_d\|_{11\cdot7\mu}^{r_d}}{N_d\|_{0}^{11\cdot7\mu}}\right]$ Calculated
81·5	2·43	14·0	53
68·5	3·00	12·4	36
48·5	3·68	12·1	18
40·5	4·35	7·8	12
29·0	6·55	6·2	5

The concentration of paramagnetic nitrogen, N_{scc}, in the sample is given in the second column of Table 1 and is obtained by dividing the observed intensity by the total volume of diamond. It is clear that the concentration is not the same for diamonds of all sizes taken from the same batch and increases as they become smaller. One way in which the variation may be expressed is by plotting the concentrations in Table 1 against $1/r_d$, where r_d is the mean radius of the diamonds in a given sample. The graph obtained is linear (Fig. 2) and suggests that over the range investigated the number of paramagnetic nitrogen centres per cm^{-3} at a radius r cm within an individual diamond can be represented by the equation:

$$N_{cc} = \frac{K_1}{r} + K_2 \qquad K_1 = 1{\cdot}2 \times 10^{17} \qquad K_2 = 1{\cdot}8 \times 10^{18}$$

A similar relationship is obtained for 'MBS'- but not for 'RVG'-type diamonds. The latter inconsistency is believed to be caused by the irregular and elongated shape of 'RVG'-type diamonds for which the present experimental method is not suited.

Although the curve of Fig. 2 can equally well be fitted by an expression for N_{cc} involving exponentials, the foregoing expression is preferred since it provides at least as good an agreement with experiment, and is more convenient mathematically.

The observed spectra are made up of contributions from continuously varying concentrations of paramagnetic nitrogen, each element of which will have an associated line width. The surface values of N_{cc} are approximately equal to 0·7 N_{scc}. Using the foregoing expression for N_{cc} it is now possible to calculate the position within a diamond at which the paramagnetic nitrogen concentration has reached 10^{20} atoms cm^{-3}. The value obtained is 11·7μ. Exchange interaction and possible pairing of spins should be observed in this region.

The reduction of width of the central resonance (ΔH in Fig. 1) may be explained if it is assumed that an exchange narrowed resonance is obtained from nitrogen in the inner regions of a diamond at a radius less than 11·7μ. There are approximately 10^{12} paramagnetic centres per cm^3 within the 11·7μ radius sphere, and the mean concentration is 1·5 × 10^{20} cm^{-3}. The expression for N_{cc} used to fit the experimental results cannot be assumed to hold below 11·7μ but it is assumed to be valid down to this value. The fourth column in Table 1 lists calculated values of the ratio of the number of paramagnetic nitrogen centres contained within the inner sphere of radius

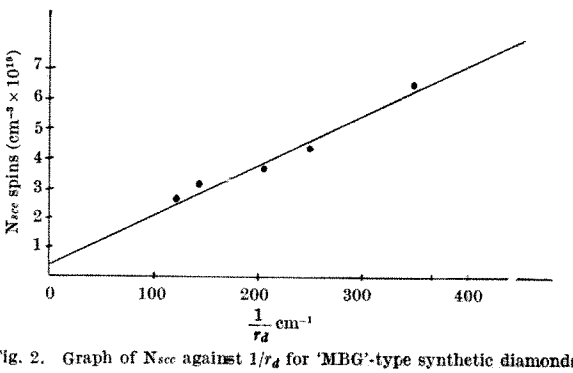


Fig. 2. Graph of N_{scc} against $1/r_d$ for 'MBG'-type synthetic diamonds

11·7μ to the number of such centres contained within the shell defined by the inner sphere and the surface:

$$N_d\Big|_{11\cdot7\mu}^{r_d} = 4\pi\int_{11\cdot7\mu}^{r_d} N_{cc}r^2dr$$

and:

$$N_d\Big|_{0}^{11\cdot7\mu} = \frac{4\pi}{3}\, r_d^3 N_{scc} - N_d\Big|_{11\cdot7\mu}^{r_d}$$

$N_d\Big|_{0}^{11\cdot7\mu}$ is constant for all samples and is of the order 10^{12}. These ratios indicate, as observed, that the contribution of an exchange narrowed signal to the total resonance is small for diamonds of $r_d = 81\cdot5\mu$ but has become important for $r_d = 29\cdot0\mu$.

For the concentrations present, the line-widths are too large to be accounted for by dipolar interactions between nitrogen centres distributed randomly throughout the lattice, using the relationship $\Delta H \sim N_{cc} \times 10^{-19}$ oersteds[7]. Ferromagnetic inclusions appear to offer a proven explanation[4].

Random orientation of a large number of diamonds will produce an asymmetrical broadening of the satellite resonances but not of the central peak. The effect, although present, is not very marked in these experiments owing to the broadening produced by the ferromagnetic inclusions which affects all three lines.

Further experiments are in progress to reduce the size of synthetic diamonds by etching and preliminary results are in agreement with the foregoing conclusions. It should be of interest to investigate small synthetic diamonds.

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FLOW PATTERNS IN A SUBMERGED LIQUID JET DIFFUSING UNDER GRAVITY

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WHEN a stream passes under a submerged sluice gate into water on the downstream side, it forms a jet which goes far downstream before it is dispersed. The physical aspects of the dispersion of such a jet have some similarity, both qualitatively and quantitatively, with the simpler problem of the diffusion of fluid jets[1]. However, the flow of an air jet discharging from a slit into the atmosphere is not affected by adjacent boundary restrictions. A sluice-way jet, on the other hand, disperses into restricted surrounding fluid and has several fundamental properties which distinguish it from a free air jet. Apart from the contraction effect due to the sharp edge of the gate, the differences are as follows.

The jet issues into a slow-moving medium and is bounded on one side by a solid boundary and on the other side by a free-water surface. The mixing process between the jet and the surrounding fluid causes part of the latter to be carried forward with the jet under conditions in which both the forward momentum and discharge are conserved. This entrainment leads to recirculation to replace the fluid entrained; thus a zone of circulation is established on top of the jet which is usually termed a 'roller'. Meanwhile, the mixing process causes the jet to expand laterally until its upper mean flow line reaches the free surface. This point is the stagnation point which defines the downstream end of the circulation zone.

In comparison with the free jet, the chief difference lies in the presence of the circulation zone. There is also the difference that in the sluice jet the upper stream or limit of the flow is a surface which is free from shear stress and slopes upward in the downstream direction, indicating that the diffusion process takes place under a pressure gradient, often of considerable magnitude.

The rise of pressure in the direction of flow is associated with the conversion of some of the kinetic energy of the high-velocity jet into pressure. The resulting difference of pressure influences the discharge through the sluice-way and also influences the nature of the flow patterns downstream as indicated in Figs. 1 and 2. The former concerns variation in downstream depth with the Froude number of the flow $F_1 = q/\sqrt{gd_1^3}$, where q is the unit discharge and d_1 is the contracted depth of the jet. The measurements were taken in a 4-in. wide glass flume having an outlet weir for adjusting the downstream water level, the upstream depth being kept constant at 7·5 in. The latter shows a number of photographs, exposure 0·2 sec, selected to describe the kind of patterns which occur under the particular conditions marked *a–e* in Fig. 1. The photographs were taken with a 'Zeiss Ikon' camera of picture size 9 cm × 12 cm, with the flow illuminated by a mercury–vapour discharge lamp flashing at 100 times a sec. The tracers were droplets of a new white emulsion discovered experimentally by the author by mixing a mixture of olive oil and nitrobenzine, prepared to the same density of water, with a suitable percentage of water. Some idea of the scale of the photographs may be obtained by noticing the sluice opening which is 2 in. deep.

At a low rate of flow, the jet flows under the gate and apparently a sharp interface exists between the jet and

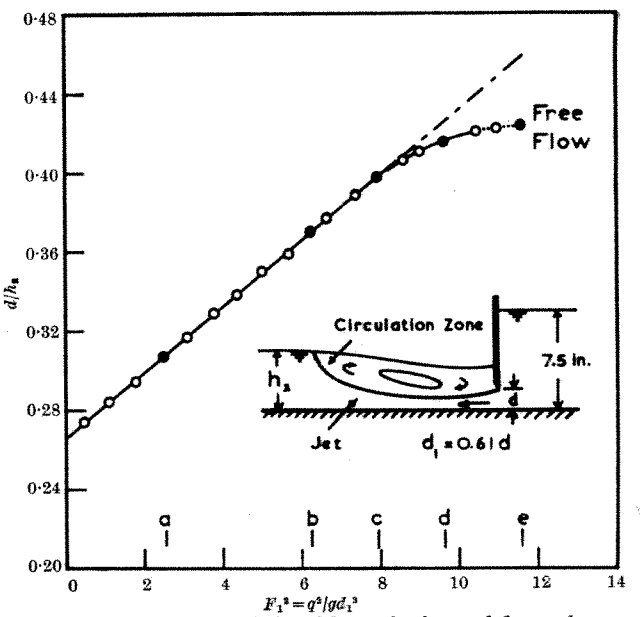


Fig. 1. Discharge characteristics of free and submerged flow under a sluice gate

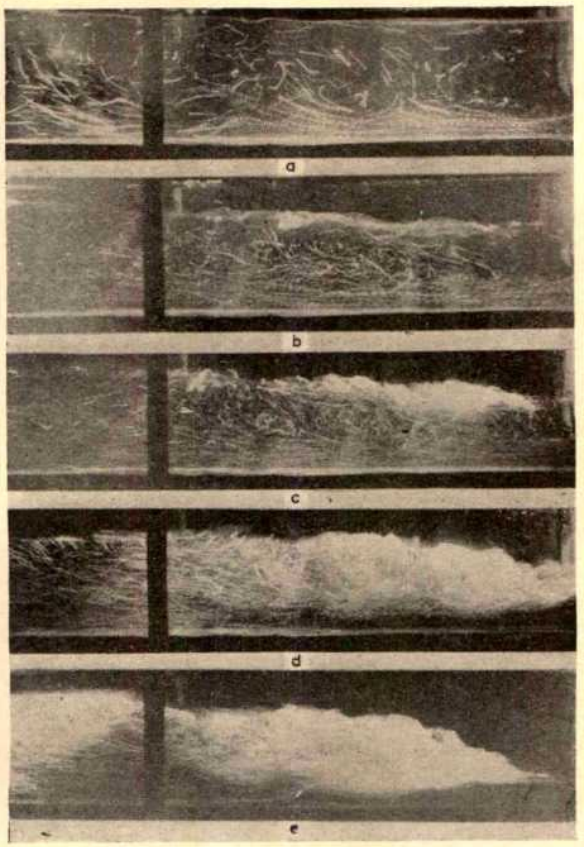


Fig. 2. Photographs of the flow patterns in the sluice-way jet for different discharges

the water above it (Fig. 2*a*). This interface persists for a distance of several sluice openings but becomes contorted with billows and hollows as a result of the development of large-scale eddies between the jet and the overlying fluid. When the rate of flow is increased by lowering the downstream level, the jet boundary breaks up into large vortices at a point nearer the gate (Fig. 2*b*). In this photograph, the diverging character of the jet is more apparent and the length of the circulation is shorter. These are typical changes though the photographs are momentary. At the next higher rate of the flow (Fig. 2*c*) the jet continues to behave in the same characteristic manner, though the large eddies now extend up to the free surface, with the result that a new surface effect comes into play: air is entrained at the occasional discontinuities produced by the strong vortex motions. Further, it is at this point that the straight line law of the discharge curve (Fig. 1) terminates and transition begins and continues until the jet discharges freely into the air.

In the transition zone, a further increase in the rate of flow results in the formation of a train of vortices, as shown in Fig. 2*d*. The vortices appear practically at the gate and they are packed with air bubbles, which swell their volumes. With distance, the vortices appear to increase in bulk and to decrease in translational velocity, until at the end of the circulation zone they appear to lose distinction and are merged in the general turbulence. A region of violent turbulence thus extends throughout the flow the structural features of which, however, remain similar to those shown in Fig. 2*a*, and resemble those of a wake flow behind a two-dimensional bluff body.

With decreasing downstream level, the depth at the gate becomes less and less until at a certain discharge the jet carries the water forward and the jet issues free from the gate. Diffusion then occurs further downstream under considerable pressure gradient, involving the phenomena called traditionally the 'hydraulic jump' (Fig. 2*e*). The dotted part of the discharge curve, Fig. 1, represents flows where the front of the jump is somewhere between the gate and the vena contracta section, about two gate openings downstream. It is interesting to note that the slope of the water surface, forming the upper boundary of the jump circulation zone, is about 1 in 4; this is practically the same as the rate of spreading of the outer boundary of a plane air jet.

Recently[2], I have investigated the diffusion of the jet under submerged conditions using a photographic technique. Owing to the entrainment of air bubbles, the flow under transition conditions could not be included. For the same reason, Rouse, Siao and Nagaratnam[3] investigated the turbulence characteristics of the hydraulic jump by stimulating the flow pattern in an air duct.

I thank Prof. C. M. White, Imperial College of Science and Technology, London, for advice.

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INVESTIGATION OF RIBONUCLEASE ISOZYMES BY AN ELECTROPHORETIC ULTRA-VIOLET METHOD

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CHROMATOGRAPHIC and immunoelectrophoretic investigations have demonstrated that a given tissue may contain a number of different molecular species of ribonuclease[1]. Immunoelectrophoretic analysis characterizes individual isozymes on the basis of mobility, but requires the preparation of antiserum, and does not provide direct visualization of the individual isozyme bands. Poort and van Venroy have referred to a method for detecting nucleases after electrophoresis[2]. It involves incubating the electropherogram while in contact with an agar slide containing dissolved substrate. The substrate slide is then placed in an acid solution, which precipitates the unreacted ribonucleic acid (RNA), but leaves the areas of nuclease activity as transparent spots. This procedure has the disadvantages that it may provide only a fleeting view of isozymes in low concentrations, and various proteins diffuse into the substrate layer. This also produces clear areas, which may be difficult to distinguish from areas of enzyme activity. However, the method has been useful when carried out with that described here.

Markham and Smith previously published a dialysis method for separating products from the reaction of RNA with ribonuclease[3]. The nucleotides produced, of smaller molecular size than the unreacted RNA, diffused through the dialysis membrane and were collected in the dialysate.

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It therefore seemed possible that, by permitting the separated isozymes to react with RNA while in contact with dialysis membrane, the reaction products would selectively diffuse through the membrane and could be detected in an agar layer on the opposite side.

Human tissues were obtained at autopsy, and were homogenized in 2 ml. of distilled water per gm. Leucocytes were prepared by a modification of the method of Christlieb *et al.*[4]. Human serum or tissue homogenates were subjected to electrophoresis essentially as previously described[5]. Agarose was added to the buffer to a concentration of 0·4 per cent. It was dissolved by heating to a boil. 100 ml. of this solution were poured over a 6 × 10 in. glass plate, which had been bordered by gluing plastic strips along the edges. The plate was connected by electrode vessels, containing the same buffer as the agarose layer, by means of thick filter paper strips (Schleicher and Schuell No. 900). After the agarose layer cooled, a separate strip of Whatman No. 17 filter paper about 2 × 10 mm was wet with each sample to be applied, lightly blotted, and then inserted in the agarose layer. The plates were covered to prevent evaporation and current was applied overnight (16 h).

At the end of this period, 40 ml. of a 0·1 M phosphate buffer, *p*H 7·25, in which 1 per cent Difco 'Noble' agar had been dissolved, was poured over a 6-in. square quartz plate (also bordered at the edges). After the agar hardened cellulose dialysis tubing (Viscose process, 0·0016 in. thick) was cut along one side, spread open as a single sheet, washed in water, and placed over the agar layer. Air bubbles between the agar and dialysis membrane were avoided.

A solution containing 1 per cent yeast ribonucleic acid (Nutritional Biochemicals Corporation), 0·1 per cent agar, and 0·1 M sodium phosphate, at a *p*H of 7·25, was added and uniformly spread over the portion of the agarose layer in which the samples had been electrophoresed. The agarose, coated with substrate, was then inverted and placed on the dialysis membrane upside down. This was done by supporting the plate containing the agarose layer, upside down, about 0·5 in. above the dialysis membrane, and sliding a fine wire between the agarose layer and the glass plate until the agarose dropped on the dialysis membrane.

Fig. 1 presents a diagrammatic representation of the arrangement. The entire preparation was covered to prevent evaporation, and incubated at 37° C for 3 h. The incubation provided the opportunity for products from the reaction of RNA with ribonuclease to diffuse down through the dialysis membrane into the underlying agar layer. After the incubation, the dialysis membrane and agarose layer were removed and discarded. The agar, layered over the quartz plate, was then photographed at a wave-length of 260 mμ using monochromatic light and 'commercial' Kodak film placed below the plate.

To detect acid instead of alkaline ribonuclease, the *p*H's of the ribonucleic acid solution, and of the agar layer over the quartz plate, were adjusted to 5·2. To facilitate bringing the agarose layer to this *p*H, it was first covered with a 0·1 M acetate buffer, *p*H 4·6. The ribonucleic acid solution at a *p*H of 5·2 was then spread over the agarose.

Ribonuclease isozymes were also visualized by the procedure referred to by Poort and van Venroy[2]. 60 ml. of a warm solution containing 1 per cent agar dissolved in a 0·1 M phosphate buffer was added to and mixed with 32 ml. of a 1·8 per cent solution of ribonucleic acid in a 0·1 M phosphate buffer. The *p*H's of the aforementioned solution had been adjusted to 5·2 or 7·25 for the acid or alkaline ribonucleases, respectively. The mixture was poured on to a bordered 6 × 10 in. glass plate. After the gel had hardened, the agarose layer containing the samples separated by electrophoresis was inverted and placed on the gel, as already described. The plate was sealed, and kept 16 h at room temperature. The agarose layer was then removed and discarded. The plate was inserted in a solution of 0·5 N hydrochloric acid. The unreacted ribonucleic acid formed a white precipitate in the hydrochloric acid. Clear areas where the precipitate did not form indicated the presence of ribonuclease isozymes.

Total ribonuclease-levels were determined by the method described by Levy and Rottino[6]. The *p*H of the ribonucleic acid solution was adjusted to 7·25 after the nucleic acid had been added rather than before. (This was also done in the preparation of the solutions described here.) For total levels of acid ribonuclease, the *p*H was adjusted to 5·2. All determinations were undertaken in duplicate. Total ribonuclease-levels were also determined for sections of agarose, along the migration path. A section of the agarose was added to each tube, which was diluted to 1 ml. with the buffer used for electrophoresis. The substrate was then added, and the procedure was as before. Blanks and standards were also prepared in the same manner (except for the omission of agarose, which, as control experiments demonstrated, did not affect the results).

After electrophoretic separation, a sample of human serum was analysed for ribonuclease distribution by the ultra-violet absorption procedure described previously, by the method of Poort and van Venroy, and by measuring total ribonuclease-levels of agarose sections along the migration path. The results obtained by the three procedures are consistent, and are presented in Fig. 2. The electrophoresis was conducted in a buffer containing 0·05 M/l. of glycine and 0·03 M/l. of lithium chloride. It had been adjusted to a *p*H of 8·7 with sodium hydroxide. 55 V were applied. The densitometer curve of the ultra-violet photograph was obtained by printing the negative on a transparent base, and measuring the optical density of this print along the migration path. The curve from the plate obtained by the procedure of Poort and van Venroy was obtained by inserting the ribonucleic acid gel strip in a densitometer, and measuring the relative percentage of transmission along the migration path. Although neither of these latter two curves is quantitative, both indicate the same general ribonuclease isozyme distribution as the optical density curve of total levels of individual sections; that is, a doublet on either side of the site of sample application (not clearly resolved in this separation, but seen more clearly below), and a less concentrated isozyme behind the γ-globulin. The electrophoretic curve of the doublet is not grossly different from that obtained by Rabinovitch and Dohl with ribonuclease purified from guinea-pig serum[7].

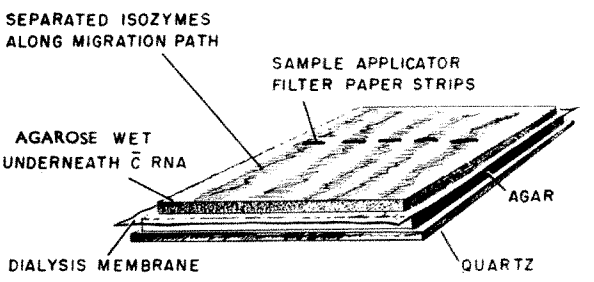


Fig. 1. Arrangement for the incubation of separated ribonuclease isozymes with RNA

Agar had originally been used, instead of agarose, for the electrophoretic separation of the samples. In this case, the principal isozymes had a variable mobility which ranged from that of an α-globulin to that of a pre-albumin. It was also observed that isozymes migrated less rapidly towards the anode when more concentrated. The difference in the results obtained by substituting agarose for agar suggests that the isozymes were interacting with an anionic component of the agar, as has previously been observed with lactic dehydrogenase isozymes[8,9]. Ribonuclease

interactions, however, are apparently more extensive, since such interactions would not occur with lactic dehydrogenase under the already-described conditions.

Kidney homogenates were subjected to electrophoresis in the glycine buffer already used, in the same buffer diluted 1 to 2, and in a 0·1 M phosphate buffer of *p*H 7·25. Agarose was used on all occasions. The results are presented in Fig. 3. In the diluted glycine buffer, interactions are extensive, even with agarose, and all isozymes move as a smear towards the anode. In the more concentrated glycine buffer, these interactions are greatly reduced, although some of the isozymes still appear to be displaced by interactions towards the anode. In the phosphate buffer, interactions are no longer apparent. Isozyme mobility was found to be independent of its concentration with this buffer, but not with the glycine buffer.

Ribonuclease isozymes have also been detected after separation by starch-gel electrophoresis. The gel strip was incubated in contact with the dialysis membrane as done with the agarose strips. The bands obtained were not sharp and the starch gels did not appear to offer any advantage over agarose for this application.

Fig. 4 shows a human serum ribonuclease isozyme pattern. The most intense band (strip *a*) has a mobility approximately that of normal adult haemoglobin, while less-intense bands can be detected near the site of application, and behind the γ-globulin. The forward band is more intense at a *p*H of 7·25, while the band behind

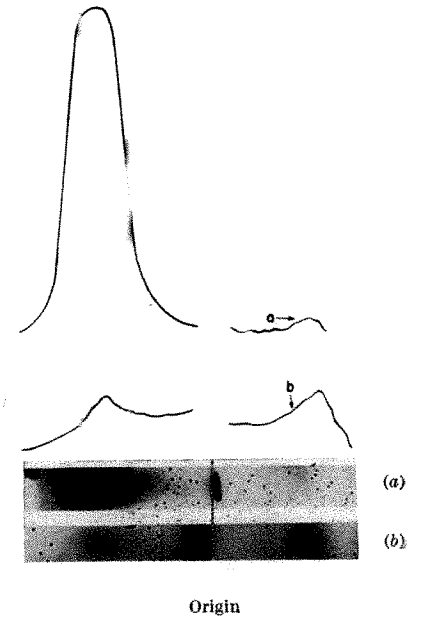


Fig. 4. Ribonuclease distribution in human serum, after incubation at a *p*H of 7·25 (*a*) and 5·2 (*b*). Electrophoresis had been conducted in the 0·05 M glycine buffer. Densitometer curves, depicted here, were obtained from prints of ultra-violet negatives

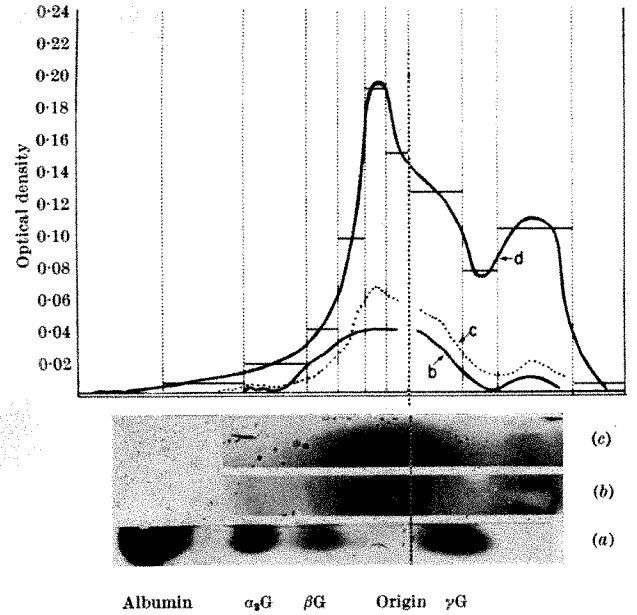


Fig. 2. Comparison of methods for detection of ribonuclease distribution in human serum. Bottom strip (*a*) is stained for total proteins. Strip (*b*) and curve (*b*) (relative percentage of transmission) were obtained by the method of Poort and van Venroy. Strip (*c*) and curve (*c*) (relative optical density) were obtained by the ultra-violet method. Curve (*d*) (optical density, scale on the left) represents determinations undertaken on sections along the migration path, cut as indicated by the horizontal lines. Migration towards the anode is to the left, throughout

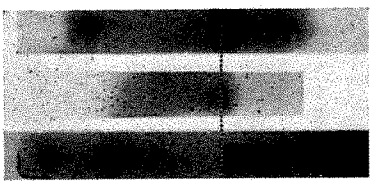


Fig. 3. Ribonuclease distribution in human kidney homogenates, (ultra-violet method), after electrophoretic separation in a 0·05 M glycine buffer *p*H 8·7 (*a*), in a 0·025 M glycine buffer *p*H 8·7 (*b*), and in a 0·1 M phosphate buffer (*c*), *p*H 7·25. (Speckles were caused by air bubbles)

γ-globulin is relatively more intense at a *p*H of 5·2. Various sera have been investigated, but no isozymes in addition to those shown previously have been detected.

The type of sera used, and their total ribonuclease-levels, are presented in Table 1. The values obtained with normal sera are similar to those reported by Levy and Rottino[6] and by Herriott *et al.*[10]. So far as we can trace, acid ribonuclease has not been detected in human serum previously. Its concentration in normal serum is so low that it might not exhibit a discernible peak when the *p*H is plotted against activity.

Table 1. RIBONUCLEASE-LEVELS IN HUMAN SERUM

Patient No.	Alkaline ribonuclease (μg per ml.)	Acid ribonuclease (μg per ml.)	Alkaline–acid ratio	Diagnosis
1	0·21	0·05	4·3	Apparently normal
2	0·25	0·04	6·1	Apparently normal
3	0·22	0·05	4·4	Apparently normal
4	0·18	0·03	5·9	Apparently normal
5	0·22	0·04	5·5	Apparently normal
6	0·255	0·05	4·7	Pernicious anaemia
7	0·37	0·08	4·6	Macrocytic anaemia
8	0·225	0·04	5·3	Atherosclerotic heart disease
9	0·29	0·08	3·7	Laennec's cirrhosis
10	0·54	0·25	2·15	Disseminated cancer of the liver, haemolytic anaemia
11	0·31	0·14	2·2	Chronic granulocytic leukaemia
12	0·37	0·17	2·1	Chronic myelogenous leukaemia, anaemia
13	0·45	0·14	3·2	Disseminated cancer of the colon

Ribonuclease patterns found with solutions obtained from cells of erythrocytes and leucocytes are presented in Fig. 5. A preliminary comparison was made of the leucocytes from two patients with leukaemia to the leucocytes from two apparently normal individuals. No obvious difference was found except for the possibility that the isozyme most rapidly migrating towards the anode was relatively more intense in the leucocytes from the two leukaemic patients than in the leucocytes from the normals. This isozyme would seem to be comparatively

Table 2. RIBONUCLEASE-LEVELS IN ERYTHROCYTES

Patient No. (corresponding Nos. refer to the same patients as in Table 1)	Alkaline ribonuclease (μg per g of haemoglobin)	Alkaline ribonuclease (μg per ml. of whole blood)	Acid ribonuclease (μg per g of haemoglobin)	Acid ribonuclease (μg per ml. of whole blood)	Alkaline–acid ratio
1	0·43	0·069	0·455	0·073	0·94
2 Normal	0·71	0·114	0·81	0·130	0·90
4 Controls	0·20	0·032	0·09	0·014	2·10
5	0·20	0·032	0·11	0·018	1·93
6	7·40	0·511	7·60	0·524	0·98
7	4·35	0·374	3·89	0·335	1·10
8	1·20	0·156	0·99	0·129	1·2
9	0·66	0·071	0·85	0·092	0·78
10	3·55	0·241	3·91	0·266	0·91
11	0·99	0·099	1·50	0·150	0·67
12	1·25	0·097	1·52	0·119	0·82

unstable, and its intensity decreases during storage at 4° C at a greater rate than the other isozymes.

Some examples of total ribonuclease-levels of haemolysates of erythrocytes are shown in Table 2. The concentrations of ribonuclease in normal erythrocytes were relatively low. In certain cases of anaemia, however, the concentrations were considerably elevated.

Ribonuclease isozyme patterns of some normal human tissue homogenates are presented in Fig. 6. The kidney and liver, in particular, have isozymes with similar mobilities. In this connexion, the same five lactic dehydrogenase isozymes are believed to occur, in different relative concentrations, in the various tissues of the body[11], and it is possible that the same ribonuclease isozymes may also occur in more than one tissue.

Houck and Berman have suggested that the ribonuclease in serum is derived from leucocytes[12]. In some instances (such as cases of congestive heart failure), they did not find any apparent correlation between the white blood cell count and the serum ribonuclease-level. There also did not seem to be any correlation between the serum ribonuclease-level and the white blood cell count in the cases presented in Table 1.

The mobility of the most concentrated ribonuclease isozyme in serum does not correspond to that of the most intense band of leucocytes, Fig. 5. The isozyme in serum which migrates behind the γ-globulin towards the cathode (an acid ribonuclease) does, however, correspond in mobility to the most intense band of the leucocytes. (Kidney, liver and erythrocytes also exhibit an isozyme with the same mobility.)

The isozyme distribution in erythrocytes is similar to that in leucocytes. The low level of alkaline ribonuclease in normal erythrocytes makes it unlikely that serum ribonuclease is derived from this source. The alkaline–acid ratios are different in serum than in erythrocytes, and a high erythrocyte acid ribonuclease value may occur with a low acid ribonuclease level in the serum (see, for example, patient No. 6 in Tables 1 and 2).

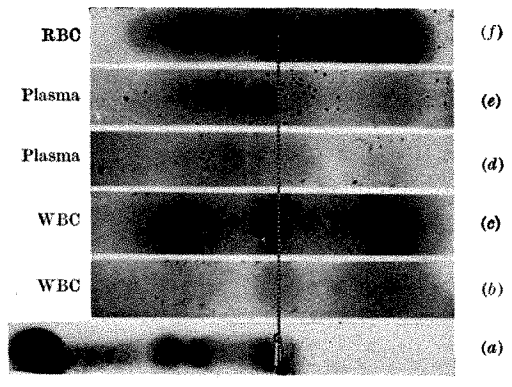


Fig. 5. Ribonuclease distribution in leucocytes and erythrocytes. The bottom strip (*a*) was stained for total proteins. Strips (*b*) and (*c*) were obtained from leucocytes, strips (*d*) and (*e*) from plasma, and strip (*f*) from erythrocytes. Strips (*b*), (*c*) and (*d*) were prepared by the method of Poort and van Venroy. Strips (*e*) and (*f*) were prepared by the ultraviolet method. All samples were from patient No. 12 in Table 1, except strip (*b*) which was from subject No. 4. The electrophoresis had been undertaken with the 0·1 M phosphate buffer, and the incubations had been conducted at a *p*H of 5·2

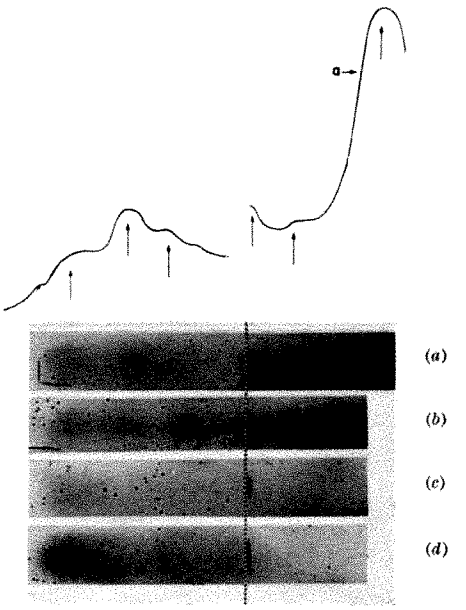


Fig. 6. Ribonuclease distribution in tissue homogenates. The homogenates were from kidney (*a*), liver (*b*), heart (*c*), and testes (*d*). The densitometer curve of the kidney homogenate was made from a print of the ultra-violet negative. Peaks are indicated by arrows. The electrophoresis was done with a 0·1 M phosphate buffer, and the incubations were conducted at a *p*H of 7·25

The procedure described here may also be applicable to the detection of isozymes of deoxyribonuclease, and we hope to investigate this possibility in the near future.

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CELLULAR CONTROL SYSTEMS AND RADIOSENSITIVITY

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RADIOBIOLOGICAL experiments and clinical experience suggest that cells do not necessarily respond to irradiation as individual autonomous units and that local interactions controlling proliferation of normal cells in tissue can be altered. Theory and experiment together reveal a strong association between temporal properties of cellular control systems and radiosensitivity.

It is generally accepted clinically that fractionation is beneficial and that the shape of the time-dose curve for most tissues is convex upwards. More precise experimental data on the skin response in pigs have confirmed this form of time-dose curve[1]. However, the usually accepted shapes for cell survival curves are not in accord with these observations. For example, the two survival curves in Fig. 1*a* can be considered to apply to populations of normal and tumour cells in solid tissue. Fractionation régimes can be applied to both populations which always result in the same survival of the tumour cells. The effect of such fractionation is shown in Fig. 1*b* to be only slightly beneficial, in that the increase in the survival of the normal cells is small. Also the time-dose curve for the normal population (Fig. 1*c*) is concave upwards. Agreement with observation is obtained, however, if the survival curves are assumed to have the shapes shown in Fig. 2*a*, where the curve for the normal cells has a further bend. If the same fractionation régimes are used, the curve in Fig. 2*b* shows that fractionation has been very beneficial and the time-dose curve (Fig. 2*c*) is convex upwards. The need for the extra bend to produce time-dose curves of this shape has been recognized for some time[2]. Hornsey and Vatistas[3] have shown with 4-day death in mice that the D_Q at the survival level used is greater than 400 r. This implies a curve with a lower bend since the damage at 200 r. is still reasonably large[4]. With fractionated doses the lower bend would be expected at a fixed survival rather than at a fixed total dose, if the mechanism causing it is subject to a repair process. Therefore the survival curve for normal cells in Fig. 2*a* is considered as representing only single dose results, and the curves of Fig. 2*b* and Fig. 2*c* have been derived by considering the bend to occur at a fixed survival.

There is evidence that this shape of survival curve arises largely from a change in the local interactions between cells, and that an absence of local interaction is associated with an absence of the bend. For example, Stoker[5] has shown that a confluent layer of irradiated polyoma-transformed cells can inhibit colony formation

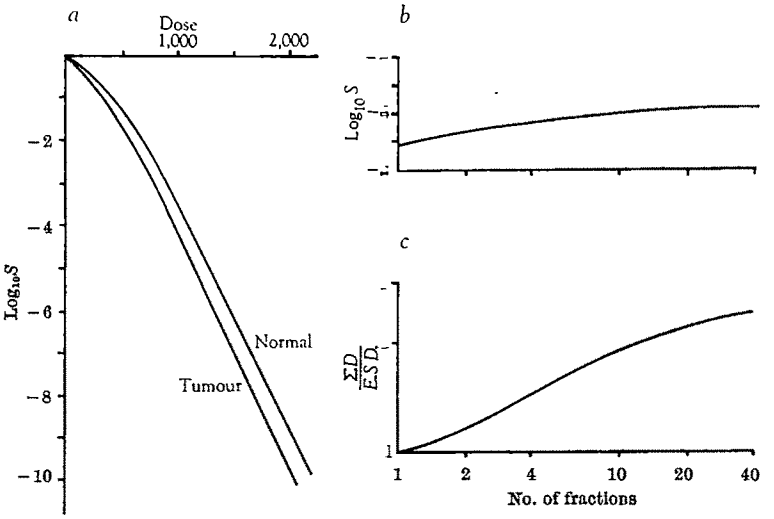


Fig. 1. (*a*) Assumed survival curves. (*b*) Effect of fractionation on survival of normal cells. (*c*) Time-dose curve for normal cells

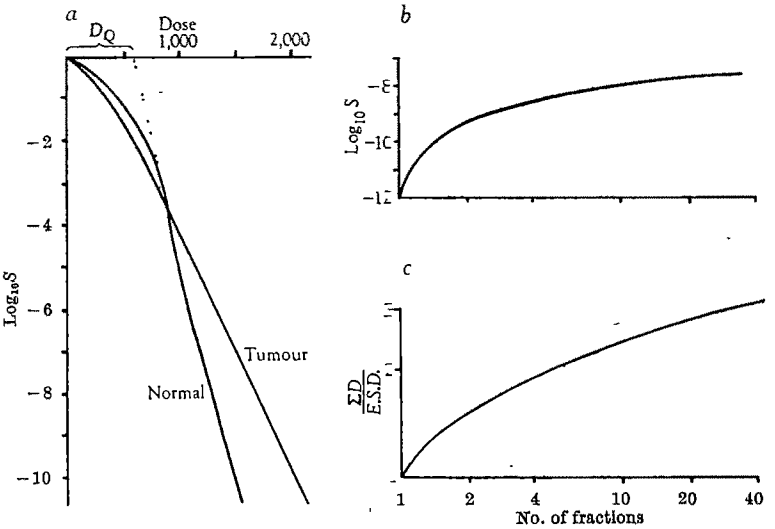


Fig. 2. (*a*) Assumed survival curves. (*b*) Effect of fractionation on survival of normal cells. (*c*) Time-dose curve for normal cells

by non-irradiated cells from the same clone. When the non-irradiated cells were separated from the irradiated layer by 0·6 mm of agar medium, the inhibition almost disappeared. A survival curve deduced from this behaviour has a lower bend. Furthermore, highly anaplastic cells do not show this inhibition after irradiation, and the lower bend is not found in survival curves obtained from solid tumours of such cells[6]. There is very little evidence on the nature of this effect on cellular interactions, although, as the effect is an inhibition of mitosis, it may be mediated by chalones of the normal intercellular control system[7].

A tendency to show periodic oscillations is a natural consequence of the interdependence of the parts of an organized biological system, and forms a cogent relationship between a number of aspects of cellular control. A theory of intracellular control has been developed mathematically by Goodwin[8], based on equations derived from recent evidence about the molecular mechanism of the control. This theory shows how oscillations can arise in cellular control systems.

A striking experiment by Harker[9] suggests the great importance of the phases of such oscillations. The experiment was performed on cockroaches, which have a well-defined neuro-secretory gland maintaining a strong autonomous periodicity in hormone secretion. When individuals received successive transplants of the glands of other individuals which had been living under similar conditions, there were no ill effects. When, however, the donors of the transplants had been living in an environment with a reversed day and night cycle, nearly all the recipients rapidly developed metastasizing transplantable tumours in organs remote from the site of implant. In this experiment malignancy has been caused by phase perturbation. Further, the relative lengths of the parts of the cell cycle are different in normal, hyperplastic and malignant cells from the same tissue[10], which again associates differences of cellular phase with important differences in cellular control. The control system defect manifested by neoplasms of progressive degrees of malignancy in ageing organisms may be a gradual slip in these relative phases.

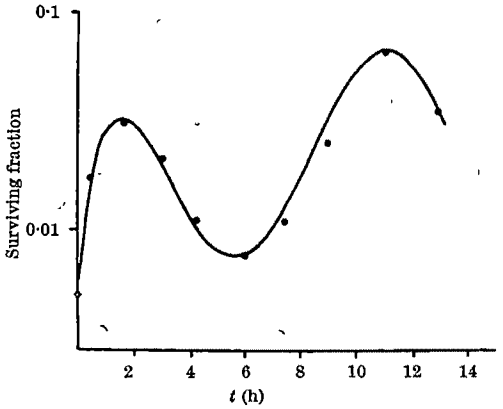


Fig. 3. Curve derived from the model for the variation in surviving fraction after two irradiations separated by t (h). The points are from the experimental results of Elkind *et al.* (ref. 13)

A growing volume of evidence supports the association of radiosensitivity with cellular control systems. In almost all systems investigated[1,3,11], an oscillation in radiosensitivity can be exhibited by suitable fractionation experiments on a short time-scale. Good agreement with experimental two-dose results can be obtained from a model in which the viability of the cell is linked to a substance represented by one of the variables in equations like Goodwin's, as suggested by Pohlit[12]. The assumptions are made that the amplitudes of the oscillations are fairly small compared with their mean value, and that the number of lethal injuries produced by irradiation is inversely proportional to the concentration of the substance. This last-mentioned assumption bears on the important, but rather neglected, problem of the mechanism of lethality. For the sake of simplicity, the equations are regarded as being linear with a solution of the form $[A \cos (wt + \psi) + B]$, where the capital letters in this and later expressions represent suitable constants. Most of the potential survivors of the first irradiation will be those in which the initial concentration was high, so ψ can be taken as zero. Therefore, the number of lethal injuries per cell produced by a first irradiation at time zero and a second at time t is proportional to $[C - D \cos wt]$. It has been shown that the initial rise in the radiosensitivity curve is due to recovery, which is a cellular process of a different type to the main oscillation[13,14], so C is taken to be $C\infty + e^{-at}(Co - C\infty)$. The number of lethal injuries per cell is therefore proportional to $[C\infty + e^{-at}(Co - C\infty) - D \cos wt]$, and as their distribution is a Poisson distribution, their number is proportional to minus the natural logarithm of the surviving fraction. Fig. 3 shows that when suitable values are inserted for the constants, the equation produces a curve which is a good fit to the experimental results of Elkind, Whitmore and Alescio[13]. The second dip in the radiosensitivity curve is recorded in published experimental results, despite the implication from the curves commonly drawn that it should not exist.

The association between control and radiosensitivity can be demonstrated in site and in biochemistry as well as in behaviour. The cytoplasm of the algae *Acetabularia* contains a sufficiently autonomous control system to maintain a rhythmic activity without a nucleus[15], and is also the site of most of the radiosensitivity of the organism. A low concentration of actinomycin D has been found to inhibit luminescence rhythms[16] and is known to reduce radio-resistance and inhibit recovery from irradiation[13]. Since actinomycin is believed to act specifically by the inhibition of *m*RNA synthesis, these results implicate *m*RNA in both rhythmic control and radiosensitivity. Irradiation also causes a phase shift in cells, indicated by a lengthening of the synthetic period[17]. Harker[18] has found, on a multicellular scale, that a stable phase shift of increasing magnitude is produced as the dose given is increased from 100 r. to 10,000 r. At less than 100 r. the phase did shift, but was unstable and soon reverted to its original position.

Intracellular and intercellular control systems must be closely linked, and therefore irradiation experiments which affect and indicate the phasing of the first should provide a tool for studying the second. Of theoretical interest is the possibility that this approach may provide a clue towards the formulation of fundamental temporal principles of cellular organization.

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DELAYED FLUORESCENCE OF CHLOROPHYLL *IN VITRO* AND *IN VIVO*

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WE have recently made measurements of the delayed fluorescence of chlorophyll, both as the pure substance in dilute solution and in the complex pigment system of living leaf cells. This article presents a summary of the results of the measurements in solution and compares them with measurements in leaves.

The spectra of prompt and delayed fluorescence of a deoxygenated 10^{-6} M solution of chlorophyll in ethanol are shown in Fig. 1 (curves 1 and 2). With high rates of light absorption at room temperature in this solvent, the intensity of delayed fluorescence was found to be approximately proportional to the square of the rate of absorption of exciting light, indicating that it was produced mainly by means of the process of triplet–triplet annihilation[1] (that is to say, it was mainly *P*-type delayed fluorescence[2]). At lower rates of light absorption there were indications that a small proportion of *E*-type delayed fluorescence[2] was also present. The lifetime of the delayed fluorescence at room temperature was 0·4 msec. For the *P*-type mechanism this would correspond to half the triplet lifetime (τ_t).

The energy of the chlorophyll triplet was determined by measurement of its delayed fluorescence in propylene glycol. The higher viscosity of this solvent reduces the rate of encounter between triplets, and, within the temperature range 20°–80° C, the intensity of *P*-type delayed fluorescence was small in comparison with that of the *E*-type delayed fluorescence. The latter is produced by thermal activation of molecules from the triplet level to the first excited singlet level[3]. From measurements of the intensity and lifetime of the delayed fluorescence as a function of temperature the activation energy was found to be 10·2 kcal per mole. The lifetime of the delayed fluorescence in propylene glycol at 21° C was 0·8 msec. For *E*-type delayed fluorescence, this should be equal to the lifetime of the triplet and it is equal to the triplet lifetime in ethanol calculated from the lifetime of *P*-type delayed fluorescence in this solvent. It agrees with the value in propylene glycol reported by Livingston and McCartin[4].

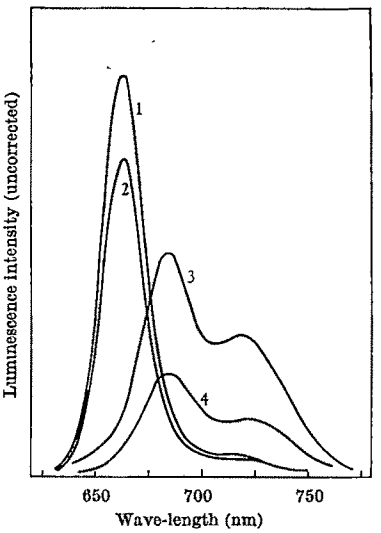


Fig. 1. Prompt and delayed fluorescence spectra. Curves 1 and 2: 10^{-6} M chlorophyll *a* in ethanol at 21° C with a rate of light absorption of 7×10^{-6} einstein $l.^{-1}$ sec^{-1} at 405 nm and band-width of 10 nm: intensity scale for delayed fluorescence (curve 2) is 2,100 times greater than that for prompt fluorescence (curve 1). Curves 3 and 4, Rhododendron leaf with incident intensity of 3×10^{-9} einstein cm^{-2} sec^{-1} at 436 nm, with band-width of 20 nm: intensity scale for delayed fluorescence (curve 4) is 500 times greater than that for prompt fluorescence (curve 3)

For the measurements of fluorescence *in vivo*, freshly plucked leaves of a variety of plants were used. Each leaf was mounted so that the beam of exciting light made an angle of about 60° with the upper surface of the leaf. The luminescence was observed from a direction at right angles to that of the beam of exciting light. Because the efficiency of prompt fluorescence from chloroplasts in living cells is small (estimated by various authors to be between 0·1 and 1 per cent[5]) the prompt fluorescence spectra had to be measured with a comparatively high instrumental sensitivity and precautions were required to prevent scattered exciting light from entering the monochromator used for measuring the emission spectra. The required wave-length of exciting light was isolated from a mercury lamp by means of a single monochromator. The beam was passed through a 2-cm depth of 10 per cent w/v $CuSO_4 \cdot 5H_2O$ in 0·1 N sulphuric acid to remove the small intensity of wave-lengths greater than 630 nm arising from scatter within the monochromator. The luminescence from the leaves was viewed through a 2-cm depth of 20 per cent w/v $Na_2Cr_2O_7 \cdot 2H_2O$ which removed all wave-lengths shorter than about 560 nm. Prompt fluorescence spectra could be measured at comparatively high resolution (3·3 nm band-width) and these spectra could be approximately corrected for the spectral sensitivity variations of the instrument[6]. To obtain sufficient light to measure the spectra of delayed fluorescence a band-width of 20 nm had to be used; because of the rapid variation of spectral sensitivity within the wave-length region of interest, correction of these spectra was not worth while.

The corrected prompt fluorescence spectra observed with a rhododendron leaf are compared in Fig. 2 with that of chlorophyll *a* in ethanolic solution. The spectrum of the leaf showed two bands at 1·46 and 1·35 μm^{-1} (685 and 741 nm) the relative intensity of which varied with the wave-length of excitation. The relative intensity of the 685-nm band was reduced considerably with excitation at 546 nm. This effect is attributed mainly to greater self-absorption caused by the greater depth of penetration of 546 nm as compared with 436 nm or 405 nm. The spectra at the latter wave-lengths are in general agreement with those reported for green leaves by other workers[5] and are probably due mainly to chlorophyll *a*. The relative intensities of the bands varied somewhat with different specimens and it is probable that the observed intensity of the 685-nm band is reduced by self-absorption even with 405- or 436-nm excitation. The prompt fluorescence spectra of the leaves from other plants showed the same two bands, although their relative intensities again varied (column 2 of Table 1).

Delayed fluorescence was observed with all types of leaf examined. Typical spectra (uncorrected and at low resolution) are shown in Fig. 1 (curves 3 and 4). Very similar results were obtained with the other types of leaf. The shapes of the delayed fluorescence spectra were always similar to those of the corresponding prompt fluorescence, whatever the wave-length used for excitation. There were pronounced transient effects, both in prompt and delayed fluorescence, on changing the level of illumination on the leaf. The spectra in Figs. 1 and 2 were obtained with steady illumination at the indicated

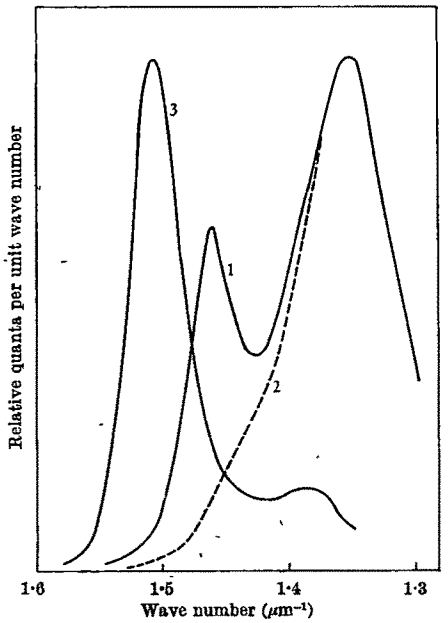


Fig. 2. Corrected prompt fluorescence spectra. Rhododendron leaf with excitation by: (1) 3×10^{-9} einstein cm^{-2} sec^{-1} at 436 nm; (2) $1{\cdot}4 \times 10^{-9}$ einstein cm^{-2} sec^{-1} at 546 nm; (3) 10^{-6} M chlorophyll *a* in ethanol. Band-width was 3·3 nm for (1), (2) and (3)

intensities. Variation in the intensity of the steady illumination produced comparatively little change in spectral shape, but this was not investigated in detail.

On switching on the illumination after a period of darkness, high initial levels of prompt and delayed fluorescence were recorded. These fell to steady values after a few minutes of irradiation. Typical results obtained with a 1-sec pen-speed recorder are shown in Fig. 3 (curves *C*–*F* full lines). The fall in intensity was greatest at the highest intensity of exciting light and the fall in the intensity of delayed fluorescence was greater than that of the prompt fluorescence. The ratio of the efficiencies of delayed to prompt fluorescence in various types of leaf after stabilization at one intensity of exciting light are given in the third column of Table 1. The values are remarkably similar and the delayed fluorescence thus seems to be a property characteristic of leaf chloroplasts in general. When the intensity of illumination was increased by a factor of ten, the ratio of the efficiencies of delayed and prompt fluorescence decreased by a factor of about two. This range of intensities of illumination overlaps that in which photosynthesis becomes saturated and the prompt fluorescence efficiency increases[5].

The period immediately after the start of illumination was investigated separately, using an oscilloscope to record the transient effects (Fig. 3, curves *A* and *B*). The intensity of delayed fluorescence (in normal air) always showed the maximum between 1 and 2 sec (curve *B*). It is obvious that both prompt and delayed fluorescence are affected by more than one process occurring in the illuminated leaves. One such process is no doubt the re-orientation of chloroplasts on illumination[5] (phototaxis), although this cannot be the sole cause since prompt and delayed fluorescence are not affected in the same way.

The rates of decay of the delayed fluorescence were observed with the use of pairs of rotating sectors, synchronously driven at a frequency appropriate to the life-

Table 1. EMISSION FROM VARIOUS TYPES OF GREEN LEAF

Plant	Ratio of corrected prompt fluorescence intensity at 685 nm and 741 nm	Ratio of delayed to prompt fluorescence intensity with steady illumination $\times 10^2$
Rhododendron	0·68	1·3
Silver birch	0·81	1·1
Rose bush	0·90	1·3
Lupin	0·72	1·6
Fern	0·77	1·5
Grass	0·82	1·5

Intensity of illumination was 3×10^{-9} einstein cm^{-2} sec^{-1} at 436 nm.

Table 2. RHODODENDRON LEAVES

Atmosphere	Approximate intensity of exciting light (einstein cm^{-2} sec^{-1})	Period of measurement (msec)	Lifetime of delayed fluorescence: Mean lifetime (msec)
Normal air	$\sim 10^{-7}$	0–1	2·4
			2·8
			2·9
			2·9
Normal air	$\sim 10^{-7}$	10–15	27
Normal air	$\sim 10^{-7}$	100–150	>100
Normal air	$\sim 10^{-8}$	0–1	3·6
Air + 40 per cent carbon dioxide	$\sim 10^{-8}$	0–1	5·9

time to be observed[6]. The decay was non-exponential (see Fig. 4). Some mean lifetimes calculated from the mean slopes of the log-plots over the indicated periods of time are shown in Table 2. The fastest rates of decay (taken over the period 0–1 msec) corresponded to lifetimes of 2–3 msec. Later, in the course of the luminescence decay, much greater lifetimes were observed although the intensities were very low.

Change in the water content of the surrounding atmosphere from about 50 per cent to 100 per cent relative humidity, or change in the oxygen content from 20 per cent to 100 per cent, produced only small changes in the intensities of prompt and delayed fluorescence. In comparison, the effects produced by changing the carbon dioxide content of the air were quite pronounced (Fig. 5). Small concentrations (0·03 per cent) appear to quench the

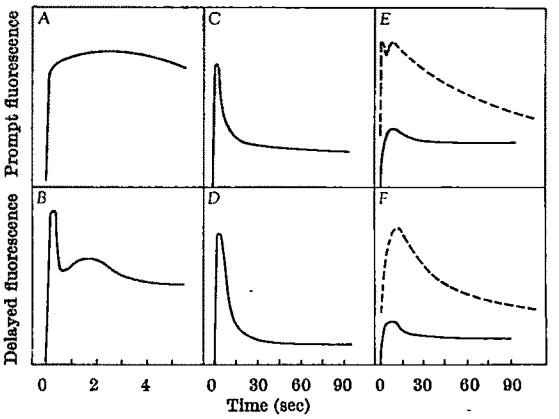


Fig. 3. Transient effects with rhododendron leaves after a period of darkness. Full lines refer to normal air, pecked lines to 40 per cent carbon dioxide. Intensities of illumination were: *A* and *B*, $\sim 10^{-7}$ einstein cm^{-2} sec^{-1} of 436 + 546 + 578 nm; *C* and *D*, 3×10^{-8} einstein cm^{-2} sec^{-1} of 405 nm; *E* and *F* full lines, 3×10^{-9} einstein cm^{-2} sec^{-1} of 405 nm; *E* and *F* dashed lines, 3×10^{-9} einstein cm^{-2} sec^{-1} of 436 nm. Intensity scales for different curves are not comparable

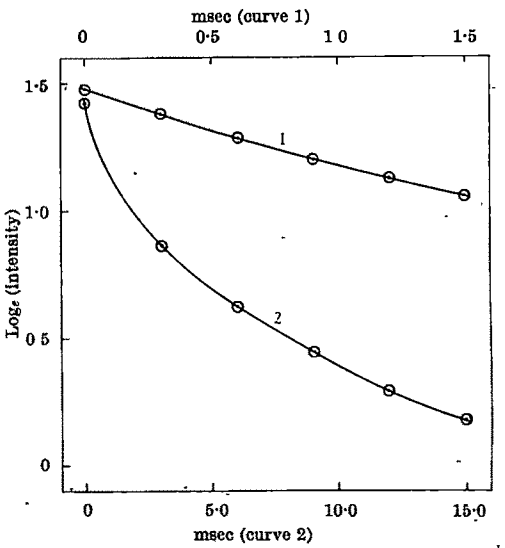


Fig. 4. Decay of delayed fluorescence of rhododendron leaves after switching off illumination. Curve (1) (upper time scale) with 100 c/s sectors. Curve 2 (lower time scale) with 25 c/s sectors

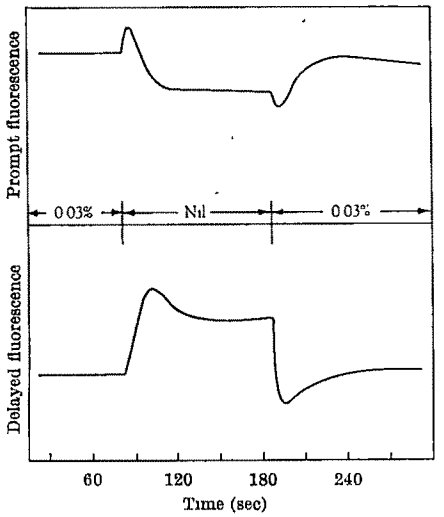


Fig. 5. Effect of low concentration of carbon dioxide. Air saturated with water vapour at 22° C and containing the indicated concentration of carbon dioxide was passed over the rhododendron leaf while it was illuminated with an intensity of 3×10^{-3} einstein cm^{-2} sec^{-1} of 436 nm

delayed fluorescence. The effects are related to, although not the same as, the changes in prompt fluorescence. The effect of high carbon dioxide concentrations is quite different. After a period of darkness in an atmosphere containing about 40 per cent of carbon dioxide, illumination produced an initial intensity of delayed fluorescence several times greater than that observed in normal air. The intensity then slowly fell (Fig. 3, dashed lines) and after some minutes attained a level similar to that in normal air. The initial increase in intensity was accompanied by an increase in the lifetime (Table 2).

It is clear that the delayed fluorescence of chloroplasts is affected in a variety of ways by the complex processes occurring in the living cell. It would be premature to attempt a discussion of the present limited observations in terms of present-day theories about the nature of the primary processes of photosynthesis, for example, their relationship to the two pigment systems described by Duysens[7]. However, some general observations about the possible origin of the delayed emission are called for. At least two kinds of mechanism have to be considered, namely: (1) *P*-type of *E*-type delayed fluorescence arising from chlorophyll triplets; (2) chemiluminescence arising either from recombination of the primary products of photo-reaction of chlorophyll itself, or from the interaction of chlorophyll with some energy-rich products of the photosynthetic reaction. We shall discuss only mechanisms of the first kind. Bertsch, who observed delayed fluorescence from *Chlorella*[8], attributed it to a back reaction following chlorophyll reduction, that is, to a mechanism of the second kind.

The fact that in fluid solution the chlorophyll triplet is strongly quenched by exceedingly low concentrations of molecular oxygen might at first sight suggest that the triplet lifetime in the living cell would be very small. However, the chlorophyll in chloroplasts is in a highly organized state and the situation may in some respects be analogous to that of concentrated solutions in rigid glasses where the degree of vibrational and rotational freedom is reduced and the triplets are shielded from collisional quenching by the high viscosity. Indeed, the non-exponential decay of the delayed fluorescence and its intensity relative to that of the prompt fluorescence are both reminiscent of the effects observed with phenanthrene in rigid media at low temperature[9]. These effects were explained in terms of triplet–triplet annihilation with the establishment of a non-random distribution of triplet molecules. In such a system the observed initial rate of decay of delayed fluorescence is considerably greater than the rate of decay of the mean triplet concentration. Applied to chloroplasts it would require the triplet lifetime of chlorophyll to be greater than 100 msec, that is, considerably greater than that observed in solution even at low temperature.

The following alternative involves the emission of *E*-type delayed fluorescence and does not require the chlorophyll triplet to have such a long lifetime. It is now generally accepted that the transfer of both singlet and triplet energy could readily occur within the chloroplast[10] so that the absorbed energy is ultimately transported to the chemically reactive centres in the photosynthetic unit. It might then be supposed that at these centres both kinds of energy are degraded to a triplet level having a much longer lifetime, and thus a better chance of encountering a substrate molecule. Reaction with substrate would result in quenching of these triplets and the non-exponential decay of delayed fluorescence could simply be a reflexion of the presence of a variety of reaction centres having different degrees of accessibility to the substrate. The reactive centre could be a molecule other than chlorophyll provided that its triplet level was sufficiently close to allow a small degree of re-population of the excited singlet levels of adjacent chlorophyll molecules by thermal activation. The observed quenched lifetimes would then correspond not to the triplet state of chlorophyll, but to the triplet state of the molecules at the reaction centres. With such a mechanism the delayed fluorescence would be directly related to a primary photosynthetic act, and would not, as Bertsch[8] suggested, be merely a result of the imperfect operation of the photosynthetic machinery.

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EFFECT OF DESASPIDIN ON PHOTOSYNTHETIC PHOSPHORYLATION AND RELATED PROCESSES

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THE uncoupling of oxidative phosphorylation by desaspidin and related phlorobutyrophenone derivatives was described in 1962 by Runeberg, who later showed that these substances also affect myosin ATPase[1] and the latent mitochondrial ATPase[2].

Baltscheffsky and de Kiewiet[3] investigated the activity of desaspidin in photophosphorylation by isolated chloroplasts. They found that the phosphorylations accompanying cyclic electron flow catalysed by two different redox agents, phenazine methosulphate (PMS) and

dichlorophenol-indophenol (DCPI), were as sensitive to desaspidin as was oxidative phosphorylation in mitochondria. The lack of net electron flux did not permit recognition of this inhibition specifically as an uncoupling effect, although this was inferred.

It is convenient to recall at this point that much of the electron transport pathway in photophosphorylation with artificial cofactors, or with the natural system reducing NADP from water, is unknown. Consequently the location and number of energy-coupling sites remain to be elucidated. A major step was taken in solving a comparable problem in the phosphorylating apparatus of bacterial chromatophores when Baltscheffsky *et al.*[4] found that the antibiotic valinomycin (an uncoupler of oxidative phosphorylation) partially inhibited synthesis in a 'physiological' electron flow system, but did not affect photosynthetic phosphorylation involving a cyclic electron flow sequence promoted by PMS. This led to the recognition of two coupling sites and their appropriate locations in *Rhodospirillum rubrum* photophosphorylation. Valinomycin proved to be rather ineffective with plant chloroplast systems, but the hope was maintained that some other selective inhibitor might be found which would also allow a definitive recognition of plural coupling sites in this case. Desaspidin appeared to be such a compound, for when Baltscheffsky tested the activity of low concentrations in non-cyclic electron flow systems involving NADP, FMN or ferricyanide reduction, the associated ATP synthesis was unaffected[3]. Hundred-fold higher concentrations were required to inhibit these systems compared with the PMS and DCPI cyclic processes mentioned earlier.

These findings were amplified in Arnon's laboratory[5,6] and led to the proposition that not only are there three coupling sites, but more notably that the transfer of an electron from water to NADP is driven by a single red light quantum. While studying the effects of desaspidin on photo-induced light-scattering changes in isolated spinach chloroplasts, the author observed lag phenomena suggesting photo-destruction of the inhibitor. This paper attempts to show that perhaps all the differential sensitivity exhibited by the various chloroplast systems to desaspidin may reflect nothing more than the relative stabilities of this compound under the various reaction conditions.

Uncoupling by desaspidin. Both Baltscheffsky and Arnon assert as evidence for uncoupling activity that desaspidin inhibits ATP synthesis but not electron flow during the photo-reduction of NADP using reduced DCPI as electron donor. Phosphorylation in this system is, however, thought to be cyclic, and not correlated with the NADP reduction rate[7–9].

The ability of almost all uncoupling treatments to increase the rate of decay of X_E (a possible non-phosphorylated intermediate of photophosphorylation[10,11]) can be used to test for uncoupling of a cyclic system, not otherwise possible. Table 1 shows that roughly 0·5 μM desaspidin is needed to half-inhibit X_E accumulation catalysed by pyocyanine in air. As the concentration is increased, the ATP yield and the X_E half-life decrease, strongly indicating an uncoupling effect of desaspidin.

Effect of light-scattering changes. Photo-dependent light-scattering changes have been classified into three general types[11]: (1) a low amplitude, dark-reversible increase in scattering (chloroplast shrinkage) observed at *p*H values close to 6·0; (2) a high amplitude, dark-reversible increase induced by illumination in the presence of chlorpromazine (CPZ), and almost independent of *p*H; and (3) an irreversible decrease in scattering associated with vacuolation and induced by illumination with amines or ammonia. Table 2 presents the effects of desaspidin on the foregoing processes, using pyocyanine as the catalytic cofactor. Its effect on chloroplasts illuminated alone at *p*H 8·0 is also studied (line 3) to check whether desaspidin itself can promote shrinkage or swelling. This proved not to be the case, and desaspidin inhibited in all four systems, resembling in this respect the carbonyl cyanide phenylhydrazone uncouplers (CCPs) (ref. 12). The greater resistance of the swelling at *p*H 8 to desaspidin is noteworthy: 1·0 μM inhibits ammonia swelling by 45 per cent, and *p*H 6·2 contraction by 86 per cent (without CPZ) or 94 per cent (with CPZ). Desaspidin, especially with CPZ, appeared to inhibit the 'on' response significantly more than could be attributed to its enhancement of the competing reversion rate. This behaviour simulates an inhibition of electron flow rate but, as will be shown, is not so caused.

Table 1. EFFECT OF DESASPIDIN ON X_E

	X_E decay, $t_{0.5}$ (sec)	ATP yield (mμmoles/mg chlorophyll)
Control	26	120
Control + DES, 0·5 μM	18	46
Control + DES, 1·0 μM	5	26
Control + DES, 2·0 μM	—	0

Illumination reaction mixture: *tris*-hydrochloric acid 0·01 M, sodium chloride 0·02 M, dimethylglutarate 2·5 mM, pyocyanine 25 μM, desaspidin (DES) as indicated, chlorophyll 250 μg/ml. Samples illuminated in red light for 2 min at *p*H 6·0 and 5° C in air. X_E trapped as ATP and assayed as previously described (ref. 10).

Table 2. EFFECT OF DESASPIDIN ON LIGHT-SCATTERING CHANGES WITH PYOCYANINE AS COFACTOR

	'On' response $t_{0.5}$ (sec)	'Off' response $t_{0.5}$ (sec)	Extent (*ΔO.D.*)
*p*H 6·2 contraction			
Control	14	71	+0·073
Control + DES, 0·5 μM	23	10	0·057
Control + DES, 1·0 μM	40	6	0·010
Control + DES, 2·0 μM	—	—	0·00
*p*H 6·2 + CPZ* 10 μM contraction			
Control	36	22	+0·226
Control + DES, 0·5 μM	360	18	0·095
Control + DES, 1·0 μM	—	—	0·014
*p*H 8·0 contraction			
Control	—	—	+0·007
Control + DES, 0·2 μM	—	—	−0·005
Control + DES, 2·0 μM	—	—	−0·002
*p*H 8·0 + ammonium chloride 6 mM swelling			(*ΔO.D.*/min)
Control	—	—	−0·038
Control + DES, 1·0 μM	—	—	0 021
Control + DES, 2·0 μM	—	—	0·007
Control + DES, 10·0 μM	—	—	0·002

Reaction conditions as for Table 1 but with chlorophyll 25 μg/ml. Sample absorbance measured with Cary 'Model 14' Spectrophotometer as previously described (ref. 11).
* CPZ; chlorpromazine.

Release from desaspidin inhibition. According to the postulates of Baltscheffsky and of Gromet-Elhanan and Arnon, it should be possible to restore scattering changes to the inhibited systems of Table 2 on addition of a cofactor of non-cyclic electron flow such as ferricyanide. Ferricyanide prevents cyclic phosphorylation[13] and would allow electron flow through the desaspidin-insensitive 'second' coupling site. Fig. 1 shows the inhibition of ammonia-induced swelling by desaspidin with pyocyanine as cofactor (curves 1 and 2). Adding ferricyanide gives rapid release from the inhibition and swelling rate becomes that of the control with ferricyanide plus pyocyanine without desaspidin (omitted for clarity from Fig. 1). Curve 3 demonstrates that the released system is still sensitive to CCP inhibition.

Table 3 summarizes similar curves showing the release by ferricyanide of the desaspidin-inhibited *p*H 6·2 low amplitude contraction. The inhibition falls from 95 per cent with pyocyanine alone to 25 per cent on adding ferricyanide. However, the 'on' response after adding ferricyanide was here much slower than the control and and was preceded by a long lag.

Table 3. LIGHT-SCATTERING CHANGES DRIVEN FROM TWO APPARENT SITES

Additive	'On' response $t_{0.5}$ (sec)	'Off' response $t_{0.5}$ (sec)	Extent (*ΔO.D.*)	Extent (%)
PYO, 25 μM	10	26	0·046	100
PYO, 25 μM + DES, 1·0 μM	—	—	0·002	4
PYO + ferricyanide, 1 mM	19	27	0·041	100
PYO + ferricyanide + DES, 1·0 μM	43*	26	0·030	75

Reaction conditions as for Table 1.
* Curve extrapolated to exclude initial lag period of about 20 sec.

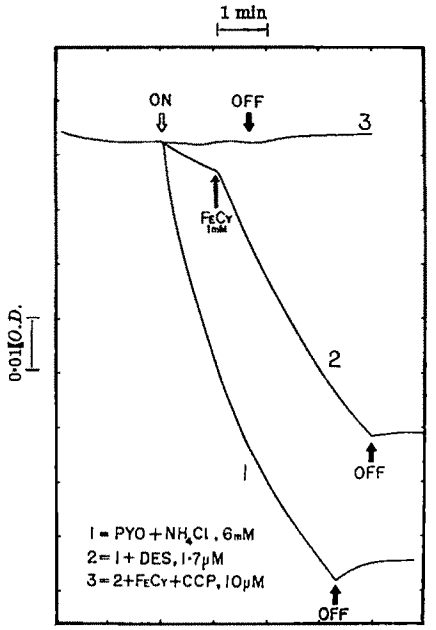


Fig. 1. Inhibition of chloroplast swelling by desaspidin. Ferricyanide added to cuvette at arrow (curve 2). Reaction conditions as for Table 2

Investigation of the lag period revealed several disturbing features, namely: (1) the lag was only present the first time a sample was illuminated—subsequent 'on' and 'off' sequences essentially resembled the control system with no desaspidin, both in the absence of lag and in their rapid 'on' response; (2) a lag was reintroduced by adding desaspidin to a sample already illuminated sufficiently to overcome the lag from an initial addition of the inhibitor; (3) the total duration of the lag was the same whether illumination was continuous or split into flashes of 5 sec separated by dark intervals of 2 min; and (4) the lag duration was roughly proportional to the desaspidin concentration at levels many times greater than needed to inhibit completely the pyocyanine-catalysed system. Table 4 illustrates some of these data.

Table 4. Concentration Dependence of Lag Duration

μM DES	0	0·7	1·5	3	7·5
Max. 'on' rate ΔA/min	0·20	0·12	0·10	0·08	0·05
Initial lag (sec)	3	11	18	23	56
Corrected lag	0	8	15	20	53
Corrected lag/μM DES	—	11·4	10·0	6·7	7·1

Reaction medium contained *tricine* 0·01 M, sodium chloride 0·02 M, dimethylglutarate 2·5 mM, potassium ferricyanide 1 mM, chlorophyll 20 μg/mL, and desaspidin as indicated above. All data refer to the first illumination period of each sample.

The evidence led to the suggestion that desaspidin might be removed or destroyed during the lag period. This latter was confirmed when it was shown that the lag period was decreased by pre-illuminating a sample with colloidal chlorophyll and ferricyanide before adding the chloroplasts (Table 5).

Table 5. Photosensitized Destruction of Desaspidin

μg/ml. colloidal chlorophyll	1·2	1·2	2·4
Minutes pre-illumination	0	10	10
Max. 'on' rate ΔA/min	0·029	0·025	0·016
Lag (sec)	35	18	6

Reaction mixture as for Table 4, with 3 μM desaspidin, except that chloroplasts were added only after the above pre-illumination in the presence of colloidal chlorophyll. Chlorophyll was extracted and adsorbed on sucrose by the method of Vernon (ref. 14) and added as an aqueous suspension. All data refer to the first illumination period. Both pre-illumination with chlorophyll and illumination with chloroplasts were conducted in red light at 5° C and *p*H 6·2.

In another type of experiment, chloroplasts and pyocyanine were illuminated without and then with added desaspidin (Table 6, columns 1 and 2). The inhibition of light scattering was then released by illumination with ferricyanide (Table 6, columns 3*a* and 3*b*). At this point addition of ascorbate sufficient to reduce all the ferricyanide did not restore the expected desaspidin-sensitive pyocyanine-catalysed system, but gave a released response (column 4) sensitive, none the less, to fresh addition of desaspidin. The addition of ascorbate (column 4) actually improved the maximal rate of scattering change (see column 3*b*). The control in column 5 shows that adding ferricyanide and excess ascorbate did not affect the scattering changes greatly (see column 1) and did not confer insensitivity to desaspidin (column 6).

Table 6. Inactivation of Desaspidin by Ferricyanide

Column System	1 Control	2 +DES	3*a* +Ferri-cyanide	3*b*	4 +Asc	5 +Ferri-cyanide +asc	6 +Ferri-cyanide +DES +asc
Extent, ΔA	0·068	0·023	0·054	0·060	0·053	0·058	0·018
Max. rate ΔA/min	0·312	0·007	0·038	0·108	0·219	0·348	0·006
Lag (sec)	0	1	42	1	0	0	7

Columns 1–4 represent successive treatments of the same sample. After adding ferricyanide, two successive scattering responses (3*a*, 3*b*) were studied. Reaction mixture contained *tricine* 0·01 M, sodium chloride 0·02 M, dimethylglutarate 2·5 mM, pyocyanine 25 μM, and where indicated: potassium ferricyanide 0·3 mM, sodium ascorbate (asc) 1 mM, and desaspidin 3 μM. Chlorophyll 20 μg/ml., *p*H 6·2.

The foregoing data show that the sensitivity of the pyocyanine-catalysed responses varies with the prior history of the desaspidin. We are observing, therefore, not the differential uncoupling of chloroplast reactions by desaspidin, but the differential destruction of desaspidin by chloroplast systems.

Effect of desaspidin on electron transport. Fig. 2 shows the variation in electron flow rate during the first illumination of a sample containing desaspidin and ferricyanide. The rate declines steadily to about 25 per cent of its initial value and remains at this lower level throughout subsequent illumination periods. Light-scattering measurements show that the lag during the first illumination period is roughly coincident with the high initial ferricyanide reduction rate, and correspondingly, the second 'on' and 'off' sequence with low initial electron flux is free of light-scattering lag. Both curves are interpreted as indicating that desaspidin, an uncoupler of the type inhibitory to light-scattering changes, is destroyed during illumination with ferricyanide.

Fig. 3 was obtained with the same chloroplast preparation. Here the electron transfer was fully uncoupled with

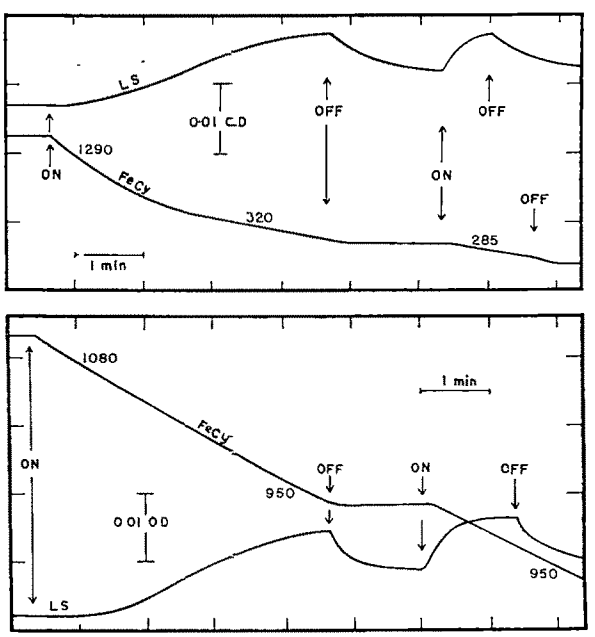


Fig. 2 (above) and Fig. 3 (below) Correlation of ferricyanide reduction at 420 mμ with light-scattering (LS) changes. Numbers refer to reduction rate in μmoles/mg chlorophyll/h. CPZ 90 μM, potassium ferricyanide 1 mM, *p*H 6·2

CPZ, the rate of 1,080 μmoles/h/mg being close to that of 1,290 μmoles seen at the start of the first illumination in Fig. 2. The rate did not decline during illumination in this case. Nonetheless, a lag in the 'on' response of the initial light-scattering curve was still present. These data show that the effect of desaspidin on turbidity changes is not specifically related to an effect on electron flux.

pH dependence of lag. It will be appreciated from Fig. 1 that the release of desaspidin-inhibited swelling by ferricyanide is virtually immediate. Yet we have seen in the foregoing section that both low and high amplitude contractions are released only after a substantial lag.

Since the swelling reaction is studied at *p*H 8 and the contractions at *p*H 6·2, the possibility arises that the *p*H condition determines whether a lag will be observed. Therefore, the CPZ-induced high amplitude contraction was studied at both these *p*H values with the uncoupler concentration adjusted for each *p*H to provide identical electron flow rates. The 'on' and 'off' turbidity responses were similar at high and low *p*H values. Adding desaspidin did not affect the electron flow rates, but did induce lag in the first contraction cycle at *p*H 6·2 (Table 7). There was no corresponding induction of lag at *p*H 8·0.

Table 7. *p*H DEPENDENCE OF LIGHT-SCATTERING 'ON' RESPONSE WITH FERRICYANIDE AS OXIDANT

*p*H	6·2	6·2	8 0	8 0
μM DES	0	5	0	5
Extent, ΔA	0·15	0 11	0·13	0·07
$t_{0.5}$ (sec)	43	80	30	56
Lag (sec)	0	30	0	2

Reaction conditions as for Table 4, with addition of CPZ 75 μM (*p*H 6·2) or 50 μM (*p*H 8 0). Electron flow rate 900 μmoles ferricyanide/mg chlorophyll/h.

Considering the phenolic nature of desaspidin and the data of Table 7 and Fig. 1, it seems reasonable to propose that at alkaline *p*H the destruction of desaspidin in an oxidizing medium is so rapid as to give an immeasurably brief lag period in light-scattering changes. One might further predict that in oxidizing media the time-course of photophosphorylation at *p*H 8·0 would be free of detectable lag. This proved to be the case.

Desaspidin activity with FMN as cofactor. Both the Stockholm and the Berkeley groups[3,5] have shown that non-cyclic photophosphorylation with FMN as cofactor is insensitive to desaspidin uncoupling. FMN is not in itself a strong oxidant akin to ferricyanide, and so the necessary conditions for making the FMN phosphorylation system patent to desaspidin are of special interest to our interpretation.

The first such condition is apparently an alkaline *p*H. Table 8 shows that light-scattering changes at *p*H 8·0 show an initial lag of 41 sec (see Table 7), but quickly recover and attain high maximum initial rates. At *p*H 6·2 the extrapolated lag is longer and the initial rate improves only slightly between illumination sequences. Table 8 also presents data for a system catalysed by PMS. In this case the desaspidin appears to be stable for there are no obvious lag periods. The maximum initial rates are close to the minimum initial rates seen with FMN: inhibition is much more severe.

Our interpretation of the foregoing data is that FMN-catalysed systems, perhaps by generating hydrogen peroxide, are capable of destroying desaspidin fairly rapidly at *p*H 8·0. However, the presence of a lag even at *p*H 8·0 suggests that in a phosphorylation run the time of assay will be critical in determining the percentage inhibition. Gromet-Elhanan and Arnon used 10-min

Table 8. *p*H DEPENDENCE OF LIGHT-SCATTERING 'ON' RESPONSE WITH FMN OR PMS AS COFACTOR

Cofactor	FMN				PMS			
*p*H	8 0	8 0	6·2	6 2	8·0	8·0	6·2	6 2
μM DES	0	1·3	0	1·3	0	1·3	0	1·3
Lag (sec)	0	41	0	63	0	0	0	0
Minimum rate	0·42	0 01	0·57	0·01	0 55	0 01	0·78	0 02
Maximum rate	0·42	0·22	0 57	0·05	0 55	0 01	0·78	0·02
Maximum extent	0·115	0·110	0 135	0·098	0 130	0 047	0·130	0·045

High amplitude contraction. CPZ 30 μM, FMN 8 μM, PMS 75 μM, chlorophyll 20 μg/ml. Initial rates are ΔA/min, and are given from two complete on/off sequences. Lag period found by extrapolation.

Table 9. TIME-COURSE OF PHOSPHORYLATION WITH FMN AND DESASPIDIN

Illumination (min)	0	0·25	1	3	10	20
Control*	0	6	28	76	199	358
Control + DES, 1·1 μM	0	0	0	13	111	251
Inhibition (per cent)	—	100	100	83	44	30

* mμmoles ATP yield. *Tricine* 0 01 M, sodium chloride 0·02 M, ADP 1 5 μmoles, magnesium chloride 5 μmoles, sodium phosphate-^{32}P 10 μmoles, FMN 17 μM. Volume 2·5 ml. Red light, 5° C, *p*H 8·0. Gas phase: air.

illumination periods and found no desaspidin sensitivity with FMN as cofactor. What is the sensitivity of the same system in brief time-courses?

Table 9 shows time-courses with and without desaspidin for phosphorylation catalysed by FMN. The percentage inhibition is 100 for the first minute and gradually falls to 30 per cent at 20 min. With slightly less uncoupler and longer incubations it has proved possible to pass from almost complete inhibition to full activity.

Before examining further the time-dependence of inhibition it is necessary to refer to Fig. 4. FMN-catalysed photophosphorylation conducted in evacuated Thunberg tubes is completely inhibited by 1·3 μM desaspidin and remains so during illumination for 10 min. Admitting air after 4 min allows phosphorylation to begin. It is not known whether the oxidant removed by evacuation is oxygen or hydrogen peroxide. Peroxide is formed by aerobic or anaerobic FMN reduction thus:

$$\text{FMN} + \text{H}_2\text{O} \xrightarrow{\text{chl.hv}} \text{FMNH}_2 + 1/2\,\text{O}_2$$
$$\text{FMNH}_2 + \text{O}_2 \longrightarrow \text{FMN} + \text{H}_2\text{O}_2$$

FMN reduction rates. Anaerobic FMN reduction rates can be measured by following the decline in FMN absorbance at 450 mμ. It is then found that enhanced reduction rates are detectable with as little as 0·09 μM desaspidin. Fig. 5 shows concentration curves for this effect of desaspidin on FMN reduction rates.

Fig. 5 also shows curves for the inhibition of photophosphorylation with FMN or PMS as cofactors, and with long and short illumination periods. In confirmation of Table 9 the FMN tubes assayed after 10 min of light showed roughly a ten-fold decrease in sensitivity to desaspidin. However, the 50 per cent uncoupling points as measured from (i) anaerobic FMN reduction rates, (ii) brief time-courses of FMN-catalysed photophosphorylation, and (iii) PMS-catalysed photophosphorylation are very similar, lying between 0·1 and 0·2 μM. Inhibition of electron flow by high desaspidin concentrations was confirmed[3].

Ferredoxin-catalysed reactions. Arnon *et al.*[6] present data for the effect of desaspidin on anaerobic ferredoxin-catalysed cyclic and non-cyclic photophosphorylation. The cyclic system, with CMU present to inhibit oxygen evolution, was half-uncoupled by about 0·2 μM desaspidin. In contrast, 3 μM desaspidin was required for the same

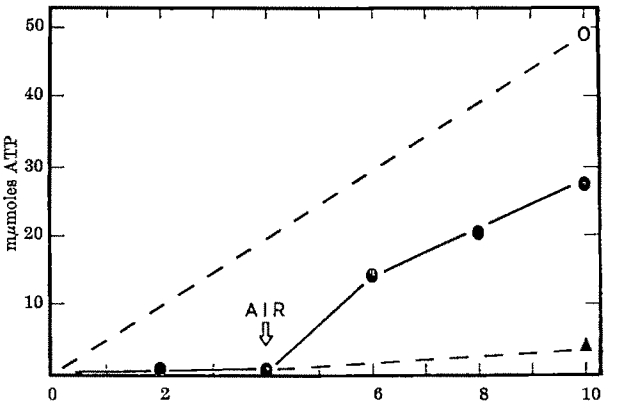


Fig. 4. Inhibition of FMN-catalysed anaerobic phosphorylation by 1 3 μM desaspidin. ○, Evacuated then shaken with air before illumination; ▲, anaerobic throughout; ●, aerated after 4 min anaerobic illumination. FMN 170 μM, other reaction conditions as for Table 9

effect on ATP synthesis accompanying the non-cyclic reduction of NADP in the absence of CMU.

The data for NADP reduction in the same paper suggest that the rate is scarcely affected by desaspidin below 5 μM. However, it is common to find such a result when electron flow is already enhanced by the coupling reagents[15].

Fig. 6 confirms that phosphorylation accompanying NADP reduction is less than 50 per cent inhibited by 1 μM desaspidin during a 10-min illumination period. Over a shorter time-course, however, 0·1 μM desaspidin gives half-inhibition. The situation is thus similar to that described for FMN (Fig. 5). A point of difference arises with the electron flow rates: FMN reduction rates measured anaerobically at *p*H 8 are enhanced by desaspidin, NADP reduction rates followed aerobically at *p*H 8 are not. Light-scattering studies have shown that during aerobic illumination at *p*H 8·0 with ferredoxin and NADP, high-amplitude contraction soon recovers from desaspidin inhibition, but does not do so at *p*H 6·2. Under the assumption that the NADP system destroys desaspidin rapidly at *p*H 8·0 but much less so at *p*H 6·2, the effect of desaspidin on NADP reduction at low *p*H was investigated. The result presented in Fig. 6 affirms that NADP reduction is 50 per cent enhanced by less than 0·2 μM desaspidin under conditions of desaspidin stability.

Evidence has been presented for the lability of desaspidin under alkaline conditions and in oxidizing media. It is noteworthy that even during storage in 60 per cent ethanol at 3° C, desaspidin gradually loses potency so that fresh solutions must be prepared every 7–14 days. The breakdown products appear to be inactive as uncouplers of photophosphorylation, but may act as inhibitors of electron flow. Their composition is being determined.

Photosynthetic reactions are studied in the presence of three broad categories of redox environment. The first category includes more or less oxidizing conditions due to the presence of an oxidant such as ferricyanide, manganese dioxide, or hydrogen peroxide. In long time-courses these media appear to support desaspidin-insensitive photophosphorylation as a result of photo-oxidation of the uncoupler.

The second class comprises reducing situations created by ascorbate or anaerobicity. In these media one finds the true susceptibility of photophosphorylation to desaspidin which gives 50 per cent uncoupling around 0·2 μM. Runeberg found that oxidative phosphorylation was not affected at this concentration and required 1·0 μM desaspidin for half-uncoupling[16].

In the third category lie cofactors having mid-point potentials close to zero which can support desaspidin-sensitive photophosphorylation, as with pyocyanine; or an apparently partially sensitive reaction as found with PMS. Gromet-Elhanan and Arnon found the PMS system to be 70 per cent inhibited in argon and 35 per cent inhibited in air, using 0·5 μM desaspidin (cf. Fig. 5). They could not explain this result since, even in air, PMS is well documented as catalysing a cyclic electron flow. Our interpretation is that, as shown for the FMN system, the destruction of desaspidin requires oxygen. It is probable that variability in the natural ascorbate level of spinach chloroplast suspensions may also affect the apparent susceptibility of PMS-catalysed phosphorylation.

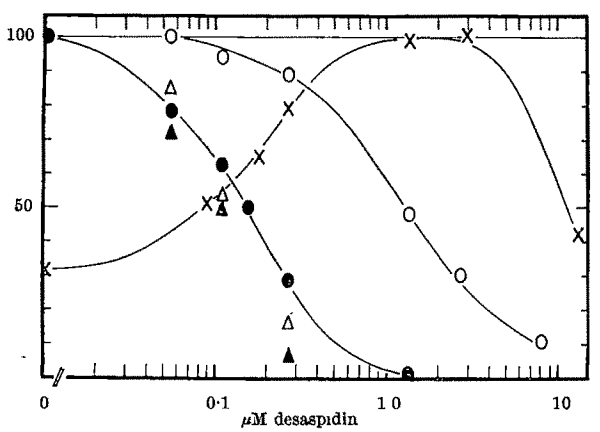


Fig. 5. Uncoupling of FMN-catalysed phosphorylation by desaspidin. ×——×, Anaerobic FMN reduction rate at *p*H 8·0 measured as decline in 450 mμ absorbance; ordinate, electron pairs transferred/mg chlorophyll/32 min. ●——●, aerobic phosphorylation with FMN, 30 sec illumination. ○——○, the same with 10 min illumination; ordinate, percentage of control rate. ▲——▲, △——△, aerobic PMS phosphorylation with 30 sec and 10 min illumination respectively, FMN 17 μM, PMS 25 μM

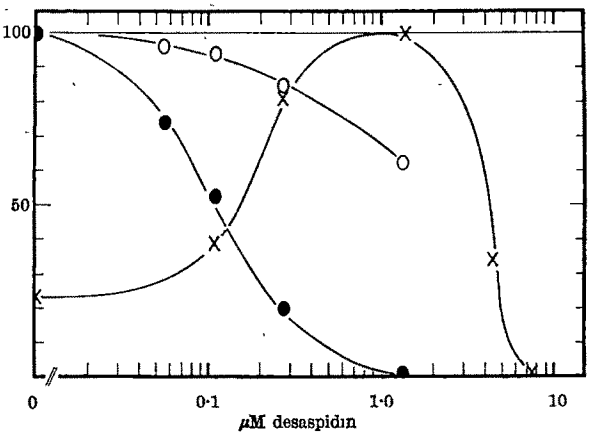


Fig. 6. Uncoupling of phosphorylation accompanying NADP reduction. ×——×, Basal NADP reduction rate at 340 mμ; ordinate, μmoles NADP reduced/mg chlorophyll/27 min. ●——●, ○——○, phosphorylation rate as percentage of control, after 30 sec and 10 min illumination respectively. NADP 1 μmole, ferredoxin 0·3 mg. Reduction rates measured at *p*H 6·2, phosphorylation rates at *p*H 8·0

The cause of desaspidin destruction during anaerobic electron transfer from water to NADP by way of ferredoxin[6] is unknown. Possibly ferredoxin, which is unstable in illuminated chloroplast preparations[17], liberates ferric ion which in turn oxidizes the uncoupler. Using 3 mg ferredoxin per sample and 0·5 μM desaspidin[6], only 0·2 per cent of the iron protein need release Fe^{+++} for the desaspidin to be so oxidized (assuming 1 : 1 stoichiometry for the desaspidin/Fe^{+-+} interaction). Such a mechanism would also account for the lack of desaspidin destruction in the highly sensitive NADP reduction system using ascorbate as electron donor[3].

Our demonstration of aerobic desaspidin-sensitive NADP reduction makes totally unnecessary the proposed phosphorylation site close to the splitting of water, and the ensuing revision of the electron transfer pathway[6].

Comparable effects of desaspidin to the CCP derivatives in inhibiting the scattering responses induced by other uncoupling agents suggest that desaspidin may also occupy a place at the top of the 'uncoupler hierarchy'[12]. Discrimination between two coupling sites by CCP has also been proposed[15]. Avron observed that in this case the cyclic systems appeared to be less sensitive than the non-cyclic. It was also found that the severity of CCP uncoupling increased markedly as the light intensity was lowered. The general criticism may be raised that conditions producing a change in the steady-state level of the redox carriers close to the coupling site (for example, light intensity and added cofactor) might be expected to modify the susceptibility of photophosphorylation to those uncouplers acting closest to electron transport and possibly with the redox components themselves.

There seems to be no concrete evidence for plural coupling sites in higher plant chloroplasts. Even if slight differences in the desaspidin sensitivities of phosphorylation do exist with alternative electron flow patterns, any conclusions will be subject to the same doubts as were voiced earlier with regard to CCP.

The misinterpretations caused by the lability of desaspidin illustrate that the best uncoupling agents, meaning

those to which photophosphorylation is most sensitive, are probably the worst from the point of view of reliability. Extra vigilance should be exercised in their employment with so reactive a system as the chloroplast photosynthetic apparatus. Perhaps we should re-examine the instances in which photosynthetic energy transduction has been thought to differ from that in the mitochondrion on the basis of its insensitivity to certain potent uncouplers and inhibitors of oxidative phosphorylation.

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REACTION KINETIC STUDIES OF THE ANTIGEN–ANTIBODY REACTION

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THE primary reaction between a soluble protein antigen (Ag)* and its antibody (Ab) can be followed by physical techniques such as light scattering[1,2], fluorescence polarization[3] or fluorescence quenching[4], or by chemical techniques as the equilibrium dialysis[5] or the Farr test[6,7]. Most of these techniques are applicable only for special Ag and Ab systems. Light scattering, on the other hand, could be applied for a great variety of soluble Ag–Ab systems. The measurement of light scatter in an antigen–antibody reaction gives direct information on the rate of the reaction, which depends on the combining properties of the Ag and Ab. Reaction kinetic data, obtained by light transmittance measurements, were used earlier to determine the association constant of an Ag–Ab system[8].

In the investigation reported here we attempted to obtain information on the rate of the formation of antigen–antibody complexes by comparing light scatter measurements with fluorescence quenching and saturated ammonium sulphate (SAS) precipitation measurements in the fluorescein-labelled conalbumin–anti-fluorescein-labelled conalbumin system and in the conalbumin–anticonalbumin system.

The conalbumin (CA) was a five-times crystallized preparation of about 95 per cent purity (Sigma Chemical Co.). The fluorescein isothiocyanate (FITC) adsorbed on 'Celite' (about 10 per cent FITC content) was obtained from Calbiochem. Fluorescein-labelled CA (FCA) was prepared by a technique described earlier[9]. The molar degree of conjugation of the 'Sephadex *G*-25' purified preparation was 1·92 (moles sodium fluorescein per mole CA), with a molar fluorescence quenching of 79 per cent and an apparent dissociation constant: $Ka = 4{\cdot}5 \times 10^{-12}$ (M sodium fluorescein)[9]. Iodine-125-labelled CA (ICA) was prepared according to the method of Talmage *et al.*[10]. The activity of the preparation was $5{\cdot}4 \times 10^8$ c.p.m. μg (ICA) at the beginning of the experiment. Anti-CA (ACA) and anti-FCA (AFCA) were prepared by injecting cows weighing 600–700 kg with 80 mg CA or FCA in Freund's complete adjuvant in two successive injections separated by a 2-week interval. A booster shot of 40 mg CA or FCA was given 3 weeks after the second injection. Blood was collected on the fifth day after the last injection. The Ab content of the AFCA was 4·6 per cent of the total serum protein, that of ACA was 0·9 per cent by the quantitative precipitin test. Quantitative precipitin tests were performed according to the method described by Kabat and Mayer[5].

Light scatter was measured in an Aminco Bowman spectrophotofluorometer at 90° angle using a 1-cm rectangular quartz cuvette. The incident beam was of 334 mμ and the scattered beam was of 668 mμ (secondary scatter). In the 320–350 mμ region the output of the xenon lamp was relatively high and changed little with the wave-length. By using the secondary light scatter the interference by the self-absorption of the reactants could be largely avoided. The light intensity of the scattered beam was directly recorded on a Lead and Northrup linear recorder with a 3 in./min chart speed. All measurements were made at room temperature (21°–22° C).

Fluorescence quenching was measured in the same instrument and cuvette as the light scatter at the 485 mμ excitation and 519 mμ emission peaks of the FITC (uncorrected, as found in this instrument).

All reagents and diluents were filtered through a 0·45μ pore size 'Millipore' filter before the light scatter measurements. If the reagents were kept carefully capped after filtration, low-background scatters were observed for several hours. The Ab solutions were diluted with 400 times diluted normal bovine serum (NBS) in 0·1 M borate buffer, *p*H 8·2. The use of NBS assured a relatively standard protein level in the reaction mixtures and gave a standard scatter background.

The antisera were diluted to give a uniform 0·146 mg/ml. Ab concentration in the light scatter titrations. This Ab working solution is called 'Ab-W' throughout this article. The Ag was diluted in 0·1 M borate buffer, *p*H 8·2. The borate buffer at this relatively high *p*H assured a good stability of both Ag and Ab. In physiological buffered saline both the Ag and Ab showed a slight increase in light scatter when standing at room temperature.

In the light scatter titration 1·0 ml. Ab-W was mixed with 0·1 ml. of Ag of various concentrations. The mixing was rapidly done in the quartz cuvette in 3–5 sec and recording started immediately. The same procedure was followed for fluorescence quenching measurement.

The amount of Ag bound to Ab in the form of soluble Ag–Ab complexes during the course of the immunoreaction was determined from the amount of labelled antigen precipitated by 50 per cent saturated ammonium sulphate (50 per cent SAS), essentially the same way as described in the Farr test[6]. A 1·0 ml. portion of the reaction mixture, containing ICA or FCA and AFCA, was withdrawn into a test-tube, containing 1·0 ml. SAS, at every 10 sec

* Abbreviations used in the text: Ab, antibody; ACA, bovine anti-CA serum; AFCA, bovine anti-FCA serum; Ag, antigen; CA, conalbumin; FCA, FITC labelled CA; FITC, fluorescein isothio-cyanate; ICA, iodine-125 labelled CA; NBS, normal bovine serum; SAS, saturated ammonium sulphate.

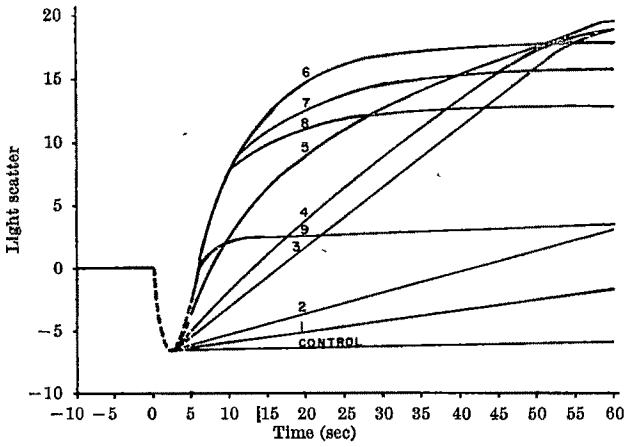


Fig. 1. The effect of antigen concentration on light scatter in the FCA–AFCA system. Protocol: 1·0 ml Ab-W (0·146 mg Ab)+0·1 ml. Ag mixed in 3–5 sec, light scatter recorded. The light scatter values are corrected for the light scatter of the control. The curves shown are redrawn from actual recorder tracings. (1), 2·0 μg FCA; (2), 5·0 μg FCA; (3), 10·0 μg FCA; (4), 15·0 μg FCA, (5), 20·0 μg FCA; (6), 25·0 μg FCA, (7), 30 μg FCA; (8), 40 μg FCA, (9), 200 μg FCA; control: 0·1 ml. borate buffer, pH 8·2. Maximum precipitation in the quantitative precipitation test was achieved when 0·020 mg FCA was added to AFCA, containing 0·146 mg Ab (Ag/Ab = 0·27 mole/mole)

after the start of the reaction. The 50 per cent SAS precipitated all soluble Ag–Ab complexes and serum globulins but not the unreacted ICA or FCA. The amount of ICA in the precipitates was determined from iodine-125 counts[11]; the amount of unreacted FCA was determined by measuring the fluorescence of the supernates by the method developed in this laboratory[12].

The reaction kinetic information obtainable from light scatter measurements is illustrated in Fig. 1 for the FCA–AFCA system. The initial reaction rate is proportional to the concentration of the Ag and the light scatter intensity increases with time in the Ab excess zone. The initial reaction rate reaches a maximum value in the slight Ag excess zone, but the value of maximum light scatter decreases in the Ag excess zone. The Ag–Ab reaction is about 90 per cent completed in 60 sec near the optimum Ag/Ab precipitation ratio, although a very slow increase in light scattering may occur over an hour period.

The rate of Ag binding by Ab in the FCA–AFCA system was followed by fluorescence quenching and by SAS precipitation, as already described. The results of this experiment are shown in Fig. 2. As can be seen, 92 per cent of the Ag was bound to Ab and precipitated by SAS 10 sec after the start of the reaction. The fluorescence quenching, which is caused by the juxtaposition of anti-FITC Ab molecules to the FCA molecule, was also practically complete in 10 sec. The data shown in Fig. 2 were taken at the optimum precipitation ratio of the FCA–AFCA system (Ag/Ab = 0·27 mole/mole); the fluorescence quenching and labelled Ag precipitation by SAS also reached maximum value within 20 sec even in the large Ab excess zone (Ag/Ab = 0·05–0·10 mole/mole). These data indicated that the primary reaction of Ag with Ab, at least in this system, occurred within seconds. The fluorescence quenching reached a maximum value in this initial phase of the reaction, and this quenching did not change in the further course of the reaction. Since in the average two molecules of FITC were bound to CA, we must assume more than one Ab molecule attached to the protein in the primary Ag–Ab complex in order to account for the maximum quenching. Since there was no further fluorescence quenching in the course of the growth of the Ag–Ab complexes, and since practically all Ag molecules were bound to Ab in the first few seconds of the reaction, the growth of the Ag–Ab complex may be visualized as linking together the initially formed Ag–Ab complexes.

The light scattering, as it was measured in these experiments, thus measured the growth of the initially formed Ag–Ab complexes. The rate of this growth must depend on the available combining sites of Ag and Ab, and probably on the structure and configuration of the primary Ag–Ab complex. The rate of the reaction as measured by light scattering was significantly different for the various Ag–Ab systems used in this investigation. Similar sets of curves as shown in Fig. 1 for the FCA–AFCA systems were also obtained for the FCA–ACA, CA–AFCA and CA–ACA systems. The maximum reaction rates attained at various Ag concentrations in the above systems are shown in Fig. 3.

The rate of the formation of the primary Ag–Ab complexes, as measured by SAS precipitation of the bound FCA or ICA, was practically identical in the ICA–ACA, FCA–ACA, ICA–AFCA and FCA–AFCA systems at the optimum Ag/Ab precipitation ratios. This was also true in the Ab excess zone, though slightly more time (10–20 sec) was necessary to achieve maximum SAS precipitation.

These findings indicate that the light scatter is a sensitive indicator for measuring differences in reaction rates between different Ag–Ab systems. The primary Ag–Ab complex formation occurred within 10 sec in all the systems studied at the optimum Ag/Ab precipitation ratios; this time interval was inconveniently small to detect differences in the rate of formation of the primary Ag–Ab complex by the techniques used. The light scatter changes, which according to our interpretation measure the rate of growth of the primary Ag/Ab complex, are more convenient to measure and, as the data of Fig. 3 show, give significantly different values for closely related Ag–Ab systems. Since the rate of the reaction

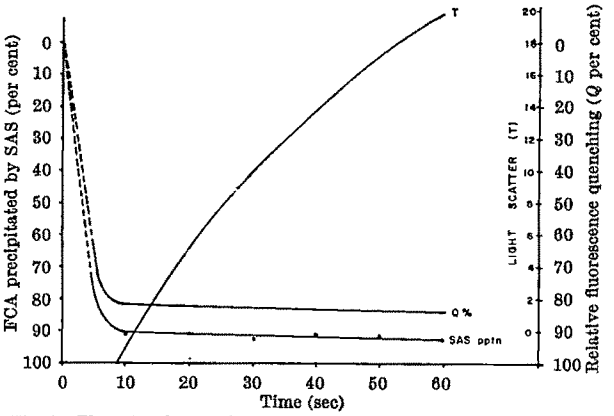


Fig. 2. The rate of formation of soluble Ag–Ab complexes in the FCA–AFCA system at the optimum Ag/Ab precipitation ratio. Protocol: 10 ml. Ab-W (1·46 mg Ab) – 1·0 ml. Ag (0·20 mg): 1·0 ml. aliquots precipitated with 1·0 ml. SAS in 10 sec intervals. 1·2 ml. aliquots used for fluorescence quenching or light scatter measurement. $Q\% = \frac{F_f - F_b}{F_f} \times 100$ where F_f is the fluorescence of free FCA and F_b is the fluorescence of the Ag–Ab mixture. The fluorescence quenching and light scatter curves are redrawn from actual recorder tracings

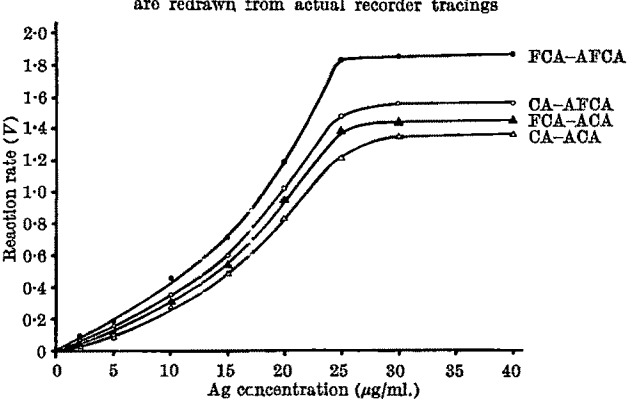


Fig. 3. The effect of Ag concentration on the rate of the Ag–Ab reaction. $V = \frac{dT}{dt}$ sec^{-1}; where T is the light scatter and t is the time in seconds. V was determined graphically from the tangent drawn to the steepest part of the light scatter curves such as shown in Fig. 1

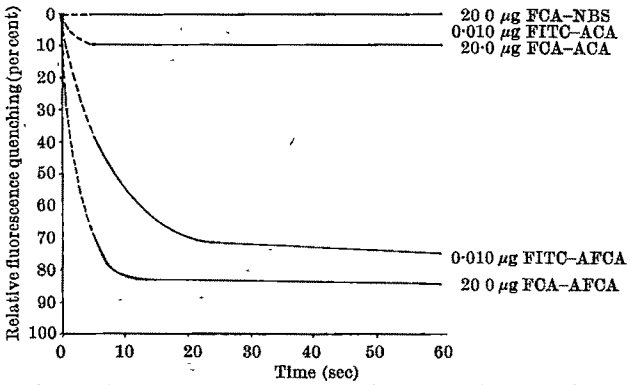


Fig. 4. Fluorescence quenching in the FCA–AFCA, FCA–ACA, FITC–AFCA and FITC–ACA systems. Protocol. 1·0 ml. Ab-W (0·146 mg) + 0·1 ml. Ag, mixed in 3–5 sec, fluorescence quenching recorded. The curves are reproduced from actual recorder tracings

depends on the Ag binding sites available on the Ab molecule, the light scatter changes during an immunoreaction are indicative of the combining properties of the Ag–Ab system.

According to this interpretation the higher reaction rate in the FCA–AFCA than in the FCA–ACA system could be explained by the existence of a combining site for the FITC label in the AFCA molecule. The existence of such a combining site was demonstrated by the observed strong quenching of the fluorescence of FITC or FCA in the presence of AFCA but not in the presence of ACA, as shown in Fig. 4.

In conclusion, the data presented here demonstrate the usefulness of the method of combining light scattering, fluorescence quenching and SAS precipitation for studying the rate of formation of Ag–Ab complexes. It also shows that the measurement of light scatter change, which depends on the rate of growth of the initially formed Ag–Ab complex, is a convenient way to determine differences in the relative combining properties of closely related Ag–Ab systems. For this reason light scatter measurements should be eminently suitable for studying structural differences in proteins or other soluble antigens by their relative combining properties with a standard antibody.

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BLOOD SAMPLES FROM THE PITUITARY STALK OF THE RAT: METHOD OF COLLECTION AND FACTORS DETERMINING VOLUME

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THE neurohumoral hypothesis of the control of the anterior lobe of the pituitary gland involves the concept of specific releasing factors which are secreted by nerve endings in the median eminence and carried by way of portal vessels into the anterior lobe[1]. There is an overwhelming amount of indirect evidence to support this hypothesis, but with the exception of the work of Porter and co-workers[2,4] there is no direct evidence that there are substances in the portal blood which do not occur in equal or higher concentration in blood in other regions. Direct evidence that such factors exist in the portal blood is urgently needed.

The principal reason for this gap in the mass of evidence which supports the neurohumoral theory is the technical difficulty of obtaining portal blood for assay. The purposes of this communication are to describe a surgical technique which makes possible the collection of samples of pituitary stalk blood which are large enough to be assayed, to discuss those factors which determine the success or failure of attempts to obtain portal blood samples, and to describe briefly some vascular phenomena in the pituitary stalk of the rat.

A Technique for obtaining Pituitary Stalk Blood Samples. With the exception of eleven Lister rats used for electrical stimulation, female albino rats of the Wistar strain were used in all experiments. Anaesthesia is induced with 1,000 mg/kg of urethane in 10 per cent solution, administered intraperitoneally, and supplemented with 1/8 to 1/4 that amount if spontaneous movements of the lower jaw or vibrissae persist. Only as much urethane is used as is required for adequate anaesthesia. The animal is immobilized on a standard rat operating board, and the skin and subcutaneous tissues infiltrated with 2 per cent procaine in the midline from the inter-ramal vibrissae to the sternal notch. The skin and subcutaneous tissues are incised in the midline and these tissues, together with the salivary glands, are separated in the midline and removed from the muscles overlying the trachea. Procaine is introduced beneath the pretracheal muscles (passing the needle anteriorly into the region of the larynx), these muscles are separated in the midline along their entire length and a silk suture is passed under the trachea. A small nick is made in the trachea and a polythene tube placed in position and tied.

The midline incision is carried through the lower lip with scissors and haemostasis is secured by the judicious use of a small eye cautery. The floor of the mouth is infiltrated with 2 per cent procaine deeply along the mandibular margin, and a tight suture is placed around each mandibular ramus. The latter procedure secures a degree of haemostasis when the mandible is divided. Long silk sutures are placed in the two sides of the divided lip and used for lateral retraction of the two sides of the jaw. Before dividing the jaw the tongue is infiltrated down to the base with 2 per cent procaine and a suture placed through the tip of the tongue for ease in subsequent manipulation.

The lower jaw is divided in the midline with heavy sharp scissors (not rongures) and the musculature of the floor of the mouth and oral mucosa are divided along the left side of the mandible. If possible, an assistant should be at the operator's left to clamp bleeders at the base of the tongue with 'mosquito' forceps and assist in tying them. A similar cut is made on the right side and any additional bleeders clamped and tied. The incisions along

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the medial border of the mandible should be extended posteriorly but, if the cut is made too deeply and too far posteriorly between the tongue and mandible, the lingual artery will be cut and profuse haemorrhage may occur. The muscles of the floor of the mouth should be cut further posteriorly, layer by layer, the fauces cut, and the tongue retracted. When the soft palate is entirely exposed and the epiglottis is clearly visible adequate exposure has been obtained. Before retracting the tongue permanently, it is wrapped with oxidized cellulose to secure complete haemostasis and, in addition, small squares of oxidized cellulose are placed on the cut edges of muscle and bone where the mandible and the floor of the mouth have been cut.

The soft palate is infiltrated with procaine and incised in the midline with an eye cautery used as a knife. After enough of the soft palate is removed to expose the posterior pharyngeal wall clearly, the mucosa of the posterior pharyngeal wall is scraped away with a small pledget of oxidized cellulose. This is better than cotton wool or bare metal because it reduces bleeding from the mucosa. If bleeding persists, the entire cavity should be lined for five or ten minutes with oxidized cellulose. The rest of the procedure is carried out under a Zeiss binocular dissecting microscope.

Since minutely detailed instructions for the removal of bone cannot substitute for experience, the following general description of the procedure is supplied. A hole is made in the outer table of bone and partially through the spongy bone of the diploë about 1 mm behind the transverse sinus of the basisphenoid bone. This is filled with neurosurgical bone wax and the drill gradually worked forward toward the transverse sinus while the defect is packed with bone wax at frequent intervals. The drill is actually moved gradually through the transverse sinus, pushing bone wax and ground fragments of bone ahead of the drill to pack it. Great care should be used in this stage of the procedure to avoid sudden massive haemorrhage. If such a haemorrhage occurs, a fairly large pledget of cotton wool carrying a mass of bone wax should be at hand for immediate packing into the wound. This massive packing sometimes stops the bleeding and allows time for more definitive packing of the defect in the transverse sinus.

The inner table should be exposed fully as a smooth glistening plate and ground with a small burr to a uniform thinness which 'yields' when touched with the tip of a forceps. This remaining portion of inner table is removed with a fine chisel. A piece of oxidized cellulose is placed on the exposed dura and on the soft tissues lining the incision for a few minutes before proceeding in order to secure complete haemostasis. Occasionally, troublesome dural arterial or large venous bleeders occur. These must be stopped before the dura is removed. If oxidized cellulose fails to stop them, very small pieces of a mixture of finely ground silver nitrate crystals in bone wax may be applied. These applications should be limited to as small an area as possible. Some degree of fixation of the outer surface of the dura can take place without doing damage to the pituitary stalk, but an excess will fix not only the dura but also the arachnoid and the stalk beneath.

When haemostasis has been secured, the dura mater is incised in a V-shaped incision (the point of the 'V' lying just anterior to the median eminence) and the flap is retracted posteriorly with a No. 5 jeweller's forceps. The arachnoid usually must be incised separately and is retracted in a similar manner. 0·5 ml. heparin (1,000 I.U. ml.) is given by femoral vein after a final check of haemostasis, and the pituitary stalk is cut through with irredectomy scissors at the point of junction with the gland. Initially a small amount of cerebrospinal fluid appears. This is aspirated and discarded.

The blood sample is collected by aspiration into a heparinized length of polythene tubing holding about 0·1 ml. When it is full, the blood is emptied into a heparinized collection tube in an ice bath, by means of a syringe attached to the suction line by a three-way stopcock.

From the incision in the stalk a mixture of blood from three sources is obtained. These are: (*A*) the proximal cut ends of portal vessels (portal blood), (*B*) the distal cut ends of portal vessels (portal back-flow), and (*C*) arterial terminations (arterial blood). There is a marked difference in colour between arterial and portal blood on the one hand and portal back-flow on the other. Portal back-flow is dark blue venous blood and appears to flow freely backward from the dural sinuses through the sinusoids of the pars distalis, and out of the cut ends of portal trunks. Both portal blood and arterial blood are bright red, and, although there is a suggestion of a colour difference, it is slight. Careful preliminary estimation of the apparent amounts of blood from the various sources suggests that portal blood from the median eminence and stalk make up at least 3/4 of any sample, and perhaps more. Any preparation in which a vessel from the dura mater or elsewhere is contributing more than a negligible trickle to the collection is discarded.

General and Surgical Factors affecting Volumes of Blood from the Pituitary Stalk: A. Condition of Animal. In so far as it may be consistent with the design of the experiment, animals in prime condition without prior treatment or manipulation should be used. In this series rats which appeared to be under weight for their group, to have poor coat condition, or the slightest sign of respiratory infection, failed to bleed satisfactorily from the stalk. Those animals which fail to give satisfactory results, even when external signs are absent, usually show some visceral lesions at autopsy.

B. Anaesthesia. Although a wide range of anaesthetic agents has not been used, the choice of anaesthetic agent appears to be important. Sodium pentobarbital anaesthesia is useless since it is known to reduce hypophysial portal blood flow in mice[5], and flow from the cut ends of the pituitary stalk have been negligible when it has been used in the rat. Ether as a supplement to urethane anaesthesia reduces blood flow from the cut ends of the stalk, but urethane anaesthesia alone seems satisfactory. The dose of 1,000 mg/kg gives satisfactory anaesthesia and is usually associated with a preparation from which adequate samples may be collected. In hypophysectomized animals a reduced dose may be necessary. It should be emphasized that very deep levels of anaesthesia make it impossible to collect useful volumes of blood from the stalk. Local procaine anaesthesia during the surgical procedure eliminates reflex responses when the pharynx and trachea are being manipulated and appears to reduce the incidence of shock, circulatory depression and low volumes of stalk sample.

C. Haemorrhage. It has been demonstrated that single large haemorrhages or numerous small haemorrhages reduce portal blood flow in the mouse[5]. This finding is confirmed for the rat. Any animal in which a haemorrhage in the order of 0·25 to 0·5 ml. has occurred is likely to give poor results and if the estimated blood loss during the procedure is as much as 1·0 ml. the preparation should be abandoned.

D. Mechanical Factors. The meninges should be cut through well in front of the median eminence. If the dura is removed too far posteriorly a bulging stalk may exert pressure against the edge of the dura, constricting the portal vessels and reducing flow. Furthermore, attempts to place small cotton wool pledgets between bone and the distal stump of the stalk reduced flow from both the proximal and distal stumps. Any pressure between the bone and pituitary or brain appears to affect the vasculature of the median eminence and stalk sufficiently to cause reduction in flow.

Results of Experimental Procedures on Volumes of Blood from the Pituitary Stalk. A. Hypophysectomy. Pituitary stalk blood samples were obtained from previously hypophysectomized animals. This is important because in such animals the effects of anterior lobe hormones do not have to be considered in assays of stalk blood for

releasing factors, and the necessity for biochemical fractionation is thereby eliminated. Further, it has been found that hypophysectomy increases the amount of luteinizing hormone releasing factor (LRF) in the median eminence of the rat[6] and that in rats hypophysectomized for 45 days LRF is detectable in peripheral blood[7]. Assays of LRF in stalk blood from hypophysectomized animals would not only confirm the presence of LRF in blood in high quantities, but also at an earlier period following the operation. The possible effects of hypophysectomy on other hypothalamic releasing factors is an important area for future investigation and the theoretically higher concentration of such factors in portal blood should make blood from this source superior to peripheral samples.

Hypophysectomized animals present much greater difficulties in obtaining samples of satisfactory volume than intact animals. Even in animals maintained on glucose, saline and a meat diet, the general condition is poor and blood pressure appears much reduced. A moderate to severe intravascular agglutination ('sludging') is present. The blood flow from the stalk is usually so low that 0·5 ml. in 2 h is considered a good yield, and 1·0 ml. an excellent one. During collection, an intravenous infusion of 1:100,000 adrenaline, B.P., at a rate of 0·085 ml./min may increase the flow.

Hypophysectomy also increases the technical surgical problems. The hypophysectomy opening must be avoided when the bone is being removed and the remaining stalk is adherent to the meninges at its cut end, making it difficult to expose as much of the stalk as is possible in the normal animal.

The vessels of the stalk appear to be functional in as short a time as two weeks after removal of the gland. The superior hypophysial arteries have greater diameters than in the normal animal, the capillaries are somewhat engorged and the number of portal vessels appears to be reduced. Occasional abnormally oriented or tortuous portal vessels are encountered. However, despite the intravascular agglutination of the red cells, no thrombosis of capillaries or portal vessels was seen and the blood flow in the portal vessels is in the normal direction (that is, from the median eminence toward the lower stalk). A new secondary capillary network develops in the connective tissues of the sella turcica to replace the anterior lobe sinusoids.

B. Electrical Stimulation. Stimulation of the hypothalamus by electrodes implanted 1 mm. above the floor of the skull and 4·5–5·5 mm anterior to the intra-aural line increased the rate of flow from the stalk. Eleven animals with implanted electrodes were subjected to the procedure described above and stalk blood was collected before and during stimulation at 0·5–1·0 V (25–50 μamp) by a fifty cycle sine wave alternating current. The current was alternatively 'off' and 'on' every 15 sec during the period of stimulation, and the volume of the sample over the total of each time period was determined. The mean value before stimulation of 1·44 ml./h increased with stimulation to 2·28 ml/h. Student's *t*-test shows this difference to be significant ($P < 0.01$).

Assay of Stalk Samples. An assay of pituitary stalk blood samples from proestrus animals for luteinizing hormone activity by the ovarian ascorbic acid depletion method[8,9] shows that 'stalk' blood samples deplete ovarian ascorbic levels significantly and that peripheral blood samples from the same animals do not. This effect is attributed to a mixture of luteinizing hormone and luteinizing hormone releasing factor in the stalk sample. Preliminary studies of blood samples from hypophysectomized animals suggest that they are equally effective. In the latter case the effect must be due entirely to luteinizing hormone releasing factor.

That some back-flow contamination with anterior pituitary hormones is occurring was indicated by determinations of thyroid stimulating hormone (TSH). When 'stalk' blood samples were assayed for this hormone directly by *in vitro* guinea-pig thyroid assay[10], they showed about ten times the concentration of TSH in peripheral blood from the same animal and 5–10 times the level found in normal unoperated animals kept under the same conditions.

In summary, a surgical procedure for obtaining samples of pituitary stalk blood from the rat is described together with some operative, physiological and experimental factors contributing to success or failure in this effort. The importance of obtaining such samples is discussed, and some preliminary results of assays of stalk blood are briefly described.

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DEFLUORINATION AND HYDROXYLATION OF 4′-FLUORO-4-DIMETHYLAMINOAZOBENZENE BY RAT LIVER *IN VIVO* AND ITS POSSIBLE RELEVANCE TO MECHANISMS OF CHEMICAL CARCINOGENESIS

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THE elucidation of the biological action of any chemical carcinogen requires, in the first instance, a determination of the position(s) in the molecule which, presumably as a result of an interaction with certain tissue elements, is responsible for its activity. An elegant approach towards a solution of this problem was suggested by Miller, Miller and Finger[1]. It involved the use of fluorine-substitution as an indicator of the biologically active or inactive positions in a molecule. The small size of the fluorine atom made it unlikely that the substitution would alter the activity for purely steric reasons. Furthermore, from the fact that the fluorine-carbon bond is among the strongest known, except where the carbon atom is electron-deficient, it was reasoned that any metabolic involvement at a fluorine-substituted position would be highly improbable. These considerations led to the premise that, if a fluorinated molecule was active, the position substituted by the fluorine atom was probably not directly involved in

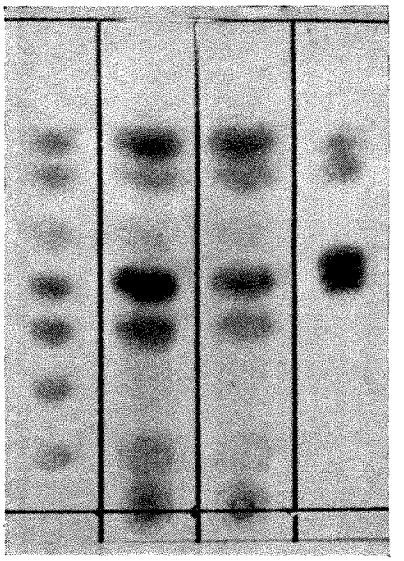
the biological activity in question. Conversely, if a fluorinated molecule was inactive, then the position blocked by the fluorine atom might participate in the biological activity of the non-fluorinated molecule.

The Millers and their associates have made systematic investigations of the carcinogenic activity of the fluorine-substituted rat hepatocarcinogen, 4-dimethylaminoazobenzene[1,2], and of two other types of carcinogen, 2-acetylaminofluorene[3] and 10-methyl-1:2-benzanthracene[4]. The activity of fluorine-substituted derivatives of the carcinogenic heterocyclic compound, tri*cyclo*quinazoline[5], has been investigated by others. From these extensive investigations, conclusions were drawn regarding the positions in these molecules which most probably were concerned in their carcinogenic activity. These conclusions have achieved a wide measure of acceptance since, hitherto, the belief in the metabolic inertness of a fluorine-substituted position in these compounds has not been questioned by evidence to the contrary. Such evidence, albeit relating to only one of the carcinogens used in the work above, forms the subject of this report. It arose as an incidental finding during an investigation into the nature of the dye metabolites in the liver of rats dosed with 4-dimethylamino-azobenzene.

4-dimethylaminoazobenzene (DMAB) was administered intragastrically in a dose of 20 mg in 1 ml. olive oil/100 g body-weight to adult Wistar rats (usually groups of 4 to 6) which were killed by decapitation 5 h later. The pooled livers were extracted repeatedly by maceration with ether in a blender until no further free dye could be removed. The total ether extract was evaporated to dryness, the residue dissolved in benzene, and the dyes extracted into dilute hydrochloric acid. The dyes were thereby freed from lipids which otherwise interfered with their subsequent chromatographic separation. After adjustment of the acid extract to *p*H 3 with potassium hydroxide and potassium dihydro-*ortho*phosphate, the total basic and amphoteric (hydroxylated) dyes were re-extracted into benzene. This solution was concentrated so that the metabolites from 1 g liver were contained in a volume of 10 μl. This quantity was spotted on silica gel *G* plates and submitted to thin-layer chromatography using the technique described elsewhere[6].

The dye administered, DMAB, together with its known metabolites[7], 4-methylaminoazobenzene (MAB), 4-aminoazobenzene (AB), 4′-hydroxy-DMAB and 4′-hydroxy-MAB, were readily identified (Fig. 1*B*). Two further metabolites were evident and they were shown to be 4′-hydroxy AB and 4′-hydroxy-N-acetyl AB from their chromatographic identity with authentic specimens. Confirmation of this was afforded since the latter metabolite could be converted into the former by mild acid hydrolysis and both, on reductive cleavage with tin and hydrochloric acid, gave the required *p*-aminophenol and *p*-phenylene diamine which were detected by Böniger's reaction as used by the Millers[8].

Groups of rats were treated similarly with 4′-fluoro-DMAB and the metabolites in the liver were isolated and examined in the same way. It was expected that 4′-hydroxylation would be blocked in this compound with the possibility that hydroxylation might occur elsewhere in the molecule. Surprisingly, the chromatographic pattern of the metabolites (Fig. 1*C*) was the same, qualitatively, as that already described for the DMAB-treated rats. The spots which corresponded to the four hydroxylated derivatives obtained from the DMAB-dosed rat liver were removed, in replicate, from the chromatograms. Each of them, on reductive cleavage, yielded *p*-aminophenol (Böniger's reaction), which established that the liver of rats treated with 4′-fluoro-DMAB contained the same 4′-hydroxylated metabolites as those treated with the unsubstituted dye. The possibility that hydroxylation had occurred at a position different from the 4′-position was unlikely because the 2′-, 2- and 3′-hydroxy derivatives of DMAB were easily separable and



A *B* *C* *D*

Fig. 1. Thin-layer chromatogram: solvent, 10 per cent methanol in benzene, 10 cm run. Dyes listed in order of decreasing R_F. Stable colours of spots after exposure to hydrochloric acid given in parenthesis. *A*, Mixture of DMAB (rose), MAB (orange), AB (orange), 4′-hydroxy-DMAB (purple), 4′-hydroxy-MAB (purple), 4′-hydroxy-AB (purple-red) and 4′-hydroxy-N-acetyl AB (orange); *B*, dye metabolites in liver of DMAB-dosed rats; *C*, dye metabolites in liver of 4′-fluoro-DMAB-dosed rats. The 4′-hydroxy-AB, though readily seen on the original plate, does not show in the photograph; *D*, mixture of 1 μg each of 2′-hydroxy-DMAB (mauve), 2-hydroxy-DMAB (yellow), 3′-hydroxy-DMAB (rose) and 4′-hydroxy-DMAB (purple)

distinguishable from 4′-hydroxy-DMAB in the chromatographic system (10 per cent methanol in benzene) used (Fig. 1*D*).

Although the chromatograms obtained with this system suggested that rats treated with either 4′-fluoro-DMAB or DMAB gave the same non-hydroxylated metabolites, namely DMAB, MAB and AB (compare *B* and *C*, Fig. 1), this was shown not to be the case. Thin-layer chromatography with xylene as developing solvent permitted the separation of 4′-fluoro-DMAB from DMAB (Fig. 2*D*). In this system the hydroxylated dye metabolites remained at, or near, the origin. It may be seen (Fig. 2*B* and *C*) that the three non-hydroxylated dyes in the 4′-fluoro-DMAB-treated rat liver extract had mobilities increased by a relatively constant amount over those of DMAB, MAB and AB, respectively, present in the extract from the DMAB dosed rats. The spot of greatest mobility was evidently the administered dye, 4′-fluoro-DMAB, and the two others were assumed to be the *N*-demethylated metabolites, 4′-fluoro-MAB and 4′-fluoro-AB. Usually, spots corresponding to DMAB, MAB and AB also were seen in chromatograms of 4′-fluoro-DMAB-treated rat liver extract but in barely detectable amounts. It would appear, therefore, that hydroxylation is concomitant with defluorination or that the latter is a distinct reaction followed very rapidly by hydroxylation at the position vacated by the fluorine atom.

The quantitative distribution of the administered dyes and their metabolites in the liver of rats treated with either DMAB or 4′-fluoro-DMAB is shown by a typical experiment (Table 1). The metabolite spots (replicated where necessary) were scraped off the plates and eluted into alcoholic hydrochloric acid. The amount of each metabolite was estimated by spectrophotometric comparison with known quantities of synthetic samples which were similarly chromatographed on, and eluted from, a silica gel *G*-coated plate. Estimates of the total 4′-hydroxylated metabolites also were obtained by a quantitative adaptation of Böniger's reaction for the determination of the *p*-aminophenol obtained on reductive cleavage of total dye extracts. The *p*-aminophenol formed was compared with that obtained when known amounts of 4′-hydroxy-DMAB were taken through the same pro-

Table 1. FREE DYES IN LIVERS OF RATS DOSED WITH DMAB AND 4′-FLUORO-DMAB

Dye	μg Free dye/g liver: DMAB-dosed rats	4-Fluoro-DMAB-dosed rats
DMAB	1·5	—*
MAB	0·4	—*
AB	2·2	—*
4′-Fluoro-DMAB	—	3·4
4′-Fluoro-MAB	—	1·2†
4′-Fluoro-AB	—	0·6‡
Total non-hydroxy dyes	4·1	5·2
4′-Hydroxy-DMAB	4·2	0·8
4′-Hydroxy-MAB	1·9	0·3
4′-Hydroxy-AB	2·8	1·7
4′-Hydroxy-N-acetyl AB	3·1	0·9
Total hydroxy dyes	12·0	3·7
Estimated total hydroxy dyes§	11·5	3·9

* Trace detectable on chromatograms, but below level of estimation.
† Assuming recoveries and E_{max} values similar to those for MAB.
‡ Assuming recoveries and E_{max} values similar to those for AB.
§ Values calculated as 4′-hydroxy-DMAB from determination of yield of *p*-aminophenol obtained on reductive cleavage of the total dyes in the liver extracts.

cedure. Good agreement between the two methods of estimation of the 4′-hydroxylated metabolites was obtained. The total amount of non-hydroxylated dye in the liver was about the same when the rats received either 4′-fluoro-DMAB or DMAB. Rats treated with the fluorinated dye, however, showed a liver content of the total hydroxylated dye metabolites only about one-third of that of those given DMAB itself.

It might be objected that the demonstration of 4′-hydroxylated metabolites in the liver of 4′-fluoro-DMAB-dosed rats might have arisen through the adventitious presence of DMAB in the 4′-fluoro-DMAB used. This was discounted for two reasons. (1) Chromatography of 1 μg of 4′-fluoro-DMAB gave one spot only. A mixture of 1 μg of the fluoro-dye and 0·025 μg of DMAB was easily resolvable into two spots. Therefore, the level of contamination of the former dye by the latter was certainly below 2·5 per cent. (2) Even on the assumption of a 5 per cent level of contamination, a rat dosed with 20 mg of 4′-fluoro-DMAB would receive only 1 mg of DMAB. Dye metabolites, hydroxylated or otherwise, could not be detected by the standard procedure in the livers of rats given this amount of DMAB.

Clearly, the present report by itself cannot be construed to invalidate the conclusions regarding the molecular positions which have significance for the activity of those chemical carcinogens which have been investigated by the method of fluorine substitution. Nevertheless, it does suggest the possibility of metabolism at fluorine-substituted positions in other molecules in which, on theoretical grounds, it would not be expected. It might be considered that regard to this point should be paid to the organic fluoro-compounds which are being introduced increasingly into commerce[9].

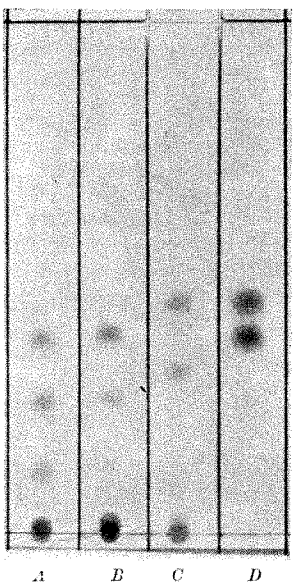


Fig. 2. Thin layer chromatogram: solvent, xylene, 15 cm run. Dyes listed in order of decreasing R_F. Stable colours of spots after exposure to hydrochloric acid given in parenthesis. *A*, Mixture of DMAB (rose), MAB (orange), AB (orange) and their OH-derivatives, which latter remained at or near the origin (purple); *B*, dye metabolites in liver of DMAB-dosed rats; *C*, dye metabolites in liver of 4′-fluoro-DMAB-dosed rats; *D*, mixture of 1 μg each of 4′-fluoro-DMAB (rose) and DMAB (rose)

With regard to the defluorohydroxylation of 4′-fluoro-DMAB, it may be significant that the 4′ position of DMAB is a favoured site for the *in vivo* hydroxylation of the carcinogen. Perhaps the removal of a fluorine atom having a presumed blocking role is effected only under these conditions. It will be of some interest to see if the *in vivo* defluorohydroxylation of the carcinogenic 7-fluoro-2-acetylaminofluorene occurs also. The situation here is analogous to that in the case of 4′-fluoro-DMAB since the 7 position in 2 acetyl-aminofluorene is a site of *in vivo* hydroxylation and, with respect to the substituted amino group, an extended para position.

The defluorohydroxylation of 4′-fluoro-DMAB may have significance in carcinogenesis additional to that mentioned heretofore. Preliminary results have indicated that the very weakly carcinogenic 4′-methyl-DMAB does not undergo appreciable 4′-demethylhydroxylation in the rat whereas the carcinogenic 4′-ethyl-DMAB suffers a fairly substantial replacement of the ethyl by a hydroxyl group. Moreover, under the standard conditions of time and dose, the mouse, which is more refractory than the rat to DMAB hepato-carcinogenesis, 4′-hydroxylated the dye much less efficiently. Guinea-pigs, which are not susceptible to DMAB hepato-carcinogenesis, did not hydroxylate the dye to a detectable extent.

The mechanism of the 4′-defluorohydroxylation of 4′-fluoro-DMAB has not been investigated as yet and may be similar to the defluorohydroxylation of 4-fluoro-phenyl-alanine[10] which requires rat or sheep liver enzymes, oxygen, reduced NADP and tetrahydropteridine. On the other hand, it might be speculated that hydroxylation occurs initially at the substituted amino group of DMAB to produce an aryl-hydroxylamine and that this undergoes re-arrangement, possibly enzyme-mediated, to the 4′-hydroxy-derivative ousting, if necessary, a fluorine atom at this site. Arylhydroxyl-amines have been shown to be produced *in vivo* in susceptible species from many carcinogenic aromatic amines and much evidence has accumulated to suggest that they are proximate carcinogens[11]. So far, this reaction has not been firmly implicated in the carcinogenic activity of the aminoazo dyes although *N*-hydroxy, *N*-acetyl-AB has been identified recently[12] in the urine of rats dosed with AB. It is interesting that the isomeric 4′-hydroxy-*N*-acetyl AB was one of the metabolites found in the liver of our DMAB and 4′-fluoro-DMAB-treated rats.

We thank Dr. W. J. P. Neish, of these laboratories, for gifts of some of the aminoazo dyes, Miss J. Oates for technical help and the Yorkshire Council of the British Empire Cancer Campaign for Research for their financial support.

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ACCELERATING AND DECELERATING ACTIONS OF ADRENALIN ON EPIDERMAL MITOTIC ACTIVITY

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FROM a series of investigations of the action of adrenalin on mouse epidermis it has been concluded[1-3], first, that this hormone inhibits the entry of cells into mitosis, and second, that it prolongs the duration of mitosis in those few cells which manage to overcome the inhibition. The antimitotic action of adrenalin on epidermis was first reported by Friedenwald and Buschke[4] and has since been confirmed, for example, by Chaudry, Halberg and Bittner[5], Chiakulas, Scheving and Winston[6], and Bogoyavlenskaya and Dobrokhotov[7].

Recently, however, Evensen[8] and Evensen and Heldaas[9], using the epidermis of hairless mice, have come to a directly contrary view that adrenalin increases the mitotic rate and decreases the mitotic duration. Evensen[8] has further concluded that with a high mitotic rate colcemid loses its power to arrest cells in metaphase when in the presence of adrenalin, and if this is true then much earlier work is invalidated. It has thus become necessary to re-examine the whole problem and to obtain further evidence.

In this new investigation the mice used were all adult males of the Kreyberg *WLL* and Strong *CBA* strains, and only slight differences were found between them. They were killed by neck-pulling and small pieces of ear were immediately transferred to a phosphate-buffered saline medium with glucose (for techniques see refs. 10 and 11). After various periods of time the pieces of ear were fixed and sectioned at 7μ, and the epidermal mitoses were counted in unit section lengths.

An investigation was first made of the effect of adrenalin on those mitoses that were already in existence when the ears were separated from the bodies. Each Warburg flask contained five pieces of ear from different mice and the adrenalin was placed in the side arm. After all the flasks had been set up and were in the constant temperature bath, the adrenalin was tipped in. Then, at each interval of 5 min, one flask was opened and the pieces of ear were immediately fixed. The results are given in Table 1. The figures, taken together with the observations on the phases of mitosis, indicate: (1) that the many mitoses present when the adrenalin was added passed quickly to completion—all prophases disappeared in about 10 min and all metaphases in about 20 min; (2) that in the presence of adrenalin, the time taken for the whole process of mitosis was probably less than 1 h, as compared with a normal *in vitro* time of about 2·8 h (see Table 2); (3) that after about 30 min small numbers of new mitoses began to appear.

Table 1. EFFECT OF ADRENALIN ON THE MITOTIC ACTIVITY OF MOUSE EAR EPIDERMIS DURING THE FIRST HOUR *in vitro*

Time (min)	No. of mitoses	Phases of mitoses
0	6·8 ± 0·23	All phases present
5	6·7 ± 0·43	All phases present
10	5·1 ± 0·54	No prophases
15	4·8 ± 0·37	Few metaphases
20	3·0 ± 0·41	All in telophase
25	2·0 ± 0·17	All in telophase
30	1·4 ± 0·09	Few pro- and metaphases
35	1·3 ± 0·29	Few pro- and metaphases
40	0·9 ± 0·15	Few pro- and metaphases
45	0·5 ± 0·19	All phases present
50	0·3 ± 0·11	All phases present
55	0·4 ± 0·37	All phases present
60	0·2 ± 0·08	All phases present

Average numbers of mitoses in unit lengths of 1 cm. ear epidermis sectioned 7μ thick.

N, Five mice. Adrenalin concentration, 0·25 μg/ml.

Note: In control ear epidermis the mitotic count falls only slightly and at 60 min = 5·6 ± 0·42 (N = 5).

Table 2. EFFECT OF ADRENALIN ON THE MITOTIC ACTIVITY OF MOUSE EAR EPIDERMIS DURING THE SECOND TO THE FIFTH HOUR *in vitro*

Culture medium	Metaphases arrested by colcemid in 4 h		All phases of mitosis at hourly intervals (no colcemid)		Duration of mitosis $\Sigma M_2/M_1$
	N_1	M_1	N_2	M_2	(h)
Control	10	7·6 ± 0·18	50	4·7 ± 0·23 5·1 ± 0·27 5·5 ± 0·32 4·8 ± 0·22 0·5 ± 0·18	2·6
With 0·25 μg adrenalin/ml.	15	0·5 ± 0·14	15	0·4 ± 0·11 0·6 ± 0·21 1·4 ± 0·29	5·8

Average numbers of mitoses in unit lengths of 1 cm of ear epidermis sectioned 7μ thick. *N*, Number of mice.

This indication of an increased speed of completion of mitosis is in agreement with Evensen[8] and Evensen and Heldaas[9] and contradicts the conclusion[3] that the addition of adrenalin can prolong mitosis to almost 6 h. However, this conclusion did not refer to already existing mitoses but only to mitoses that commenced between the second and fifth hour after the addition of adrenalin. In Table 2 the behaviour of epidermal cells at this time is shown by the combined results of ten separate experiments. These figures suggest: (1) that adrenalin inhibits mitotic activity by preventing cells from entering mitosis; (2) that with the concentration used, adrenalin causes an increase in mitotic duration from about 2·8 h to about 5·8 h.

However, these suggestions can be challenged if Evensen[8] is correct in concluding that adrenalin so interferes with the action of colcemid that in the adrenalin-treated epidermis few, if any, mitoses are arrested in metaphase. This conclusion suggests the possibilities: (1) that the lower mitotic counts obtained with adrenalin in the absence of colcemid were the combined results of an increased mitotic rate and a reduced mitotic duration; (2) that the lower mitotic counts obtained with adrenalin in the presence of colcemid were due in addition to the neutralization of the colcemid action.

Both these possibilities can be countered if it can be shown that adrenalin has no effect on colcemid action. In the present colcemid experiments (Table 2) it was found that in both the control and the adrenalin-treated epidermis almost all the mitoses were indeed arrested in the metaphase, but a further test of colcemid action was made using wounded epidermis. Such epidermis, both *in vivo* and *in vitro*, shows a high mitotic rate and a short mitotic duration, both of which are relatively unaffected by adrenalin (for an explanation of this phenomenon see refs. 2 and 12). The effects of adding both adrenalin and colcemid to such epidermis *in vitro* are seen in Table 3. This shows that since no significant differences exist between the figures for the control and the adrenalin-treated

Table 3. EFFECT OF ADRENALIN AND COLCEMID ON THE MITOTIC ACTIVITY IN WOUNDED MOUSE EAR EPIDERMIS *in vitro*

Culture medium	Metaphases arrested by colcemid in 4 h		All phases of mitosis at hourly intervals (no colcemid)		Duration of mitosis $\Sigma M_2/M_1$
	N_1	M_1	N_2	M_2	(h)
Control	56	11·4 ± 0·29	34 34 22 28	6·2 ± 0·27 5·2 ± 0·29 4·1 ± 0·27 4·7 ± 0·22	1·8
With 0·25 μg adrenalin/ml.	40	10·5 ± 0·47	31 32 32 36	5·6 ± 0·41 5·3 ± 0·27 4·0 ± 0·21 4·5 ± 0·24	1·8

Average numbers of mitoses in unit lengths of 1 mm of sections cut 7μ thick of epidermis immediately adjacent to a 2-day-old wound edge. *N*, Numbers of wounds.

epidermis, and since in both the colcemid-treated groups all the mitoses were arrested in metaphase, the action of colcemid was not affected by the adrenalin. This is an important conclusion since most previous investigations of adrenalin action on mitosis have involved the use of either colcemid or colchicine.

The most important conclusion emerging from these results is that adrenalin has a double action on the mitotic process. It accelerates the rate of completion of already existing mitoses and it decelerates both the entry of new cells into mitosis and the rate of completion of such mitoses. Adrenalin is known to accelerate many physiological processes and the stimulus to existing mitoses may perhaps be part of this general action. The blockage of mitosis in antephase (or G_2) has been ascribed to the joint action of adrenalin with a tissue-specific chalone, which forms an essential part of the mitotic homeostatic mechanism of the tissue[2]. It has been further suggested that the chalone–adrenalin inhibitor operates on the mitosis genes[12], in the manner of a bacterial effector substance, and that mitotic duration may be inversely related to the concentration of essential metabolites formed under gene instruction in antephase[13]. If this is true then the reduced antephase syntheses caused by an excess of chalone-adrenalin inhibitor could lead to the slower completion of mitosis, or even to complete inhibition. These are matters for further investigation.

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RELATIVE GROWTH OF TROPHOBLAST AND TUMOUR CELLS CO-IMPLANTED INTO ISOGENIC MOUSE TESTES AND THE INHIBITORY ACTION OF 'METHOTREXATE'

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THE first person to direct attention to the similarities between trophoblast and tumour cells was Beard[1] in 1902; this author[2] later described cancer cells as "irresponsible trophoblasts". Since this time, the many properties which trophoblast cells have in common with tumour cells have been frequently reiterated[3–5]. Some of these properties are listed in Table 1.

Table 1. COMPARISON OF SOME GENERAL PROPERTIES OF TROPHOBLAST AND TUMOUR CELLS WHEN BOTH ARE RAPIDLY PROLIFERATING

Trophoblast	Tumour
Highly invasive	Invasive
Can metastasize	Many can metastasize
Can resist homograft rejection	Can resist homograft rejection
Cannot be serially transplanted	Can be serially transplanted
Uncontrolled trophoblastic growth →	Chorionepithelioma

The trophoblast is one of the most primitive of embryonic cells, being an extra-embryonic derivative of the blastocyst. Trophoblast cells, during their invasive period, are the most highly invasive and destructive cells known; they are more active in both these respects than any known malignant cells. Conversely, tumour tissue has been described as an analogue of the implanting mouse embryo[6]. The greatest difference between the two cell types is in the limited period of invasive growth and overall life span of the trophoblast due to termination of pregnancy; in contrast, many tumours possess an autonomy which enables them to be serially transplanted. When trophoblastic growth becomes uncontrolled the neoplastic trophoblast (chorionepithelioma) is still histologically and endocrinologically identical with normal trophoblast except in its uncontrolled behaviour.

It has been suggested[7] that benign tumours exhibit all the characteristics of tumour growth except that of malignancy, so that comparison of benign and malignant tumours should yield information on the malignant properties of the cancer cell. Comparison of the trophoblast with malignant tumour cells will take us a stage further than this in enabling a direct comparison to be made of the 'malignant' properties of invasiveness and destruction of normal embryonic cells with those of malignant cells.

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Few experiments on the co-implantation of trophoblast and tumour cells have been reported. Runner[8] co-implanted mouse blastocyts with mammary carcinoma into the anterior chamber of the mouse eye and observed that both implanted tissues grew for 5–7 days, but unfortunately no further observations were made. The trophoblast grows more rapidly in organs such as the spleen[9] and testis[10] than in the uterus; the testis is the most favourable host organ for growth that has yet been tried[10]. In the present article, the behaviour of trophoblast and tumour cells rapidly growing together in isogenic mouse testis is described. The effect of administering 'Methotrexate' to mice bearing testicular single implants or co-implants of trophoblast and tumour has also been studied. 'Methotrexate' was chosen because it is known to inhibit growth of sarcoma 180 in mice[11], and this compound has some beneficial effects in cases of human chorioncarcinoma.

Ectoplacental cones were obtained from *CBA* mice at 7·5 days of gestation. Deciduomata were taken from the uterus, sliced both sides of the centre to reveal the ectoplacental cone, which was then removed with a very sharp needle. Ectoplacental cones were kept in sterile normal saline for 1–2 h before implantation. Crocker tumour (sarcoma 180) was passaged in *CBA* mice for 6 months by subcutaneous implantation. Tumour tissue was obtained by repeatedly cutting a portion of the external area of the subcutaneous implant until pieces of tumour were just small enough to be drawn into a glass syringe. The bore of this syringe was just large enough to take an ectoplacental cone; in this way fragments of tumour of similar size to the ectoplacental cones were obtained.

The right testis of adult *CBA* males, anaesthetized with ether, was withdrawn after incision of the skin and body wall. The tunicae of the testis were pierced with a sharp needle and the glass syringe inserted. The tumour and ectoplacental cone were ejected by blowing through the syringe. The hole in the tunicae was sealed with a drop of collodion and the testis returned to the scrotum. Skin and body wall were clamped with two small metal clips.

Histological examination of the implanted testis was made from 3–10 days after implantation by selecting every fiftieth section of the testis and staining with haematoxylin-eosin. Growth was estimated by the total area occupied in all the serial sections. The results of 75 successful implantations are reported. 'Methotrexate' tablets (Lederle Laboratories) were dissolved in sterile normal saline, the solution filtered and injections made intraperitoneally.

The growth pattern of single implants of trophoblast or tumour into the testis were first studied. Proliferation of trophoblast cells in the absence of tumour cells does not begin until between 3–4 days after implantation; at 5 days, the trophoblast cells are dividing rapidly and invading the testicular tissue. At this time, the trophoblast area is filled with typical giant cells (Fig. 1). Two days later, about one-quarter to one-third of the testis has been eroded away and the giant cells line a sac containing chiefly red cells (Fig. 2). To test whether the 'mass action' transplantation phenomenom applied, two ectoplacental cones were implanted together. There was mutual enhancement of the rate of growth of both trophoblasts, the areas of which were very much greater at 3 days after implantation than those of single implants. Seven days after implantation the two trophoblasts occupied the entire testis (Fig. 3).

Implants of tumour alone appeared to grow somewhat less rapidly than those of trophoblast alone during the first 5 days after implantation. At 7 days, the area of testis invaded by tumour cells was usually considerably less than that occupied by trophoblast derived from a single ectoplacental cone.

Co-implantation of tumours and trophoblast resulted in a very small focus of proliferating cells at 3 days after implantation. This consisted chiefly of trophoblast cells in active mitosis and a few giant cells were also visible. This was a similar stage of growth to that of trophoblast implanted alone after 5 days. Tumour cells were also rapidly dividing, but occupied only about one-twentieth of the area of the trophoblast cells. There was some evidence of phagocytosis of a few of the tumour cells by the invading trophoblast. Seven days after implantation the trophoblast had eroded the same area of testis as that due to a trophoblast implanted alone; however, the area occupied by the tumour was greatly increased above that normally found for a single implant of tumour alone (Fig. 4). Examination of the junction of trophoblast and tumour cells (Fig. 5) showed some degree of phagocytosis of the tumour cells but otherwise both giant and tumour cells appeared normal. Ten days after implantation the

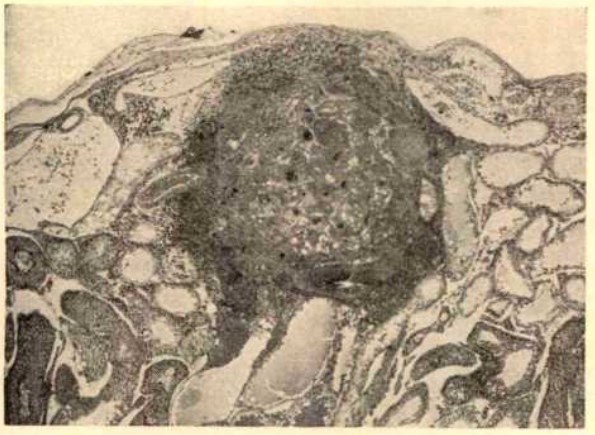


Fig. 1. Typical early stage in proliferation of trophoblast alone (5 days after implantation)

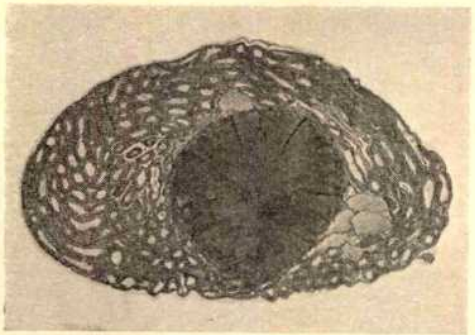


Fig. 2. Typical erosion of the testis by trophoblast at 7 days after implantation

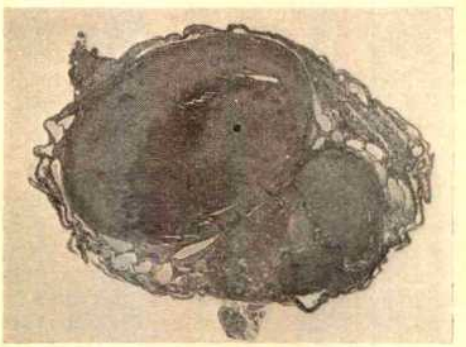


Fig. 3. Almost complete erosion of the testis at 7 days after implantation of 2 ectoplacental cones. Note enhancement of one trophoblastic area

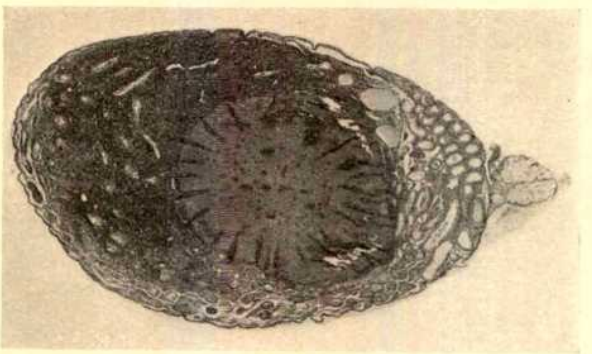


Fig. 4. Growth of tumour and trophoblast cells at 7 days after co-implantation. Note the large area occupied by tumour cells

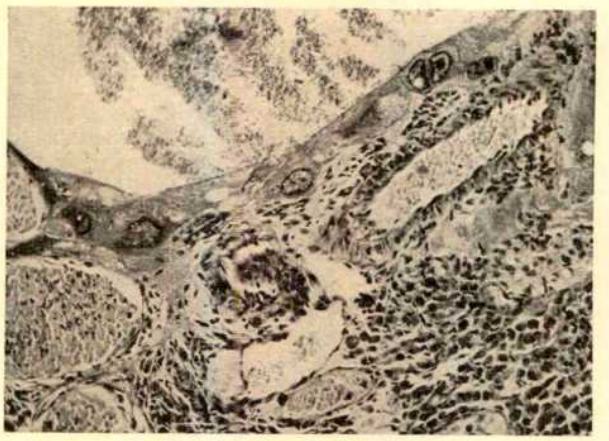


Fig. 5. Neighbouring trophoblast and tumour cells

trophoblast area had diminished considerably and appeared to have been occupied by tumour cells.

In experiments designed to inhibit trophoblast and tumour growth, 'Methotrexate' was administered 2 h after implantation and then daily for the following 3 days. Testes were removed 6 days after implantation. Doses of 'Methotrexate' are expressed as the total dose given during the experiment. Small doses of 'Methotrexate' (0·15 mg) had no effect, but double this dose (0·3 mg) produced some slight inhibition of tumour growth but did not inhibit trophoblastic growth. Larger doses of 'Methotrexate' (1·0 mg) completely inhibited growth of single implants of tumour in 80 per cent of the mice; in the remainder, tumour growth was only just discernible. At this dose level and a higher level (2·0 mg) the trophoblast area was reduced about four-fold (Fig. 6), but was histologically identical with trophoblasts in normal mice. No tumour cells were visible at these higher dose levels in the co-implanted testes.

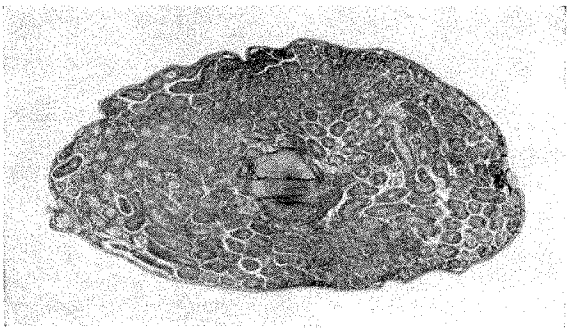


Fig. 6. Effect of 'Methotrexate' (1·0 mg) administered to host mice on the growth of trophoblast and tumour. Trophoblast growth has been severely inhibited and no tumour growth can be seen

The phenomenon of enhanced tumour growth in the presence of rapidly dividing embryonic cells is well documented[12–14]. However, adult cells when transplanted with tumour cells fail to enhance tumour growth. It has been suggested that embryonic cells provide a primary stroma[15] or a more favourable matrix[14] for the initiation of tumour growth. In the present work it has been shown that not only is tumour growth enhanced by the presence of actively dividing trophoblast cells but that initial growth occurs sooner after implantation. In the same way, one trophoblast may enhance the growth of a neighbouring trophoblast. The milieu of a rapidly dividing cell population may induce more rapid proliferation in a neighbouring cell population. How this occurs is not yet known.

The invasiveness of trophoblast in the testis has been fully described[10]. Destruction of testicular tissue occurs after infiltration of trophoblast cells between the seminiferous tubules and takes place by lysis rather than by phagocytosis. Kirby[10] suggests that a cytolytic agent diffuses ahead of the advancing trophoblast as he observed a layer of cell debris and extravasated blood between the trophoplast and the seminiferous tubules. No phagocytosis of unchanged testicular tissue has been observed in either Kirby's or the present work, but a few tumour cells appear to be ingested by the trophoblast cells. Generally, both trophoblast and tumour cells appear to coexist quite normally at the junction of the two-cell types. The essentially normal appearance of host tissue next to the invading trophoblast has also been observed in other host organs such as the spleen[9]. Not all cells cohabit with trophoblast tissue; in the same way some organs such as the liver are not suitable sites for implantation of trophoblast (D. R. S. Kirby, private communication). Tao[16] has shown that, whereas autologous trophoblast–lung cell mixtures are compatible *in vitro*, trophoblast–liver mixtures are mutually incompatible and necrosis of both cell types occurs.

In the present work, observations have generally been made quite soon after implantation during the maximal growth and invasive phase of the trophoblast in order to avoid studying testes in which implant growth has been restrained due to lack of *lebensraum*. As trophoblast is the first cell to begin actively proliferating and invading the testicular tissue it is somewhat surprising, under these circumstances, that nearly all the tumour implant is not engulfed and destroyed. However, as soon as the trophoblast begins to proliferate the milieu of active cell division appears to stimulate earlier division of the tumour cells and, although some are undoubtedly phagocytosed by trophoblast cells, sufficient survive to produce eventually greater tumour growth than normal. The ingestion of tumour cells does not appear to occur frequently, as the tumour seeks to evade the rapidly approaching 'front' of giant cells by invading neighbouring testicular tissue. In this way, both tumour and trophoblast cells make full use of the *lebensraum* of the host organ. This impression is confirmed by the work of Cole and Paul[17], who found that trophoblast cells from mouse blastocysts implanted on to a monolayer of HeLa cells migrated outwards in typical fashion and that the HeLa cells preferred to retreat rather than be engulfed. When ectoplacental cones were implanted into spontaneous mammary carcinomata the trophoblast cells actively invaded and eroded the tumour tissue[19]. It appears that trophoblast always has the strategic advantage of rapid proliferation even in an environment, such as a tumour, when it might be expected to be destroyed during the latent period immediately following implantation, before proliferation of the implant begins.

The present trophoblast–tumour model offers an ideal situation for testing drugs for specific anti-tumour effects. It is an essential of this model that the tumour cell be nearly as rapidly dividing as the trophoblast cells. In the case of the Crocker tumour (originally a spontaneous mammary carcinoma), although it proliferated less rapidly than trophoblast it was nevertheless more susceptible to 'Methotrexate' than the trophoblast. Such a result suggests some specific anti-tumour action as trophoblast was used as a control. This model may pave the way for future chemotherapeutic tests. In this way, it may now be possible to test for specific anti-tumour drug action rather than general growth-inhibitory effects.

I thank Lederle Laboratories for their donation of 'Methotrexate' and Prof. A. Haddow for his active interest in this work. This investigation has been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council and the British Empire Cancer Campaign for Research, and by the National Cancer Institute, U.S. Public Health Service.

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LETTERS TO THE EDITOR

PHYSICS

Sparkover in Long Uniform-field Gaps

THE object of the work described in this communication was to measure the static breakdown voltages of long uniform-field spark gaps in atmospheric air, and to investigate the mechanism of sparkover. The measurements were made using electrodes of the Bruce profile[1], for which sparkover occurred in a uniform electric field at electrode spacings up to 17 cm.

The gap voltage was obtained from a high-stability electrostatic generator, and measured using a resistance divider of approximately 800 MΩ total resistance: to minimize the effect of the temperature variations on the divider ratio, both arms of the divider were constructed from resistors of the same type, mounted in the same oil-filled container. The output voltage from the divider was measured to within ±0·05 per cent using a digital voltmeter.

All measured sparkover voltages have been corrected to 20° C, 1,013·25 mbars and a humidity of 11 g/m³. In the temperature and pressure correction, the curve given by Bruce[1] was used, and in the humidity correction a factor of +0·2 per cent per g/m³ increase in humidity[2,3]. The comparative accuracy of measurements made under different atmospheric conditions is considered to be ±0·17 per cent while the absolute accuracy is better than ±0·25 per cent.

Preliminary experiments carried out with the gap in the open atmosphere of the laboratory produced an unacceptable scatter in sparkover voltage measurements which was presumably due to dust. The measurements reported here were made using the arrangement of Fig. 1, with the gap in filtered atmospheric air within an aluminium enclosure (8-ft. cube): the inside walls of the enclosure were frequently cleaned.

The following experimental procedure was adopted: After the electrodes had been cleaned and adjusted, air was blown through the enclosure at ~200 ft.³/min for about 2 h; at the same time, a voltage of value ~10 per cent below the expected sparkover voltage was applied to the gap to allow the potential divider to attain temperature equilibrium. Sparkover voltages were recorded 1 h after switching off the blower: after each sparkover, the output voltage of the generator fell to about 10 per cent of the sparkover voltage (V_s) of the gap, before automatically resetting to a value ~0·5 per cent below sparkover voltage. By means of a motor-driven potentiometer, the gap voltage was then increased at approximately 0·5 per cent V_s/min until sparkover again occurred. The time interval between succeeding sparkovers was about 2 min.

At each gap setting, about twenty-five sparkovers were required to stabilize the gap, as shown in Fig. 2. Thereafter, a very stable breakdown condition obtained, with no 'low freak' sparkovers, and the sparkover voltage for the gap was taken as the mean of the next twenty to thirty individual breakdown measurements.

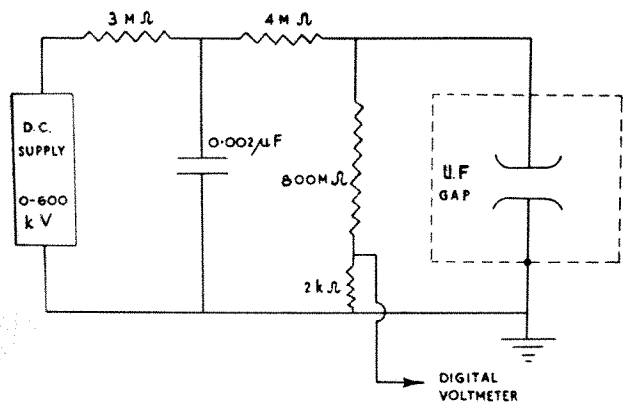


Fig. 1. Circuit diagram

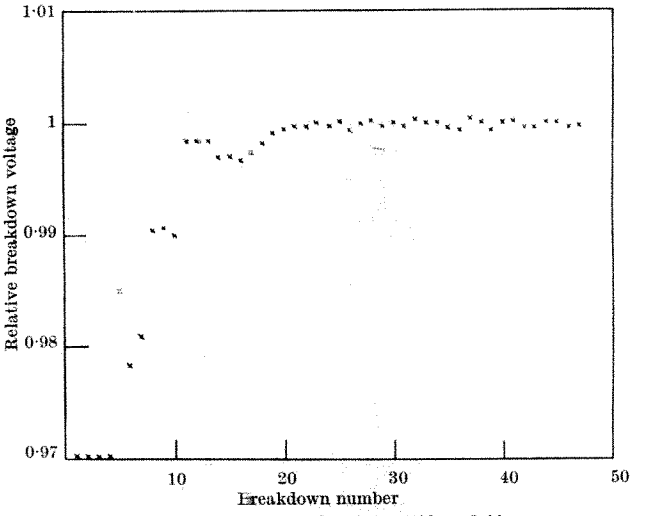


Fig. 2. Sequence diagram for 12-cm uniform field gap

Two series of measurements were made for each gap length, at different times, the mean sparkover voltage in the two cases agreeing within the comparative accuracy of ±0·17 per cent already quoted. The results obtained lie within 0·2 per cent of the values given by:

$$V = 24{\cdot}49S + 6{\cdot}61\sqrt{S}\ \text{kV}$$

where V is the sparkover voltage at 20° C, 1,013·25 mbars and humidity of 11 g/m³, for an electrode spacing of S cm.

In Table 1, the present results are compared with those of Schröder[3], who conducted similar experiments with filtered air and gap lengths of up to 9 cm. The results are in good agreement, but the mean deviations reported by Schröder are considerably greater than in the present work and, in addition, occasional 'low freak' sparkovers were observed during his calibration.

The reduced scatter and absence of 'low freaks' in the present investigation are, we think, due to improved experimental conditions, in particular with regard to dust: the result is significant because of the statement made by Schröder that the scatter in his results was to be expected at the longer gap lengths (> 6 cm), where, he claimed, the Townsend mechanism of breakdown was replaced by a modified 'kanal' mechanism. This claim was supported by oscillograms obtained using a photomultiplier to record the growth of ionization in the discharge gaps at or near the sparking threshold[4].

We have carried out some preliminary work of this kind using a photomultiplier: two forms of oscillogram are obtained, and are shown in Fig. 3. Fig. 3*a* shows succeeding avalanches produced presumably by photoelectric effect at the cathode (Townsend mechanism), while Fig. 3*b* shows an initial avalanche followed by an 'aftercurrent' without evidence of successive Townsend-type secondary avalanches. The 'aftercurrent' was stated by Schröder to

Table 1

Gap length (cm)	Present results: Mean breakdown voltage (kV)	Present results: Deviation (per cent) Mean	Present results: Deviation (per cent) Max.	Schröder's results (ref. 3)* Mean breakdown voltage (kV)	Schröder's results (ref. 3)* Mean deviation (per cent)
3·5	98·0	0·04	0·12	98·24	0·12
6	163·4	0·05	0·09	163·24	0·4
8	214·69	0·05	0·13	214·37	0·5
12	316·49	0·07	0·12		
15	392·83	0·04	0·12		

* Schröder's results have been adjusted to an absolute humidity of 11 g/m³.

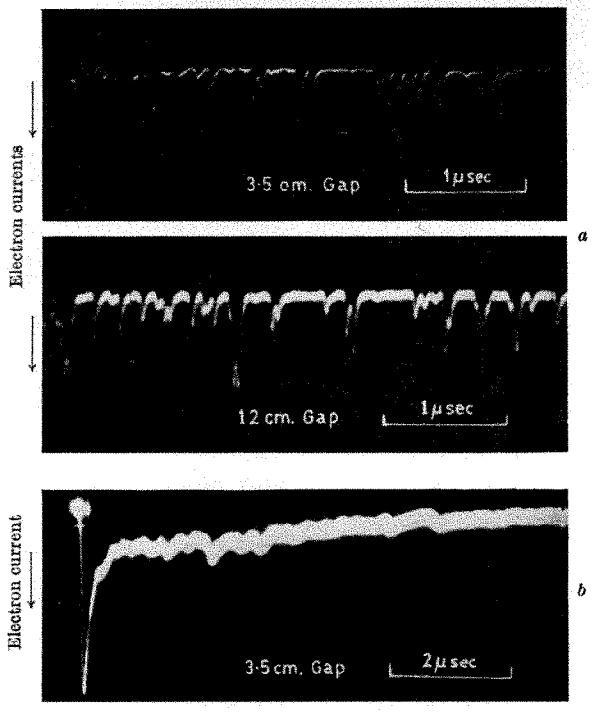


Fig. 3. Oscillograms obtained using a photomultiplier to record the growth of ionization in the gap. *a*, Oscillograms showing generations of avalanches; *b*, oscillogram showing 'aftercurrent'

be due to delayed electrons, probably produced by detachment, which, avalanching towards the anode, increase the positive ion concentration and lead to a streamer breakdown: as he obtained oscillograms of type A for $d < 6$ cm and of type B for $d > 6$ cm, he concluded that a transition in mechanism took place in air at a value of pd (pressure × gap length) ≈ 4,500 mm mercury cm. In the present work, however, both types of oscillogram were recorded at all gap lengths investigated (that is, from 3·5 to 12 cm), their relative proportion depending on the surface condition of the cathode, the proportion of type A increasing after polishing and conditioning. It is suggested, therefore, that the large avalanches which gave rise to aftercurrents and apparent streamer breakdown in these experiments may have been initiated, not by single electrons, but by large numbers of electrons released simultaneously from microprojections on the cathode surface and/or dust particles: a similar explanation was proposed by Schröder for the 'low freak' sparkovers observed in his experiments in the non-irradiated condition, and was supported by oscillograms of type B being recorded at all gap lengths when an α-particle source in the cathode was used as irradiating agent.

Further detailed investigations of these concepts, together with knowledge of the elementary ionization, attachment and detachment processes, are necessary before a region for the so-called Townsend-streamer transition can be defined for air. The present experiments are being extended to obtain the necessary data.

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Very High Precision X-ray Diffraction

VERY high precision has been obtained in the measurement of X-ray diffraction angles, such that if translated into terms of changes in the crystal lattice constant, a precision of 1 part in 10,000,000 can be attained. All indications are that the limit of sensitivity can be extended even further. Fig. 1 shows the application of the method to measurements of the coefficient of thermal expansion of magnesium oxide over a temperature range of 1° C, and gives a clear idea of the sensitivity that is at present being obtained.

The technique involves the use of a specially designed instrument, the 'automatic precision X-ray goniometer'; precise measurements of intensity and angle are made, and special treatment is given to various systematic errors. Tests show that if care is taken to ensure that there is no variation in the atmospheric absorption of the X-rays, due to variations in atmospheric pressure and humidity (preferably by having the instrument in a vacuum enclosure), then the intensity can be kept constant to at least 0·02 per cent for many hours, and so measured to this accuracy—provided certain further precautions are also taken with the X-ray generating and counting equipment.

The angles are measured by counting pulses to a stepping motor connected to a worm and gear as shown in Fig. 2. The crystal is fitted to a holder connected to the gear shaft. The system has the following properties. Originally around the gearwheel, there is an angular error of ±15 sec of arc, but this can be corrected for by means of a cam and the residual error is reduced to ±5 sec. However, tests over ranges of some hundreds of seconds show that the gear is stepped on correctly to better than 0·1 sec, and this represents the basic sensitivity of the method. On the gear, 0° and 360° occupy the same position, so that the diffraction peaks for a reflecting crystal, rotated through 360°, should be in the same position with regard to the gear, with an accuracy that reflects the basic sensitivity, and not the gross overall error. Tests show that this is the case. Thus there is a closed error loop around the gear (that is to say, the sum of the errors is zero), so that if, when an angle is being measured, each part of the gear is used in turn (for example, an angle of 21° would have to be offset $\frac{360}{21} = 17$ times around the gear during measurement), this should also reflect the basic sensitivity of the method in the precision of the result. Tests, consisting of the measurement of angles in this manner, but starting at differing positions around the gear, indicate that this is so. This closed error loop strategy cannot normally be carried out to perfection, and a small overlap at each new position on the gear will usually occur. The effect of this, however, is small, due to the small overall error.

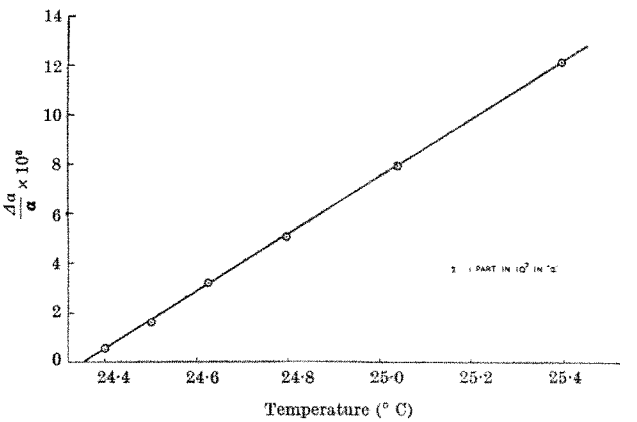


Fig. 1. Thermal expansion of magnesium oxide over a temperature range of 1·0° C

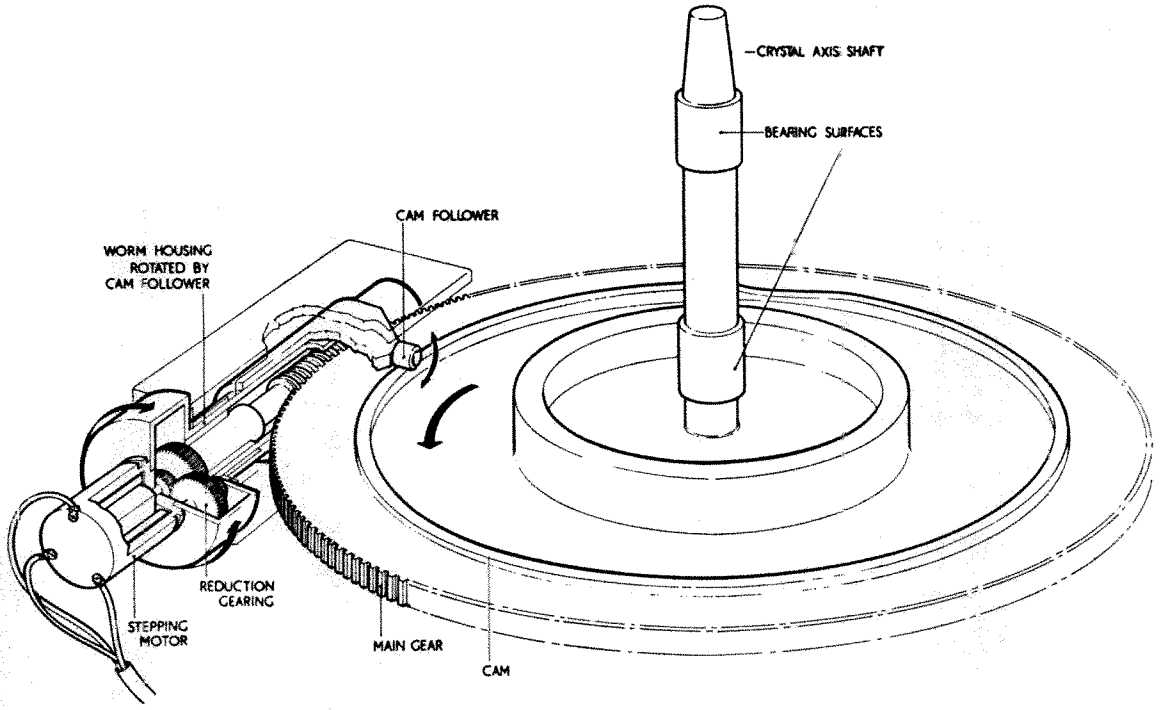


Fig. 2. Method of angle measurement

When applied to problems of diffraction, the method is to measure the angle between two reflecting positions of the crystal, under which conditions, as has been pointed out by Bond[1], errors associated with measurements of the diffracted beam (eccentricity of specimen, absorption, and zero error) do not occur. The method used is basically that of the Bragg spectrometer—the Braggs[2] were clearly aware of the directness of the method.

At the degree of precision dealt with here, there is a further systematic error which requires more stringent treatment than hitherto reported. This is due to the fact that the plane of diffraction may not be sufficiently parallel to the plane of measurement, so that the angle measured is only a projection of the true angle. The plane of diffraction is defined by the directions of the incident beam (that is, the collimator) and the normal to the crystal plane. For a measuring circle in the horizontal plane, errors in the measured angle will occur if either the collimator or crystal has a vertical tilt. A simple procedure has been developed, however, that allows the non-planar conditions, introduced by a collimator tilt of small but unknown magnitude, to be tolerated and which at the same time adjusts the tilt of the crystal to the optimum value. If the crystal is progressively tilted in the vertical plane, the angle between the two reflecting positions of crystal passes through a maximum value, and this maximum value is very close to the correct one, for although non-planar conditions will hold good, the projected angle is very close to the true value. (The reason for this is that in these circumstances the projection function is only changing very slowly.) In practice, the crystal is merely set on the side of a peak, and its tilt is varied until maximum intensity occurs—such conditions result in the maximum angle being obtained. Fairly large tilts of the collimator can be tolerated. For example, at a Bragg angle of 80°, a collimator tilt of 10 min introduces an error of only 0·25 sec in the measured angle, which is equivalent to an error of 1 in 10,000,000 in the crystal lattice constant.

In offsetting the crystal holder around the gear, it is inevitable that small errors in centring and tilting will occur; however, these are allowed for by the method. The instrument is maintained in a thermostatically controlled enclosure and manipulated by remote control; in this way it is protected from outside influences during its operation—an important consideration if the closed error loop method is to be effective. The fact that the procedures can be automatically carried out makes the method particularly convenient. It is arranged that the results are fed into a computer, and a curve is fitted to the profile of the peak, so that small changes of position or shape can be properly described. Graphical constructions of the mid-chord peak can also be used.

Since the peak can be described to 0·02 per cent and 0·1 sec from the point of view of intensity and angle, respectively, it can be used for purposes of calibration, and its subsequent movement measured with, say, changes of temperature and pressure, by intensity measurements alone—provided, of course, that due regard is paid to any changes in shape or intensity that arise.

The high sensitivity which can be obtained with these methods can be applied to experiments where changes of dimension or angle are normally measured macroscopically.

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Photomultiplier Studies of the Temporal Growth of Ionization during the Formative Time of Spark Breakdown in Nitrogen

The purpose of the present communication is to report the use of a photomultiplier technique to record the temporal growth of ionization in a pulsed Townsend discharge in nitrogen, with high values of the parameter pd (pressure × gap length) of ~1,000 mm mercury cm. The object of the investigation was to examine the

secondary ionization processes leading to the sparkover of uniform-field gaps, and to test current growth theory[1] under the aforementioned conditions, where the formative time lags are of the order of microseconds at low overvoltages and evaluation of the growth constant λ[1] requires measurement of the rate of growth of the discharge for currents up to 10^{-5} amp at which space-charge distortion becomes important.

A previous investigation in which formative time lags and transient current growth were measured simultaneously was confined to low values of pd (< 15 mm mercury cm), with a small pulse voltage superimposed on a static approach voltage of up to 3 kV (ref. 2): under these conditions the capacitance charging current flowing in the gap on application of the voltage pulse would be small, and the discharge current could be measured using a resistor in series with the gap. In the present work, in order to satisfy the condition that there should be no initial current in the gap[1], full impulse voltages of up to 50 kV were applied to the uniform-field gap; these had a rise time (10–90 per cent) of 0·2 μsec and time to half value of 500 msec. Consequently, the gap capacitance charging currents had peak values of the order of 1 amp. This, and the much faster growth of ionization obtaining at high values of pd, made direct measurement of the discharge current extremely difficult, and an alternative technique for measurement of the growth constant λ by observation of the radiation from the discharge was used.

Photomultipliers have been used in observations of individual avalanche pulses using static voltages close to the breakdown voltage, and it has been shown[3] that the rise of the electron current recorded by this means is identical with that recorded by the current measurement technique. In the present experiments, the output of the photomultiplier would therefore be proportional to the electron component of the discharge current and, as only the time constant of the current growth was required, calibration of the optical system was unnecessary.

The photomultiplier tube (EMI type 6256S) was mounted outside the glass discharge vessel about 50 cm from the centre of the discharge gap. Suitably placed screens shielded the tube from the light from an ultraviolet lamp used to irradiate the cathode and eliminate statistical time lags. The outputs from the photomultiplier and applied impulse voltage divider were viewed simultaneously on a double-beam oscillograph.

In measurements of the growth constant λ, the photomultiplier was operated at 1,000 V, and the temporal growth of ionization was seen as a smooth exponential curve from which accurate measurements could be made: typical records are shown in Fig. 1. Records were taken at several overvoltages, using various sensitivities on the oscillograph; the corresponding formative time lags (t_f) lay in the range 1·5–15 μsec. The output of the photomultiplier was of the order of 15 V at the end of the formative time, and sensitivities of 0·2–5 V/cm were used, the lower limit being set by the certainty with which the

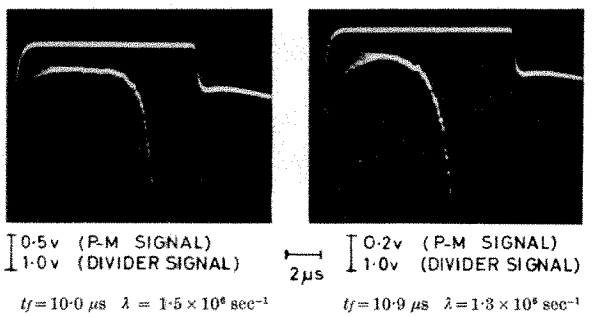


Fig. 1. Oscillograms showing simultaneous records of gap voltage (upper trace from impulse voltage divider output) and discharge current growth (lower trace from photomultiplier output). Photomultiplier tube voltage 1,000 V

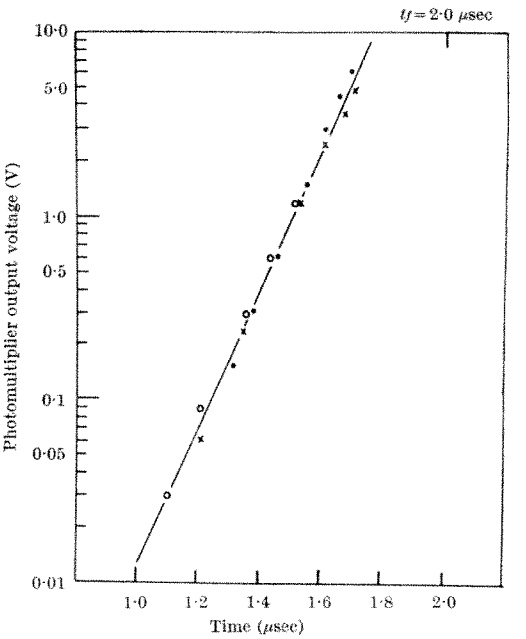


Fig. 2. Composite curve, showing the temporal growth of ionization current as recorded, for the same value t_f, from three oscillograms covering different ranges of the photomultiplier output. ($d = 3·0$ cm, $p = 200$ mm mercury, $\Delta V = 2·9$ per cent, $\lambda = 9 \times 10^6$: Oscillograph sensitivities were as indicated, ●, 5 V/cm; ×, 2 V/cm; ○, 1 V/cm)

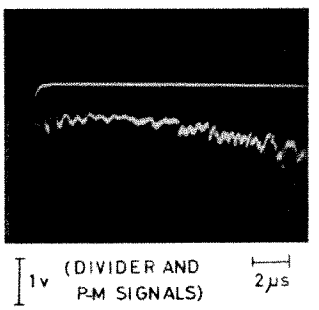


Fig. 3. Oscillogram of photomultiplier output at high sensitivity, showing individual electron avalanches in the early stages of the discharge current growth. Photomultiplier tube voltage, 1,500 V

mean signal could be determined, and the upper by saturation of the photomultiplier circuit.

The records were replotted on a logarithmic scale, and the growth constant evaluated at various overvoltages (ΔV). The accuracy with which λ may be determined depends on the amplitude of the noise superimposed on the growth signal and, with the photomultiplier technique, the output voltage was high enough at gap currents well below space-charge distortion level for measurements to be made at different sensitivities over several orders of magnitude. Fig. 2 shows results from three records for a time lag of 2·0 μsec, obtained at different sensitivities, and plotted to give a composite growth curve over three orders of magnitude. It is seen that the growth is exponential, giving a well-defined value of λ even for very short time lags which could be especially difficult to examine with the electrical technique. The values of λ thus obtained in nitrogen were found to be in excellent agreement with those calculated from ionization data measured in the same system under identical conditions[4].

As formative time-lag calculations indicated that hyperexponential current growth commences at $\sim 10^{-5}$ amp, and as the records obtained were sensibly exponential and referred to times $< 0·8\ t_f$, it is considered that this technique enables the growth constant to be measured when the discharge current is increasing in the range 10^{-9}–10^{-5} amp, compared with a lower limit of $\sim 5 \times 10^{-7}$ amp for the current measurement technique[2].

When the photomultiplier was operated at 1,500 V, the increased sensitivity enabled individual electron avalanches to be recorded in the early stages of the discharge current growth as shown in Fig. 3. If statistical effects were eliminated by providing initiating electrons by means of an α-particle, or short-duration pulse of ultra-violet light, such measurements would be useful in investigations of the earliest stages of impulse breakdown, and could be related to the exact solutions for the temporal growth of ionization[5].

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Negative Temperatures

RECENTLY[1], through mapping the relation:

$$T \leftarrow e^{\psi} \qquad (1)$$

where $\psi = \int g(\theta)d\theta$ and depends on the thermal properties (θ) of the system, a relation was presented between the linear (Kelvin) and exponential thermodynamic temperature scales. It was shown through relation (1) that the absolute zero, $T = 0°$ K, is an inaccessible limit in the linear Kelvin temperature scale[1] defined by the asymptotic behaviour of its own explicit argument, the integrating factor[2], e^{ψ} ($\psi = -\infty$).

As a further consequence of the presented relation (1) it follows that if the so-called 'abnormal' negative energy thermodynamic states are admitted (Fig. 1) introducing negative temperatures[3–6] only the linear Kelvin temperature scale permits an extension, that is, formation of continuous sequence of numbers:

$$+0°\text{K} \ldots +273{\cdot}16°\text{K} \ldots \pm\infty°\text{K} \ldots -273{\cdot}16°\text{K} \ldots -0°\text{K} \qquad (2)$$

including both the positive and negative temperatures.

Thus, the acceptance of an equivalent exponential temperature scale (right-hand side of relation (1)), based on thermal properties of the system as shown by Caratheodory[2] in defining the integrating factor e^{ψ}, contradicts in effect the recently established existence of negative temperatures[3–6]. As a matter of fact, the exponential scale makes it possible to describe only the left-hand side of Fig. 1, with temperature, T, where T is defined:

$$T = \left(\frac{\partial S}{\partial E}\right)^{-1}_{X_i} \gtreqless 0 \qquad (3)$$

where X_i is any thermodynamic property, and $i = 1 \ldots k$.

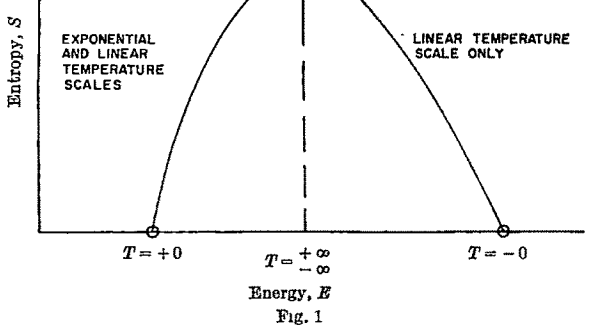


Fig. 1

This contradiction is real, and although no apparent violation of the Second Law of Thermodynamics is brought about by the introduction of negative temperatures—that is, negative energy states[7]—the existence of such 'abnormal' thermodynamic states is of a somewhat artificial nature, for they occur only in special subsystems. That this is so is evidenced by the experiment in which a change from positive to negative temperatures was achieved in an ingenious way which did not correspond to any ordinary reversible process[3–4].

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Observations of Power Station Plumes using a Pulsed Ruby Laser Rangefinder

As part of a comprehensive investigation of the dispersion of chimney gases from a power station, we have been much concerned with studying the behaviour of the buoyant plume during its initial rise. In order to understand its behaviour it is also desirable to have some knowledge of the atmospheric thermal structure. Since plumes from modern power stations are commonly too tenuous for optical observation and rise to heights above the range of convenient surface-based temperature sounding techniques, it has been necessary to develop new techniques. Collis *et al.*[1,2] have already pointed out the value of the pulsed ruby laser rangefinder for studying the atmospheric aerosol.

The necessity of detecting tenuous plumes well away from their source, coupled with the necessity simultaneously to detect the stable layers which profoundly influence plume behaviour, led us to investigate the use of the pulsed ruby laser rangefinder as a tool in pollution investigations.

Observations were made on July 6, 1965, at the 360-MW Tilbury power station with the prototype pulsed ruby laser rangefinder described in Table 1. A series of probes was made through the plume at five angles of elevation from 30°–10°, at 1-min intervals, on a constant bearing of 070° T. Oscilloscope traces from three of these probes are shown in Fig. 1. The regions of enhanced backscatter due to the plume have been marked. The envelope of the plume deduced from the series of probes is shown on a vertical section in Fig. 2. The pulsed ruby laser rangefinder is set up some 200 m from the stacks of the power station. The plume was emitted at a height of 100 m, and its axis intersected the section obliquely at a range

Table 1. G. AND E. BRADLEY PROTOTYPE PULSED RUBY LASER RANGEFINDER (1964)

Laser: 17 cm × 1·3 cm, 60°/C-axis ruby crystal (water cooled). Passive Q-switch using kryptocyanine solution.
Pulse length: 20 nsec
Peak power: 200 MW.
Beam width: 1·5 mrad.
Receiver: 20-cm diameter reflecting telescope with 5-mrad field of view.
Detector: Eleven-stage EMI photomultiplier 9558C.
Receiver bandpass: 20 Å.

of about 1 km. The wind speed was 5 msec^{-1}. The top of the plume has levelled out abruptly at a height of 600 m. From Fig. 1, it is apparent that the character of the environmental aerosol also changes abruptly at this height. Below 600 m the signal shows rapid fluctuations of the order of 10 per cent or more of the mean signal. Above 600 m the fluctuations do not exceed 2 per cent. This transition is strongly indicative of a stable layer overlying the adiabatic mixing layer. The rise of the plume had evidently been limited by this stable layer.

It should be emphasized that the tenuous plume was barely visible to the eye at the time of these observations, even though there were portions scattering up to ten times as much light as the environmental aerosol. The threshold of detection of a plume is clearly determined by the concentration and variability in the environmental aerosol, but we have found that plumes which scatter only 50 per cent more than the environment are normally detectable. The present equipment is capable of detecting such plumes at ranges of several kilometres.

Although the observations that have been described are of a single plume, we often observe up to four separate plumes on a single section from our pulsed ruby laser rangefinder. The sources vary in height from 60 m to 150 m,

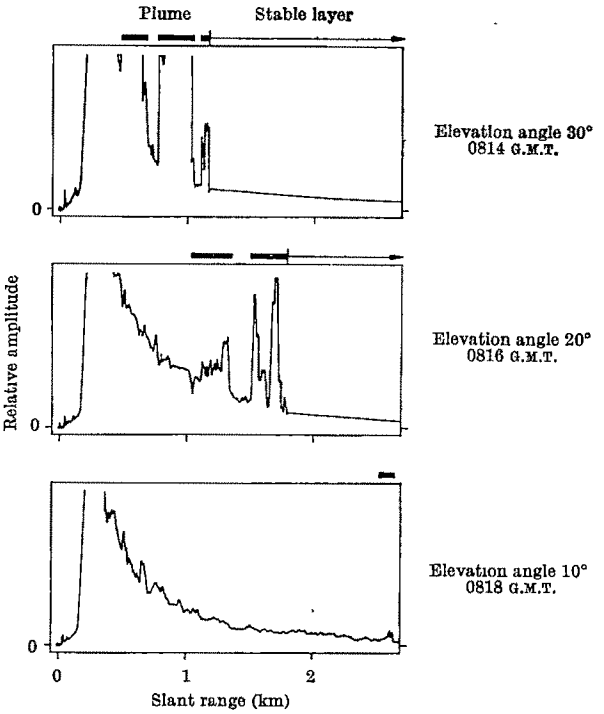


Fig. 1. Pulsed ruby laser rangefinder probes through Tilbury plume, July 6, 1965

and we have been able simultaneously to monitor the quite different behaviour of these plumes under changing atmospheric conditions. This is yet another aspect of the power of pulsed ruby laser rangefinders in pollution research, which judging from the present results will prove to be a powerful and versatile tool.

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Size Analysis in the Sub-sieve Range by Electronic Counter

RECENTLY, Harris and Jowett have discussed an inherent shortcoming of the electronic counter which is able to count only those particles above a certain threshold size[1]. Thus a substantial portion of the sample remains unmeasured and in order to express the results by a conventional cumulative percentage, it is necessary to know the total amount of material in the suspension being counted. The authors point out that this could, in principle, be done gravimetrically, but reject this idea as unsuitable, remarking that "one of the potential advantages of the Coulter counter is that, in the suspension prepared for analysis, the concentration of solids need not be known; additionally, the gravimetric analysis would have to be highly sensitive". They go on to discuss several statistical methods for estimating the volume of solids that lie below the threshold of measurement of the counter.

Because we have been confronted by the same problem in our laboratory, we think it pertinent to describe our solution to it. We have found that the falling-drop method of determining the density of small amounts of solution or soil suspensions is admirably suited to the purpose.

A small sample of known volume is drawn from the suspension to be counted and injected into a column of an immiscible liquid with a density slightly less than that of the suspension. If the particles are suspended in water, the organic liquid anisole ($C_6H_5OCH_3$) is most suitable. The drop slowly sinks and, after the terminal velocity has been obtained, the time required for it to fall a specified distance is measured. The terminal velocity of a drop falling in such a system is a function of the difference in density between the drop and the liquid, according to an equation of the type established by Stokes. By comparing the measured time with times observed for drops of identical size, but consisting of salt solutions of various concentrations (and known densities), it is easy to find the

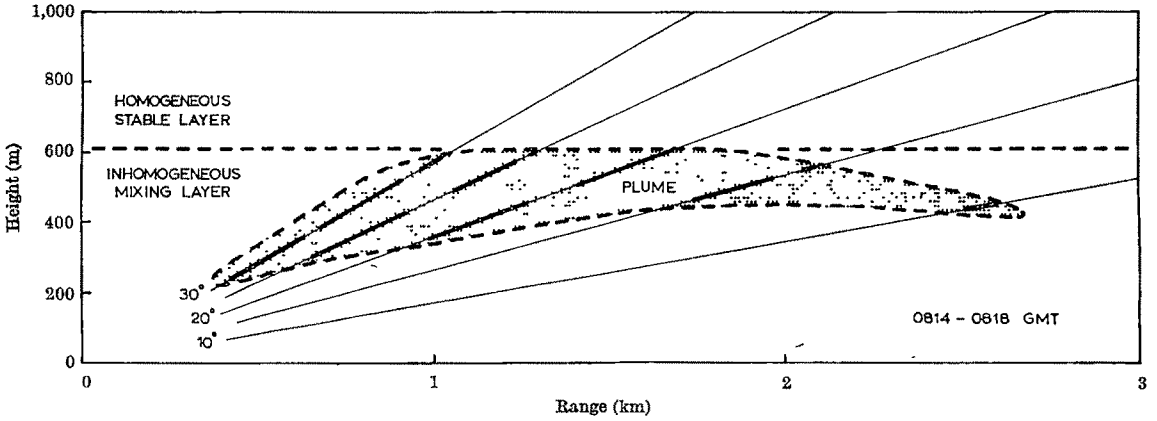


Fig 2. Vertical section from pulsed ruby laser rangefinder probes, Tilbury, July 6, 1965

average density of the drop of suspension. If the particle density is known, the particle concentration of the suspension is then easily computed, either by weight or volume. The calibration procedure makes it unnecessary to know the precise volume of the drops—so long as they are identical—and if drops of standard density are interspersed with 'unknowns' during a series of analyses, temperature and shape corrections to Stokes's law are avoided. The determination requires less than a minute per drop. Density measurements performed in this manner are usually accurate to the fourth decimal place—based usually on the observed mean of three drops. The error introduced by small differences in the volume of the drop is small; using a drop size of about 40 mm^3, the necessary accuracy is ± 1 mm^3 in order to obtain reliable results.

As the determination is simple and rapid, the falling-drop method can easily be applied to the complete grain-size analysis by sedimentation velocity procedures, and the method can be used to determine the density versus depth and time function in a sedimenting column with convenience and precision.

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GEOLOGY

Permanency of Continents and Ocean Basin Lineations

Carr[1] recently proposed that the close correspondence between lineations on continents and the ocean floor and a theoretical shear net calculated by Vening Meinesz[2] militates against the hypothesis that the continents have drifted since Pre-Cambrian time. The long linear sea floor fracture zones, all discovered since Vening Meinesz drew his net, are admirably suited for testing such a correspondence. Some appear on the charts used in Carr's investigation, but many newly discovered ones do not. The whole pattern of fracture zones does not show a significant correlation with the shear net.

The Mendocino and Murray fracture zones follow the shear net in a very remarkable way[3], but they are only two of a vast system of sub-parallel and relatively evenly spaced fracture zones extending to the south. The Molokai, Clarion, Clipperton, Galapagos, Marquesas, and Easter fracture zones[4] have the same westerly to south-westerly trends across an enormous area of the eastern Pacific spanning 60 degrees of latitude. In the same region, the Vening Meinesz shear net changes direction by about 90 degrees. A two-step migration of the poles was capable of explaining the trend of the first four zones discovered[3], but it is not adequate to produce the pattern now known.

Numerous fracture zones have also been discovered in the Atlantic Ocean basin[5]. Almost all trend roughly west. The shear net, on the other hand, trends every direction in the Atlantic, and the only westerly trends are in high northern latitudes. Considering that the intensity of shearing is supposed to be at a maximum in the equatorial Atlantic, the total lack of correspondence appears crucial.

Fracture zones are also common in the Indian Ocean basin[6] where the intensity of shearing should be weak. Some follow the shear net and some do not.

In sum, the Vening Meinesz shear net shows a close correspondence with the margins of continents, but this is scarcely surprising because it was drawn to do so. It shows little correspondence with the whole pattern of enormous faults discovered on the sea floor during the past 15 years. It appears that this net should be allowed to rest in peace. What could be fruitful would be further application of the pioneering ideas and techniques developed by Vening Meinesz for the analysis of global shear patterns resulting from possible changes in speed of rotation and polar wandering indicated by geophysical data.

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Global Network of Mobile Belts

Prof. J. Tuzo Wilson[1–3] has suggested that mountains, mid-ocean ridges, and major horizontal shear faults are not isolated features, that few come to dead ends, but that they are connected into a continuous network of mobile belts around the Earth which divide the surface into several large rigid plates.

This communication directs attention to a possible continental continuation of the network, based on the interpretation of the British–Scandinavian Caledonian chain[4–5], and the Mediterranean and Persian Alpine mountains as narrow mountain systems[6].

According to new investigations, the graben zones of the British–Scandinavian Caledonian chain (Fig. 1) originated as horizontal shear faults, caused by the main Caledonian stress[7]. The horizontal movements continued in post-Caledonian time, but as a result of epeirogenic Hercynian and Alpine movements between the blocks[8]. These movements have given the fault lines their character of graben zones.

With regard to the Alps, de Sitter[9] has concluded that in origin the Rhine trend and the Hercynian trend are a simple conjugate set of wrench faults, brought about by the Alpine stress.

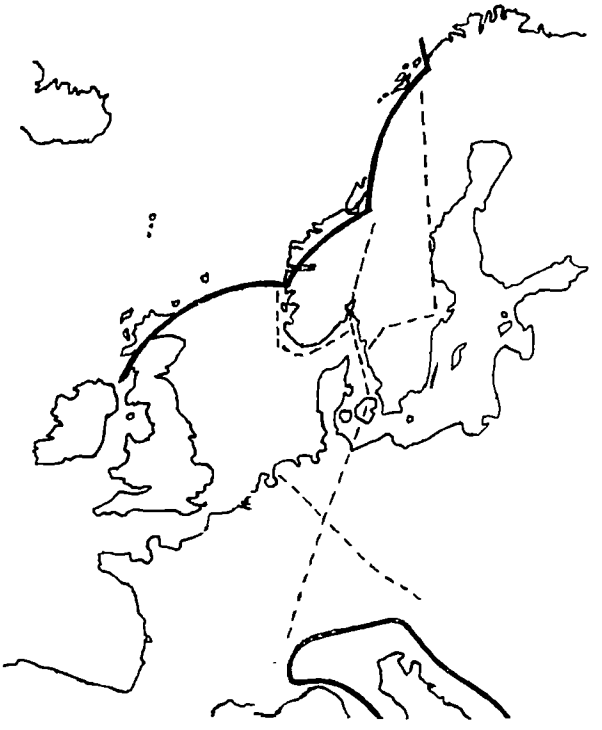


Fig. 1. Connexion between the graben zones of the British–Scandinavian Caledonian and the Mediterranean Alpine narrow mountain systems. Heavy lines represent axes of primary arcs and dashed lines fracture zones

An important feature is the connexion between the major fault lines in Europe[10]. They form continuous fracture zones between the hinterlands of the mountain systems, they have repeatedly been exposed to fault movements, and they terminate between the primary arcs (Fig. 1).

The fracture zones in the forelands are mainly submarine. They tend to terminate near the junctions of the primary arcs. The position of the partly continental fracture zones in the foreland on both sides of the Persian primary arc[1] supports this view. The conclusion seems warranted that they represent a continuation of the fracture zones in the hinterlands.

According to this interpretation, the junctions of the primary arcs form important cross-points between the mobile belts of mountain chains and major faults.

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Orogenic Belts of the Northern Uganda Basement

THE Tectonic Map of Africa is being revised. It is appropriate, therefore, to direct attention to certain conclusions, produced at the second Symposium on African Geology held in March 1965, at Bedford College, London, regarding the status of previously established orogenic belts in the Northern Basement of Uganda.

Holmes[1] postulated the 'West Nile orogenic belt' after the predominant north-north-easterly trend of the gneissose rocks occurring in the district of that name in Uganda. He supposed that in the north the belt is either terminated by, or swings around to join the north-westerly trending Chua and Madi belts of de la Vallée Poussin[2] and Combe[3], respectively. Holmes further postulated that to the south the West Nile belt 'disappeared beneath' the 'Kibale-Toro' rocks.

Cahen[4] later used the term 'La formation du Nil occidental' for rocks in the Congo Republic which underlie the Kibalian and which are continuous with the gneisses of West Nile.

The existing Tectonic Map of Africa[5] places the West Nile rocks in the division 'Precambrian I Anterieur a 3 milliards d'années'. The Chua belt is not shown.

Mapping in West Nile by Hepworth[6,7] and Macdonald[8] has in part confirmed and in part modified Holmes's postulates. Thus Macdonald recognizes the north-westerly trending, Madi folding in the north of the district as a late tectonic event, and Hepworth has shown that in the south rocks of Kibalian trend are unconformable on, and of lower metamorphic grade than, the north-north-easterly striking rocks which they overlie. Although the axial trends of the Kibalian and Madi folds are similar they affect rocks of different groups and there is no evidence that they are the same age.

In the country which lies between these north-westerly trending belts in West Nile we have established three main tectonic groups. These are distinguished by characteristic structural styles, axial trends and metamorphism, and each may be orogenic in status. Macdonald has named them the 'Watian', 'Aruan', and 'Mirian'. The earliest, or Watian, is characterized by an east–west axial fold trend and a charnockite–granulite facies, partly anatectic metamorphism. This group has been retrogressively metamorphosed and partially re-worked in the ensuing Aruan tectonism. A time break between the Watian and Aruan is indicated by a dyke phase in the Watian which is absent in the other groups, or is represented by amphibolites in re-worked Watian rocks. The Aruan folds plunge gently north-north-easterly with steep axial planes and are associated with amphibolite facies metamorphism. The north-north-easterly axial trend of the Aruan and re-worked Watian rocks is the dominant structural direction in western and southern West Nile. The third tectonism, the Mirian, is characterized by recumbent isoclinal folds overturned from the south-east and south-south-east with flaggy axial plane cleavages and epidote–amphibolite facies metamorphism. This tectonic group is predominant in the north and east of the district.

Throughout much of the district the flaggy Mirian folding is succeeded by a fourth tectonic event with folding on north-westerly trending axes. Macdonald has shown that these folds, which in the north pass locally into mylonitic shear zones, increase in amplitude towards the Madi belt with which they are approximately co-axial, and so refers to them as 'Madian'.

In southern West Nile the Watian and Aruan rocks are cut by structural belts of tightly folded and foliated rocks with thick mylonites in the middle. Hepworth has termed these 'Refoliation Zones' and considers that they are the depth equivalent of the Mirian. He has pointed out that, although there is a difference of direction (north-east and north-west, respectively) and perhaps age, there is a strong structural resemblance between these 'Refoliation Zones' and the Aswa Zone.

In north central Uganda the Madi belt is continued south-eastwards by the Aswa Zone. This consists of a 6-mile-wide belt of strongly-foliated Basement rocks tightly folded on gently north-westerly plunging axes. The cover rocks of the Madi belt in West Nile are absent. The middle of the belt is mylonitic. A late kinematic granite extends for 30 miles along the northern part of the Zone. Some of the mylonites are refolded to form steep southward-plunging minor folds and these, together with a displacement of the granite by related cross-faults, indicate a late stage of transcurrent movement.

We propose to use 'Chuan' as a general term to include the Madian of West Nile and the Aswa Zone deformation, following Holmes's use of the name especially in his 'provisional map of the Pre-Cambrian orogenic belts of . . . Africa'[1].

Macdonald's[9] work in Karamoja, north-eastern Uganda, showed a suspected three-fold tectonic grouping with mylonitic shear zones which is remarkably similar in structural style and metamorphism to that now established in West Nile. The third group in apparent age in Karamoja, represented by the Flaggy Moroto Granulites and the Karasuk Group, is of similar style and lithology to the Turoka Series of Kenya and the Kenya 'Basement Complex System' in general and may correspond to part of the geosynclinal sediments of the Mozambiquian orogeny. At the same time, this third group in the Karamoja area strongly resembles the Mirian of West Nile in structural style and metamorphism.

Isotopic potassium/argon age determinations of micas from a Watian charnockite and an Aruan quartzite in West Nile give ages of 541 ± 22 m. yr. and 659 ± 26 m. yr., respectively[10]. These are ages of Mozambiquian range. As it is now known that the 'West Nile belt' comprises rocks affected by several orogenic events, the question arises as to which one is responsible for these apparent ages. Both the Aruan and Mirian produced pervasive metamorphisms in West Nile and the Chuan tectonism was also effective in northern West Nile and north-central Uganda. Thus the apparent age might be related

Table 1. TECTONIC EVENTS IN NORTHERN UGANDA

Southern West Nile	Northern West Nile	Aswa Zone	East-central Karamoja	
[KIBALIAN]← Relationship not known →MADIAN		CHUAN		
Folds trending NW No associated mylonites	Folds trending NNW–NW Mylonites trending approx E–W	Folds trending NW Mylonites trending NW		
MIRIAN Overthrust folds with NE–ENE axes			Kerasuk Group	? MOZAMBIQUIAN
Mylonites in 'Refoliation Zones' of the 'foreland' area, trending ENE	No evidence for mylonites in 'foreland' areas	(Note resemblance between southern W.N. 'Refoliation Zones' and Aswa Zone)	Flaggy Moroto Granulites Mylonites in 'foreland' area	400–650 m. yr.
ARUAN Folds trending N or NNE		Northerly trending gneisses cut by the Aswa Zone	Central Karamoja gneiss tectonism	? ?
WATIAN Folds trending E–W		Present as reworked granulites	Chernockite Group tectonism	? DODOMAN

to any of these tectonisms. It seems likely, however, that the Mirian, as expressed by the flaggy refoliated gneisses throughout the district and by the 'Refoliation Zones' in the south, was the dominant tectonism which imposed a Mozambiquian age on Aruan and Watian rocks. This correlation is perhaps supported by the strong tectono–metamorphic resemblance between the Mirian and the 'Mozambiquian' rocks in Karamoja. It is suggested that the Mirian may represent an early phase of the Mozambiquian and that the Chuan is a later phase, the age range of the Mozambiquian (*c.* 400–650 m. yr.) being enough to span both events (see Table 1).

If these suggestions are correct the 'pervasive event' invoked by geochronologists for the ubiquitous Mozambiquian ages in the African Basement acquires an identity as far as northern Uganda is concerned in the form of the Mirian and Chuan orogenies. The presence of granites in the Aswa Zone part of the Chuan lends support to this supposition.

As a result of these studies several considerations arise:

(1) The 'West Nile orogenic belt' is not a valid entity as it consists of four tectonic elements, the Watian, Aruan, Mirian and Chuan. Its status on the revised tectonic map should be reconsidered.

(2) The Chuan orogenic belt, which was omitted from the 1958 tectonic map and which crosses the West Nile belt, should be re-instated. If it is not sufficiently known from ground mapping it is now possible to delineate its extent by photogeological means.

(3) The Mozambiquian orogeny pervaded irregularly the 'foreland' of the northern Uganda Basement and is probably equivalent in age to the Chuan and Mirian tectonisms. This 'foreland' to the Mozambiquian, which is made up of congealed earlier orogenic belts, was thus fragmented by the Mozambiquian movements.

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Recrystallization of Mica in Crenulated Schists

DR. NICHOLSON, in his recent communication[1], quite rightly states that in strain-slipped phyllites micaceous minerals commonly concentrate on alternate limbs of crenulations. This observation has been made by Clough[2] and repeated by King and Rast[3]. In my more recent paper[4] I was essentially concerned with the mechanics of growth of isolated matrix mica flakes under conditions involving annealing recrystallization, and not with the mechanism of chemical transport involved in chemical differentiation, which will be attempted elsewhere. My point, however, that in mimetic recrystallization mica flakes grow so as to become stain-free is amply illustrated by Dr. Nicholson's photographs. In particular his Fig. 2 shows clearly the completely stain-free mica flakes at the closures of microfolds. My other point that at a subsequent stage of recrystallization mica flakes grow parallel to axial planes of folds is again shown by Dr. Nicholson's Fig. 1. At no stage in my discussion did I claim that abundant new mica grows along the axial plane, but I did point out mica flakes growing parallel to axial planes. As my Fig. 4*d* shows, these micas, like Dr. Nicholson's Fig. 1, grow on the limbs of microfolds.

I have no objection to the explanation of quartz migrating from one site in the strain-slipped fabric to another, and I think his is an idea worth stating.

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DR. RAST'S letter is best answered by using his own text[1] which proceeds as follows. "Thus the first stage of [annealing] recrystallization . . . is . . . the regeneration of mica with approximate . . . orientation of the micas involved in the flexure. This regeneration . . . involves a nucleation step. It is an elementary assumption that where flexure of first generation micas is most intense the second generation mica will nucleate more rapidly [than elsewhere]. Assuming . . . initial post-nucleation growth is [the] most rapid, *it is likely that the points of maximum flexure will be the positions of maximum growth of phylloblastic minerals thus producing the phenomenon of metamorphic differentiation* (Fig. 4*b*). . . ." (My square brackets and italics.)

Thus Dr. Rast's text suggests this differentiation is a product of annealing recrystallization while I argued in my communication[2], largely on petrographic grounds, that it is produced during deformation. Since that

communication was written a modern petrological account of the south-west Highland rocks described by Clough[3] has been published[4] and in it there is a very interesting survey of the experimental information on quartz solubility which supports a view supposing silica mobility during deformation rather than the mobility of the components of mica. Secondly, it is difficult not to read the quotation as directly implying mica concentration along axial planes (taking the axial plane to be the surface joining 'points of maximum flexure'[1]). It is true that the caption to Dr. Rast's Fig. 4*b* (ref. 1) does refer "to concentration of mica on the limbs of microflexures", but this arrangement is referred to only once and then indirectly in the quotation given here where it seems to clash with the sense of the text.

I agree that my Fig. 2*b* (ref. 2) shows strain-free micas on fold hinges and this might well be a product of annealing recrystallization; this is not to say that this process produces differentiation. Fig. 1 of my communication[2], contrary to Dr. Rast's suggestion, does not show micas with cleavage and length parallel to fold traces. Instead it shows a schistose fabric continuous from a high mica concentration in one fold limb to a much lower concentration in the adjacent limb. As Dr. Rast observes, this has been described on many occasions, one recent example being in Prof. De Sitter's text-book, *Structural Geology*[5].

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[1] Rast, N., in *Controls of Metamorphism*, edit. by Pitcher and Flinn, 89 (1965).
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CHEMISTRY

Oxidation of Gaseous Formaldehyde

IN their investigation of the oxidation of gaseous formaldehyde at 337° C, Axford and Norrish[1] found that the reaction, which started immediately, was accompanied by a pressure increase. The rate of pressure rise gradually diminished as the reactants became consumed. The major overall reactions were considered on the basis of product analysis to be:

$$2H_2CO + O_2 \rightarrow 2H_2O + 2CO \quad (1)$$

and

$$H_2CO + O_2 \rightarrow H_2O + CO_2 \quad (2)$$

with the former predominating. The propagation steps, as suggested then and modified later by McKellar and Norrish[2], were:

$$CHO + O_2 \rightarrow CO + HO_2$$
$$HO_2 + H_2CO \rightarrow H_2O + CO + OH$$
$$OH + H_2CO \rightarrow H_2O + CHO$$

which together are equivalent to reaction (1). To explain the production of carbon dioxide the following step was postulated:

$$CHO + O_2 \rightarrow [HCO_3] \begin{cases} \rightarrow CO_2 + OH \\ \rightarrow CO + HO_2 \end{cases}$$

Recently, we have found that if the reaction vessel is allowed to mature from run to run and not treated with hydrofluoric or nitric acid between reactions, a delay develops in the attainment of maximum rate of pressure rise. During this stage and later we find considerable quantities of hydrogen peroxide are produced indicating that the reaction:

$$H_2CO + O_2 \rightarrow CO + H_2O_2$$

which proceeds without change of pressure, has been occurring. A cylindrical quartz reaction vessel which had been used for ethylene oxidations for several months without treatment was used for oxidizing a mixture of 100 mm formaldehyde and 200 mm oxygen at 320° C. In this, the hydrogen peroxide which formed rose to a maximum of 70 mm after the reaction had progressed for 30 min implying a 70 per cent conversion of formaldehyde to hydrogen peroxide, while the total pressure had only risen 8 mm. Over a period of several hours the pressure continued to rise by a further 24 mm and the hydrogen peroxide finally disappeared.

New reaction vessels of quartz or 'Pyrex' previously washed with hydrofluoric or nitric acid gave completely different results closely approximating to the observations of Axford and Norrish. The rate of pressure rise was much greater; it commenced almost immediately at its maximum rate and less than 2 per cent conversion to hydrogen peroxide occurred. After three or four reactions had been performed the rate of pressure rise was decreased and its maximum rate delayed, and increasing quantities of hydrogen peroxide began to be formed. Washing the vessels again with nitric acid restored the original conditions of pressure rise and low hydrogen peroxide concentration, but merely evacuating between runs had no effect of this kind; a gradually increasing quantity of hydrogen peroxide was formed as maturing proceeded. In the quartz vessel this process seemed more rapid.

In the experiments of Axford and Norrish, a pressure of about 1 mm of mercury was present in the reaction vessel—due to the use of heated mercury cut-offs—and in repeating this work, Scheer[3] concluded that similar results could be obtained either by introducing mercury at this pressure or by cleaning the silica vessel with nitric acid before each run. On the other hand, use of a matured vessel gave results similar to ours in that there was a delay before the pressure rise reached its maximum rate. His analysis by mass spectrometry showed unidentified peaks with mass numbers in the range 30–95. He ascribed these to peroxides and in particular performic acid was tentatively identified by peaks at 62, 61 and 60. A mass spectrum of reaction products frozen out of our matured reaction vessel showed no such peaks, but we found a conspicuous peak at mass number 34 which we attributed to hydrogen peroxide. Scheer's mechanism for propagation of the reaction was:

$$CHO + O_2 \rightarrow HCO_3$$
$$HCO_3 + H_2CO \rightarrow HCOOOH + CHO$$

and these two steps are equivalent to:

$$H_2CO + O_2 \rightarrow HCOOOH$$

Final products were supposed to arise from decomposition of performic acid or by its reactions with more formaldehyde, but it is evident from the stoichiometry of the reaction that if performic acid is a true intermediate an initial pressure decrease would be observed, contrary to experience.

We have shown that a clean surface is very effective in destroying hydrogen peroxide as it is formed, whereas a series of reactions performed without treating the surface causes this to alter, becoming more inert so that the hydrogen peroxide concentration can build up. We consider that these results can be explained by the following propagation step which can be compared with those outlined above:

$$CHO + O_2 \rightarrow [HCO_3] \rightarrow CO + HO_2$$
$$HO_2 + H_2CO \rightarrow CHO + H_2O_2$$

The net result of these two steps is:

$$H_2CO + O_2 \rightarrow CO + H_2O_2$$

Decomposition of hydrogen peroxide occurs according to:

$$H_2O_2 \rightarrow H_2O + \tfrac{1}{2}O_2$$

Under the conditions which operate in a matured vessel, this last reaction is very slow so that the main course of the reaction proceeds with only a small pressure change. Subsequent decomposition of hydrogen peroxide causes the pressure to continue to rise slowly, though by this time most of the formaldehyde will have been oxidized.

Since the oxidation of ethylene and methanol gives rise to formaldehyde directly, we consider that large quantities of hydrogen peroxide should be formed in suitably conditioned reaction vessels when these substances are oxidized. Recently we have found a 20 per cent conversion of ethylene to hydrogen peroxide at 320° C in a matured quartz vessel. It seems probable that with flow methods even higher yields of hydrogen peroxide may be possible.

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Factors affecting the Mobilities of Anions in Nylon

IT has been reported[1,2] that the diffusion coefficients of anions in nylon rapidly increase with increasing internal concentration. In the case of the trisulphonic dye naphthalene scarlet 4*R*, McGregor, Peters and Petropolous[2] attributed this result to a proportionality between the diffusion coefficient and some power of the activity coefficient of the anion. Atherton and Peters[3] modified the Gilbert-Rideal theory[4] (originally developed to explain the sorption of inorganic acids by wool) so as to cover polyvalent dyes in nylon; they obtained anion activity coefficients of the form $[1/(1-\theta_A)]$, where θ is the fraction of occupied amine sites.

We wish to direct attention to the importance of distinguishing between the influence on diffusion of direct interactions between anion and substrate, and interactions involving the accompanying cations (usually protons). These associate with specific sites in the nylon so that the proton activity coefficient takes the form $1/(1-\theta_H)$; if coupled bulk transport of dye acid occurs, proton activity variation leads to relationships between diffusion coefficient and concentration of the type observed. This effect can only be satisfactorily eliminated by using radiotracers, which enable anion mobilities to be measured. Even when this is done, there remains the possibility that anion mobility can be increased by co-operative interaction with mobile cations. Such effects have been observed in keratin[5]. For acid sorption, the number of free protons is $\theta_H/(1-\theta_H)$, so proliferation occurs near the site-saturation point, and gives rise to a similar experimental result.

The following tracer experiments illustrate these points. The ions investigated were a simple inorganic ion, Br^-, and a monosulphonic dye anion, naphthalene orange *G* (*NOG*, C.I. acid orange 7).

The internal concentration range investigated was the so-called 'amine-dyeing' region. The amine end group content, 0·028 m.equiv./g, was obtained by dissolving the nylon in 70 : 30 per cent phenol : methanol solution and titrating with N/20 hydrochloric acid solution using thymol blue as the indicator. The titration curve of each acid has a shoulder at approximately 0·026 m.equiv./g (at the temperature at which the diffusion runs were carried out), before rapidly rising at low *p*H's due to acid sorption on the amide sites.

It can be seen from Fig. 1*A* that the diffusion coefficient of the inorganic ion in the amine-dyeing region varies only slightly with internal concentration; it is impossible to explain in this case the variation of diffusion coefficient with concentration merely in terms of an idealized activity coefficient of the form $\frac{1}{(1-\theta)}$.

Two curves are shown for the NOG anion. Fig. 1*B*, curve *b*, at internal concentrations less than 0·02

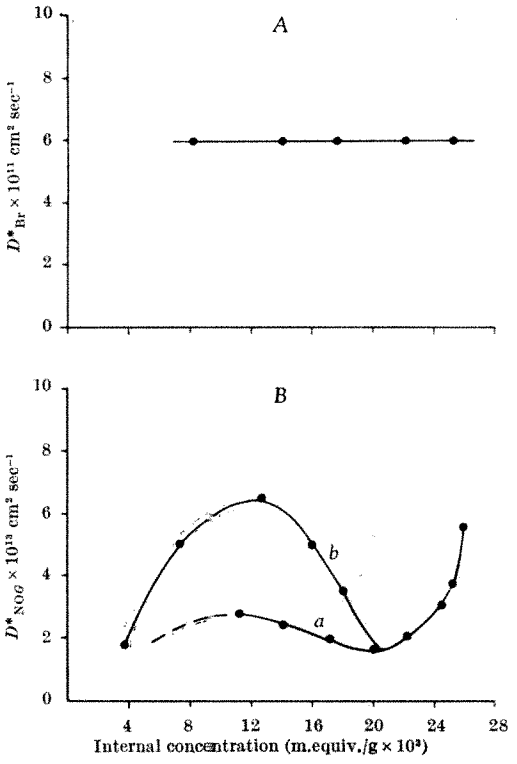


Fig. 1

m.equiv./g, was obtained using buffered solutions (buffer concentration M/100 mixed phosphate) whereas curve *a* was obtained under free acid conditions. Free acid points could not be obtained for internal concentration < 0·01 m.equiv./g since these correspond to external free acid concentrations of 10^{-6} N and lower. The general effect of the use of buffer is to produce a pronounced peak at an internal concentration of about 0·012 m.equiv./g.

In the absence of buffer, the height of the peak is reduced. It has been shown previously that the presence of inorganic electrolytes accelerates NOG in keratin and that even more pronounced effects are observed with polyvalent ions. The pronounced peak in the presence of buffer may well have a similar cause and it is, therefore, of considerable importance to exclude all ions other than those of the free dye acid from the system in investigations of this kind. After allowing for possible ion-acceleration effects, there remains the residual negative slope on curve *a* in the intermediate internal concentration range; such an effect was previously observed in keratin also[6], and was attributed to internal agglomeration. In view of this complex behaviour the $\frac{1}{(1-\theta)}$ term is inadequate at intermediate concentrations.

The dye self-diffusion coefficient increases rapidly with increasing concentration as the available amine sites become saturated ($\theta \rightarrow 1$) as was observed by Peters *et al.*[2] with naphthalene scarlet 4*R*. It remains to be seen whether this is solely due to interactions between the anion and the fixed positive sites, or whether interactions with the hydrogen ions also play a part, analogous to the salt acceleration effect already described.

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Absorption Spectra of Excited Atoms and Excited Ions produced by Isothermal Flash Photolysis of Metal Carbonyls in the Gas Phase

SPIN-ORBIT multiplets of atoms in ground electronic states are particularly interesting for the investigation of electronic energy transfer; simple theoretical considerations indicate that the relaxation rates correlate with the magnitude of the internal energy changes[1]. Measurements of the formation and decay of excited selenium (4^3P) and iodine (5^2P) have already been reported[2,3]. We have recently shown that the flash photolysis of iron pentacarbonyl ($Fe(CO)_5$), which has been investigated in some detail, and of nickel carbonyl ($Ni(CO)_4$), can be used to produce excited atoms in gases under isothermal conditions. Spin-orbit relaxation rates can be measured by kinetic spectroscopy, before polymerization of the normal atoms occurs. The method is general to volatile transition-metal compounds, and the observations therefore reveal numerous experimental systems for energy transfer studies.

A flash-photolysis apparatus of standard design produced a 1700-J light pulse in 5×10^{-5} sec, and absorption spectra were recorded with a Hilger Medium Quartz Spectrograph. In experiments with $Fe(CO)_5$ at pressures of 0·005–0·001 mm of mercury, and argon added to a total of 10–50 mm of mercury, the disappearance of the far ultra-violet continuum of the carbonyl was complete in the first 3×10^{-5} sec of the flash. The absence of any significant rise in temperature may be shown by calculation, assuming each $Fe(CO)_5$ molecule to absorb five light quanta at 2000 Å, or else demonstrated experimentally by deducing the equilibrium concentration in the a^5D_4 and a^5D_3 states from the observed line intensities in absorption and the published oscillator strengths[4].

Table 1 shows the states of atomic iron detected in absorption in flashed $Fe(CO)_5$/argon mixtures. The energies correspond to the lowest of the spin-orbit multiplets[5].

The highly excited levels rapidly decay to populate the spin-orbit components of the a^5D state; relaxation of the a^5D multiplets occurs in 10^{-4} sec. The calculated temperature rise, the rapid decay of the excited atoms, and the increased rate of decay caused by addition of traces of polyatomic gases prove that the extent of electronic excitation is greatly in excess of a Boltzmann distribution at the translational temperature, which remains close to 300° K. (With partial pressures of $Fe(CO)_5$ of the order of 0·1 mm of mercury, relaxation is extremely fast and the population in the lower electronic states is due to a substantial temperature rise.) Provided the $Fe(CO)_5$ is completely destroyed by the flash, the populations in the a^5D states are independent of the energy of the flash, and the most highly excited distributions occur at the shortest delay times. Photodissociation evidently produces atomic iron in a range of electronic states, for example:

$$Fe\,CO + h\nu \rightarrow Fe^* + CO$$

Our original objective was to measure the rate of the $a^5D_3 \rightarrow a^5D_4$ (416 cm^{-1}) transition in argon, and the probability of deactivation has been recorded as $0{\cdot}2 \times 10^{-4}$ at 300° K. This is significantly slower than vibrational relaxation of non-hydrides, if the same energy is transferred to translation.

Flash photolysis of $Fe(CO)_5$ provides the first example of the production of ions in a gas at concentrations ($\sim 10^{12}$ ions/c.c.) suitable for measurement by kinetic absorption spectroscopy. Fig. 1 shows the formation and decay of Fe^+ (a^6D). The relative populations of the five multiplets

Table 1

State	Energy (cm^{-1})	State	Energy (cm^{-1})
a^5D	0	a^3H	19,360
a^5F	6,928	b^3F	20,641
a^3F	11,976	a^3G	21,715
a^5P	17,550	Z^7F	22,650
a^3P	18,378	b^3P	22,838
Z^7D	19,350	Z^7P	23,711

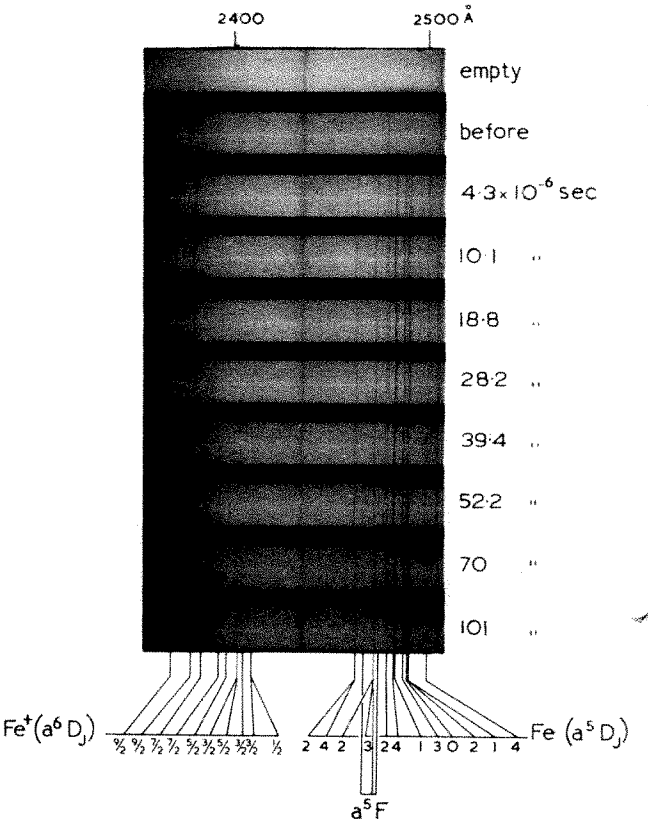


Fig. 1. Formation and decay of $Fe^+(a^6D)$ in flashed $Fe(CO)_5$ vapour

vary during and following the flash. We have also shown that the total concentration of ions increases linearly with flash energy. Ultra-violet radiation at the 1850 Å oxygen cut-off is 10,000 cm^{-1} short of the 7·40 eV ionization potential of atomic iron in the a^5D_4 state. However, all states above a^5F suffer ionization by secondary light absorption. The decay rate of the ions increases with increasing pressure, which may suggest that removal is by three body processes.

We have made preliminary measurements of the non-Boltzmann electronic-distribution of atomic nickel, produced by flash photolysis of $Ni(CO)_4$.

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Dynamic Fatigue in Glasses

THE static fatigue of glasses is a well-known and well-documented phenomenon (see, for example, ref. 1), but the only investigation of cyclic loading fatigue known to the authors is that of Gurney and Pearson[2], who reported a negative result. Perhaps in view of this result and of the near ideal brittle behaviour of glasses the investigation of cyclic loading fatigue appears to have been neglected. Recent results obtained in these laboratories as part of an extensive investigation of the strength of fused silica have indicated that a real cyclic fatigue effect may exist in this material. Circumstances have prevented a full investigation and confirmation of the effect, but in view of the importance of the problem the preliminary results would seem worth reporting here.

In the upper part of Fig. 1 a comparison is made between the static fatigue life under constant tensile

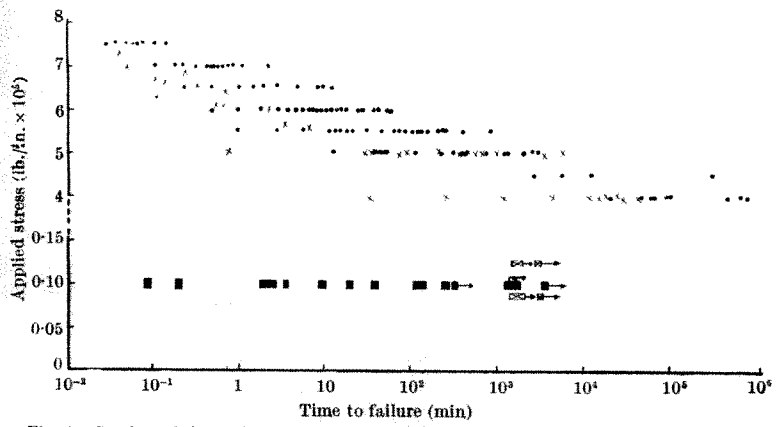


Fig. 1. Static and dynamic fatigue of silica. ●, Static fatigue of silica fibres in tension; ×, dynamic fatigue of silica fibres in tension; ⊠, static fatigue of silica rods in bending; ■, dynamic fatigue of silica rods in rotating bending. ■→ and ⊠→ indicate samples surviving life shown

stress, and the fatigue life under cyclic tensile stress, of virgin silica fibres[3]. The repetition rate of cyclic loading was in the range 30–75/min and the stress varied from zero to the nominal stress plotted on the graph. Loading was achieved by picking up and releasing a dead weight with a cam and the pulse shape was a flat-topped half sine curve so that stress was only applied to the fibre for less than one half the plotted times. Particularly significant are the early failures at 400,000 lb./in.^{2}.

In the lower part of Fig. 1 a comparison is made between static fatigue in cantilever loading, and rotating bending fatigue in cantilever loading, of mechanically damaged 1 mm diameter fused silica rods at much lower strength-levels. The cycling rate here was very much higher (3,700/min) and the cycle approximated to a full sine curve with the stress reversing from the maximum tensile value shown in Fig. 1, through zero, to the same value in compression. At the same stress and over the same time scale, no failures were observed with the comparable static bending fatigue specimens.

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BIOCHEMISTRY

Acetylcholine-like Activity of Acetyl-l-carnityl CoA in the Brains of Narcotized Rats

Numerous investigators[1–5] have shown that the administration of narcotic drugs to animals causes an increase in the brain acetylcholine-like activity. Most of these authors[1–4] believed that the material extractable from such brains was acetylcholine and this was based largely on the results obtained from experiments performed with the parallel bio-assay technique. Hosein and Koh[6] and Hosein, Rambaut, Chabrol and Orzeck[7] have shown that the method of parallel bio-assay is incapable of identifying acetylcholine in mixtures of substances which possess acetylcholine-like activity. In addition, these latter authors showed that the various test preparations used in the method of parallel bio-assay could not discriminate between mixtures of substances which possessed acetylcholine-like activity, even when non-natural substances were used.

Hosein and his associates[8,9] have shown by microchemical analysis that, of the material with acetylcholine-like activity extractable with trichloroacetic acid from brain, 20 per cent of this activity was due to a mixture of choline esters and the remainder to a mixture of butyrobetaine CoA esters. Numerous investigators[10–13] have since performed chromatographic separation of similar brain extracts and, although the peak of acetylcholine-like activity on the chromatogram differed in each such attempt (range from R_F 0·05 to 0·9), in every instance it was concluded that the active material was due solely to acetylcholine. Recently, Maslova[14], in contradicting the findings of Birks and MacIntosh[15] while confirming those of Hosein and Proulx[16], has shown that chemically identifiable acetylcholine in trichloroacetic acid extracts of the superior cervical ganglia accounted for only one-fifth of the amount of such material determined by bio-assay.

Hosein and Ara[5] suggested, on the basis of chromatographic analysis of trichloroacetic acid extracts of narcotized rat brain, that the material with acetylcholine-like activity was probably a butyrobetaine CoA ester rather than a choline ester. Hosein and Koh[17] showed on the basis of chromatographic and chemical analyses that the material with acetylcholine-like activity extractable from narcotized brain was acetyl-1-carnityl CoA. The experiments described here represent an extension of this work.

Sixty rats were deeply narcotized with pentobarbital (65 mg/kg) for 30 min. After that time the animals were killed and the brains were homogenized (fifteen at a time) in 5 per cent trichloroacetic acid. This extract was prepared for bio-assay with the frog rectus preparation as previously described[5,8]. The total activity found in the sample was equivalent to 276 μg acetylcholine chloride which was then divided into two equal parts, *A* and *B*. A solution containing 138 μg of crystalline acetylcholine chloride was added to sample *B*. The two samples *A* and *B* were evaporated to dryness and then converted to their tetrachloroaurate derivatives[8]. In each sample there was material which formed an immediate precipitate with the auric chloride and which could easily be collected by centrifuging as crystals fraction (i). When the supernatant fluid of each sample was left in the refrigerator for a further period of 4–5 days, crystals fraction (ii) was collected. The substances in the various fractions were identified by melting-point determination.

The results obtained from such analyses are shown in Table 1. It can be seen that the added acetylcholine was readily recovered and identified in crystals fraction (i) of sample *B*. No such material could be found in the corresponding fraction of sample *A*. Crystals fraction (ii) of both samples *A* and *B* contained material that melted at 120°–124° C which, in other work, we have shown to be acetyl-1-carnitine[9]. It was observed in previous work that preparation of the tetrachloroaurate derivatives of the CoA ester of the butyrobetaines in tissue extracts destroys the thiol ester linkage. It was assumed that a similar reaction took place in this extract.

In these experiments a small amount of materials in crystals fraction (i) only was found to have a melting-point of 130° C. Hosein and Proulx (unpublished) have found that the tetrachloroaurate of γ-butyrobetainyl choline also melts at 130° C. Further chemical analyses are, however, required to identify these particular crystals.

Table 1. Melting Point Determinations of Tetrachloroaurate Derivatives present in Trichloroacetic Acid Extracts of Narcotized Rat Brains

Sample	Melting point (° C) of crystal fractions (i)	(ii)	Probable substance
A	—	120–124	Acetyl-1-carnitine
	130	—	(γ-Butyrobetainyl choline) ?
B	—	120–124	Acetyl-1-carnitine
	130	—	(γ-Butyrobetainyl choline) ?
	165–167	—	Acetylcholine

The results described above indicate that, if acetylcholine were the sole material with acetylcholine-like activity in the brain extract, then it would have been possible to isolate and identify it, as was done for the equivalent amount of added crystalline acetylcholine chloride. This was not observed. It is therefore unlikely that the original material could have been acetylcholine. The results obtained, however, strongly support the previous findings of Hosein and Koh[17] that the material with acetylcholine-like activity in narcotized rat brain extracts is probably acetyl-1-carnityl Co*A*.

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Effect of Adenosylethionine on 5-Methyltetrahydrofolate-homocysteine Transmethylase

THE 5-methyltetrahydrofolate-homocysteine transmethylase of *Escherichia coli* requires catalytic quantities of *S*-adenosyl-L-methionine[1] and a vitamin B_{12} derivative for activity[2]. It was of interest to determine the effect of *S*-adenosyl-L-ethionine on this reaction since this compound can substitute for adenosylmethionine in certain enzyme-catalysed reactions such as the formation of the ethyl derivatives of phospholipid choline, creatine, histidine and carnosine[3,4]. Adenosylethionine bromide was isolated from rat liver after ethionine feeding[5]. It behaved as a single spot on a paper chromatogram with 1-butanol–acetic acid–water (60:15:25, v/v/v) as the solvent. After boiling in 0·1 N sodium hydroxide for 1 h a chromatogram of the digest yielded spots for ethionine, homoserine and adenine[5]. Adenosylmethionine iodide was purchased from Calbiochem, Los Angeles, Calif. It was found (Table 1) that adenosylethionine could not replace adenosylmethionine in the reaction under investigation. When added simultaneously at equal concentration little or no effect was observed. Significant inhibition was observed when adenosylethionine was pre-incubated with the enzyme or when higher concentrations of adenosylethionine were employed.

Table 1. EFFECT OF ADENOSYLETHIONINE ON 5-METHYLTETRAHYDROFOLATE-HOMOCYSTEINE TRANSMETHYLASE

Reaction mixture: *d,l*-L-5-methyltetrahydrofolate, 3·5 μmoles; D,L-homocysteine, 20 μmoles; DPHN, 1 μmole; FAD, 0·4 μmole; K_2PO_4 buffer, *p*H 7·4, 200 μmoles; 5-methyltetrahydrofolate-homocysteine transmethylase (partially purified from *E. coli* (ref. 2)), 2·7 mg; protein containing 150 mμg vitamin B_{12}. The total volume was 2 ml. Incubation was carried out at 37° C under hydrogen in Thunberg tubes for 1·5 h. Methionine was determined microbiologically with *Leuconostoc mesenteroides* (ATCC 8042). The assay was not affected by adenosylethionine.

Adenosylethionine (μmoles)	Adenosylmethionine (μmoles)	L-methionine (μmoles)	Inhibition (per cent)
	Experiment I		
—	—	0	—
—	0·03	0·50	—
—	0·06	0·77	—
—	0·12	0·81	—
0·03	—	0	—
0·06	—	0	—
0·12	—	0	—
	Experiment II		
—	0·06	0·62	—
0·06	0·06	0·62	—
0·06*	0·06	0·46	26
0·06	0·06*	0·57	8
0·12	0·06	0·36	42
0·24	0·06	0·32	48

* Pre-incubated with enzyme for 15 min. In all the other experiments both adenosylethionine and adenosylmethionine are added before enzyme.
A zero time value of 0·12 μmole L-methionine has been subtracted from each of the above values.

At present no compound is known which will replace adenosylmethionine in this system. Adenosylhomocysteine, thiomethyladenosine and *S*-methylmethionine were inactive[6]. The precise role of adenosylmethionine in this reaction remains uncertain.

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Trans to *Cis* Isomerization of Urocanic Acid

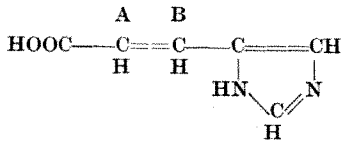
IRRADIATION of natural and synthetic urocanic acid with ultra-violet light results in the formation of a new product which has been identified by melting-point determination, ultra-violet and infra-red spectroscopy, and chromatography[1–3]. By analogy with cinnamic acid this has been thought to be a *trans* to *cis* isomerization. A similar change occurs in the urocanic acid of mammalian epidermis *in vivo* when the animals are exposed to ultra-violet light[4]. Using nuclear magnetic resonance it has been possible to demonstrate more conclusively that this ultra-violet-induced transformation is a *trans-cis* isomerization.

Commercial urocanic acid was shown by paper chromatography to contain trace amounts of a contaminant with the same R_F as the product formed by irradiation with ultra-violet light. A 0·1 per cent solution of urocanic acid at *p*H 6·0 was irradiated with stirring at 0° C with a General Electric sunlamp (1×10^6 ergs cm^{-2} sec^{-1}). Paper chromatography was performed on the concentrated solution in propanol and ammonia (3 : 1), and showed two spots with R_F values of 0·51 and 0·70—the former being identical to the major spot in the original material and the latter to the trace component. These compounds were separated by differential water solubility; their physical properties are shown in Table 1. Nuclear magnetic resonance spectra were obtained on a 10 per cent solution in deuterium oxide with a Varian *A*60 instrument. The chemical shifts of the doublets corresponding to the various olefinic protons and the spin–spin coupling constants are indicated. The structural assignments are based on the known effect of structure on the physical properties enumerated. Cinnamic acid, for example, shows a change in the coupling constant from 15·8 c.p.s.

Table 1

	R_F	M.p. (° C)	λ Max (mμ) at pH 1	Chemical shifts (p.p.m.*) HA	HB	Coupling constant c.p.s.
Trans	0·51	224	267	6·37	7·27	16
Cis	0·70	180–184	270	6·18	7·07	13

* Down field relative to 3-(trimethylsilyl) propylsulphonic acid (Na salt) as internal standard.



to 12·3 c.p.s. for the olefinic protons of the *trans* and *cis* isomers respectively[5]. It is of interest that the extinction coefficient of the *cis* isomer of cinnamic acid is much less than the *trans*[6], while those of urocanic acid are quite similar.

Because of the large amount of material needed for the nuclear magnetic resonance studies it was necessary to use isotope techniques to identify the new ultra-violet light absorbing product formed in epidermis following irradiation. Guinea-pigs were injected with 20 μc. of L-histidine-^{14}C (uniformly labelled) intraperitoneally and 24 h later they were depilated with wax and given a minimal erythema dose of ultra-violet light with a 'Hanovia' hot quartz lamp. The epidermis was separated by heating the skin to 50° C for 1 min and the urocanic acid isolated on a 'Dowex-1 formate' column as previously described[7]. Two radioactive compounds were identified by chromatography and autoradiography. The spots were eluted from the paper and chromatographed with the *cis* and *trans* isomers to which they corresponded exactly.

The data presented confirm that natural urocanic acid consists of the *trans* isomer contaminated with some *cis* and that following irradiation there is a *trans* to *cis* isomerization. This same reaction also occurs in the epidermis where urocanic acid is present at a high concentration.

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Cortisone-induced Lipaemia and Hepatic Steatosis in the Male Rat

It has previously been reported that female rats develop lipaemia and fatty livers during treatment with cortisone[1], and, more recently, the pathogenesis of this has been traced to a markedly enhanced mobilization of fatty acids from adipose tissue[2]. The mechanism thus seems quite different from that accounting for the fat accumulation in the livers of female, but not of male, rats during ethionine administration[3], and it is not reversed or prevented by measures which are effective during ethionine treatment[2]. Nevertheless, it is important to clarify whether the effect could also be observed in male rats.

Male albino 190–200 g rats were treated in a manner identical to that used previously for female rats[1]. All rats received tube feedings three times daily of a liquid diet; control rats gained weight well (average weight 247 g after 3 weeks). Experimental rats received 6·25 mg cortisone intramuscularly daily, while controls received saline. The experimental rats lost weight (average weight 170 g after 3 weeks). Animals were killed after 3 weeks; serum and liver lipids were extracted and total glycerides estimated by the van Handel–Zilversmit procedure[4]. Liver protein content was determined on a saline homogenate by the method of Oyama and Eagle[5].

Results of lipid analysis are shown in Table 1. The magnitude of rise of serum and hepatic lipids is similar to that seen in female rats[1]. Thus, it seems clear that no sex difference can be demonstrated for the fatty acid mobilizing properties of cortisone.

Table 1

	Serum glyceride*	Liver glyceride†
Untreated controls (6)	1·42 ± 0·24	0·208 ± 0·06
Cortisone-treated, pair-fed animals (3)	3·42 ± 0·18	0·850 ± 0·30

* mmoles/l. of serum.
† mmoles/g of liver protein.
All figures are given ± one *S.D.* Figures in parenthesis represent number of animals.

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In vivo Conversion of γ-Hydroxybutyrate into γ-Aminobutyrate

γ-Aminobutyrate (GABA) transaminates in normal brain to succinic semialdehyde[1,2] which in turn oxidates to succinic acid[3].

Moreover, Fishbein and Bessman[4] report that in the soluble fraction of brain homogenate, succinic semialdehyde is reduced to γ-hydroxybutyrate by action of a DPN^+ depending enzyme, indistinguishable from lactic-dehydrogenase; γ-hydroxybutyrate is then lactonized to γ-butyrolactone[5]. γ-Hydroxybutyrate and γ-butyrolactone have been recognized in large amounts in the normal nervous system and they seem the only physiological compounds in the nervous system which have an anaesthetic activity[6].

This communication reports on the variation of γ-aminobutyrate content in rat brain after injection of γ-hydroxybutyrate.

Male albino rats (200 g weight) were injected intraperitoneally with 500 mg/kg of γ-hydroxybutyrate. The rats were killed 2 h after injection and the brain removed and homogenized with 70 per cent alcohol (20 ml./g brain). After centrifugation and extraction with chloroform (10 ml./g brain), the alcohol layer was dried *in vacuo* at 40° C; the residue was dissolved with 1 ml. water/g brain.

γ-Aminobutyrate was separated by paper electrophoresis; 25 or 50 μl. of residue were utilized. Paper: Whatman No. 1; pyridine–acetic acid–water, buffer (250 : 50 : 900); *p*H 6, 12 V/cm, for 1·5 h. Paper strips, dried with hot air, were sprayed with 0·1 per cent ninhydrin in butanol (Fig. 1). A Chromoscan apparatus was used for reading the quantitative measurements. The standard curve

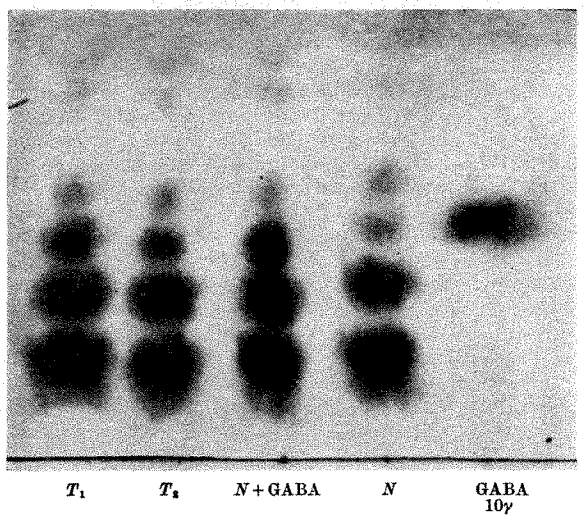


Fig. 1. Paper electrophoresis of rat brain extract (25 μl.). *N*, Control; T_1–T_2, brain of rat injected intraperitoneally with 500 mg/kg of γ-hydroxybutyrate, killed 2 h after injection; *N* + GABA, control + 200γ of GABA added per g brain wet weight after deproteinization

was made with known amounts of γ-aminobutyrate on the same paper.

The results of these measurements showed that after injection of γ-hydroxybutyrate, the γ-aminobutyrate content of rat brain rose from 2·2 to 3·0 μmoles/g brain wet weight (Table 1).

Table 1. γ-AMINOBUTYRATE CONTENT IN RAT BRAIN

	μmol/g wet weight
Controls	2·2
Rats treated with 500 mg/kg γ-hydroxybutyrate	3·0
Percentage increase	36·3

This work was carried out under the auspices of the Impresa Enzimologia of the Italian National Research Council.

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Absorption Changes in Blue-green Algae at the Temperature of Liquid Nitrogen

THE light-induced bleaching of a pigment at 700 nm in blue-green algae has been observed by many investigators, beginning with Kok[1]. It has been suggested that this absorption change is a manifestation of a primary photochemical event: the oxidation of a specialized chlorophyll component, perhaps the 'sink' of the long wavelength pigment system. In this connexion the behaviour of this change at low temperatures is important. Rumberg, Müller and Witt[2] and also Chance and Bonner[3] have reported the occurrence of an irreversible photo-induced decrease in absorption at 700 nm in chloroplasts maintained at the temperature of liquid nitrogen. On the other hand, Kok and Hoch[4] alluded to a reversible change at liquid nitrogen temperature. Data that show clearly the partial reversibility of the 700 nm change at low temperatures have not heretofore been published.

Measurements of absorption change on *Anacystis* and other organisms were made at liquid nitrogen temperature and at room temperature, using a commercial version of the Britton Chance dual wave-length spectrophotometer (Aminco, Silver Springs, Md.) modified by us to accept a small dewar with the sample. The photosynthetic material was held by capillary attraction between a microscope cover slip and a flat glass plate fused to the bottom of a 2·5 cm diameter test-tube. The test-tube fitted snugly in an unsilvered dewar[5]. The sample was cooled by conduction from liquid nitrogen placed in the test-tube above the sample.

Interference filters were placed in front of the photomultiplier to protect the detector from the actinic light and to reduce the various artefacts which may be observed in the absence of this precaution[6]. Further checks were made by comparing the results obtained with the photomultiplier close to the sample and also 15 cm away from the sample. Moving the photomultiplier away from the sample should greatly diminish the size of artefacts due to fluorescence while increasing artefacts due to changes in scattering. By these procedures, we concluded that the changes observed were due to absorption.

Experiments performed at room temperature followed closely the results reported by Kok and others[1]. At liquid nitrogen temperature, a reversible decrease in absorption at 705 superimposed on an irreversible change is observed on illumination. This is illustrated in Fig. 1. The ratio of the reversible to the irreversible component varies in different experiments but is about 1 : 1 for the

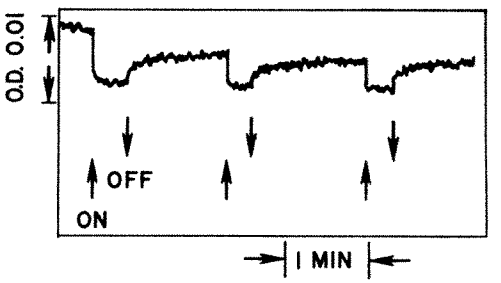


Fig. 1. Kinetics of the photo-induced bleaching at 705 nm in *Anacystis nidulans* at the temperature of liquid nitrogen

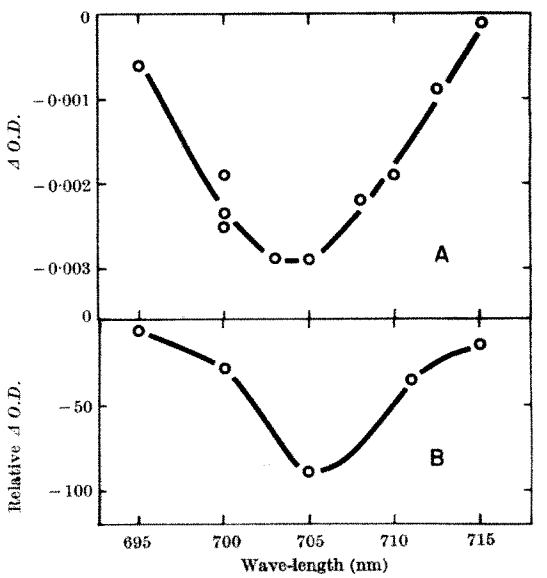


Fig. 2. *A*, Difference spectrum of the reversible photobleaching of *A. nidulans* at the temperature of liquid nitrogen; *B*, difference spectrum of the irreversible component of the photobleaching at liquid nitrogen temperature. This spectrum was obtained by normalizing the irreversible component to the magnitude of the reversible change at 705 nm

blue-green algae, *Anacystis nidulans* and *Plectonema boryanum*. For spinach chloroplasts a much smaller reversible component is observed, yielding a ratio of about 1 : 10. The reversible change is repeatable at liquid nitrogen temperatures for many consecutive measurements with little decrease in magnitude.

The difference absorption spectrum of the reversible component is shown in Fig. 2*A*. In the far red region the difference spectrum obtained closely follows that reported by other investigators[1] for *P*700 at room temperature. An accurate difference spectrum of the irreversible component is more difficult to obtain, since a different sample must be used at each wave-length. The difference spectrum of the irreversible absorption change at the temperature of liquid nitrogen is shown in Fig. 2*B*; this spectrum was obtained by normalizing the irreversible component at each wave-length to the magnitude of the reversible change at 705 nm. The precision of these measurements was less than for the reversible absorption change, but it is clear that the two components of the absorption change have similar spectra in the 705 nm region. Reversible and irreversible components of bleaching are also observed in the Soret region with a peak at 432 nm. The band in this region appears to be smaller than that at 700 nm, both in total amount and in the ratio of reversible to irreversible change.

The partial reversibility of the bleaching of *P*700 at low temperature strengthens an analogy between this pigment and the pigments *P*870 and *P*890 of photosynthetic bacteria. The latter are bleached reversibly by illumination at liquid nitrogen temperature[5].

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Effect of Age and Nephrectomy on ε-Lysine Acylase of Rat Kidney

ALTHOUGH a considerable amount of work on physiological changes has been carried out in the remaining kidney after unilateral nephrectomies, surprisingly little work has been reported concerning the change of enzyme levels in the kidney under these conditions. Thus, it was felt worthwhile to undertake an investigation into the effect of age and unilateral nephrectomy on an enzyme, ε-lysine acylase. ε-Lysine acylase was chosen as an illustration, merely because this enzyme has been identified and characterized previously in our laboratory[1].

Sprague–Dawley rats were employed throughout the present experiment and, after unilateral nephrectomy, the animals were fed with 10 per cent glucose solution in tap water. At intervals the animals were killed and the ε-lysine acylase activity was measured. In each case, the enzymatic activity in the nephrectomized kidney on removal served as control. Each kidney was homogenized in 4 volumes of water using a mechanically driven glass homogenizer. The homogenate was incubated with equal volumes of ε-*N*-acetyl-L-lysine solution containing 60 μmoles per ml. After incubation at 38° C in a water bath for 2 h, the reaction was terminated by the addition of 1 N hydrochloric acid to lower the *p*H to about 6·0. After boiling and light centrifugation to remove precipitated protein, the amount of free L-lysine in the supernatant was determined manometrically by lysine decarboxylase. Detailed description of the procedure will be found elsewhere[2]. The protein content of the homogenate was measured by the method of Lowry *et al.*[3]. ε-*N*-Acetyl-L-lysine was prepared according to the method by Neuberger and Sanger[4]. L-Lysine decarboxylase was purchased from Worthington Biochemical Inc. as acetone powder of *Bacterium cadaveris*. The enzymatic activity is expressed as μl. carbon dioxide per h per mg protein (specific activity), and 19·9 μl. carbon dioxide corresponds to 1 μmole of substrate hydrolysed.

As shown in Fig. 1, the specific activity of the enzyme gradually decreased as the age of the animals increased. Age was expressed as the increase in body-weight (the 30 g rats were two weeks old and the 250 g rats two months old). However, the total enzymatic activity increased, owing to the fact that the weight of the kidneys increased in proportion to body-weight as the animals aged. Neither starvation of the animals for up to ten days nor protein-rich diet (30 per cent casein) for three weeks influenced the level of ε-lysine acylase activity, although the high-protein diet has been shown to increase the enzyme of the urea cycle in liver[5].

Fig. 2 illustrates the change in specific activity of ε-lysine acylase in the remaining kidney after unilateral nephrectomy. The numbers in brackets indicate the number of animals used for each point. As seen in Fig. 1, the specific activity of the enzyme reached maxima increase of approximately 35 per cent in three days after the nephrectomy, and thereafter decreased slowly. This observation is similar to the finding of Tomassini[6] of the increase of acid phosphatase in the surviving kidney. Acid phosphatase in all nephrons of the surviving kidney increased sharply until the 5th day after nephrectomy and the decrease began at the 7th–10th day. In the present experiment, the weight of body after operation stayed rather below the value at pre-operation. The increase of the activity of ε-lysine acylase was more dramatic when the total enzymatic activity in the remaining kidney was considered. As also shown in Fig. 2, the total protein increased sharply during the first two days after operation to approximately 40 per cent above the control, and thereafter increased quite slowly, reaching about 47 per cent above the control at the end of the experiment.

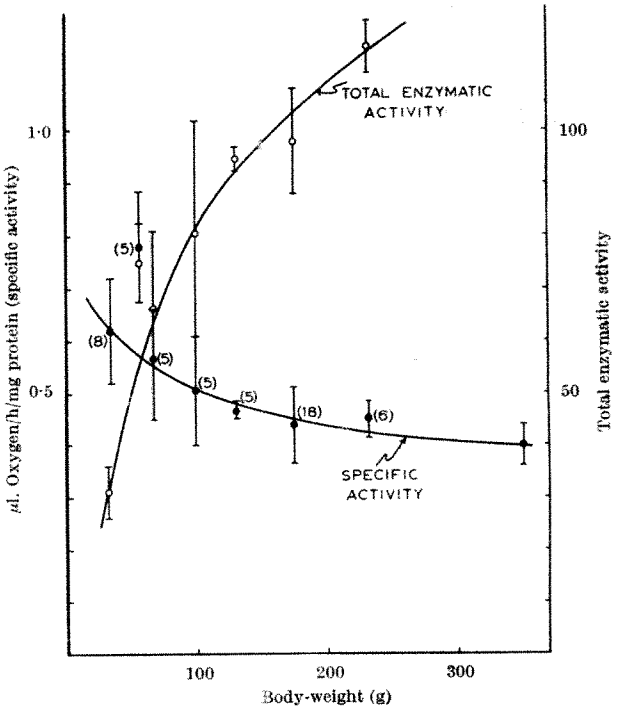


Fig. 1. Relationship between the body-weight, specific activity, and total enzymatic activity of ε-lysine acylase in rat kidney. The numbers in brackets indicate the number of animals used for each point and the body-weight is expressed as the mean value of these animals whose weights were approximately identical

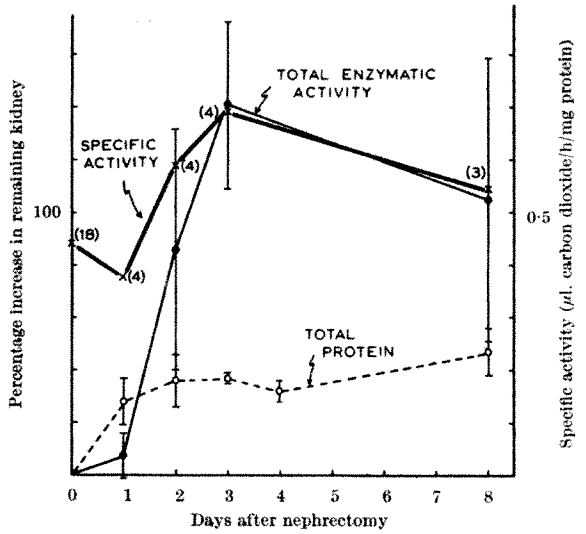


Fig. 2. The effect of unilateral nephrectomy on ε-lysine acylase activity, total enzymatic activity and total protein content in the remaining kidney. The numbers in brackets indicate the number of animals used for each point

On the other hand, the activity of ε-lysine acylase increased to approximately 140 per cent of the level observed in the control in 3 days and remained above 100 per cent even eight days after nephrectomy when the experiment was terminated. The result indicated very clearly that after nephrectomy the activity of ε-lysine acylase in the remaining kidney completely compensated for the loss due to the removal of one kidney, and that this increase in the activity was highly significant because the total protein did not increase as much as the enzyme protein. By the experiments described here, it is impossible to deduce whether there was any preferential *de novo* protein synthesis.

Thus, the present results can be summarized as follows: the specific activity of ε-lysine acylase decreased as the rat grew older, although the total enzymatic activity in the kidney increased considerably. Furthermore, the total enzymatic activity in the remaining kidney after unilateral nephrectomy doubled the amount of the control value in three days while the total protein content increased only approximately 40 per cent during this period, possibly indicating preferential synthesis of this enzyme, and the complete compensation of the function by the remaining kidney as far as the activity of ε-lysine acylase was concerned.

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Method of Recording Simultaneously the Positions of Labelled and Unlabelled Compounds on Autoradiographs

A COINCIDENCE technique for paper chromatography has recently been described[1], in which a 'shadowgram' of a chromatogram is produced by placing the chromatogram over a photographic film and exposing the covered film to radiation of a wave-length which is absorbed by the 'spots' on the chromatogram. In this way a 'shadowgram' of purine or pyrimidine 'spots' may be produced by exposing the covered film to an ultra-violet source, or of amino-acid spots (after development with ninhydrin) by exposure to visible light.

In each instance when the film is developed the separated compounds appear as light 'spots' on a dark background and such a 'shadowgram' may be conveniently superimposed on the autoradiograph of its own chromatogram for checking the correspondence of 'spots'.

In this laboratory we have made use of a somewhat similar technique, but one which has the added advantage of providing a record of both labelled and unlabelled compounds on a single film.

In this method an X-ray film is covered with a chromatogram or ionophoresis paper which is clipped firmly in position. The covered film is then illuminated with a suitable source (visible or ultra-violet) for a period sufficient to produce partial exposure of the film except in those areas screened by the 'spots'. The film, with the chromatogram or ionophoresis paper still in place, is then covered and exposure to the radio-active compounds on the paper continued as in the preparation of a normal autoradiograph.

When finally developed the labelled compounds appear as dark 'spots' and the unlabelled compounds as light 'spots' on a grey background, as illustrated in Fig. 1.

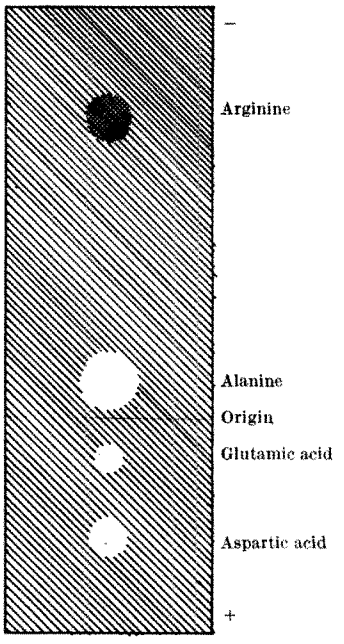


Fig. 1. Diagram showing the position of labelled arginine and unlabelled alanine, glutamic acid and aspartic acid after ionophoresis at *p*H 3·6

Using 'Kodirex' film covered with Whatman 3 *MM* paper, an initial exposure time of 10 min was found suitable for recording the position of amino-acids (developed with ninhydrin) when a Kodak 'Beehive Safelight' fitted with yellow filter ('Wratten' series *OB*), placed 30 cm from the covered film, was used as a light source.

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Metabolism of Coumarin by a Micro-organism

RECENT reports described the isolation of a soil organism (*Arthrobacter* sp.) as the source of an enzyme which reduced *o*-coumaric acid to melilotic acid[1-3]. The present communication describes further studies on this enzyme which suggest that it is a flavoprotein and that the flavin moiety is FAD.

The organism was cultured with coumarin as its sole carbon source, and collected as previously described[1,2]. To demonstrate the role of a flavin in the reaction, the enzyme purification described earlier[2] was modified as follows: The enzyme, obtained after ammonium sulphate precipitation, was treated once again with calcium phosphate gel and the mixture clarified by centrifugation at 75,000*g* for 30 min. The supernatant solution was then brought to 50 per cent saturation with solid ammonium sulphate and, after cooling for 30 min, the suspension was centrifuged at 75,000*g* for 30 min. The precipitate was redissolved in a volume of water equal to the original volume of crude extract. This enzyme was dialysed against 4 l. of water changed three times during a 24-h period. This preparation was used in all the experiments reported here.

Attempts to separate the flavin from the protein using ammonium sulphate precipitation followed by treatment with acid, as described by Strittmatter[4], were unsuccessful in that the enzyme was irreversibly inactivated. It was found that the above modification of the original purification procedure[2] separated the flavin from the apoenzyme with only minimal loss of enzyme activity.

Table 1 shows the effects of the addition of various flavins on NADH : *o*-coumarate oxidoreductase. It can be seen that FAD at $6{\cdot}6 \times 10^{-5}$ M doubled the activity of the enzyme. The other flavins were virtually without effect. When FAD was added after the addition of inhibiting concentrations of partial structural analogues of FAD or various anions (Table 2), activity was in most cases restored to greater than control levels. Other flavins such as FMN and riboflavin either had no effect, or inhibited enzyme activity. It appears, therefore, that FAD is a specific co-factor for NADH : *o*-coumarate oxidoreductase.

The reduced form of FAD ($FADH_2$) bound to the enzyme surface may function as the direct electron donor in the reduction of *o*-coumaric acid to melilotic acid.

It has been shown that the enzyme, D-amino-acid oxidase, a flavoprotein isolated from pig kidneys[5,6], was inhibited by various anions, as well as by partial structural analogues of FAD. In both cases, FAD added to the reaction mixture restored the lost activity[5,6]. The interpretation of these data was that FMN and the structural analogues exerted their inhibition by competing with FAD for binding sites on the protein. The inhibition by anions was alleged to represent competition with dissociated phosphoryl groups in FAD. The results of studies with NADH : *o*-coumarate oxidoreductase suggest that it is similar to D-amino-acid oxidase with respect to the manner in which the FAD is bound to the apoenzyme.

o-Coumaric acid and dihydrocoumarin were purchased from K and K Laboratories, Inc. Melilotic acid was prepared as described previously[2]. NADH was purchased from Pabst Laboratories, Inc. FAD, FMN, riboflavin, adenine, adenosine, adenylic acid, ADP and ATP were obtained from Mann Laboratories, Inc. Coumarin was bought from Eastman Organic Chemicals.

Table 1. EFFECT OF VARIOUS FLAVIN COMPOUNDS ON NADH : *o*-COUMARATE OXIDOREDUCTASE ACTIVITY

Co-factor added	Final concentration	mμ Moles NADH oxidized/minute
Control	—	100
FAD	$6{\cdot}6 \times 10^{-5}$ M	200
FAD	$1{\cdot}3 \times 10^{-4}$ M	116
FMN	$6{\cdot}6 \times 10^{-5}$ M	100
Riboflavin	$6{\cdot}6 \times 10^{-5}$ M	87

The basic reaction mixture contained in a total volume of 3 ml. consisted of the following: 440 mμmoles of *o*-coumaric acid; 300 mμmoles of NADH; 0·1 ml. of enzyme, and 4·8 mμmoles phosphate buffer, *p*H 7·3. When a flavin was included in the reaction mixture, there was a proportionate reduction in the volume of buffer added. Enzymatic activity was assayed spectrophotometrically by recording the decrease in absorbancy of NADH at 340 mμ over a 60-sec interval.

Table 2. (*A*) EFFECT OF ANIONS AND PARTIAL STRUCTURAL ANALOGUES OF FAD ON NADH : *o*-COUMARATE OXIDOREDUCTASE ACTIVITY

Salt or partial analogue added	Concentration	mμmoles NADH oxidized/min
Control	—	100
Adenine	5×10^{-4} M	100
Adenosine	5×10^{-4} M	99
AMP	5×10^{-4} M	69
ADP	5×10^{-4} M	75
ATP	5×10^{-4} M	75
Sodium acetate	0·1 M	63
Potassium iodide	0·1 M	67
Sodium chloride	0·1 M	29
Sodium sulphate	0·1 M	44

(*B*) EFFECT OF FLAVINS ON NADH : *o*-COUMARATE OXIDOREDUCTASE ACTIVITY IN PRESENCE OF VARIOUS ANIONS OR PARTIAL STRUCTURAL ANALOGUES OF FAD

Salt or partial analogue added	Final concentration	Flavin added	Final concentration	mμmoles NADH oxidized/min
Control	—	—	—	100
Adenosine	5×10^{-4} M	FAD	$6{\cdot}6 \times 10^{-5}$ M	188
AMP	5×10^{-4} M	FAD	$6{\cdot}6 \times 10^{-5}$ M	175
ADP	5×10^{-4} M	FAD	$6{\cdot}6 \times 10^{-5}$ M	188
ATP	5×10^{-4} M	FAD	$6{\cdot}6 \times 10^{-5}$ M	181
ATP	5×10^{-4} M	FMN	$6{\cdot}6 \times 10^{-5}$ M	99
ATP	5×10^{-4} M	Riboflavin	$6{\cdot}6 \times 10^{-5}$ M	86
Sodium acetate	0·1 M	FAD	$1{\cdot}3 \times 10^{-4}$ M	113
Potassium iodide	0·1 M	FAD	$1{\cdot}3 \times 10^{-4}$ M	128
Sodium chloride	0·1 M	FAD	$1{\cdot}3 \times 10^{-4}$ M	100
Sodium chloride	0·1 M	FMN	$6{\cdot}6 \times 10^{-5}$ M	29
Sodium chloride	0·1 M	Riboflavin	$6{\cdot}6 \times 10^{-5}$ M	19
Sodium sulphate	0·1 M	FAD	$1{\cdot}3 \times 10^{-4}$ M	100

The reaction mixtures were essentially the same as those of Table 1. To maintain the total volume of 3 ml. when a flavin and/or inhibitor were included in the reaction mixture, proportionately less buffer was added. Enzymatic activity was assayed as indicated in Table 1.

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PHYSIOLOGY

Extracellular Recording of Converging Input on Cortical Neurones using a Flexible Microelectrode

THE convergence of different modalities of sensory input on restricted areas of the cerebral cortex has received much experimental attention in recent years[1-5]. Such investigations are valuable for an understanding of the integrative capacities of the cerebral cortex.

One cortical association area studied in detail at the cellular level[4,5] showed differential responsiveness to various peripheral and central stimulations. Intracellular recordings indicated that excitatory and inhibitory postsynaptic potentials would, for many cells, determine the responsiveness of a cell, or the lack thereof, to converging input. Other convergences occurred before the level of the impaled cortical cell.

It seems likely, because of strong convergence of input, that non-primary, association areas may demonstrate especially good integrative capacities. Furthermore, a cell which is unequally responsive to different modalities of sensory stimulation may be able to alter its pattern or extent of responsiveness under different conditions. It will be necessary ultimately to consider the likelihood of a differentially responsive cortical cell changing its discharge characteristics during certain behavioural situations.

More descriptive information about convergence on neurones in association cortex is needed, and before long-term investigation of neuronal activity is possible, means must be developed of recording unit discharges for days and weeks. Previous microelectrode studies of extracellular cortical activity have been successful for minutes or several hours and these experiments have yielded valuable information about unit discharges to sensory stimulation under several conditions in the unanaesthetized mammal[6–8].

In the present investigations, carried out on twenty-four cats anaesthetized with chloralose (55 mg/kg initially), a simple microelectrode was used for stable recording from neurones in an association area of the cerebral cortex (anterior marginal gyrus). Extracellular recordings could be routinely obtained for many hours with the flexible electrode and thus high reliability of neuronal discharges to the converging inputs could be established.

Microelectrodes were made from 80-μ nichrome wire, electrolytically sharpened to a tip of less than 5μ, with a taper of about 300μ, and insulated with lacquer to within about 25μ of the tip. The experimental procedure consisted of inserting the flexible microelectrode through a small opening in the skull and through the slit meninges at an angle which permitted electrode movement through the cortex approximately horizontal to the cortical surface and roughly parallel with the cortical cell layers (Fig. 1). With the flexible electrode inserted several millimetres, no provision for controlling brain movement was necessary in most cases. The electrode, held in the microdrive about 2 cm from the tip, had sufficient flexibility to allow it to move with brain pulsations without being dislodged from the vicinity of an active cell.

Extracellular recordings from well-isolated cells could be made for exceedingly long periods. Figs. 2 and 3 are from a cell which was studied for nearly 4 h with little change in spike configuration. In one cat, with electrodes sealed at the opening in the skull with dental acrylic, single-cell discharges were observed for 30 h, before the experiment was deliberately terminated.

Data were collected on eighty-one cells in the anterior marginal gyrus at an average location of A 20·5 (ref. 9) and with the entrance made at about A 15. Recordings and stimulations were used as previously described[3,4]. As the microelectrode was advanced, animals were simultaneously stimulated with a photic flash (P, 5 msec), an auditory click (A, 0·01 msec), and contralateral forepaw shock (CFP, 0·01 msec) every 2 sec. Nearly all cells selected for study were fired by the combined stimuli. Hubel's criteria[10] for distinguishing between cell and fibre spikes were used. Because one purpose of the investigation was to determine the reliability of unit recordings using the flexible microelectrode, no analysis of convergence was attempted until the cellular activity had been observed for at least 15–20 min without any sign of injury. Nearly all cells were investigated for at least 45 min, many for several hours.

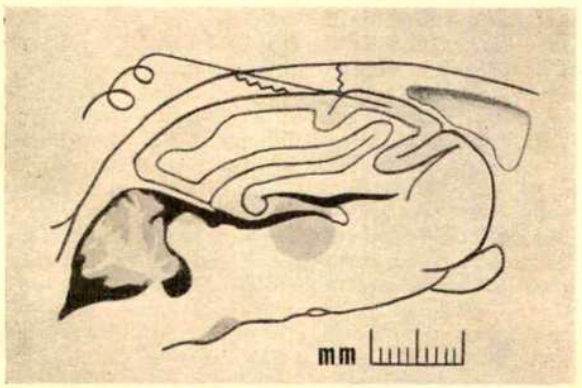


Fig. 1. Drawing of longitudinal section of cat brain and skull about 3 mm from midline. Flexible microelectrode seen in the angle approach to cortex of the anterior marginal gyrus

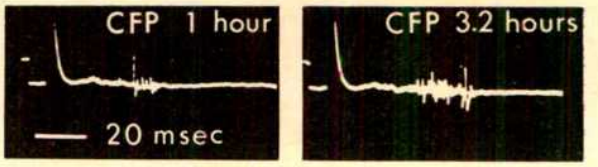


Fig. 2. Extracellular recording from a cortical neurone firing to contralateral forepaw shock 1 h and 3·2 h after its initial isolation. Latency remained constant at about 35 msec. Later recording shows spikes from another cell which appeared as depth of anaesthesia decreased. Foot shock intensity just below that which produced motor twitch. CFP probability 40 min after finding the cell was 0·52, at 3 h it was 0·74. Bar at first break in baseline in 200 μV. Second break is point of CFP shock. Downward deflexion positive here and in Fig. 3

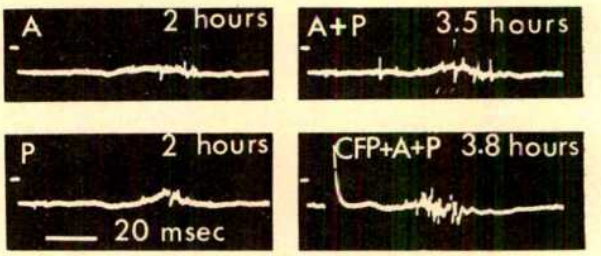


Fig. 3. Convergence of input on the same cell as in Fig. 2. Cell discharged to auditory and photic stimulation at 2 h with latencies of 49 and 53 msec and probabilities at 1·8 h of 0·48 and 0·48. Pairing A and P at 3·5 h produced other cellular spikes and slightly increased the probability of firing by the principal cell to 0·54. All three stimuli together at 3·8 h resulted in multiple spikes and further summation for a probability of 0·98. Calibration as in Fig. 2. A and P artefacts occur as downward deflexions in baseline

Rates of cellular discharge, as observed previously under chloralose, were low. Only sixty-four of the eighty-one cells were spontaneously active; nine of these could not be fired by any of the three inputs. Average spontaneous frequency of the fifty-five cells was slightly less than 2 sec.

Of the eighty-one cells, eight were exclusively monosensory, 2 P, 2 A, and 4 CFP. Fifteen were bisensory, that is, they responded to each of two inputs. Since the study of convergence of all three inputs on a particular cell was intended, attention is directed to the forty-nine cells which fired to each of the three inputs. Table 1 shows that cells in the anterior marginal gyrus had low probabilities of firing, much lower than did those in the anterior middle suprasylvian gyrus[3,4].

Table 1. AVERAGED PROBABILITIES OF FIRING (p) AND LATENCIES IN MSEC (lat) FOR FORTY-NINE CELLS FIRING TO ALL THREE INPUTS. CELLS CLASSIFIED AS PREFERENTIALLY RESPONSIVE OR EQUALLY RESPONSIVE (ER)

	P		A		CFP		
Class	p	lat	p	lat	p	lat	Signif.
11 P	0·67	61 (8)*	0·38	48 (7)	0·30	47 (6)	$\ll$0·001†
8 A	0·32	93 (4)	0·74	41 (6)	0·33	53 (4)	$<$0·001†
5 CFP	0·36	74 (3)	0·32	43 (4)	0·60	29 (4)	0·02†
25 (ER)	0·52	73 (18)	0·51	46 (18)	0·52	36 (16)	$\ll$0·001‡

* Number of latency measurements in parentheses.
† Significance level for six t ratios of mean p differences, for example, 11 P cells compared with A and CFP inputs.
‡ Significance level for three t ratios of latency differences for the twenty-five equally responsive cells. Numbers too small for latency difference tests on the other cells.

Twenty-four cells were classified as preferentially responsive on the basis of highest firing probability. As indicated in Table 1 these cells had a significantly higher probability of firing to a single input than to either of the other two. (It is worth emphasizing, as noted before[3,4], that chloralose in light to medium concentrations does not produce equal responsiveness of all cortical neurones to all peripheral stimuli.) Preferential responsiveness was not directly reflected in latency differences. For the twenty-five equally responsive cells though, latencies were significantly different with the order from shortest to longest,

being CFP, A, P. There were also differences in latencies between the two general categories. Table 1 shows that latencies of cells in a preferentially responsive class were in all three instances less than latencies of equally responsive cells firing to the same modality of stimulation.

These data are all consistent with the interpretation that an important portion of a given population of cells in association cortex can be expected to demonstrate differential responsiveness to converging sensory input. Another portion would show equal convergence. This fact is supported by the results given in Table 1 and also by instances of occlusive interactions observed when firing probabilities were sufficiently high for such tests to be made.

Simultaneous pairing of two stimuli yielded unequivocal data on cellular integrative mechanisms. Sixty cells were tested with pairings of supra- and/or sub-threshold stimuli. Fifteen cells showed summation of input, seven facilitation (one stimulus of the pair not producing any cell discharge alone), and five inhibition. Summation, as illustrated in Fig. 3, could be seen in some cells only with pairing of all three inputs. With the cell illustrated a distinct latency decrease occurred, plus the appearance of additional cell spikes.

These results show that with a simple flexible microelectrode reliable extracellular recordings can be obtained from cortical neurones for at least many hours. The data also indicate that the anterior marginal gyrus of the cat contains neurones unequally responsive to different modalities of sensory input and it has cellular discharge characteristics reflecting summation, facilitation and inhibition.

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Inhibition of β-D-Glucosiduronic Acid Conjugation by Eugenol

IT has been claimed that eugenol, 4-oxy-3-methoxy-1-allylbenzoic acid, has mucinogenic activity and that it has a beneficial effect on gastric ulcers[1,2]. The gastric wall contains various uronic acid containing mucopolysaccharides, the formation of which is possibly dependent on a proper coupling capacity of β-D-glucosiduronic acid (UDPGA). Evidence has been obtained which indicates that the amount and type of mucopolysaccharides in tissues probably have some bearing on the mechanism of ulcer formation[3]. This investigation was undertaken in order to determine whether eugenol has any effect on the glucuronation of an exogenic substance. The effect on the whole conjugation capacity of the tissue, as well as on the UDPGA transferase enzyme, was also considered in the investigation.

Wistar rats and guinea-pigs were used as test subjects. The whole conjugation was followed using a modification of the method developed by Levvy and Storey[5,6]. The UDPGA transferase activity was determined by the method of Bogell and Leloir[7]. This method requires washed microsomes, UDPGA (Sigma) and *p*-nitrophenol. Eugenol was dissolved in 0·2 per cent 'Triton *X*-100' solution. The oxygen consumption was measured in a Warburg apparatus.

The effect of eugenol on the formation of *o*-aminophenol glucuronide by liver slices is shown in Fig. 1. It can be seen that a 50 per cent inhibition is obtained with a eugenol concentration of 2 μg/ml. in the incubation solution. The same result was obtained using 50 and 100 μg aminophenol. At a concentration of 200 μg/ml. or above, eugenol constantly caused an activity less than 5 per cent of that of the controls; 250 μg resulted in some cloudiness. At a concentration of 100 μg/ml. the oxygen consumption was identical to that of both the controls and also to the controls without the tissue specimens.

Several additional experiments were carried out in order to elucidate the nature of the inhibitory effect of eugenol. When eugenol (100 μg) was added after the tissue had been incubated for 1 h, a 70 per cent reduction in glucuronide synthesis was produced as compared with the control.

Eugenol, when added just before deproteinization and at subsequent stages, had no effect. This eliminates the possibility of there having been any interference in the analytical procedure.

In the *in vivo* experiments, 3 ml. of 5 per cent eugenol was given to the animals and this virtually resulted in a complete inhibition in rinsed stomach, and about a 75 per cent inhibition in duodenal slices. The results with liver were just above normal. No effect could be detected after 24 h, but it was still complete 6 h after the eugenol had been administered; the effect was variable after 12 h. Histological studies of the stomach revealed desquamation of the surface epithelium and punctate haemorrhages in the pylorus and glandular stomach. Cellular exudate was present on the surface; this was visible to the naked eye. No attempt was made to find the least effective dose *in vivo*. When tissue slices were placed in a solution of 0·025 per cent eugenol in Ringer's solution for 2 min before they were rinsed and transferred to the Warburg vessel, the synthesis dropped to less than 25 per cent of the controls. The effect was the same for stomach, duodenum and liver. No attempt was made,

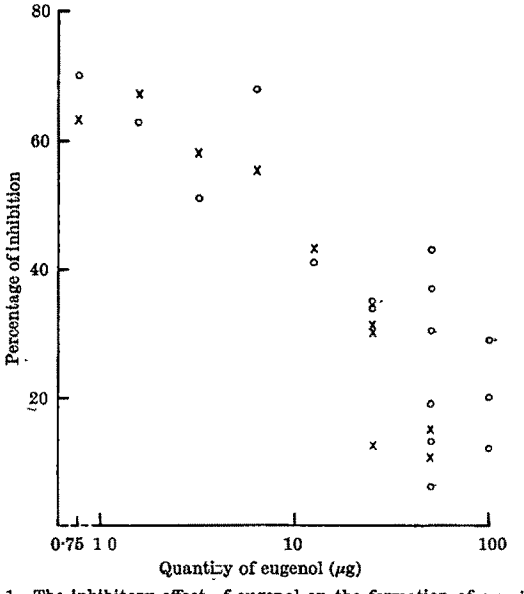


Fig. 1. The inhibitory effect of eugenol on the formation of *o*-aminophenolglucuronide by liver slices

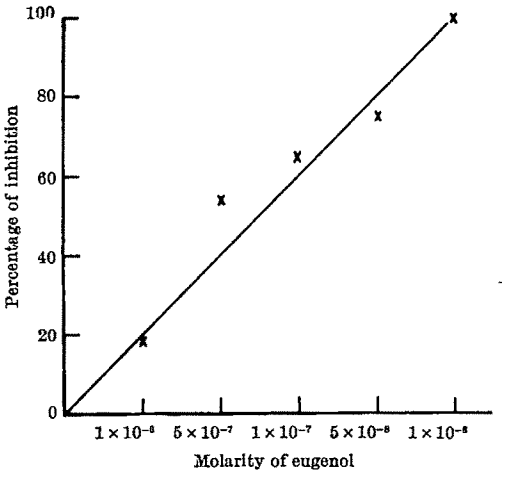


Fig. 2. The inhibitory effect of eugenol on the glucuronyl transferase activity of the liver

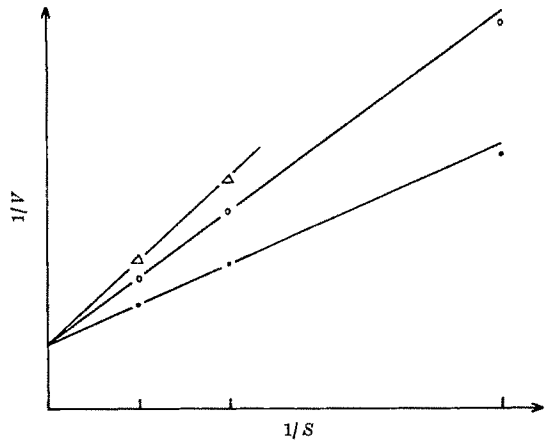


Fig. 3. The nature of the glucuronide conjugation inhibition by eugenol according to Lineweaver-Burk. △, 2.5×10^{-7} M eugenol, ○, 1×10^{-7} M eugenol; ●, control

however, to find the shortest effective time and lowest effective concentration.

When the specific effect of eugenol on the enzyme in the last stage of the conjugation reaction system (that is, on the glucuronyl transferase) was studied, it was found that for this enzyme the inhibition is K_{50} 2.5×10^{-7}. The correlation between the degree of inhibition and the concentration of eugenol is shown in Fig. 2. The nature of this inhibition has been studied by preparing a Lineweaver-Burk plot of the results as shown in Fig. 3. It can be considered that the inhibition is of a competitive nature. The concentration of *p*-nitrophenol was also varied, but with the methods used no satisfactory results were obtained. The 'Triton *X*-100' was used in the experiments in order to increase the solubility of eugenol. It had no inhibitory effect at a concentration of 0·2 per cent. However, if the concentration was increased to more than 1 per cent, a very small inhibitory effect was obtained.

It has been possible to demonstrate the inhibition of β-D-glucosiduronic acid conjugation with eugenol. An important point in the reaction system is that UDPGA transferase would seem to be inhibited. It could be that this is not the only enzyme in the glucuronic acid conjugation system the activity of which is inhibited.

These results are in agreement with our earlier observations that eugenol can lower the quantity of salicylamide glucuronide formed in dogs when it is fed to them together with salicylamide[8]. It is difficult at present to correlate these observations with the mucinogenic effect of eugenol demonstrated by Hollander *et al.*

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Presynaptic Inhibition in Primate Lateral Geniculate Nucleus

SINCE their discovery[1-4], the effects of midbrain stimulation on excitability of the visual system have been studied extensively. Work on the squirrel monkey[5,6] has shown that the centrencephalic system exerts a powerful control over transmission through the lateral geniculate nucleus (LGN) for both photically and electrically elicited responses. Initial experiments showed facilitation following electrical stimulation of the mesencephalic reticular formation or natural alerting stimuli. Recent experiments, however, have shown that a brief period of inhibition precedes the facilitation and, as set forth below, strongly suggest that this inhibition is presynaptic in nature.

The phenomenon of presynaptic inhibition has been thoroughly reviewed by Eccles[7]. Among the characteristics which, in the mammalian central nervous system, reliably distinguish presynaptic from postsynaptic inhibition are susceptibility to different pharmacological agents. Strychnine, for instance, blocks most types of postsynaptic inhibition without affecting presynaptic inhibition, whereas picrotoxin strongly depresses the latter while leaving postsynaptic inhibition intact.

In the present investigation, 32 squirrel monkeys (*Saimiri sciureus*) were lightly anaesthetized; 20 with 'Diabutal' (sodium pentobarbital), 25 mg/kg, and 12 with 'Dial', 0·3 ml./kg. Pairs of electrodes made from 0·25 mm diameter 'Nichrome' wire were introduced into the optic tract (stereotaxic co-ordinates [8] : *A*9, *L*5, −1), optic radiation (*A*1, *L*10, +3), mesencephalic reticular

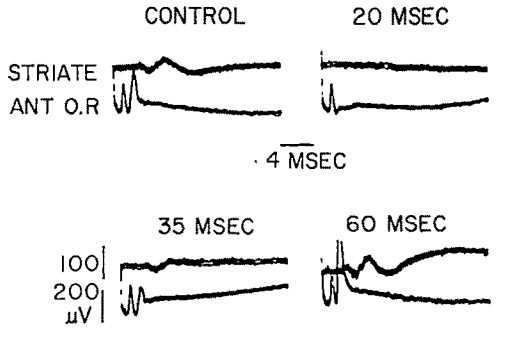


Fig. 1. Squirrel monkey *R*291. Control level of response in anterior optic radiation (Ant. O.R.) and striate cortex to stimulation of optic tract (10 V, 0·03 msec), bipolar electrodes. First peak in Ant. O.R., presynaptic or optic tract component; second peak, postsynaptic response conducted from LGN. Application of single pulse (15 V, 0·1 msec) to mesencephalic reticular formation 20 msec prior to optic tract stimulation abolishes responses in radiation and cortex. At 35-msec interval, inhibition not complete. Note at 60 msec, three-fold enhancement of postsynaptic responses. Presynaptic component unchanged throughout

formation (*A2, L2,* +2 to ±0), and posterior area striata. All electrode sites were confirmed histologically.

From the specified position in the optic radiation, both pre- and post-synaptic components[5,9] for the response of the LGN can be seen following an electrical pulse applied to the optic tract (Fig. 1). In 23 of the 32 monkeys, application of a single pulse (10 V, 0·1 msec) to the midbrain site prior to stimulation of the optic tract produced the sequence of inhibition and facilitation seen in Figs. 1, 2 and 3. The inhibition was always earlier than the facilitation. Facilitatory effects alone were found in six animals, and inhibition alone in three. The inhibition had a latency of 5–8 msec, was maximal at 20–30 msec (completely abolishing responses in some preparations), and then usually passed gradually into facilitation at about 40–80 msec. Two spikes representing contributions from magno- and parvo-cellular components of the LGN can often be recorded from the radiation following stimulation of the optic tract. Both spikes appeared equally influenced by mesencephalic stimulation. Responses in area striata varied roughly *pari passu* with those in the radiation, although adequate analysis of cortical excitability *per se* has not yet been made. Bilateral enucleation (Fig. 2) or removal of most of area striata did not change these effects beyond altering the background against which they occurred[10].

In twelve of the monkeys in which inhibition was found, strychnine sulphate or picrotoxin were given in progressive doses, up to convulsive levels. Strychnine (up to 0·4 mg/kg) did not alter the inhibitory effects in any of the monkeys used, whereas picrotoxin (1·0–1·5 mg/kg) consistently abolished all the inhibitory effects in each of the nine monkeys tested. The effects of picrotoxin subsided after about 2 h, at which time inhibitory effects returned (Fig. 3). The lack of effect of strychnine could then be demonstrated in the same preparation. Injection of additional pentobarbital prolonged and augmented the inhibitory effect (Fig. 2) and could cut short the action of picrotoxin. Intravenous injection of mephenesin (80 mg/kg) in one animal also abolished the inhibition.

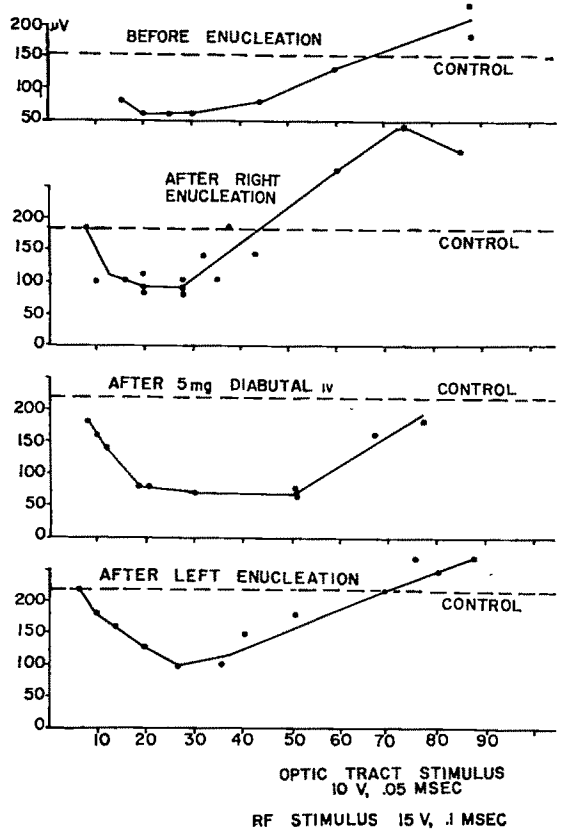


Fig. 2. Amplitude of response in optic radiation under various conditions. Ordinate, response amplitude in μV; abscissa, interval by which mesencephalic precedes optic tract stimulus. Solid line, postsynaptic response in optic radiation at varying intervals following mesencephalic stimulation; dotted line, control levels. Note degree of inhibition unchanged by removal of both eyes although control level of excitability shifts. Additional dose of sodium pentobarbital lengthens and potentiates the inhibition

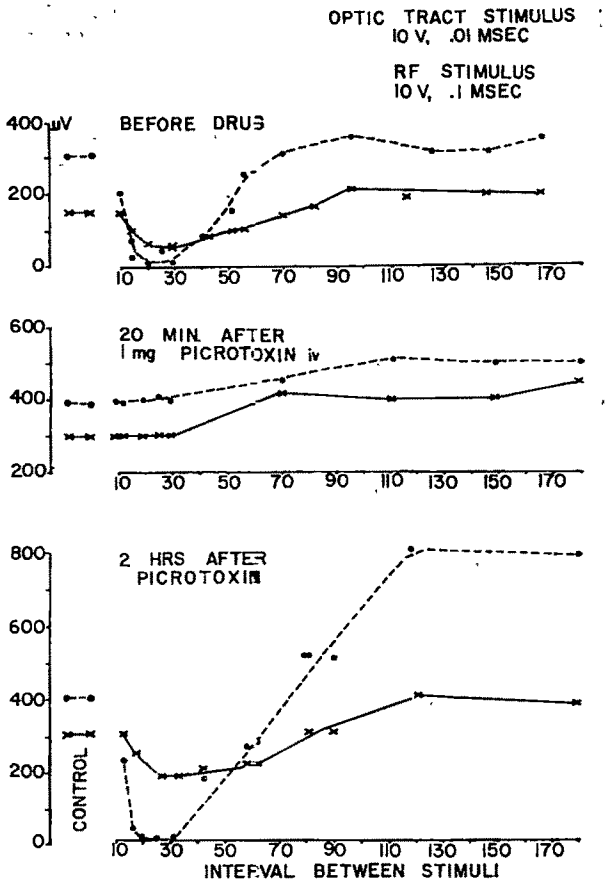


Fig. 3. Excitability curves of responses in optic radiation and striate cortex before and after intravenous picrotoxin (1·5 mg/kg). Ordinate and abscissa as in Fig. 2. Solid line, postsynaptic response in optic radiation at varying intervals dotted line, cortical response (peak to peak). At left, control responses to optic tract stimulus (without mesencephalic stimulus). Note abolition of inhibition 20 min after picrotoxin administration, and recovery after 2 h. Increased responsivity of cortex in latter is partly from diminishing barbiturate anaesthesia

The pharmacological effects on this inhibition in the LGN are thus identical with those seen for presynaptic inhibition in the spinal cord[7,11], and the latency and time course are similar at the two sites. The inhibition endures for a shorter time in the LGN, but this is probably because it is overwhelmed by later-developing facilitation. Slow potential changes which might correspond with those recorded from dorsal roots or cord dorsum in association with presynaptic inhibition can be registered in the optic radiation and optic tract, but the probability of mixed facilitatory and inhibitory effects makes their interpretation difficult at this time. Morphologically, axo-axonic endings which could function in presynaptic inhibition have been described in the spinal cord[12] and there are preliminary reports[13,14] that such endings are also abundant in the LGN of cats Axo-axonal endings have been described for the LGN of macaques[15], although the apparent functional relations here are difficult to interpret. In any event, the electrophysiological and pharmacological evidence is all concordant with the possibility that the inhibition exerted by the centrencephalic system on transmission in the LGN is presynaptic in nature.

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Note added in proof. We have found that potentials antidromically elicited in the optic tract by submaximal stimulation of LGN increase 10–20 per cent during the period of inhibition. Such manifestation of increased excitability in presynaptic terminals is exactly concordant with expectations for presynaptic inhibition[7].

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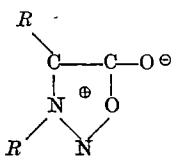
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PHARMACOLOGY

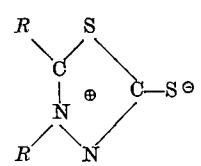
A New Class of Hypotensive Agents

FOR several years we have been engaged at these laboratories in an investigation of the physical, chemical, and pharmacological properties of various mesoionic heterocyclic ring systems. In the first of these systems investigated—the sydnones (I)—we found marked central nervous system stimulation[1–6].



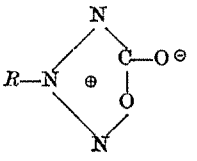
(I)

More recently, we have investigated a series of sulphur-containing mesoionic heterocyclic compounds with a ring isoelectronic with the sydnones. These compounds—the mesoionic 1,3,4-thiadiazoles (II)—were found to have antimicrobial activity[7,8].



(II)

Our continuing interest in the potential pharmacological activity of the mesoionic compounds, as well as the possibility of structure-activity correlations, led us to synthesize and study a number of mesoionic compounds known as the ψ-oxatriazoles (III). This class of heterocyclic compounds had been known since 1933[9], although the structure and nomenclature at present accepted were not established until more recently[10,11]. Later investigations have yielded additional methods of synthesis[12,13].



(III)

Several of these compounds have been synthesized by means of the procedure previously described[10] with minor modifications. The compounds made and tested were the isopropyl, the secondary butyl, the 3-pentyl, the *cyclo*hexyl, the 1,3 dimethyl butyl and the 1,2,2-trimethyl propyl derivatives of the basic structure. They all produced a hypotensive effect when injected intravenously into anaesthetized dogs, in a dose of 5 mg/kg as a 1 per cent solution in a 1 : 1 mixture of polyethylene glycol and normal saline. The fall in blood pressure ranged from about 6 per cent with the 1,3-dimethyl butyl compound, to 30 per cent with the *cyclo*hexyl compound, and the duration of action ranged from 1 to 2 h. The polyethylene glycol vehicle by itself was found not to affect blood pressure. Pretreatment with the antihistamine pyribenzamine did not prevent the depressor effects of the compounds, so that release of histamine is considered to be an unlikely mechanism of action. Pretreatment with the compounds appeared to potentiate responses to epinephrine and to decrease responses to norepinephrine. It is possible that some of the hypotensive effects are due to competition with norepinephrine for adrenergic receptors.

This preliminary investigation indicates that the pseudo-oxatriazoles as a group possess interesting hypotensive effects. We are now in the process of investigating these properties more intensively.

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Antagonism of Digitalis Arrhythmia by Pronethalol : a Neural Phenomenon?

THE depression by pronethalol of ventricular arrhythmia induced by digitalis, and of ventricular escape during vagal stimulation, is apparently not related to the capacity of this drug to block the cardiac effects of exogenous catecholamines[1–3]. Roberts *et al.*[3] pointed out

that the actions of pronethalol on the digitalis-induced arrhythmia and on vagal escape closely resemble those of reserpine and β-TM10—agents which act on sympathetic nerve terminals—and suggested that pronethalol may also affect the activity of sympathetic post-ganglionic nerve terminals.

To determine whether pronethalol acts on nerve terminals, we have examined the effects of the drug on cat soleus motor nerve terminals. Soleus axons normally respond to a single stimulus with a single action potential, but after a period of high-frequency stimulation the response to a single stimulus is repetitive. The post-tetanic repetitive activity (PTR) which is generated in the motor nerve terminal[4] is highly sensitive to neurotropic drugs[5–7]. Furthermore, the repetitive activity is transmitted to the soleus muscle where it causes an increase in isometric contraction strength (post-tetanic potentiation (PTP))[8]. Because of this direct link between PTR and PTP, soleus muscle PTP provides a simple method for assessing drug action on nerve terminals. The pharmacological similarity of motor and sympathetic nerve terminals is indicated in a recent report[9] which states that *d*-tubocurarine, dihydro-β-erythroidine and decamethonium block sympathetic post-ganglionic activity; these drugs profoundly depress the motor nerve terminal[10,11]. Conversely, Burn and Seltzer[9] found that bretyllium and guanethidine exert a blocking action in the rat phrenic-nerve–diaphragm preparation.

The details of our methods have been published[4,8]. In brief, cats were anaesthetized with 80 mg α-chloralose/kg and the popliteal fossa dissected to expose the soleus muscle and its nerve and blood supplies. The leg was mounted in a modified Brown–Shuster myograph and the soleus tendon attached to an isometric electrical strain gauge. The neural origin of the drug effects on PTP was confirmed, in several experiments, by monitoring effects on PTR in the ventral roots of soleus axons. Supramaximal rectangular stimuli, 0·1 msec in duration, were applied to the soleus nerve at a rate of 0·4 c/s. A high-frequency stimulus—10 sec at 400 c/s—was interposed every 5 min. Crystalline pronethalol (Ayerst) was dissolved in 0·85 per cent sodium chloride solution. The volume of injection was standardized at 0·1 ml./kg intra-arterially and 0·5 ml./kg intravenously, and the concentration of the pronethalol solutions adjusted accordingly.

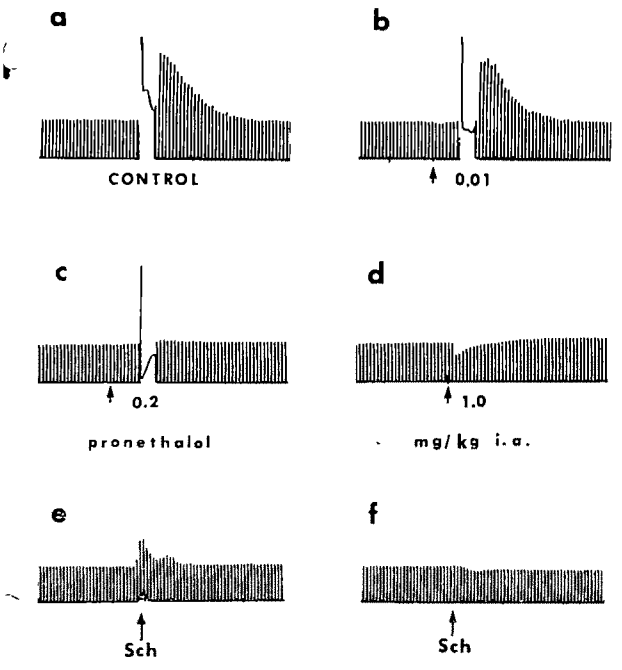


Fig. 1 Pronethalol depression of PTP (*a*, *b*, *c*) and neuromuscular block (*d*). *e* and *f*, from another experiment, show the effects of 0·2 μg succinylcholine/kg intra-arterially before and 80 min after 5 mg pronethalol/kg intravenously

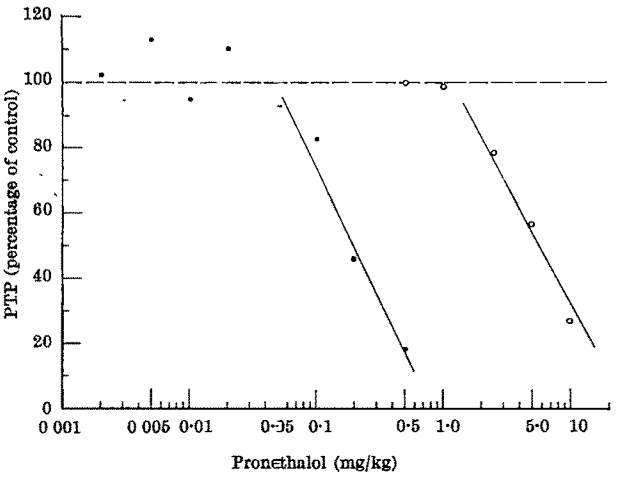


Fig. 2. Dose-response relationships between pronethalol and PTP. ●, Intra-arterial; ○, intravenous

The principal effects of pronethalol, illustrated in Fig. 1, are reduction of PTP and—with higher doses—blockade of neuromuscular transmission. The former results from a pronethalol action on the motor nerve terminal; recordings from ventral root axons confirm that pronethalol reduces PTP by attenuating PTR. The neuromuscular blockade is seldom produced by sub-lethal intravenous doses of pronethalol, but is readily produced by intra-arterial injections. It is presumably caused by depression of the motor nerve terminal as, on a dose scale, it follows directly on the abolition of PTP. The blockade is increased by edrophonium.

The dose–response relationships between pronethalol and PTP suppression are shown in Fig. 2. The results with intravenous administration are of particular interest because they indicate that depression of neural activity occurs with pronethalol doses which afford protection against the ventricular arrhythmia induced by digitalis[1–3]. It is of further interest that pronethalol is about three times as potent in suppressing PTP as diphenylhydantoin[5,12]—another neurotropic drug which has been observed to suppress digitalis arrhythmias[13].

The maximum pronethalol effect on PTP occurs within 2 min, and its duration is a function of dose. Apparently, complete recovery from the intravenous administration of 2·5, 5 and 10 mg/kg occurs in about 25 min, 35 min and 60 min, respectively. Re-administration of the drug at the time of apparent recovery, however, results in a greater effect than that of the initial injection—an indication of continued presence of sub-threshold concentrations of drug.

An additional pronethalol effect on PTP is indicated in Fig. 2. Small, rapidly administered intra-arterial doses produce a transient increase in PTP in roughly two-thirds of the experiments. The effect does not seem to occur with intravenously administered drug. Given by the latter method, however, the drug occasionally produces a small (about 10 per cent) increase in the muscle response to single stimuli. This effect, which has a latency of about 30 min and a duration of several hours, is not accompanied by muscle or nerve repetitive activity and apparently results from a drug effect on the muscle. (For possible mechanisms, see ref. 14.)

Pronethalol also suppresses drug-induced neural repetitive activity. Drugs such as physostigmine, 3-hydroxyphenyltriethylammonium ion (3-OHPTEA) and succinylcholine effect repetitive activity in the motor nerve terminal and potentiation of soleus muscle contraction strength[15,16]. The enhanced muscle activity, like

PTP, is caused by the neural repetitive activity, and consequently its suppression, like that of PTP, indicates a drug effect on the motor nerve terminal. Fig. 1 shows the effects of 0·2 μg succinylcholine/kg before and 80 min after the intravenous administration of 5 mg pronethalol/kg. The effects of physostigmine and 3-OHPTEA are similarly but less strongly reduced.

There is abundant evidence of pronethalol action on other neural systems. Convulsions are one of the principal toxic effects of the drug[17,18]. Recently, Vaughan Williams *et al.*[18,19] have described a variety of spinal reflexes which are affected by pronethalol. These effects, which are probably best interpreted on the basis of pronethalol depression of intra-medullary nerve terminal activity, occur with the same range of doses producing antagonism of the digitalis-induced arrhythmia.

Other drugs, such as β-TM10 and procaine, which depress digitalis arrhythmias, also affect nerve terminals[20,21]. The accumulated evidence therefore indicates that drugs which depress the digitalis-induced ventricular arrhythmia also depress nerve terminal activity. This correlation suggests that some of the digitalis arrhythmias and their antagonism by pronethalol and other drugs may be neural phenomena.

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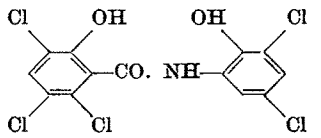
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A New Drug for the Treatment of Fascioliasis in Sheep and Cattle

ALTHOUGH a number of drugs are available for the treatment of liver fluke disease in sheep and cattle, they are all liable to produce unpredictable toxic effects, with occasional deaths, when used under farm conditions. This is particularly true in cattle, and the precautionary measures often required during their use are such that valuable animals may not be treated routinely for the condition. These untreated animals act as important reservoir hosts for the parasite and thus help perpetuate the disease.

The problem has led us to conduct a search for a safe and reliable treatment for fascioliasis, and we now report the discovery of a compound which appears to satisfy this need. This compound (I) is 2,2′-dihydroxy-3,3′,5,5′,6-pentachlorobenzanilide (U.K. patent applications Nos. 32129/63 and 32130/63), which is a white crystalline solid melting at 208° C, virtually insoluble in water but moderately soluble in the common organic solvents.



(I)

The activity of this compound was first demonstrated in a series of tests against adult and immature *Fasciola hepatica* infections produced experimentally in rats and mice. The definition of liver fluke maturity which we feel important in relation to the disease process, and which we have therefore adopted in describing drug activity, refers to the actual location of the parasite in the liver and not to the onset of egg production. Thus our test against 'mature' liver flukes refers to all flukes present in the bile duct of rats 12 weeks after an experimental infection, irrespective of whether these parasites are producing eggs or not. Our 'immature' test is carried out against 3-week-old *F. hepatica* infections in mice because the parasites are still migrating through the liver tissue at this time.

The results achieved with 2,2′-dihydroxy-3,3′,5,5′,6-pentachlorobenzanilide in this test system are compared in Table 1 with those of other drugs, known to be effective against liver flukes.

It can be seen that 2,2′-dihydroxy-3,3′,5,5′,6-pentachlorobenzanilide is highly effective against adult flukes in the rat and has a therapeutic safety margin which exceeds that of the other drugs. However, like the other drugs, its action against 'immature' flukes in mice is relatively poor, and consequently near-toxic levels of compound have to be administered before worthwhile activity is obtained.

Table 1. COMPARATIVE ACTIVITY AND MAMMALIAN TOXICITY OF VARIOUS FASCIOLICIDES FOLLOWING ORAL ADMINISTRATION TO RATS AND MICE

Fasciolicide	Fasciocidal activity* 'Adult' fluke (rats)	'Immature' fluke (mice)	Toxicity (approx. $L.D._5$ in rats)
Carbon tetrachloride	250	No action†	8 g/kg
Hexachloroethane	250	No action	6 g/kg
1.1:1:2 Tetrachloro-2,2-difluoroethane	500	No action	> 8 g/kg
1:4-*bis*-Trichloromethyl benzene	100	No action	6 g/kg
2-Hydroxy-3,4′,5-tribromobenzanilide (3 parts) + 2-Hydroxy-4′,5-dibromobenzanilide (1 part) (Mixture)	100	200 × 2 (100 × 3 no action)	600 mg/kg
2,2′-Dihydroxy-3,3′,5,5′,6,6′-hexachlorodiphenylmethane	10	100 × 1 (50 × 3 no action)	100 mg/kg
2,2′-Dihydroxy-3,3′,5,5′,6-pentachlorobenzanilide	15	100 × 3	1 g/kg

* Activity expressed as lowest dose in mg/kg showing definite action.
† Highest dose tested was 1 g/kg administered on three successive days.

A number of possible reasons for the relative resistance of 'immature' flukes to 2,2′-dihydroxy-3,3′,5,5′,6-pentachlorobenzanilide have been examined. Experiments showed that both 'adult' (12-week-old) and 'immature' (3-week-old) flukes are equally susceptible to the action of the compound when investigated under identical conditions *in vitro*, which suggested that the 'immature' flukes must receive some sort of 'protection' from their particular habitat *in vivo*. We know of two ways in which this 'protection' might arise. First, the drug is excreted as an active metabolite (glucuronide) into the bile duct, which provides a mechanism for it to become preferentially concentrated around 'adult' flukes inhabiting the bile duct. Secondly, the activity of the drug is considerably reduced in the presence of blood and serum, presumably by protein binding, and this could reduce its effectiveness against 'immature' flukes in the liver tissue. We do not know whether these are the only reasons for

the apparent resistance of 'immature' flukes or which is the most important.

The results in laboratory animals with this compound were so promising that it is now being extensively evaluated in sheep and cattle under a variety of farm conditions. Preliminary reports from a number of workers indicate that it may be of considerable value and, accordingly, it is being made generally available by Imperial Chemical Industries, Ltd., under the brand name of 'Zanil'.

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HAEMATOLOGY

Strain and Sex Dependency of Pre-albumin in Mice

THE composition of the pre-albumin fraction in the serum of several species is known to be a hereditary characteristic[1-5], but sex dependency has not as yet been described.

We used Smithies'[6] horizontal technique of starch-gel electrophoresis in a discontinuous buffer system (*tris*, citrate, borate/borate, *p*H 8·5). In this way the pre-albumin in serum of mice was separated into three clearly distinct components (PA_1—PA_2—PA_3). We were not able to express our data on pre-albumin concentration in mg/ml., since pure PA was not available to us as a reference. Instead, measurements were taken with a densitometer after we had stained our electrophoretograms with amido black 10 *B*.

Seven inbred strains of mice were investigated: *AKR*, *BALB/c*$^+$, *CBA*, *C57BL*, *C*$_3$*H*, *CO*$_{13}$ and *RF*. Groups consisting of 25–30 males of each strain were examined. Fig. 1 shows the electrophoretic pattern of serum in the different strains of mice. PA_2 is present only in the *AKR*, *BALB/c*$^+$ and *RF* strains and is not observed in the others.

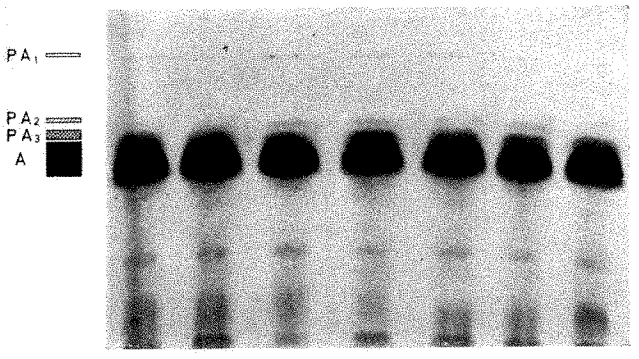


Fig. 1. Starch-gel electrophoresis of the serum of different strains of male mice. From left to right: *C57BL*, *CO*$_{13}$, *RF*, *AKR*, *C*$_3$*H*, *BALB/c*$^+$ and *CBA*

The electrophoretic pattern of female mice is significantly different from that of the males. As Table 1 shows, PA_1 is detectable only in male mice and PA_2 and PA_3 are significantly lower in females (55 per cent and 52 per cent for PA_2 and PA_3, respectively, of that of male mice).

Table 1. SEX DEPENDENCY OF PRE-ALBUMIN

Sex	No. of animals	PA_1	PA_2	PA_3
Adult male	17	7·7 ± 0·7	21·5 ± 1·5	66·5 ± 2·9
Adult female	16	Not detectable	11·2 ± 0·7	36·4 ± 1·2
Immature male	9	Not detectable	13·7 ± 1	43 ± 2
Immature female	9	Not detectable	13·5 ± 1·1	36 ± 1·6

Mean values are presented expressed in arbitrary densitometric units ± standard deviation of the mean.

No significant differences were detected in the electrophoretic patterns of immature 4-week-old male and female mice, but they are different from adult females.

We are at present investigating the influence of sex hormones on the concentration of pre-albumin in the serum of mice.

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Aggregation of Human Platelets by Dextrans

DURING the course of an investigation into the effect of dextrans on platelet aggregation induced by adenosine diphosphate, it was noted that the addition of dextrans of various molecular weights to platelet-rich citrated human plasma *in vitro* resulted in a fall in the optical density of the plasma. This effect has been examined using a turbidimetric method[1] and also phase-contrast microscopy.

Blood was obtained from normal volunteers by atraumatic venepuncture using siliconized S.G. 20 needles, plastic tubes containing 3·8 per cent sodium citrate, one part citrate to nine parts blood, and was centrifuged at 190*g* for 10 min to give platelet-rich citrated plasma (PRCP) which was used within 3 h. 2-ml. portions of PRCP were placed in siliconized glass cuvettes and were stirred with polythene-covered magnetic stirrers. The optical density of the PRCP was measured at room temperature in an EEL titrometer using a red filter (608). 20 per cent solutions of dextrans of various molecular weights were prepared in 0·895 per cent sodium chloride.

The addition of dextran to a final concentration of more than 0·5 per cent to PRCP results in an immediate optical clearing effect larger than the dilutional fall in optical density, produced either by the addition of similar volumes of isotonic sodium chloride to PRCP, or of dextran to platelet-free plasma. Resuspension of the isolated platelet button in the platelet-free plasma restores the effect just described. The effect is also seen after the addition of dextrans to suspensions of washed platelets in a saline–*tris* hydrochloride buffer solution.

Table 1. MEAN PERCENTAGE FALL IN OPTICAL DENSITY AFTER THE ADDITION OF 0·2 ML. OF 20 PER CENT SOLUTIONS OF DEXTRANS OF VARIOUS MEAN MOLECULAR WEIGHTS IN ISOTONIC SALINE, AND OF 0·2 ML. SALINE ALONE TO 2·0-ML. PORTIONS OF PRCP*

Additive	No. of exps.	Mean % fall in *O.D.*
*D*10†	22	18·8
*D*20	19	19·0
*D*40	21	20·1
*D*80	20	21·8
*D*110	19	20·2
*D*150	19	16·6
*D*250	21	16·6
*D*500	22	17·0
Sodium chloride 0·895%	17	8·1

* Final dextran concentration 1·82 per cent.
† *D*10, Dextran with mean molecular weight of 10,000; *D*20, dextran with mean molecular weight of 20,000, etc.

Examination of small aliquots of PRCP and of platelet suspensions before and after the addition of dextrans, using phase-contrast microscopy and siliconized microscope slides and coverslips, confirms that dextrans produce platelet aggregation *in vitro* (Figs. 1 and 2). The degree of platelet aggregation, according to changes in optical

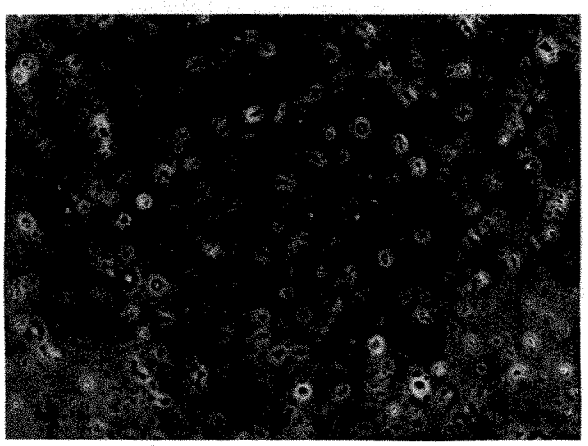


Fig. 1. PRCP before addition of dextran (phase-contrast microscopy, × c. 255)

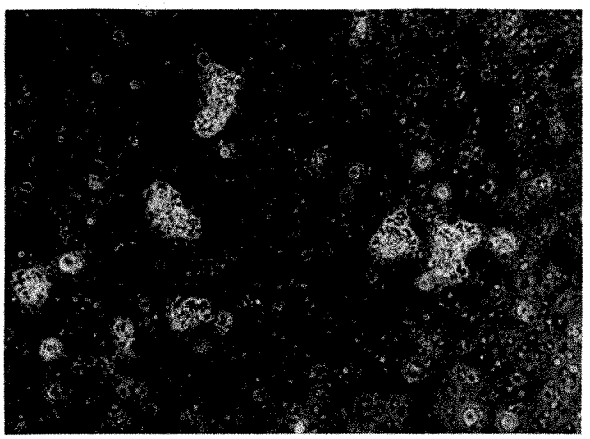


Fig. 2. PRCP after addition of dextran with mean molecular weight of 40,000; final concentration 1·82 per cent (phase-contrast microscopy, × c. 255)

density, increases with increasing concentrations of dextran, but seems remarkably independent of molecular weight (see Table 1). However, after the addition of dextrans to plasma, measurements of optical density are complicated by the precipitation of dextran–protein complexes. This effect increases with increasing molecular weight, and at the upper end of the molecular weight range of dextrans studied the net change in optical density is a summation of the optical clearing effect from platelet aggregation, and of the increased turbidity produced by protein precipitation. Thus, precise dissection of the relationship between platelet aggregation and molecular weight requires further investigation using protein-free platelet suspensions.

Previous workers have reported release of histamine from platelets by dextran[2], possibly through damage to the platelet cell membrane. Pharmacologically active substances released in this way could cause platelet aggregation, but our preliminary findings show that dextran-induced platelet aggregation is probably not mediated by any of the known chemical inducers of platelet aggregation. Dextran-induced aggregation is not spontaneously reversible on observation for up to 6 h, and slowly increases over this period; it is qualitatively different from that induced by ADP and is not inhibited by adenosine, diphenhydramine, promethazine, mepyramine maleate, imipramine or phentolamine, even in very high non-pharmacological concentrations.

We have observed a similar phenomenon with other macromolecular colloids, and early investigations of the toxicity to animals of dextran sulphate mention a platelet agglutinating activity[3]. Dextran and other colloids may induce platelet aggregation either by their adsorption on to the platelet surface or by producing physical changes in the 'atmosphere plasmatique'.

It is evident that platelet aggregation is induced *in vitro* by dextran fractions similar to those used clinically to expand the plasma volume and to improve capillary blood flow[4] and at dextran concentrations that can be attained in the blood after infusion. Further investigations to confirm that dextrans also induce platelet aggregation *in vivo* are being undertaken. The possible relationship between dextran-induced platelet aggregation and the known antihaemostatic properties of dextran remains speculative.

The dextran fractions were kindly supplied by Pharmacia (G.B.), Ltd.

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IMMUNOLOGY

Antibody Nature of the Inhibitor to Antihaemophilic Globulin (Factor VIII)

ABOUT 5 per cent of haemophilic patients develop in their blood an inhibitor to factor VIII. The most likely explanation is that this is due to an immunological response to antihaemophilic factor of human origin given therapeutically on some previous occasion, since most haemophilic patients receive multiple transfusions of human blood or plasma; but development of an inhibitor is comparatively rare and therapeutic use of factor VIII of animal origin does not increase the incidence. A similar inhibitor is known as a rare complication of other conditions in patients with no previous history of haemophilia or transfusion. No direct cause-and-effect relationship between intravenous therapy and development of inhibitor has ever been established. For these reasons, and partly because of the uncertainty as to whether the inhibitor acts in the manner of an enzyme or an antibody[1,2], there has been doubt about the antibody nature of inhibitor.

The purified inhibitor was found to be associated with immunoelectrophoretically medium fast 7*S* γ-globulin[3], as had been found for reaginic antibodies involved in asthma and hay fever[4] which were later identified as IgA-antibodies[5,6]. The greater heat stability of the inhibitor and differences in the conditions of elution from DEAE-cellulose distinguish it from reagins.

In view of the controversy concerning the mode of action of inhibitor, and because of the possibility that even our most highly purified inhibitor preparations could have contained plasma components other than medium fast γ-globulin, it seemed important to establish conclusively whether the inhibitory activity could be identified with the medium fast γ-globulin in these purified inhibitor preparations. Attempts were therefore made to precipitate the inhibitory activity by rabbit antiserum specific for human γ-globulins.

Our starting material was serum from a haemophilic patient described by Hall[7] (case 1). Coagulant proteins were removed by absorption with barium sulphate and the inhibitor was purified according to Leitner *et al.*[8] and finally concentrated by ultrafiltration (to a final solution having $E^{280m\mu}_{1\,cm} = 3{\cdot}0$).

The rabbit antiserum *R*92 was prepared by repeated multiple injections of immuno-electrophoretically pure

human IgG-globulin[8] and gave on immuno-electrophoresis against whole human serum never more than the characteristic single IgG line. *R*92 thus possessed antibodies only for IgG-globulins although being capable of precipitating IgA- and IgM-globulins, since these share determinants with IgG-globulins[8,9].

*R*92 and a control normal rabbit serum of similar age and stored in the same way were both pre-treated with barium sulphate to remove the coagulation factors (barium sulphate was added to each serum sample at a concentration of 100 mg/ml.; both samples were incubated at 37° C for 15 min with constant agitation; the barium sulphate was then spun off).

The inhibitor preparation was divided into two portions of 0·5 ml. each and rabbit anti-IgG-serum *R*92 was added to one portion in four successive amounts of 0·1 ml., until only a trace of γ-globulin remained; like amounts of control rabbit serum were added to the other (control) portion of inhibitor. The resultant supernatants were examined immuno-electrophoretically and for inhibitor potency against factor VIII of human and bovine origin. Immuno-electrophoresis showed that all but a trace of γ-globulin had been specifically removed by *R*92 from the inhibitory preparation, leaving the other serum proteins intact. Table 1 shows that the anti-IgG-antiserum had

Table 1. INHIBITOR POTENCY OF SUPERNATANTS AFTER TREATMENT WITH RABBIT SERA

Pre-treatment of inhibitor	Potency* of inhibitor (units per ml.) Against human factor VIII	Against bovine factor VIII†
With rabbit antiserum for human γ-globulin (*R*92)	0·6	2·1
With normal rabbit serum	26·0	13·3

* Assay method of Biggs and Bidwell (ref. 10).
† Supplied by Maws Pharmacy Supplies, Ltd., Barnet.

likewise removed most of the inhibitor potency. The possibility remained that the inhibitor might have been absorbed non-specifically on an antigen–antibody precipitate. To test this, a precipitate of egg albumen-rabbit anti-egg albumen was formed in the presence of inhibitor. There was no diminution in the inhibitor potency when compared with a control using normal rabbit serum. (The rabbit sera were pre-treated with barium sulphate as before.)

The near-quantitative removal of IgG-globulin by *R*92 in parallel with the near-quantitative removal of inhibitor, together with the fact that the inhibitor is potentiated by therapy with factor VIII, makes it highly probable that the inhibitor is indeed an antibody. The possibility of the inhibitor being a γ-globulin with enzyme activity cannot be excluded, but seems now somewhat unlikely.

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Antigenicity of Soluble Protein in the Presence of High Levels of Antibody : a Possible Mode of Action of the Antigen Adjuvants

IN experiments carried out to elucidate the mode of action of depot-forming antigen adjuvants, such as alum and mineral oil, several attempts[1,2] to mimic a slow release mechanism have been made by injecting daily doses of the antigen alone. The antibody responses obtained have, however, been found to be inferior to those stimulated by a single dose of the antigen with adjuvant. In these tests the quantity of antigen injected daily was relatively large; the total quantity given over a short time being arranged to be at least equal to a normal single dose given with adjuvant.

During recent work[3] with water-in-oil emulsion adjuvants it became evident that the antigen persisted in an active state within the emulsion at the injection site for very long periods, possibly years. The daily rate of release, if any, must therefore be very small and it was questionable whether it could be effective as an antigenic stimulus. To examine this, ten mice in a group were each injected subcutaneously with very small daily doses of antigen, chromatographically-purified ovalbumin[3], in saline. Doses of 64, 32, 16, 8, 4 and 2 μg were given successively over the first 6 days and thereafter 1 μg each day. On the fiftieth day, by which time a total of 171 μg of ovalbumin had been given, the injections were stopped.

The titre of antibody in individual samples of serum collected at intervals from the mice was measured by the tanned red cell haemagglutination test[4]. The geometric means of these titres and the response of a group of ten mice, each of which had been given a single dose of 2,000 μg of ovalbumin in a water-in-oil emulsion, are shown in Fig. 1. The very tiny doses of ovalbumin were as effective in stimulating antibody production as the much larger dose with adjuvant, even though the later injections were made into a highly immune animal. In particular, their activity is confirmed by the rapid fall in antibody level which occurred as soon as the injections were stopped.

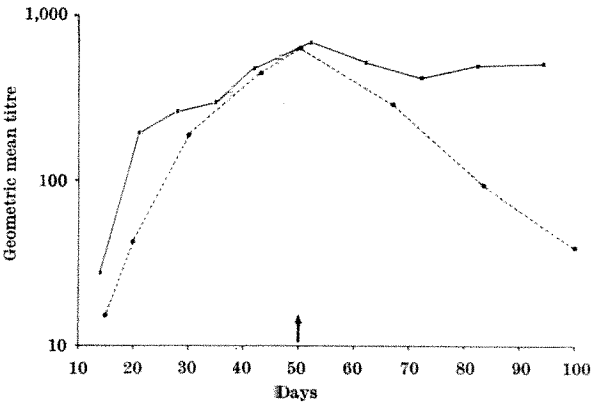


Fig. 1. The antibody responses of groups of mice inoculated subcutaneously with ovalbumin either in a water-in-oil emulsion, or as very small daily doses in saline. ——, 2,000 μg in water-in-oil emulsion; - - - -, 171 μg in saline, in divided doses, last dose at the arrow

The 2,000-μg dose of antigen given in the water-in-oil emulsion was a standard one, chosen as being the smallest which, if given in saline alone, would stimulate a measurable antibody titre. The peak antibody titre reached by mice at about the twentieth day after inoculation of 2,000 μg of ovalbumin in saline alone was less than 10 on the scale of Fig. 1. Smaller single doses of antigen given in saline or in water-in-oil emulsion produced lower peak titres and there was no evidence of any immunological paralysis at the standard dose level.

The excellent antibody response obtained by giving very small daily doses of antigen indicates that a slow release mechanism could fully explain the great effectiveness of the

depot-forming adjuvants. These results also show that a very tiny quantity of antigen, which must presumably combine with antibody immediately after injection, is still able to stimulate further the antibody-forming cells.

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HISTOCHEMISTRY

Histochemical Observations on the Lipids of Carious Human Dentine

THE presence of lipids in carious dentine has been observed, and variously considered to be of exogenous[1] or endogenous[2,3] origin, and the staining of the peritubular dentine matrix of sound dentine by histochemical techniques for lipids has been reported earlier[4].

Undecalcified ground sections of dentine, approximately 7μ thick, were prepared from human molar teeth exhibiting occlusal Class I type caries. Sections were cut through the lesion transverse to the long axis of the tooth. The caries was thus completely surrounded by sound dentine, which provided reference and support and the maximum number of tubules were cut transverse to their long axis. The centre of the lesion was its most advanced part and its periphery the advancing front. Some sections were unfixed, being prepared under iced saline; others were fixed in 10 per cent neutral formol saline or 70 per cent ethyl alcohol. Sections were stained without further treatment, or after being subjected to boiling 0·02 per cent acetic acid for 8 min. The staining methods employed were: acetone sudan black *B*[5], phosphomolybdic acid[6], nile blue sulphate[7], and Luxol fast blue[8].

After unmasking in acetic acid, dentine sections exhibited a periphery of sound dentine in which the peritubular matrix was well stained but the intertubular matrix was unstained. At the junction with the carious lesion, the staining of the peritubular matrix increased in density, and in the more advanced parts of the lesion the intertubular matrix stained with increasing intensity, until, at one level, the peritubular and intertubular matrices stained equally. With further progression of the caries the peritubular matrix became unstained, so that the appearance was the reverse of that seen in sound dentine. In the centre of the lesion no stain was retained by any part of the dentine. Similar sections, stained without previous unmasking in acetic acid, did so in a comparable manner, but the carious dentine tended to retain more stain, consequently the differentiation of the various zones was not so evident. The sound dentine, apart from the tubule contents, remained unstained. At the advancing front of the lesion the peritubular matrix was heavily stained; at a deeper level the intertubular matrix also stained, and with increasing intensity.

It appears that the carious process first unmasks the lipids present in the peritubular matrix, as shown by its heavy staining. This unmasking is accompanied by a partial release of these lipids, as following hot acetic acid they are unstained in the more advanced parts of the lesion. Comparable changes in the staining of intertubular dentine matrix, though phased later, presumably indicate the presence of lipids. However, probably because suitable unmasking techniques have not been utilized to date, they have not been convincingly demonstrated in this matrix in sound dentine.

This evidence, showing that the development of lipid staining in carious dentine follows a similar pattern to that which can be obtained in sound dentine, suggests that much of the lipid component of caries is of intrinsic origin, though it does not exclude the possibility of an extrinsic component as well.

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RADIOBIOLOGY

Relation between Extrapolation Number and Apical Cell Number in Gemmae of *Marchantia polymorpha* L.

THE gemmae of *Marchantia polymorpha* L. are approximately discoidal with two apical notches 180° from each other[1,2]. An apical notch marks the location of paired apical cells. Radiobiological data on gemmae of *Marchantia* indicate that the apical growth of a gemma need not necessarily be the only type of growth occurring in the proliferation of a viable outgrowth from a gemma[3–5]. In addition, it has been shown that cell survival within the irradiated gemma is correlated with nuclear volume differences among the different cells[4,5]. These results are similar to those reported for the radiosensitivity of 'higher plants' by Sparrow and co-workers[6–8], Osborne *et al.*[9,10], and Lunden[11], although the former authors[6–8] state that the volume of the chromosome at interphase is a better index of radiosensitivity than nuclear volume.

The present investigation was conducted in an attempt to determine whether or not the production of a viable apical outgrowth from an irradiated gemma is dependent on the destruction of both apical cells.

Gemmae of *Marchantia* were irradiated with 250 kVp. X-rays and aseptically cultured according to a previously published method[12]. At the control and lower exposures 130 gemmae were used for each treatment; at the 35·8 kr and 39·4 kr exposures 2,080 gemmae were used for each exposure. The percentage apical survival for each treatment was plotted against exposure on a semilog graph (Fig. 1) according to the method of Atwood and Norman[13]. The survival curve in Fig. 1 is sigmoid and fits the expression $S = 1-(1-e^{-kD})^n$ where S is the survival at dose D, n is the extrapolation number that can represent the number of sites (or cells) of a particular type that must be inactivated in order to halt the increase in cell number[13]. This parameter, n, may be determined by extrapolation of the exponential portion of the survival curve if survival is plotted against dose on semilog paper, or it may be computed according to the equation $\log S = \log n - kD$ (see derivation in Atwood and Norman[13]). Extrapolation of the straight line portion of the curve in Fig. 1 to $D = 0$ gives $n = 2{\cdot}7$. A t-test showed this number not to differ significantly from 2 or 3 at the 5 per cent level, but to differ significantly from 1. Thus, the survival curve for apical survival gives an extrapolation number of approximately 2, which on a target hypothesis indicates that both apical cells must be inactivated in order to stop apical growth of a gemma.

Additional research has shown that both apical cells are active in DNA synthesis and mitosis. These results tend to support further the idea that both apical cells

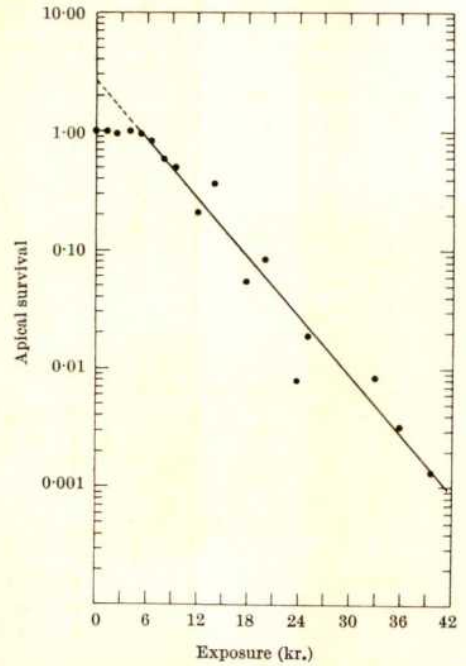


Fig. 1. Radiosensitivity of the apical notch to acute gamma irradiation. Measurements for survival of the apical notch were made at the end of one week of growth. The linear portion of the curve is extrapolated to D (exposure) = 0. Log $Y = 0{\cdot}43746 - 0{\cdot}08296\ X$

must be inactivated to halt apical survival. Further investigations are now in progress.

This research was carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.

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Ultrastructural Aspects of Duodenal Crypts in X-irradiated Mice after Chemical Protection

AFTER X-irradiation the cells of the duodenal crypt of the mouse show morphological alterations which have been described with the help of optic and electronic microscopy[1,2]. The protective effect of aminoethylisothiourea (AET) on the intracellular structures of the irradiated duodenum has already been shown[3]. Recently, it was demonstrated by different authors that a mixture of certain radioprotectors was more effective than each alone[4,5] and even allowed an increase of the *L.D.* 100/30 by a factor of 2·4 (ref. 6). With these results in mind, we have investigated the ultrastructural aspects of the duodenal crypt after irradiation of mice protected by a mixture of glutathione, AET and serotonin.

BALB/c[+] adult male mice were given intraperitoneally 16 mg glutathione per os, 8 mg AET and 1 mg serotonin, 25, 10 and 5 min before irradiation respectively. Other mice were injected, before irradiation, with AET alone and some were irradiated controls. The conditions of irradiation were: 1,350 r., 200 kV, 10 m.amp; dose-rate, 100 r./min. The animals were killed 30, 60, 180 and 300 min after irradiation. A small piece of duodenum was immediately fixed in 2 per cent osmium tetroxide and embedded in 'Epon'.

We report here the first results we obtained of the modifications of the stem cells of the duodenal crypt. We found that the irradiated non-protected mice showed a dilatation of the mitochondria after 30 min; the number of lysosomes increased after 60 min and big cytolysomes were obvious in almost all the stem cells after 180 min[2].

The AET-protected irradiated mice exhibited some delay in the appearance of the lesions; the dilatation of mitochondria was not visible before 60 min and later was less important than in irradiated controls. It must be noted, however, that after 300 min many cytolysomes were present in the stem cells (Fig. 1).

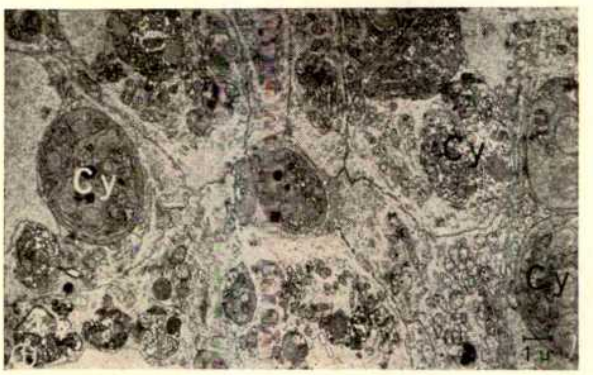


Fig. 1. Duodenal crypt 6 h after 1,350 r. in an AET-protected mouse. Many cytolysomes (*Cy*) in the stem cells (×*c.* 2,465)

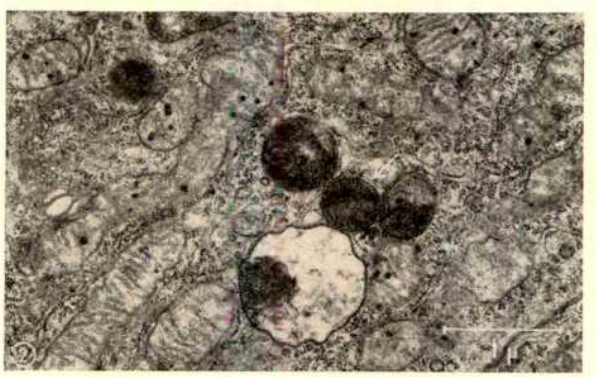


Fig. 2. Part of a stem cell 6 h after 1,350 r. in a mouse protected by the radioprotective mixture. Four vacuoles with dense or clear content; arrow points to vacuole with needle-shaped structures (×*c.* 14,815)

The irradiated mice protected by the chemical mixture did not show, even after 180 min, any important mitochondrial dilatation, but the mitochondria were twisted and elongated with shadowed cristae and frequently presented a shrinkage; after 300 min, the number of cytolysomes was still very low; at this time, in the vicinity of the Golgi zone, many clear vacuoles with a granular or fibrillar content were found; other vacuoles exhibited a dense matrix with needle-shaped structures (Fig. 2); these organites were never seen in the controls and were uncommon in animals injected with AET alone; these structures were also numerous in the non-irradiated mice injected with the mixture

The protection of the undifferentiated stem cells of the duodenal crypt at the ultrastructural level seemed to be,

in the first hours after 1,350 r., very important after the use of this chemical mixture.

It is possible that the needle-shaped structures in vacuoles, described 300 min after administration of the protectors, are formed by the collected metabolites of the injected drugs through a similar process of the accumulation of 'Triton' in the hepatocytes of the rat[7].

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BIOLOGY

Cell Cleavage in Polar Body Formation

THERE has been widespread acceptance of the generalization formulated by Conklin[1] and Wilson[2] that, when mitosis is followed by normal cytokinesis in plants and animals, the "cleavage takes place in a plane approximately at right angles to the spindle axis and primarily across its equatorial plane"[2]. (In certain cells which are large in comparison with the mitotic apparatus, the equator of the spindle becomes a part of the cleavage surface, but the latter may curve on either side of the spindle.) The generalization has been shown to be true for all cases of cytokinesis which have been studied intensively[3]. Moreover, after the nucleus has been displaced by experimental means, the subsequent cleavage has occurred through the middle of the spindle wherever the spindle might be at the late anaphase stage[1,4–8].

Polar body formation is an extreme case of unequal cleavage and so it affords a useful test case for theories of cleavage. A recent reviewer states that "it is one of the few cases in which the cleavage plane does not bisect the metaphase spindle"[9]. This statement does not contradict the generalization of Conklin and Wilson because it is the telophase spindle rather than the metaphase spindle which is bisected by the cleavage furrow. Conklin showed that in the normal maturation divisions of eggs of the gastropod *Crepidula* "the division wall forms through the equator of the spindle"[1]. In polar cleavages the spindle moves with respect to the egg surface between metaphase and the stage at which cleavage is completed. For the case of the *Cerebratulus* egg[10], the *Limnaea* egg[11], and other eggs for which the polar metaphase spindle is oriented radially at the egg surface[2], there is a translational movement of the spindle parallel to its axis and outwards from the egg centre. This is accompanied by a cleavage for which the astral relaxation theory has been proposed[9]. The theory postulates that the aster differentiates the cell membrane opposite it, where a local weakening in the consistency of the cell surface allows internal pressure to push out the polar body from the egg.

More recently, observations have been made on the formation of the second polar body of certain rodent eggs *in vitro*[12–14]. This work seems to have passed unnoticed by students of the mechanism of the cleavage process. Excellent photographs by phase-contrast microscopy show that, for eggs of the rat and the hamster, the cleavage surface grows through the mid-point of the spindle and, in so doing, bisects that aggregation of granules found at the equator of the spindle which is sometimes called the intermediary body or the mid-body. Although in certain optical microphotographs, such as Fig. 10 of Austin[13] and Fig. 5 of Odor and Renniger[15], the cleavage furrow appears to have passed just to one side of the intermediary body so that it is included in the polar body, by electron microscopy the new plasma membrane appears to be derived by the coalescence of vesicles located down the mid-line of the intermediary body[15]. The optical microscope appearance may be an illusion due to the optical depth of focus being greater than the thickness of the ultra-thin section required for electron microscopy. The cleavage furrow is initiated at the egg surface where it is first close to the equatorial plane of the spindle when the latter is in its initial chordal position (Fig. 1*a*). As the spindle pivots about one pole to assume a radial orientation with respect to the egg surface, the newly formed furrow grows through the cytoplasm in the path of the intermediary body (Fig. 1*a*, *b* and *c*). The photographs of Austin[12] were taken with the egg flattened between slide and coverslip so as to observe greater detail. Under these conditions cleavage is not completed and the path of the furrow is more deeply indented into the vitellus than would be the case for an uncompressed egg, such as that shown in Figs. 10 and 18 of Austin and Walton[16]. Other features of the polar cleavage described above are not affected by the compression. To the extent that the polar cleavage of these rodent eggs appears to be initiated at or near the equatorial plane of the spindle, and to grow from the egg surface through the cytoplasm, it appears to resemble the first zygotic cleavage of newt[17], axolotl[8] and sturgeon[8]. The astral relaxation theory does not adequately explain the polar cleavages in eggs of rodents.

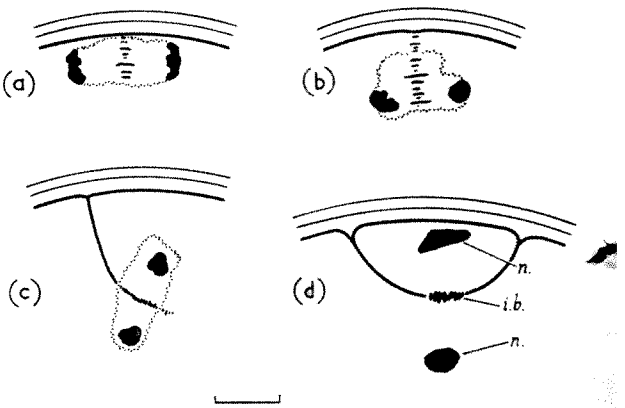


Fig. 1. Stages in the formation of the second polar body in the rat egg, traced from the microphotographs of Austin (ref. 12) (*a*, *b* and *c*) and Austin and Braden (ref. 14) (*d*); (*n*, nucleus, *i.b.*, intermediary body). *a*, *b* and *c* are from continuous observations made on a single egg. The scale represents 10μ

Several lines for future experimental work suggest themselves. It may be possible to measure changes in the optical properties of the cell membrane during polar cleavages and to compare the results with those obtained for the first zygotic cleavage of the sea-urchin egg[18]. Recently, improved methods of fixation for electron-microscopy have revealed greater ultrastructural detail within the intermediary body of dividing HeLa cells[19,20] and similar techniques should now be applied to eggs in polar cleavage. By these methods and by micrc manipulation it may be possible to obtain evidence for the existence of connexions between the mitotic apparatus and the inner layers of the egg cortex and this would throw light on the observed spindle movements.

I thank Dr. C. R. Austin for commenting on this letter and for permission to publish diagrams from his photo-

graphs. I also thank Dr. R. A. Beatty for discussion, and Prof. C. H. Waddington for his interest.

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Oxytocin and the Life-span of Male Rats

Friedman and Friedman[1] recently reported that chronic administration of a crude extract of the posterior pituitary gland extended the life-span of old male rats. They postulated that this effect resulted from changes caused by vasopressin in the metabolism of water and salts. Vasopressin alone, however, failed to produce prolongation of life in the aforementioned experiments. More recently, they have extended their investigations to the effect of aldosterone.

It seemed to us not unreasonable to assume that the effect on life span of posterior pituitary extracts is attributable, at least in part, to their content of oxytocin rather than of vasopressin. The possibility that a third, perhaps yet unknown, factor is present in the gland has not been overlooked.

In the investigation reported here, male Sprague-Dawley rats were used (*C-D* strain, obtained from Charles River Breeding Laboratories), which were 15 or 16 months old at the start of the experiment. Animals of approximately equal weight (within 15 g) and in apparent good health were selected and divided at random into three groups of twelve rats each. Rats in each group were given subcutaneous injections three times a week. One group received 1 U/kg of posterior pituitary extract prepared by suspending 'Powder Posterior Pituitary U.S.P.' (Lilly) in saline. The second group received 1 U/kg of synthetic oxytocin, 'Syntocinon' (Sandoz), diluted in saline, and the control group was treated with saline.

While no significant difference was observed between the number of surviving rats in either of the two treated groups and those in the control group during the early months of the experiments, after a year a difference was apparent. The results of our investigations are summarized in Table 1, which shows the number of survivors at the end of each month of the experiment.

Our experiment seems to confirm the findings of Friedman and Friedman concerning the life-prolonging effect of the chronic administration of posterior pituitary extract in the rat but, at the same time, points to oxytocin as the most probable factor responsible for this phenomenon. It seems to be quite possible that oxytocin, in addition to being a hormone which causes ejection of milk and contraction of the uterus, may have also an as yet undisclosed, more general role in the life processes. Its occurrence in the male and in non-mammals is also suggestive in this respect.

Table 1. NUMBER OF RATS SURVIVING AT THE END OF EACH MONTH

	Month																	
Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Saline	11	11	10	9	8	8	8	7	7	6	5	2	2	2	1	1	1	0
Post. pituitary	11	10	10	10	10	10	9	9	8	8	8	7	5	3	3	3	0	0
Oxytocin	12	10	9	8	8	8	8	8	7	7	6	6	5	4	4	3	2	0

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Peritrophic Membranes in Arachnida (Arthropoda)

PERITROPHIC membranes have been studied in insects from the microscopic[1], submicroscopic[2] and histochemical[2–4] point of view. The lack of information about this structure in arachnids has already been emphasized[5,6]. I therefore present some more bibliographical and histological data on this subject.

Tulk in 1843 (ref. 7) gives a description of a membrane which completely surrounds the excreta in the stomach in *Phalangium opilio* (Latr.). Plateau[8] indicates some of the chemical properties of such a membrane in the distal part of the midgut in *Agelena labyrinthica*, and presents figures of a membrane that envelopes the excrementa of *Argyroneta* and *Epeira umbratica* (these three species belonging to the Araneidae). The histological work of Millot[9] on spiders also gives a description of a basophil membrane, formed by the fusion of distinct droplets round the excrements in the midgut, just behind the outlet of the abdominal diverticulae. This author gives no details concerning the species in which he observed the membrane. De Mets[5] gives electron microscopic figures of the peritrophic membrane in the horseshoe 'crab' *Xiphosura polyphemus*, whereas Grainge and Pearson[6] describe a membrane of 0·5μ thick in some Phalangidae.

I observed a peritrophic membrane in the cloaca (Fig. 1) and the midgut (Fig. 2) of *Linyphia triangularis* (Cl.), (Araneidae), a web-spinning spider, as well as in the postdiverticular midgut of the garden-spider *Araneus diadematus* (Cl.) (Fig. 3).

The histological preparations (fixation in Bouin and paraffin sections of 8μ) are stained with haematoxylin at *p*H 3 and potassium alum, and counterstained with erythrosine. The blue colour of the peritrophic membrane contrasts with the reddish base-tone of the gut contents and excrements and indicates their basophilic nature. Its

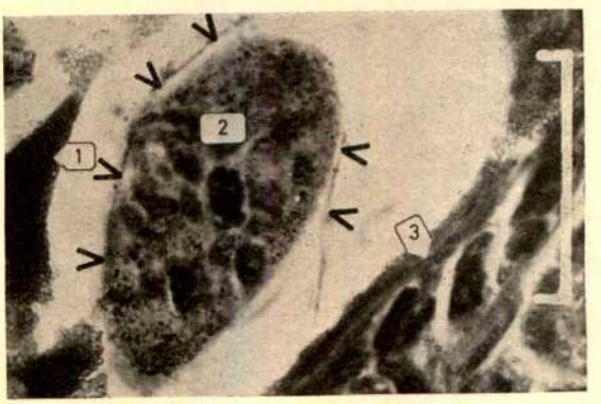


Fig. 1. *Linyphia triangularis:* guanine excretion (1); faecal pellet (2) with cellular debris; cloacal wall (3). The 'V' signs point to the peritrophic membrane. Scale = 50μ

thickness lies on the limits of optic resolution in *L. triangularis* (*c.* 0·4μ).

Some preliminary electron microscopic observations on the membrane that surrounds the faecal pellets (Fig. 4) in the cloaca of this species confirm this thickness, and suggest the composite nature of the membrane. The inner part presents a folded surface, the outer part being rather smooth and sometimes being loosened from the inner membrane (possibly due to stress during preparation: fixation in osmium oxide, embedding in 'Araldite' and sectioning with glass knives).

The complete lack of any experimental evidence for hypothetical functions of this membrane in digestion and/or excretion, in insects as well as in spiders, might stimulate physiological research into this matter.

I thank Dr. J. Hugon for the electron microscope photographs. This work was supported by grants from the Belgian Fund for Joint Fundamental Research.

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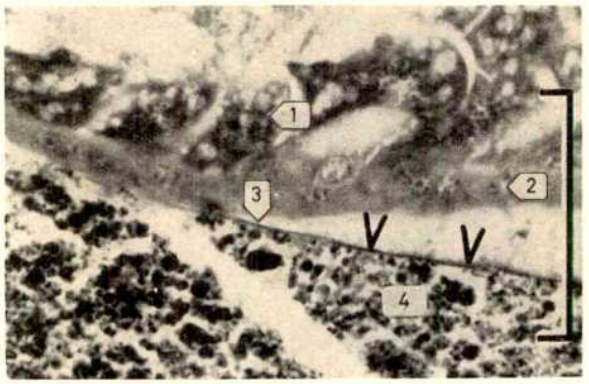


Fig. 2. Midgut of *L. triangularis:* (1) Interstitial cell. (2) Syncytial gut wall. (3) Peritrophic membrane, loosing from the gut wall. (4) Intestinal content

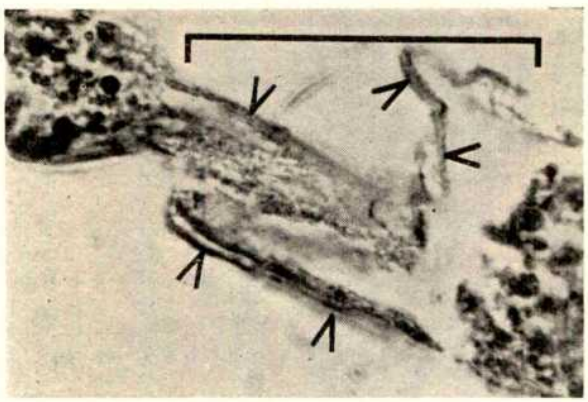


Fig. 3. Midgut of *Araneus diadematus*, phase contrast. Peritrophic membrane at a site with little or no intestinal content

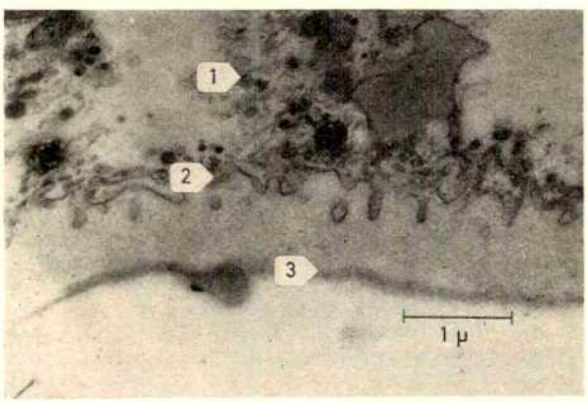


Fig. 4. Electron microscopic image of peritrophic membrane from the cloaca of *L. triangularis*. Cellular debris (1) is seen at the inside of the membrane, presenting a folded inner surface (2) and dense smooth outer part (3)

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Light Measurements in Plant Growth Investigations

THE procedure suggested by K. J. McCree[1] under the above heading would only be of use if it were intended to estimate plant growth from the time-integrated values of the transformed light measurements, and in view of the many assumptions involved it would then seem more sensible to measure the growth directly. He implies that the object of measuring daylight as a part of the microclimate of the plant is to obtain a good correlation (that is, a linear relation) between the light measurement and growth and that the ideal would be if "the response of the photocell were to be made to match that of the plant before the integration was performed". However, as he truly writes: "Though the actual relationship may vary with type of plant, the stage of growth and the growth conditions, it will practically never be linear". It is in fact just the study under natural conditions of the departures from linearity of these relationships that provides the usual reason for measuring daylight, and this study becomes impossible if an empirical transformation, based on data obtained under other growth conditions, is used as suggested by McCree. An integration with respect to time of the daylight measured on a linear scale during a period gives an unequivocal value for the amount of light available and the response of the plant during that period shows the use it makes of this under natural conditions. The response will depend on the variation in light intensity in the period, which is one of the natural conditions involved.

It is obvious that the curvilinear relationships between amount of light and plant responses can only be estimated with much precision by holding the light intensity constant at various levels for a sufficiently long period for the responses to be measured at each. This is usually impossible under natural conditions and the best that can be done is to integrate the daylight over the shortest periods suitable for measuring the particular plant response concerned. In this way approximate relations can be obtained without question-begging and can be compared with those found under controlled conditions, which are necessarily in some respects remote from the conditions found in nature. Information on the range of light intensity in each period might well assist interpretation.

It is to be expected that by no means all plant growth responses to light will show a good correlation with carbon dioxide uptake, and in McCree's system it would be necessary to use different transformations not only for different types of plant, stages of growth and growth conditions but also for various kinds of plant response. I agree with him that "the first essential is to choose a rational basis for the measurements" but not that "the instrumental details are of secondary importance". Under natural

conditions there is so much spatial variation in light intensity, this variation itself varying in time, that it is necessary to use large numbers of detectors, which must therefore be inexpensive. The question of what range of wave-lengths to include in the light measurements is also important and must depend on a knowledge of the plant processes under investigation. However, within that range the same principle applies, that it is essential to measure light on a scale with a definite physical meaning and then compare the response of the plant with the amount of light, rather than beg the question by adjusting the light scale to some assumed plant response. Thus the measuring instrument should give as nearly as possible a constant response, preferably per quantum rather than per unit of energy, at all wave-lengths within the range included, rather than be made to follow, for example, an action spectrum for photosynthesis measured under a certain set of controlled conditions. (The confusion that this last arrangement would cause becomes obvious when it is remembered that the action spectrum changes with light intensity above the range where light is severely limiting.) Changes of light quality within the periods for which plant response is to be related to the time integral of the measured light, like changes of intensity, are likely to decrease the accuracy of the estimated relationship. Changes of quality between such periods, if they can be detected by other means and correlated with changes in the plant response to amount of light, may provide clues to be followed up by experimentation under controlled conditions.

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PROF. HEATH raises the fundamental question of the purpose of making light measurements, and I am happy to give my point of view. I think that it will eventually be possible to predict the growth rates of plants from measurements on their environments, given sufficient understanding of the principles behind the interactions between plant and environment. The models will have to be complex, because plants are complex, but this presents no difficulty to an electronic computer. The limitation is not in our ability to speculate, but in our ability to describe the principles of plant behaviour in numbers rather than in words.

As more sophisticated models of plant behaviour are developed, they must be tested at every stage against facts obtained by growing plants in the field. In experiments designed for this purpose, the climate measurements will be confined to those which the theory says are relevant. I hope there will be more of this type of field experiment in the future. Even in experiments designed to determine the growth rate of a particular plant in a particular place, where climatological data are collected only as a guide to the factors which might be influencing the growth rate, there is room for a great deal more selection of climate measurements than is common at present.

For example, it is simply not true that the departure from linearity of the relationship between growth rate and the various totals of a linear light integrator is a measure of the light response of the plant. The departure could just as well be due to water stress or abnormally high temperatures, accompanying high levels of radiation. But if one makes a quantitative allowance for the non-linearity of the plant, based on laboratory measurements of the curve for carbon dioxide uptake, then the light effects can legitimately be separated from the effects of other factors.

This quantitative allowance for the response of the plant needs to be applied before the light is integrated with respect to time. The totals of the usual linear light integrator are not sufficient; one needs information about how each total was made up. In my previous communication I indicated how this information might be collected, and we are now developing an instrument for this purpose. At the same time we are conducting experiments to find out how well the readings of such an instrument can be related in practice to the growth rates of plants.

In his discussion of 'physical' and 'empirical' scales, Prof. Heath appears to be investing physical measurements with an almost mystical significance. But even in physics, a measurement has meaning only after it has been interpreted. This is the real test of the superiority of one scale over another—take, for example, the various scales which can be used for measuring spectral colours. The physicist uses a frequency scale when he is studying photoelectric effects, because he interprets in terms of quanta; in interferometry, he uses a wave-length scale, because he interprets with the wave theory; in photometry, he uses a scale which biases the strengths of the various colours in the same way as an 'average' human eye. In the same way, when measuring light for plant growth studies, it is logical to use a scale which takes account of the photosynthetic efficiency of different colours. On any other scale, the measurements would be more difficult to interpret.

Of course, in each case it is essential that everyone should agree to use the same scale. In science, an 'interpretation' is not complete unless it can be repeated. But this is not an argument against using a scale which fits the purpose of the experiment.

I have discussed elsewhere[1] the problems of choosing and agreeing on a scale for photosynthetically active radiation. The difficulties raised by Prof. Heath are just those which were raised in the 'twenties when a photometric scale was being discussed. Yet the scale which was internationally agreed in 1931 has worked very well in practice, despite its known deficiencies—in fact, a whole branch of engineering is based on it. Since I do not believe that the human eye is inherently any less complex than the photosynthetic system of a plant, I hope it will not be too long before 'green plant engineers' will agree to adopt their own scale of light measurement. I am sure it will then be easier to interpret the measurements.

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[1] McCree, K. J., *Agric. Meteorol.* (in the press).

Clarity in recording Germination Data

TIMSON[1] suggests that, compared with straight germination percentages, the following procedure would provide a more meaningful evaluation of germination data: the total percentage of seeds which have germinated to date is recorded every day for a stated number of days from sowing, and these daily values are summed. Timson terms the result thus obtained from observations up to, for example, day 10, the '$\Sigma 10$'. This scheme is possibly as successful an attempt as any at producing a unified expression of two values, germination percentage and time to germination. But any such expression is apt to obscure, rather than clarify, the situation, except when germination is both rapid and high or both slow and low. For example, with two hypothetical seed samples the percentages germinating on successive days may be 40, 20, 0, 0, 0 of sample A, and 10, 30, 30, 30, 0 of sample B. Timson's $\Sigma 4$ would therefore be $40 + 60 + 60 + 60 = 220$ for A, and $10 + 40 + 70 + 100 = 220$ for B, although the two samples are dissimilar in both germination percentage and time to ultimate germination. More-

over, a shorter period of assessment would favour the faster, a longer period the more fully germinating sample: Σ3 would be 160 for *A* and 120 for *B*, whereas Σ5 would be 280 for *A* and 320 for *B*.

Even without taking percentages into account, the time to germination, the importance of which Timson stresses, is not easy to represent unambiguously. Kotowski[2] used as his "coefficient of velocity of germination" (*Cv*) the reciprocal of the weighted mean number of days to germination, which may be written:

$$Cv = \frac{\Sigma G_n.100}{\Sigma G_n.D_n}$$

where G_n is the number of seeds germinating on day D_n, and D_n is the number of days from sowing. This figure has the advantage of indicating the average time to germination irrespective of germination percentage, but does not provide any information about the uniformity of the seeds within a population: a certain average time may result, either when all seeds germinate almost simultaneously or when some do so very early but others very late. Therefore, in order that the germination pattern may be characterized more precisely, a third piece of information, in addition to percentage and average time to ultimate germination, is required, corresponding to the variance of the frequency distribution, if this were normal, of the number of seeds recorded as having germinated over successive time intervals. But the frequency distribution of times to germination even in non-dormant seed samples is not usually normal but positively skewed, and in certain ecological situations the size and shape of the 'tail' may be of considerable importance. With plants in cultivation the seed cleaning process often further modifies any pattern that may exist in a random population by eliminating a proportion of light-weight seeds.

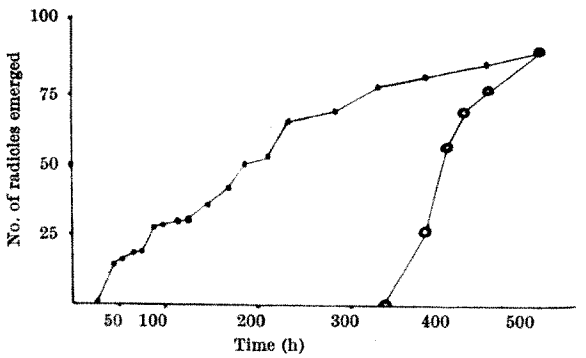


Fig. 1. Radicle emergence of lettuce seeds ('Webbs Wonderful') at 0·5° C (○), and 25° C (●). 100 seeds per 9-cm diameter Petri dish on two layers of Whatman No. 1 filter paper with 7 ml. distilled water, in the dark. Three replications

The use of quartiles, that is, times to 25, 50, 75 and 100 per cent of the ultimate number germinating, has been suggested by R. T. Clarke (personal communication) as a method of dealing numerically with such complex situations. However, cumulative curves can be very informative, even though not themselves statistically tractable.

Furthermore, the effect of environmental conditions on germination should be sufficiently stressed. It is of interest in this connexion that the same batch of seeds can differ widely, depending on the temperature, not only in its average time but also in the scatter in the time to germination. Fig. 1 presents results obtained with lettuce seeds near their minimum and maximum germination temperatures. The seeds show greater uniformity in behaviour at the cold than at the warm extreme. The genetical and ecological significance of this phenomenon is a subject for further investigation.

I thank M. A. Nichols for permission to present unpublished data.

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Comparison of the Biosynthesis of Cellulose *in vitro* and *in vivo* in Cotton Bolls

THE work of Hassid *et al.*[1-3] on the cell-free synthesis of cellulose with an enzyme system isolated from mung bean seedlings and young cotton bolls has shown that the enzyme is apparently unable to distinguish guanosine diphosphate-D-glucose from guanosine diphosphate-D-mannose. Moreover, there was a notable decrease in the amount of the synthesized cellulose using enzymes from cotton bolls older than 15 days.

Lately, I have approached the biosynthesis of cellulose in higher plants from the kinetic point of view with the following results[4-8]:

(1) The biosynthesis of cellulose consists of two distinct kinetic stages. The first proceeds very slowly and yields only little cellulose, while in contrast, the second stage proceeds very rapidly and gives nearly all the cellulose produced (Fig. 1). The two kinetic periods have been correlated with the formation of the primary and the secondary cell wall, respectively.

(2) The corresponding degrees of polymerization (weight average DP_w), and its distribution at different conversions, remain constant (Fig. 1) and practically homo-

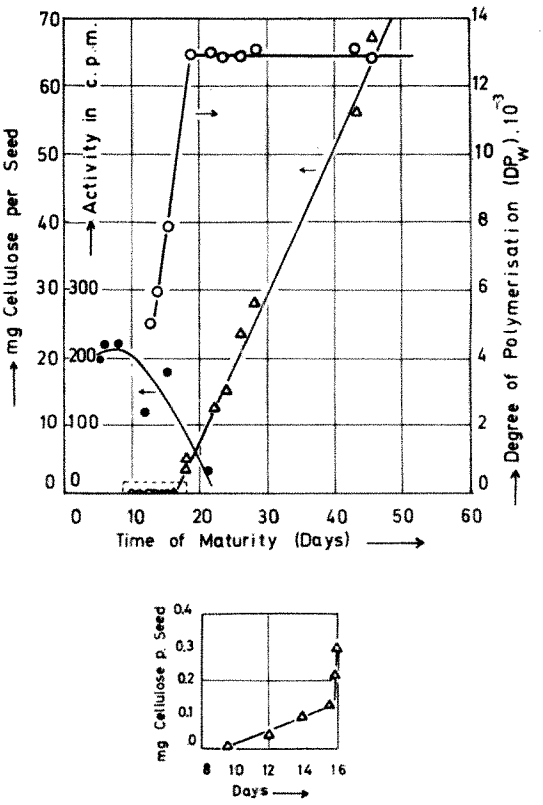


Fig. 1. Cell-free synthesis and biosynthesis of cellulose in relation to the age of the cotton bolls. ●, Cell-free synthesis according to Hassid, conversion expressed in c.p.m.; △, biosynthesis, conversion in mg per seed; ○, biosynthesis, corresponding degrees of polymerization (weight average)

geneous during the whole second reaction stage. This means that the biosynthesis of the secondary wall cellulose reacts by a structure-controlled mechanism (template mechanism).

With regard to the primary-wall cellulose I found no results which point to a similar template mechanism. The corresponding DP_w is much smaller and its distribution very broad. In this period, a high quantity of materials other than cellulose is also produced.

On the basis of these results I have formulated the following explanation for the findings of Hassid: the cell-free synthesis of cellulose by means of enzyme preparations from cotton bolls corresponds to the first reaction period leading to the primary cellulose. Since *in vivo* during this stage other compounds than cellulose are also formed, it is probable that this also happens *in vitro*. The enzyme seems to lose its activity with an increase in the age of bolls, since I have found that the synthesis of the primary cell wall is finished only a short time after the beginning of the secondary wall synthesis[6]. The enzyme responsible for the syntheses of the secondary wall cellulose may be not the same and may react only in presence of a template. Assuming that the template was not yet formed or was destroyed during preparation of the enzyme for the cell-free synthesis, one could explain the great decrease in the amount of formed cellulose using the 21-day-old cotton bolls.

Fig. 1 shows the values found by Hassid with my kinetic results. Although the conditions of growth of the cotton plants were different, the coincidence of the age of the bolls at which the cell-free synthesis loses its activity, and the biosynthesis of the primary cellulose in the cotton bolls finishes, is remarkable.

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Kinetics of the Biosynthesis of Cellulose in Cotton Bolls by Different Light Intensities

PREVIOUS kinetic investigations of the biosynthesis of cellulose in higher plants suggested that the secondary wall cellulose is synthesized by a structure-controlled mechanism (template mechanism)[1–5]. In order to confirm my assumption I changed the reaction rate by varying the intensity of illumination of the cotton plants. Cotton plants (*Gossypium herbaceum*) were grown in a glass-house at a mean temperature of 25° C (the night temperatures never were less than 18° C). The intensity of illumination amounted to 4,000, 15,000 and 60,000 lux respectively (mean values). The time of maturity was calculated from the time the flowers were fertilized. Bolls of different maturities were picked, immediately opened and the seedhairs quantitatively removed from the seeds. The methods used to determine the conversion of cellulose and the degree of polymerization have already been described[1,2].

If the secondary wall cellulose is synthesized by a template mechanism, then the degree of polymerization must be independent of any variation in the kinetics. The results in Fig. 1 show that this is the case. In spite of the considerable variation of the rate of synthesis with light intensity, the degree of polymerization remains

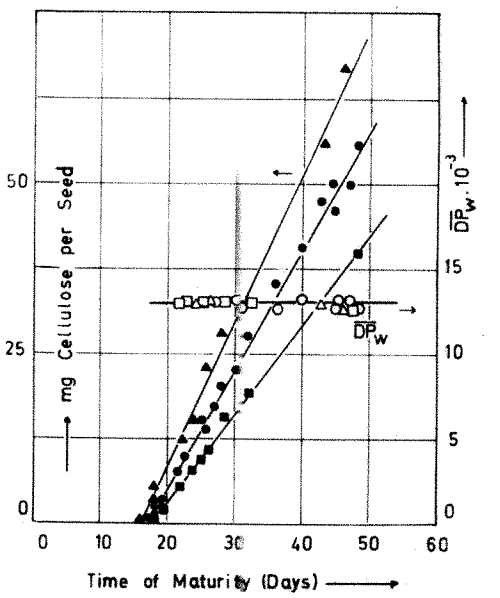


Fig. 1. mg Cellulose per seed and corresponding degree of polymerization (weight average) with time of maturity at different intensity of illumination. ▲, 60,000 lux; ●, 15,000 lux; ■, 4,000 lux; △, 60,000 lux; ○, 15,000 lux; □, 4,000 lux

constant at approximately $DP_w = 13{,}000$, independent of the conversion and the rate of synthesis.

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Bromination of Fluoresceins by the Dogfish, *Squalus acanthias*

THE natural occurrence of bromo-compounds in the coral fan *Gorgonia verrucosa* and in certain species of shellfish, especially *Murex brandaris* and *M. tranculus*, is well known[1,2]. Bromination of phenol red by an enzyme system isolated from the mould *Caldariomyces fumago* has been observed[3]. *In vitro* bromination of phenol red by uterine slices and bromination of phenol red, chlorophenol red, metacresol purple and fluorescein after intra-uterine administration of these dyes to the pregnant spiny dogfish, *Squalus acanthias*, have been reported[4,5]. The work recorded here is an extension of the latter observations.

Pregnant spiny dogfish were kept in a wooden enclosure with free access to sea water at 14°–15° C. The uterine fluid was withdrawn by pipetting through the uterine pores which open into the cloaca and was replaced with a saturated solution of the dye in sea water. Various dilutions of the dyes were also used. At the end of the experiment, generally 48 h after instillation of the dye, the uterine fluid and resultant dye were easily collected by pipetting. The spectra of the dyes in the visible region before and after instillation were recorded and compared with authentic samples. Paper chromatography was performed on Whatman 3 *MM* paper in systems previously reported[4,6].

Forty-eight hours after intra-uterine administration of fluorescein, the resultant product was found to be eosin (tetrabromofluorescein). Thus, the sulphonic acid moiety present in the phenosulphone phthalein dyes, which are brominated, could be replaced by the carboxylic group

in fluorescein without loss of bromination. The following bromofluoresceins were found to be brominated to eosin 48 h after instillation in the dogfish uterus: 4-bromo, 2,4-dibromo, 2,7-dibromo, 4,5-dibromo, 2,4,5-tribromo and 2,4,7-tribromofluorescein. After intra-uterine administration of fluorescein, various bromofluoresceins (4-bromo, 4,5-dibromo, 2,4,5-tribromo and 2,4,7-tribromofluorescein) could be recovered in 6, 12 and 24 h. However, 48 h after instillation of fluorescein only eosin could be recovered. This evidence implicates a stepwise bromination by the dogfish uterus. Because of the oxygen bridge, fluorescein differs from phenosulphone phthalein dyes in having such a rigid tricyclic structure that two monobromo, four dibromo, and two tribromofluoresceins are possible. A study of the kinetics of formation of these isomers might be of help in the elucidation of the stereochemistry of the bromination reaction. This stepwise biological bromination by the dogfish may be analogous to the biogenesis of thyroxine where tyrosine residues undergo iodination[7].

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ANATOMY

Electron Microscopic Identification of Autonomic Nerve Endings

THE endings of some autonomic axons, which are presumed to be either adrenergic or cholinergic in function, can be distinguished using the electron microscope by their different populations of vesicles. This was first pointed out by de Robertis and Pellegrino de Iraldi[1]. The cholinergic fibres, as in motor end-plates of skeletal muscle, contain uniform vesicles consisting of an outer wall of unit membrane enclosing a central core which is scarcely denser than the adjacent cytoplasm. In contrast, adrenergic fibres contain similar vesicles, but mixed with a minority possessing an electron-dense central core or granule. The first type are conveniently called agranular vesicles, and the second type granular, or dense cored, vesicles. However, some doubt has been cast on the reliability of granular vesicles as an index of adrenergic function. Whereas in the osmium tetroxide-fixed rabbit iris[2] they are present abundantly in the axons supplying the dilator muscle, they are not present at all, or only very rarely in the rodent iris with similar fixation[3,4]. They appear to be absent also in axons supplying the outer longitudinal muscle coat of the guinea-pig vas deferens[5], though they are invariably present in the circular muscle coat of many species including the guinea-pig. Isolation of granular vesicles in homogenates by differential centrifugation in sucrose gradients has proved difficult. They are seldom seen in heart homogenates, for instance, even in fractions known to contain a high concentration of norepinephrine[6], but do appear in certain fractions of homogenized hypothalamus[7]. The possibility has been raised that the granules within the granular vesicles are artefacts unworthy of serious consideration. On the other hand they may represent in miniature the large granules of adrenal medullary cells which contain high concentrations of bound catecholamine. Assuming that this is so, care must be taken in fixing tissues containing adrenergic axons to avoid severe losses of catecholamines by diffusion or extraction.

We have looked for adequate methods of fixing the iris, and have found that ice-cold 3 per cent potassium permanganate in 0·1 M phosphate buffer at *p*H 7·0, in contrast to buffered osmium tetroxide or glutaraldehyde solutions, is much more reliable for preserving granular vesicles, though the over-all quality of fixation may be somewhat inferior. With this fixative, the granular vesicles can be constantly demonstrated in the rat and mouse iris in their expected situations. The same is true for the longitudinal muscle of the guinea-pig vas deferens. Indeed it is likely that wherever autonomic axons show a specific fluorescence with the Falck and Hillarp technique they will be found electronmicroscopically to contain granular vesicles after permanganate fixation. It is interesting that while permanganate has little or no merit as a histochemical reagent for catecholamines, fixatives combining glutaraldehyde and dichromate, buffered to *p*H 6·5, for which histochemical merit is claimed[8,9], appear to wash out the catecholamines from very thin tissues like rat iris and thus fail to preserve the granular vesicles. It should be mentioned that the permanganate solution, though grossly hypertonic, does not shrink tissues unduly and the swelling of mitochondria, which is a well-known feature of the weaker solution proposed by Luft[10], is minimized but not abolished. Whether permanganate would also be the fixative of choice for pellets of homogenized tissue remains to be determined. As there may be loss of catecholamines from vesicles occurring during the various manipulations in sucrose gradients, before the fixative is applied to the centrifuged pellet, a positive result with permanganate can hardly be predicted. A further interesting possibility is that, even in cases where osmium tetroxide fixation is successful in preserving granules in some vesicles, we may not be visualizing anything like the full catecholamine content of an axon, but merely the part which is not washed out. This may be one reason why agranular vesicles are so numerous in adrenergic nerve endings.

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Mast Cells in the Central Nervous System

IN the central nervous system, the occurrence of mast cells in the pineal[1] and neurohypophysis[2] appears to be well established. They have been noted in the normal olfactory bulb[3]. They occur also in the human brain under pathological conditions, for example around infarcts and in the plaques of multiple sclerosis[4], as well as around the cerebral vessels of ageing animals[5].

We have recently examined the brains of ten hedgehogs (*Erinaceus europaeus*) in coronal, horizontal and

sagittal sections, and found cells apparently identical with mast cells which are localized to the diencephalon. These cells were observed only in the thalamus, epithalamus and metathalamus, and were located predominantly in the nucleus dorsomedialis thalami. We have not been able to confirm the previous report of the presence of mast cells in the olfactory bulb.

The basophilic cytoplasmic granules of these cells (Fig. 1) are so numerous that the nuclei are often hidden as in the mast cells of connective tissues. The cells are histochemically identical with the mast cells found in the tongue of the same species, in so far as their granules stain strongly with alcian blue, metachromatically with thionine (Fig. 2), contain peroxidase and are periodic acid-Schiff-positive and slightly sudanophilic.

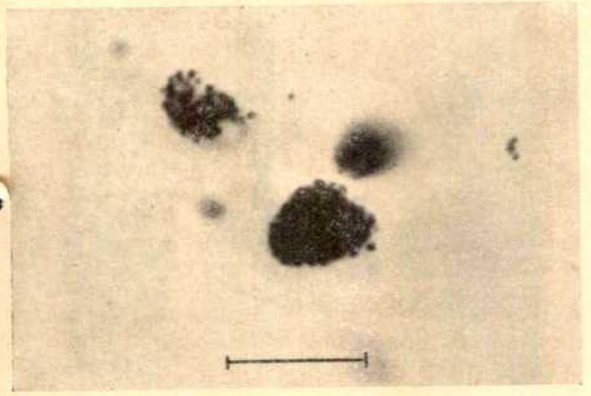


Fig. 1. Mast cells in the thalamus of the hedgehog showing basophilic cytoplasmic granules. Giemsa stain. Scale, 10μ

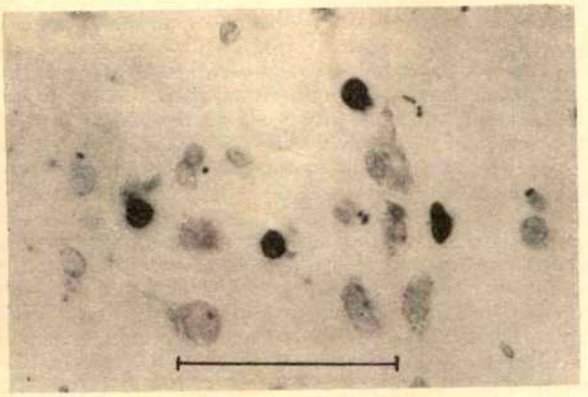


Fig. 2. Metachromatic staining of four mast cells in the nucleus dorsomedialis thalami. Schmorl's thionine technique. Scale, 50μ

Further investigations of other Insectivora and hibernating animals should reveal any physiological or taxonomic correlates of the presence of mast cells in the diencephalon. The possibility of intra-thalamic release of heparin, histamine or serotonin is of considerable interest in relation to local haemodynamic changes during hibernation.

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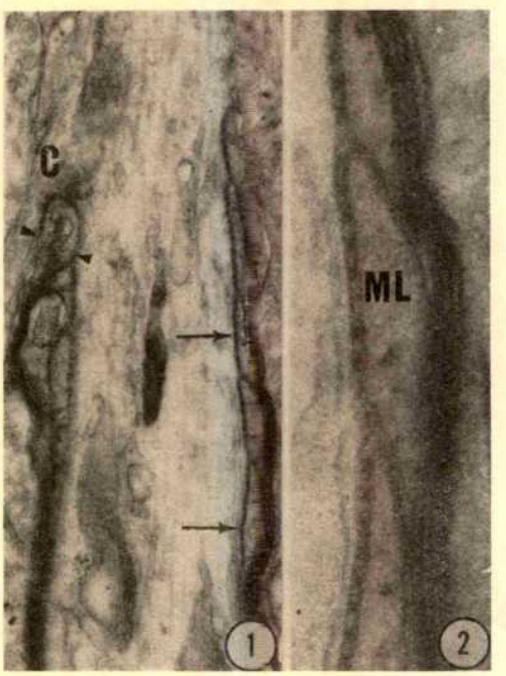
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A Structural Specialization at Nodes of Ranvier in the Central Nervous System

DURING the course of an electron microscopic study of the hippocampal region in the rat an interesting periodicity associated with the axolemma was observed in the area of termination of the myelin sheaths either at nodes of Ranvier or at the last myelinated segment of an axon. As this would seem to be a constant feature of myelinated axons, not only in the hippocampus and dentate gyrus which we have investigated most intensively, but also in other sites that we have examined, and because it does not appear to have been described elsewhere, a brief account of our findings will be presented.

The material on which these observations are based was derived from the brains of adult rats perfused under hypothermia with 3 per cent glutaraldehyde in 0·1 M sodium phosphate buffer with added calcium chloride. Small blocks of tissue were post-fixed in 2 per cent osmium tetroxide, embedded in 'Araldite' and the sections stained with lead citrate.

In longitudinal sections through the nodal region where the myelin lamellae unfold to form the characteristic loops of glial cytoplasm, a striking periodic density is seen along the outer surface of the axolemma and extending into the cleft between this membrane and that of the myelin loops (Figs. 1–3). These regular densities which are between 100 Å and 150 Å wide, repeat with a periodicity of 300–400 Å and extend over nearly the whole area of 'peeling off' of the myelin lamellae. Between six and eighteen densities may underlie a single loop—the number depending solely on the length of the individual loops. While the densities always cease abruptly at the beginning of the unmyelinated segment of the axon, they may extend for a variable distance along the first cytoplasmic loop which is formed by the innermost myelin lamella. As a rule, the only point at which the regularity of the densities is interrupted is opposite the cleft between adjoining myelin loops (Fig. 2). Oblique or tangential sections through the nodal region (Figs. 4 and 5) show a characteristic cross-striation which suggests that the



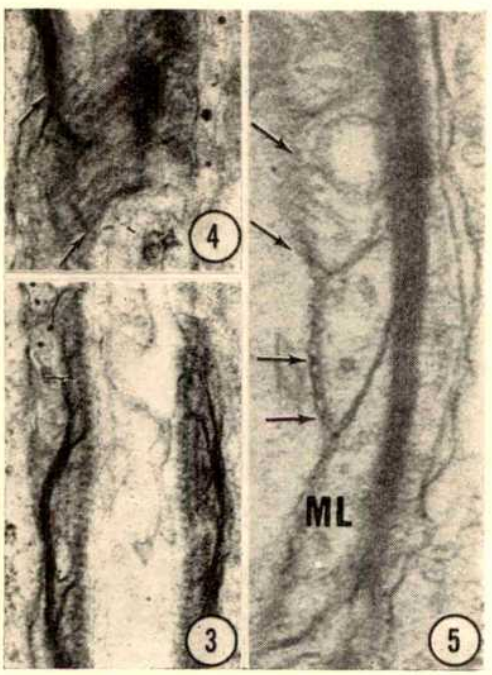
Figs. 1 and 2. Longitudinal section through part of a node of Ranvier to show the periodic densities over the region of the myelin loops (*ML*). The arrows indicate the area which is enlarged in Fig. 2. The axon gives off a collateral branch (*C*) and the arrow heads indicate a continuation of the periodic thickenings on to the proximal part of the collateral. (Fig. 1, × *c.* 41,600; Fig. 2, × *c.* 145,800)

densities either encircle or, possibly, spiral around the axon. In one instance a continuation of the periodicity has been observed in relation to a myelin loop which is wedged in the angle between an axon and a collateral branch (Fig. 1). The precise localization of the densities is rather difficult to determine; but, judging from the slight indentations which occur along the inner surface of the axolemma opposite the periodic densities, it would appear that the densities are mainly a specialization of the axolemma. Certainly, they seem to be more closely associated with this membrane than with the membrane of the myelin loops. In Fig. 6 we have attempted to reconstruct these membrane relationships as they appear in the paranodal or terminal region of a myelinated fibre to illustrate the form and position of these periodic densities.

It is difficult to account for the failure of earlier workers to recognize this very striking specialization of the nodal region, unless perhaps it is a feature which is only clearly revealed by aldehyde fixation and/or lead staining. It is not uncommon for different fixatives to give rise to variations in the appearance of structures under the electron microscope and it is conceivable that this may be the case here. But, whatever the reason may be, it is clear that some component of the related axolemma and myelin loops may appear as a series of annular or spiral densities around the nodal or terminal region of myelinated axons in the central nervous system.

Robertson[1] has described a 'fusion' or 'tight junction' between the axolemma and the membrane of the myelin loops at nodes of Ranvier in permanganate-fixed sciatic nerves of frogs and has illustrated this diagrammatically as a continuous density extending over the whole length of each myelin loop. A re-examination of the published micrographs, however, suggests that what has been interpreted as a continuous density may in fact be periodic.

In a recent investigation of the desmosome-like thickenings which occur between adjacent Schwann cell membranes in peripheral nerves, Harkin[2] has published micrographs of nodes of Ranvier in rat sciatic nerves from glutaraldehyde-fixed and lead-stained material. In one



Figs. 3 and 4. Two parts of a section through the same node to show the appearance of the periodic densities where the axon is sectioned slightly obliquely (Fig. 3) and tangentially (Fig. 4). (× *c.* 54,100)

Fig. 5. Part of an obliquely transverse section through the paranodal region of another fibre. Arrows indicate the orientation of the membrane densities related to the myelin loops (*ML*). (× *c.* 83,300)

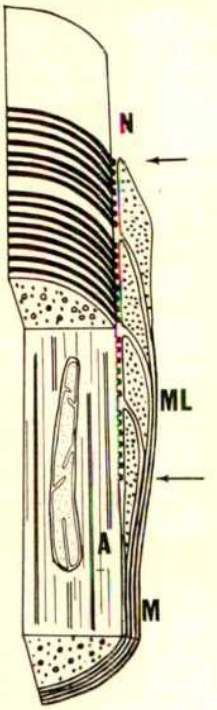


Fig. 6. A schematic representation of the axolemmal thickenings in the region of a node of Ranvier. *N*, Nodal region; *ML*, myelin loops; *M*, myelin lamellae; *A*, axon

of these the same type of periodicity as we have found is clearly seen between some of the myelin loops and the axolemma (especially over the loops labelled 1 and 2 in his Fig. 1), but unfortunately no reference is made to this in either the text or legends. In view of this, it seems likely that periodic densities associated with the axolemma are a common feature of both central and peripheral nodes and it is reasonable to suppose that their structural and functional significance will prove to be the same in the two situations.

At least two possibilities may be entertained as to the significance of these densities in the nodal and terminal regions of myelinated axons. The first is that they may provide a tight attachment for the myelin loops which would 'anchor' them to the axolemma. Quite apart from lending mechanical stability to the unfolded myelin lamellae, such an arrangement would also act as a physical barrier between the periaxonal space around the node on one hand, and the narrow cleft between the myelin lamellae and the axon on the other[3]. In support of this suggestion is the finding that the periodic densities are limited to the region of unfolding of the myelin lamellae. The fact that they usually end about half-way along the first or innermost loop may also be taken as support for the view that the separation of the myelin lamellae to form loops is the critical factor which leads to the appearance of the regular densities along the axolemma. The second possibility is that this structural specialization is in some way involved with the mechanism of saltatory conduction, possibly by concentrating current flow at the nodal segment of the axon.

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ENTOMOLOGY

'Catalysomes', or Enzyme Caps on Lipid Droplets: an Intracellular Organelle

WHILE studying the distribution of acetylcholine and other esterases in the insect *Rhodnius*[1], it was observed that every droplet of triglyceride in the cells of the fat body has a small disk-like area or 'cap', 1μ or less in diameter, which is the site of an enzyme that will hydrolyse 5-bromoindoxyl acetate (method of Holt[2]) and naphthol AS acetate (method of Pearse[3]). This esterase is not inhibited by eserine or other inhibitors of the cholinesterase group; it was believed to be a lipase and it was concluded that the active disk or cap constitutes an organelle responsible for the liberation and perhaps the uptake of fatty acids in the droplets of stored lipid[1].

More recently it has been shown by Chino and Gilbert[4] that fatty acids in insects are transported in the form of diglycerides incorporated into lipoprotein; and that liberation of the stored lipid is an energy-demanding process which involves the simultaneous oxidation of carbohydrate[5].

In the course of an investigation of the histology and histochemistry of the fat body in *Rhodnius*, which is now being written up for publication, it was observed that nicotinamide–adenine dinucleotide diaphorase ($NADH_2$ as substrate, with nitro-BT as tetrazolium compound[6]), besides its expected localization in the mitochondria, is intensely active in the lipase-containing caps on the droplets of fat (Fig. 1, *a*). In the absence of added substrate endogenous activity gives a barely detectable reaction. Of the enzymes investigated so far, the 'caps' contain also the dehydrogenases of isocitrate (+nicotinamide adenine dinucleotide phosphate, NADP, as co-enzyme), α-ketoglutarate (+NAD), succinate (without added co-enzyme), malate (+NAD), glutamate (+NAD), β-hydroxybutyrate (+NAD) and a very weak lactate. The dehydrogenases of the pentose cycle acting on glucose-6-phosphate and 6-phosphogluconate (+NADP) are virtually absent. In the G-'Nadi' reaction indophenol blue is sharply localized in the muscle mitochondria[7]. In the fat body cells the dye rapidly accumulates in solution in the droplets of lipid, but the site of its formation cannot be localized.

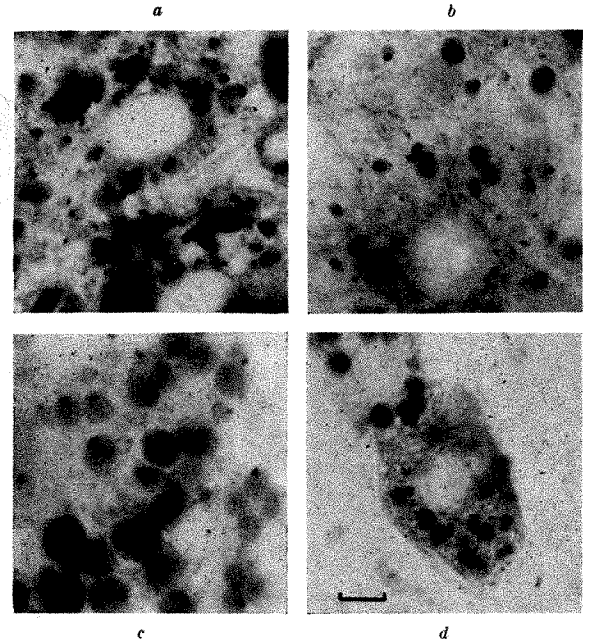


Fig. 1. Whole cells containing lipid droplets. Fixation: glutaraldehyde at *p*H 7·4. Fat staining: sudan 4. Light filter: greenish yellow. Scale (shown in *d*) equals 5μ. *a*, Fat body cells of *Rhodnius*: NAD-diaphorase. Mitochondria around nuclei, catalysomes on lipid droplets. *b*, The same, showing some catalysomes with invisibly small lipid droplets among the mitochondria. *c*, Fat cells in mesentery of mouse: 5-bromoindoxyl acetate method for esterase. Catalysomes in focus on many of the fat drops. *d*, The same: NAD-diaphorase. Mitochondria and lipid droplets with catalysomes

It appears likely, therefore, that these enzyme sites contain all the enzymes of the Krebs cycle and presumably the cytochromes. They are probably responsible for the synthesis of stored lipid (which is readily formed in the fat body cells from sugars and amino-acids[8]) as well as for its preparation for distribution in the circulating blood.

For convenience these organelles, which may be appropriately termed 'catalysomes', have been chiefly studied in the fat body of *Rhodnius*. But their presence is readily confirmed in the developing fat cells in the mesentery of the young mouse. Here they have the same appearance and contain both esterase and NAD-diaphorase (Fig. 1, *c* and *d*). In both insect and mammal they arise as intensely reactive granules in the cytoplasm between the mitochondria (Fig. 1, *b*). A minute droplet of lipid then appears in contact with the granule and as this droplet enlarges the granule becomes a crescent and finally a sharply localized and slightly raised spot on the surface of the oil drop. The fine structure of this organelle is being studied in collaboration by A. V. Grimstone.

Catalysomes must often have been seen before, but apart from their esterase activity[1], their significant association with lipid droplets has not been recognized. It was reported by Chino[9] that much of the cytochrome oxidase in the developing egg of the silkworm was contained in particles that were carried up with the lipid layer during the centrifugation of homogenates. These particles were probably catalysomes.

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Pertinacity of Host-seeking Behaviour of *Aedes aegypti*

MOSQUITOES on the hunt are persistent pests. To the best of our knowledge, this persistence has not been quantitated. Use of mosquitoes in lengthy biting or probing experiments requires that the drive to bite or probe does not fatigue during the minutes or hours of the experiment, so that the experimental variable can be reliably quantified. The following observations were made to record the pertinacity (and lack of fatigue) of *Aedes aegypti* females.

Adult female *Aedes aegypti*, var. *queenslandensis*, were reared in cages, 1 ft.^{3}, in the presence of males. They were fed 5 per cent sucrose solution until the experiment began. 5–9-day-old females were placed in small cages, 5 × 5 × 1·5 cm, made of a wire frame and covered with nylon net. In probing experiments these cages were suspended 1 cm above the inner surface of the forearm of a volunteer. The mosquitoes were observed and any attempts to probe were recorded.

Five mosquitoes in a small cage were observed for 30 min. The number probing towards the arm was recorded continuously. Ten replicates, using different mosquitoes but the same subject, were performed.

The 30-min experimental period was divided into three successive 10-min periods for purposes of comparison.

Table 1. PROBING ACTIVITY OF FIVE *Aedes aegypti* FEMALES OVER A HUMAN FOREARM IN A 30-MIN PERIOD*

	0–10 min	11–20 min	21–30 min
Percentage of time probed	87·78 ± 4·3	85·99 ± 6·4	84·5 ± 8·0
Percentage of mosquitoes which had probed	42·4 ± 4·7	45·2 ± 8·6	46·1 ± 6·7

* Each figure is a mean of ten replicates ± *S.E.*

Table 2. PROBING ACTIVITY OF A POPULATION OF *Aedes aegypti* FEMALES OVER A HUMAN FOREARM

Trial No.	Seconds to 50 per cent probing*
1	24·2
2	24·1
3	20·3
4	23·6
5	15·4
6	24·9
7	22·1
8	21·2
9	22·5
10	22·3

* Each figure is a mean of ten replicates.

Two values were determined for each 10-min period: (1) '*T*', the percentage of time one or more mosquitoes were probing, and (2) '*P*', the percentage of probing activity (100 per cent being five mosquitoes probing without interruption for 10 min).

As can be seen from Table 1, mosquitoes probed during 86·0 per cent of the available time, and during this time an average of 44·5 per cent of the mosquitoes probed. No significant difference of activity was seen when the three successive 10-min periods were compared.

We felt that a population study as mentioned here might mask activity cycles of individual mosquitoes. The experiment was therefore repeated twice with one female per cage. Probing activity was recorded continuously for 30 min. The pattern of 'probing' and 'not-probing' was completely random, as can be seen from Fig. 1. The shortest probing period was 1 sec, the longest 184 sec. Probing periods tended to be about 8–10 sec, and inactivity periods about 5–7 sec. When analysed by three successive 10-min periods no significant difference of probing activity was demonstrated.

It was planned to use the time to initiation of probing as a measure of attractiveness of human subjects, and it was presumed that a shorter initiation time indicated a more attractive subject. The variability of this initiation time with repetition and passage of time was the subject of this experiment. Ten female *A. aegypti* were placed in a small cage. The time from first exposure to the forearm to the time when at least five were probing simultaneously was measured. When five mosquitoes were probing together the arm was withdrawn from beneath the cage, the mosquitoes allowed to rest for 1 min, and then the arm replaced below the cage. One hundred successive determinations, over a 2·5-h period, were performed, using the same arm and the same mosquitoes. The results are recorded in Table 2, grouped in ten trials of ten successive determinations each. Obviously, no diminution of vigour occurred with repetition of exposure.

It was desired to know whether continuous exposure to the immediate presence of the host would lead to sensory adaptation and diminution of probing and biting. A small cage containing five female *A. aegypti* was fastened to the forearm of an experimenter, but spaced 1 cm from the skin by a rubber ring. The experimenter carried on his normal indoor activities during the 12 h of the experiment, covering the cage with his shirt sleeve. At intervals he would observe the mosquitoes for probing, and at the end of the 12 h the spacer ring was removed and the cage placed directly on the skin. Three replicates were carried out. No attempt was made to quantitate probing behaviour, but on all occasions that they were observed, probing activity was obvious. At the end of 12 h all subjects were bitten by all their mosquitoes.

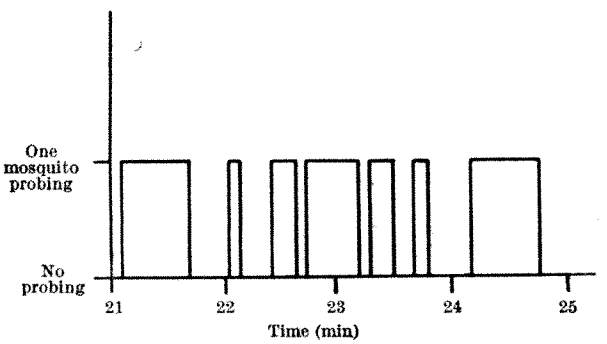


Fig. 1. The pattern of 'probing' and 'not-probing'

These experiments give indication of the untiring host-seeking behaviour of *A. aegypti*. Neither perception of the host, initiation of probing, continuation of probing, nor biting urge seemed to diminish with repeated or continued exposure to the host. The observations confirm casual ones made on free-flying mosquitoes, and also now make it possible to undertake repetitive or continued use of mosquitoes in the laboratory without fear of diminished vigour.

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MICROBIOLOGY

Location of Spore and Vegetative Antigens of *Bacillus cereus* by means of Ferritin-labelled Antibodies

FERRITIN labelling of antibodies[1] provides a method for the study of antigen/antibody reactions at the subcellular level using the electron microscope. This technique has now been successfully applied to numerous problems in bacteriology, virology and pathology[2].

Antigenic changes occurring on the surface of *Clostridium sporogenes* and *Bacillus cereus* during germination and sporulation have been investigated using fluorescent labelled antibodies[3,4]. Similar labelling of these antisera with ferritin should enable antigenic changes not only on the surface, but also within the organisms to be followed. Two approaches to this problem, namely, pre-embedding and post-embedding staining with ferritin labelled antibodies, are possible[5]. Some preliminary observations on the pre-embedding staining of spores and vegetative cells with appropriate ferritin-labelled antibodies are described here.

Antisera prepared against the spores and vegetative cells of *B. cereus*, var. 'Terminalis'[4], were coupled with ferritin using toluene 2 : 4-di-isocyanate as coupling agent[6]. 0·2 ml. amounts of a concentrated suspension of spores and vegetative cells, previously fixed for 18 h in 10 per cent phosphate buffered formalin, *p*H 7·2, were mixed with 0·1 ml. amounts of the appropriate ferritin-labelled antibody and shaken at 37° C for 0·5 h. After centrifuging the deposits were washed six times in 10 per cent phosphate buffered formol saline, *p*H 7·2, and processed for electron microscopy. The specimens were post-fixed in osmic acid and embedded in 'Maraglass'[7]. Sections were cut on an L.K.B. ultratome, using glass knives, and collected on 200 mesh carbon coated grids. They were examined in a Philips *EM* 200 electron microscope at 60 kV.

Specific staining of the appropriate antigens was observed by both spore and vegetative ferritin-labelled antibodies. In Fig. 1 it can be seen that the ferritin-

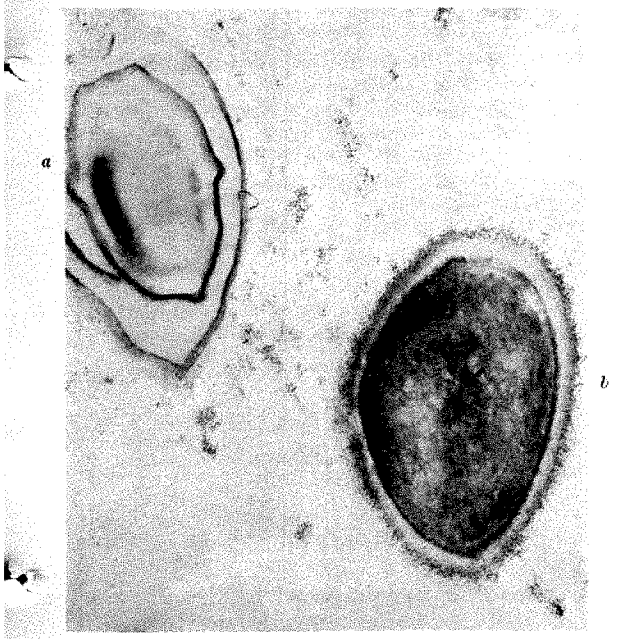


Fig. 1. Electron micrograph showing (*a*) spore and (*b*) vegetative cell of *B. cereus* var. 'Terminalis' stained with vegetative ferritin-labelled antibodies

labelled vegetative antibody is specifically located around the vegetative cell only. The background always contained a small number of ferritin particles, which presumably became detached during processing, but these did not interfere with the interpretation of the photographs.

Further intracellular location of vegetative antigens in spores has been achieved by similarly staining disintegrated spores. This will be the subject of further communications.

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Amino-acid Protection of Ultra-violet-irradiated *Bacillus subtilis*

IT has been reported[1] that cultivation of *Hansenula wingei*, prior to ultra-violet irradiation, in beef-extract glucose broth supplemented with small concentrations of either tryptophan, cystine, tyrosine, phenylalanine, leucine, cysteine, threonine, isoleucine, histidine, or the amino-acid analogues, ethionine or norvaline, protected this yeast against ultra-violet exposure. The hypothesis was made that amino-acids may be strongly active as protective or reactivating agents in Gram-positive organisms, but only slightly active in this capacity in Gram-negative organisms because of permeability differences in the two types of organisms. The present investigation was initiated to determine if amino-acids reverse or protect against the inactivating effects of ultra-violet radiation in a Gram-positive bacterium.

For experimental purposes, *Bacillus subtilis ATCC* 6051 cells were grown overnight at 37° C in shaken 125 ml. flasks of a defined broth medium containing 0·2 per cent glucose; 0·8 per cent L-glutamic acid; 10 ml. of an aqueous salt solution composed of 0·2 per cent magnesium sulphate H_2O, 0·05 per cent calcium chloride, 0·05 per cent sodium chloride and 0·0001 per cent copper sulphate; 1 ml. of an aqueous salt solution containing 0·1 per cent manganese sulphate and 0·1 per cent iron sulphate; plus 0·05 per cent of either L-phenylalanine, L-glycine, L-proline, L-tyrosine, L-serine, L-tryptophan, L-alanine, L-threonine, L-histidine, or L-methionine (Nutritional Biochemical Corporation, Cleveland, Ohio). The medium was phosphate buffered (0·15 M, *p*H 6·2 or 7·4 depending on the experimental design).

The cells from the respective cultures, including a non-enriched control culture, were collected and washed by centrifugation in sterile 0·85 per cent sodium chloride and used to prepare saline suspensions containing approximately $1·0 \times 10^6$ viable cells/ml. A 20 ml. volume of each suspension was transferred to sterile 90 × 15 mm Petri dishes; these plates (with covers removed) were placed 30 cm from a 12 in. ultra-violet lamp (A. H. Thomas Company, Philadelphia, Pennsylvania) which emitted light with a principal wave-length of 254 mμ and an intensity of 17 mW/cm² at 1 m. One ml. aliquots were removed from each suspension prior to ultra-violet treatment and after 2 and 4 min of irradiation. During the exposure period, the dishes were manually agitated. The aliquots, withdrawn at the respective intervals, were suitably diluted and plated with trypticase soy agar to allow the surviving cells to form colonies. Irradiating, sampling, diluting, and plating procedures were carried out in a dark room illuminated with a 25-watt, red photographic bulb (Westinghouse Corporation, Bloomfield, New Jersey). Recovery plates were placed in closed boxes and incubated at 37° C for approximately 36 h. The protection afforded by the amino-acids against ultra-violet inactivation was determined by counting the colonies arising on the recovery plates and calculating the percentage of survivors after 2 and 4 min of ultra-violet exposure.

A typical experiment, which could be reproduced at will, is shown graphically in Fig. 1. It is noted that

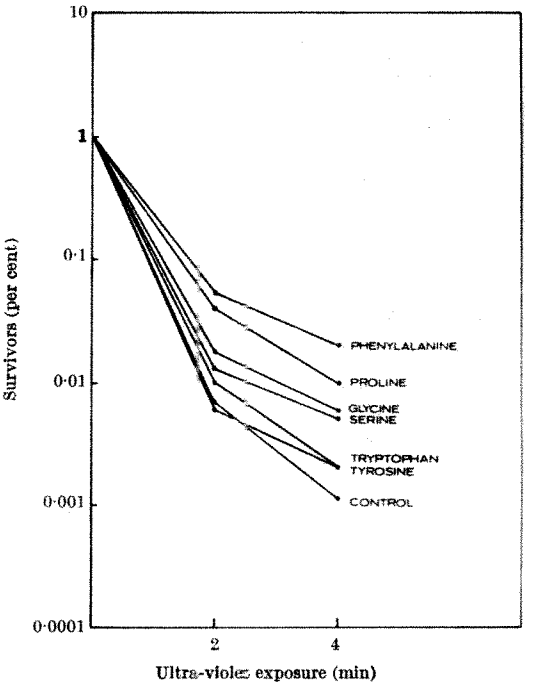


Fig. 1. Protection afforded by amino-acids (in an acid medium) against ultra-violet inactivation as shown by the percentage of cells surviving the indicated periods of radiation exposure. Prior to irradiation, all cells (except those of the control) had been grown in a defined phosphate buffered medium (0·15 M, *p*H 6·2) supplemented with 0·05 per cent of the indicated amino-acids

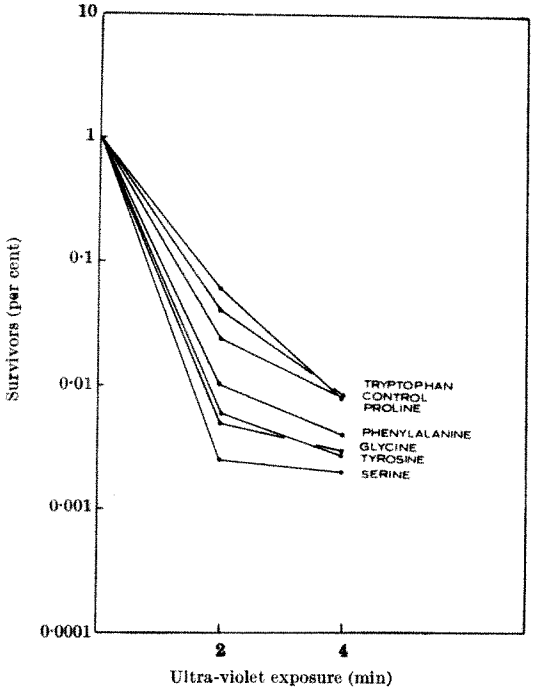


Fig. 2. Protection afforded by amino-acids (in an alkaline medium) against ultra-violet inactivation as shown by the percentage of cells surviving the indicated periods of radiation exposure. Prior to irradiation, all cells (except those of the control) had been grown in a defined phosphate buffered medium (0·15 M, *p*H 7·4) supplemented with 0·05 per cent of the indicated amino-acids

cultivation of *B. subtilis* in a defined acidic broth medium supplemented with phenylalanine, proline, glycine, or serine markedly increased the resistance of this organism against inactivating ultra-violet exposure. Tryptophan and tyrosine are less active in this capacity. Valine, methionine, and threonine (not plotted) yielded patterns of protection quite similar to phenylalanine; histidine closely paralleled proline. Fig. 2 shows the results obtained in a similar experiment in which the test organism was cultivated in an amino-acid enriched alkaline medium. Under these conditions, all the amino-acids, except tryptophan and proline, potentiated the lethal action of ultra-violet radiation. These seemingly contradictory results can be resolved in the following way: The *p*H of the acidic *B. subtilis* cultures was 6·2 which approximates the isoelectric points of phenylalanine, glycine, serine, tryptophan, proline, tyrosine, valine, and alanine[2]. As inner salts (zwitter ions) these compounds probably pass freely into *B. subtilis* cells; however, at *p*H 7·4, these amino-acids shift to their ionic form to which the cell boundaries of this organism may be relatively impermeable. This does not explain why most of the amino-acids, in an alkaline medium, increase ultra-violet sensitivity unless their presence in ionic form causes leakage of intracellular metabolites, perhaps analogous to that of surface active ionic compounds which bring about the release of purines and pyrimidines[3]. The loss of such compounds would be expected to increase ultra-violet sensitivity.

The possibility was considered that increased ultra-violet resistance in the amino-acid enriched cultures at *p*H 6·2 was partially or wholly attributable to increased sporulation. However, this explanation appeared invalid since the suspensions, which were later irradiated, were prepared directly from young rapidly growing cultures. Such cultures normally do not contain large numbers of spores[4]. In addition, microscopic examination of cells from the various cultures revealed that sporulation was negligible.

Although alanine is the only amino-acid reported to be a strong reactivator of ultra-violet irradiated *Escherichia coli*[5], the data presented here generally support the hypothesis that amino-acids may be strong reactivating or protective agents in Gram-positive organisms; however, this is equivocal until amino-acid protection against ultra-violet exposure has been demonstrated in a representative spectrum of Gram-positive organisms.

I thank Dr. R. F. Pittillo for his review of this paper and Mrs. Elwanda Johnson and Miss Carolyn Monerief for technical assistance.

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GENETICS

Chromosome Breakage with Two Isomers of *l*-Threitol 1,4-*bis*methanesulphonate in Plants

THE chromosome breaking ability of *l*-threitol 1,4-*bis*-methanesulphonate was investigated in three plant species[1]. Chemical and cytological[2,3] evidence supports the fact that this compound can transform slowly *in vivo* into an active *bis*epoxide. The differential mutagenic activity of the *l*(+) and *d*(−) isomers of *bis*epoxybutane was investigated in *Penicillium multicolor* and in *Vicia faba*[4]. The *l* isomer exerted a higher activity.

This communication attempts to estimate the activity of two isomers of *l*-threitol 1,4-*bis*methanesulphonate: *d*-threitol 1,4-*bis*methanesulphonate and -erythritol 1,4-*bis*methanesulphonate. For this purpose, seeds of *Vicia faba* (ssp. *minor* var. Åkerböna Weibull) and *Nigella damascena* (variety Miss Jekyll) were treated with several concentrations of the compounds at 21° C. The possible influence of a 21-h pre-soaking time was investigated in *Vicia*.

In the first set of experiments, seeds were treated with solutions containing 40 mg of each chemical per 100 ml. distilled water for 3 h. After washing, they were allowed to germinate in Petri dishes. Root tips were fixed at different times corresponding to the first and second mitotic cycles. Successive fixations were necessary because the mitotic cycle was found to be delayed in the previous work[3].

The results of the first set of experiments in *Vicia* are summarized in Fig. 1. For the *l* configuration these results are in agreement with previous findings[1,3]. The activity of this compound is much higher than in the case of the two other isomers.

The differences further increase for the second fixation time which corresponds principally to the second mitotic cycle.

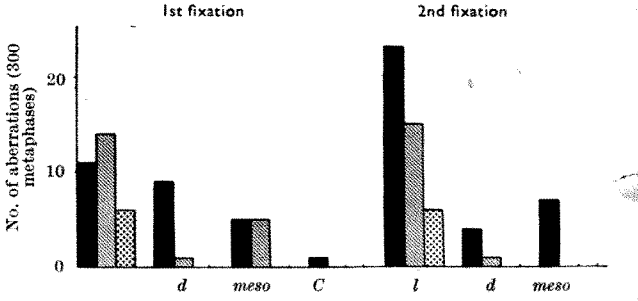


Fig. 1. *Vicia faba:* dry seed treatments. Effects of a dose (40 mg/100 ml./3 h) of the three isomers compared with control level (*C*). Black, one-hit aberrations; hatched, two-hit aberrations; dotted, minutes

No significant difference occurred between the efficiency of *d* and *meso* isomers, although the amounts of aberrations induced by these compounds were significantly higher than the control level.

A period of pre-soaking still increased the differences between the activity of the isomers, particularly for one-hit aberrations (Fig. 2). A delayed effect was observed with *l* isomer, whereas *d* and *meso* isomers seemed to exert their maximum activity during the first mitotic cycle.

In the second set of experiments, *d* and *meso* isomers were tested at very high concentrations in which the *l* isomer had a complete inhibiting effect after a few mitoses (Fig. 3). At such a high dose, the *l* configuration could not be tested on pre-soaked seeds owing to the immediate root degeneration.

Comparing these two experiments, no significant increase of the effect with the dose was found to occur (Fig. 3).

If we make a comparison with previous findings on the *l*-isomer[1,3] in which root tip fixations were carried out over a long period of time, it can be stated that the *d* and *meso* compounds are about 10–20-fold less efficient than the *l* compound. This difference is much greater than that between the *l*(+) and *d*(−) isomers of *bis*epoxybutane. In addition, some qualitative differences appeared. For example, minutes, that is chromosome fragments smaller than 1μ, were only observed with the *l* compound.

In the last set of experiments the results obtained in *Vicia* could be repeated in *Nigella* with *d* and *l* isomers (Fig. 4). For the *l* compound a proportionality between dose and amount of aberrations exists. The higher the dose, the greater the difference between the efficiency of both compounds. After a first fixation (46 h) no aberrations were observed.

On the basis of these data, it can be concluded that in some respects *d* and *meso* compounds behave as monofunctional alkylating agents, whereas the *l* compound has a difunctional action. Cytological arguments in favour of this are:

(i) The difference in activity at the chromosome level is the same as for other classes of alkylating agents, for example, between 'Myleran and ethyl methane sulphonate, and between *bis*epoxybutane (*meso* form) and ethylene oxide.

(ii) The delayed effect is much stronger for the *l* compound just as in the case of difunctional alkylating agents.

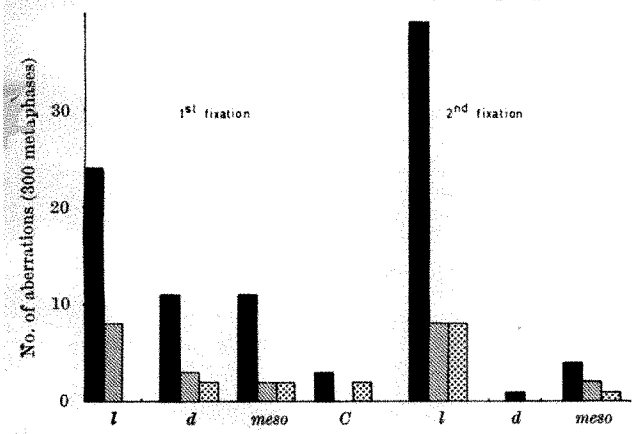


Fig. 2. *Vicia faba:* 21-h pre-soaked seed treatments. Key: same as for dry seeds

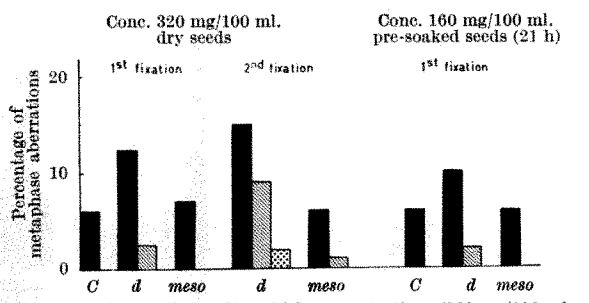


Fig. 3. *Vicia faba:* effects of two high concentrations (320 mg/100 ml. for dry seeds, 160 mg/100 ml. for pre-soaked seeds—100 metaphases analysed at each concentration)

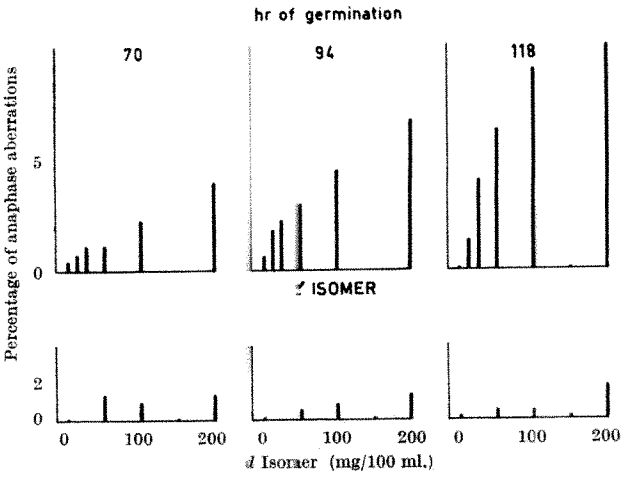


Fig. 4. *Nigella damascena:* dry seed treatments. Effects of increased concentrations of *l* and *d* isomers (300 anaphases analysed)

We thank Dr. W. Feit (Leo Pharmaceutical Products, Ballerup, Denmark) for providing the compounds.

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SOIL SCIENCE

Effect of Iron on measuring Water Content with a Neutron Meter

THE neutron moisture meter is based on the principle that hydrogen atoms, having a mass almost the same as that of neutrons and much less than that of other atoms in such media as soils, are the only atoms which reduce the kinetic energy of neutrons from high to thermal levels by collision. It is assumed that all hydrogen atoms are chemically associated with oxygen atoms in the form of water and that the detected activity of slow neutrons is proportional to the moisture content of the medium. It is recognized that an error will result if an unknown quantity of hydrogen atoms is present in some other form.

It is also generally recognized by those familiar with these instruments that there are a number of elements that, when present in only small proportions, are capable of absorbing appreciable quantities of neutrons, thereby introducing errors into the relationship. With the exception of chlorine, which may also be present in the soil moisture in the form of salts, this effect is seldom considered in practice.

All elements are, in fact, capable of absorbing neutrons to a greater or lesser degree, but natural soils do not generally contain elements with high capture cross-sections. During calibration, the aggregate result of neutron absorption is included as a background effect; if use of the instrument is confined to the same soil type, or to another having the same absorption effect, no appreciable error is evident. If, on the other hand, an element with higher than average absorption is present

in the soil, the calibrated relationship may not be valid. Only three elements usually occurring in natural soils require special attention. They are iron (2·5 barns), potassium (2·2 barns) and titanium (5·2 barns). Of these, only iron and potassium normally occur in relatively large enough quantities. Both these elements were investigated to determine their effect on the results of moisture contents determined with a scintillation-type depth probe.

The first investigation was carried out using potassium carbonate solutions as a pilot experiment. The results showed a loss of detected thermal neutron activity roughly proportional to potassium concentration. In terms of weights, potassium at 2 per cent of the dry weight of the soil caused a reduction of 4 per cent in the counting rate.

The second experiment involving iron was conducted using a substitute soil medium of silica sand, activated bentonite, and water, to which quantities of ferric sulphate were added. The purpose of using such a mixture was to provide conditions similar to those existing in a natural soil in an attempt to explain an observed discrepancy between two calibration curves[1,2].

The important parameters are: bulk density, as this affects the normal scattering of neutrons in the medium; water content, which, when kept within the range experienced in the field, provides a normal flux of thermal neutrons in the medium; and a range of water : iron content ratios equivalent to that encountered in the natural soil so that the quantities of thermal neutrons available for capture by iron would be realistic.

In the proportions necessary to meet these requirements, the mixture had a consistency of soft mortar, and blending of the components was easily accomplished in a large pigment mixer. Because some of the water was in the form of water of crystallization, both moisture content and iron content were determined by chemical analyses. As expected, the moisture contents did vary over a narrow range, but it was possible to reduce the results to the basis of a uniform moisture content by referring to the initial calibration curve.

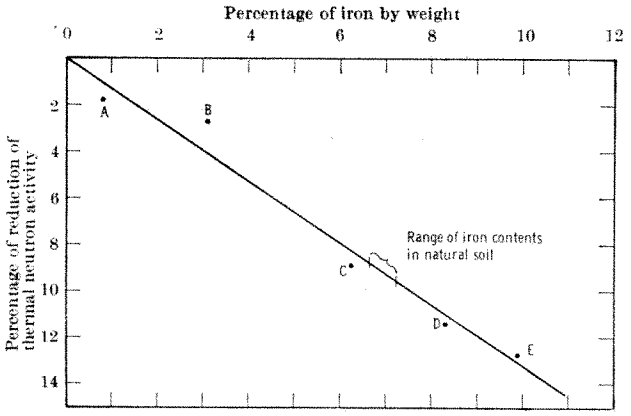


Fig. 1. Reduction of thermal neutron activity by iron absorption. Iron : water content ratios; *A*, 1·5 per cent; *B*, 6 per cent; *C*, 14 per cent; *D*, 20 per cent; *E*, 26 per cent

The results of five determinations of reduction of detected thermal neutron activity against iron content plotted in Fig. 1 show that the relationship is roughly linear. This appreciable reduction in counting rate goes far to explain the observed discrepancy. It is recommended that each calibration curve should be checked and, if necessary, adjusted for each soil type in which a neutron meter is used.

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PSYCHOLOGY

Change in Transfer Function of Joint Receptor Output

HARRIS[1] and others[2,3] have suggested that a change in the 'felt position' of the arm might be involved in the adaptation of limb movements after exposure to visual displacement in a prism-wearing situation. Efstathiou and Held[4] have concluded that ". . . adaptation to displaced vision cannot be interpreted as a change in the felt position of the arm in relation to the body". The subject matter of their article is considered in a forthcoming article[5] and will not be dealt with here, but this communication bears on the conclusion quoted above.

The experiment reported here gives clear evidence that neither the 'felt position' hypothesis nor the more specific hypothesis of 'change in the transfer function of joint receptor output' can be dismissed.

The sub-system discussed here would appear to have two major components. The first is the motor outflow to the limb. It would seem reasonable to infer from Lashley[6] that this operates on a basis of dead reckoning. The second is position feedback, probably from receptors in the joints[7]. The former is the appropriate pattern of impulses to motor units in the limb, appropriate, that is, to the given visual (or other) position information. The latter is a mechanism which can record limb excursion independent of any load on the limb.

My hypothesis is that this highly simplified system will adjust to prismatic displacement in the following manner. As a result of the first attempt to point at a target while wearing prisms, the subject will see, *inter alia*, that he has to move his arm some distance farther or less far. However, his first attempt would have been successful in reaching the target if it had been in the position which the visual information would have led the subject to expect, taking into account the proprioceptive information about the initial position of the limb. Since the result was not a success, the subject will react by assuming that the position of the limb has been wrongly assessed; this results in a change in the setting of the transfer function relevant to the output of the joint proprioceptors.

The experiment consisted in the subject having the left arm supported horizontally and clamped in the sagittal plane. Three points were marked in a straight line down this arm, near the shoulder, elbow and wrist respectively. Immediately above the limb was a plate-glass sheet. The experimenter touched each of the aforementioned points with a glass rod five times at random. Each time the arm was touched, the subject indicated where this point was by placing the index finger of the right hand on the glass sheet.

The subject was then trained to point at visual targets wearing 20-dioptre prisms, and using only his left arm. During this training, knowledge of results was obtained after each movement and the head was restrained with a bite board. After ten successive pointings accurate to within ± 0·5°, the subject was again placed in the first situation, and asked to point at the adapted arm with the unadapted arm. Table 1 gives the results of these experiments.

Table 1. DIFFERENCE SCORES BETWEEN POINTINGS BEFORE AND AFTER ADAPTATION

		Point 1 (shoulder)	2 (elbow)	3 (wrist)
Mean in centimetres:	Subject (1)	−3·03	−3·8	−4·6
	Subject (2)	−0·93	−1·27	−2·87

Best fit straight line gives 6° adaptation for subject (1) and 4·5° for subject (2).

The form of the results should be that localizations successively further from the shoulder of the adapted arm have greater and greater linear error from the true arm position. Position receptors probably operate by signalling the angle which the segment takes up with respect to the

adjacent segment. Hence, if the transfer function alters, the locus of points which the subject behaviourally defines as corresponding to his left arm should be a straight line which deviates from the true position by 11°, with its origin at the shoulder. This assumes that there is full adaptation and no decrement of effect during the time spent collecting the results.

As can be seen, the results support this contention in so far as both subjects show a greater linear error from true arm position as the distance from the shoulder increases. Full angular adaptation is not shown, however, which shows the need for more rigorous treatment of the problem.

The most parsimonious explanation of this result is that it is based on a change in the transfer function of joint receptor output. To explain the result using the motor outflow component is difficult, bearing in mind that the immobile limb shows no on-going activity as recorded by electro myographic techniques[8]. Presumably, then, there is no motor-outflow which can be used to give the subject information about limb position in this situation.

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Harris, C. S., *Science*, **140**, 812 (1963).
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Efstathiou, A., and Held, R. (unpublished results)
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Intermodal Transfer of Adaptation to Displacement

IT has been reported[1] that after adaptation of pointing behaviour in a prism-wearing situation, subjects will mislocate the position of an auditory stimulus if blindfolded and asked to point directly at it. The direction of this error of location is identical to that which would be expected if the subject were asked to point at a visual target after adaptation.

Before interpreting this result, it is necessary to know the generality of the phenomenon. If it could be shown that a subject exposed to a situation in which he received inaccurate auditory information as to the position of his pointing arm, then mislocated visual targets, there would then be strong evidence to suspect the implication of a change in the transfer function relating the output of joint receptors to some other component of the system, probably in the central nervous system, but as yet unspecified.

The experiment was carried out in three parts: (*a*) the subject pointed to visual targets without seeing his pointing limb at any time, and the investigator measured errors of location; (*b*) the subject pointed to a clicking auditory 'point-source' while blindfolded and with the pointing limb splinted. Pointing was carried out solely by horizontal abduction and adduction. Knowledge of success was given the subject when he depressed a switch which clicked a small loudspeaker which he thought was mounted directly above his pointing hand, but which in fact was offset by 12°. The task of the subject was to become as accurate as possible. After these adaptation trials, part (*c*), identical to (*a*), was carried out.

The results indicate that such auditory training leads to a change in pointing behaviour to visual targets at a level of confidence for t far in excess of 0·001.

It has been shown that there is no transfer of adaptation between limbs when the head is restrained[1]. The present cross-modal transfer of adaptation thus provides very strong evidence for change in transfer function for the output of the position receptors of the joints concerned. This is further confirmed in another article[2]. Alternative explanations could be put forward, but cannot satisfactorily explain how the subject could respond coherently if the joint was signalling information as to limb position which was incompatible with visually-derived information about the limb.

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[1] Harris, C. S., *Science*, **140**, 812 (1963).
[2] Craske, B. (preceding article).

STATISTICS

Determination of the Solid Volume Percentage below Detectable Size in Coulter-counter Analysis

IN a recent paper Harris and Jowett[1] suggest an extrapolation technique for determining the solid volume percentage below detectable size in Coulter-counter analysis. The technique is based on the assumption that the Gates–Gaudin–Schuhmann or the Rosin–Rammler distribution is valid for the particles in question, and it is presupposed that, if a mathematical distribution function fits the experimental data, this function also holds for the undetectable part of the physical distribution.

From a logical point of view there is usually no strict connexion, if any relationship at all, between a physical particle-size distribution and the mathematical equation used to fit the physical data. Frequently the point is to find a curve, a graph of some suitable mathematical function, which runs through the experimental points with satisfactory precision. Often, however, it is rather difficult to obtain complete overlap, and in such cases the temptation to believe in the smoothed curve more than in the actual experimental points will always be present. Thus the application of mathematical distribution functions in order to estimate the fine end percentage of a measured particle-size distribution may introduce errors in the total volume. First, even if complete overlap is obtained, this does not prove that the mathematical function also holds for the undetectable part of the physical distribution. Secondly, if complete overlap is not obtained the evidence that an extrapolation of the mathematical function will coincide with the physical distribution is further decreased. These points have also been emphasized by Harris and Jowett[1].

In cases where the purpose of using the mathematical function is merely to estimate the total solid volume, I have found an alternative, 'non-mathematical', method very useful. The method will be illustrated using the data of Harris and Jowett[1]. The extrapolation has, of course, to be based on a more or less 'non-quantitative' valuation, but, as the following example shows, it is possible to obtain a visual impression of the plausibility of the extrapolation.

The primary data from the Coulter-counter give a cumulative number distribution over stated size, but this distribution may easily be recalculated to a volume or weight basis, as illustrated by the data of Harris and Jowett[1]. In Fig. 1 these data are plotted as a frequency histogram, instead of applying the commonly used integral (cumulative) form of the size distribution. If $\bar{v}_P$ is the mean particle volume in a narrow size class of Δn particles and Δx the size range of that class, the frequency is equal to $\frac{\Delta n \cdot \bar{v}_P}{\Delta x}$. The different frequencies are given in Table 1. Δv is here identical with the product $\Delta n \cdot \bar{v}_P$.

In fact, the histogram in Fig. 1 includes the whole experimental information available from the Coulter-counter analysis, and some extrapolation in the small particle range is necessary to obtain the total area under the frequency curve, which is a measure of the 100 per cent

reference. The area under the extrapolated part of the curve represents the unknown volume fraction below detectable size. In Fig. 1, two different extrapolations are suggested. The first one, which in simplified form is frequently used in our laboratory, for example in Coulter-counter analysis of cement, is given by the straight line between the central point, *a*, of the top of the finest histogram column, and the origin, *e*. The other is given by the 'best' straight line through the three finest histogram columns, a somewhat inaccurate formulation which allows different investigators to arrive at different results, although the differences would be rather small. In general, it is advisable to choose a standardized, well-defined extrapolation procedure to eliminate the individual variations among different investigators.

In Fig. 2, the cumulative volume percentages above stated size are plotted in a log-probability graph with three different 100 per cent references, one of them being identical with the result of Harris and Jowett and equal to 20,600. The other two are based on the above-described procedures, and the unknown percentages below detectable size are given by the areas of the triangles *ecb* and *dcb'* in Fig. 1.

Finally, attention is drawn to the dotted horizontal line through the point *f* on the ordinate axis in Fig. 1. This line represents the top of the histogram column from zero to 5·86μ based on the 100 per cent value 20,600 of Harris and Jowett. It seems to be reasonable to expect the decreasing pattern of the three finest histogram

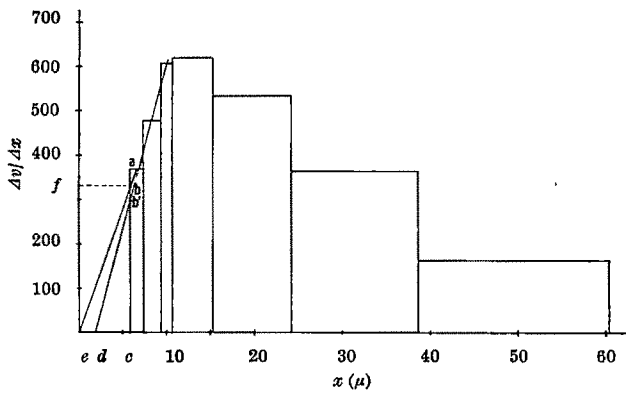


Fig. 1. Differential histogram of the Coulter-counter data of Harris and Jowett (ref. 1)

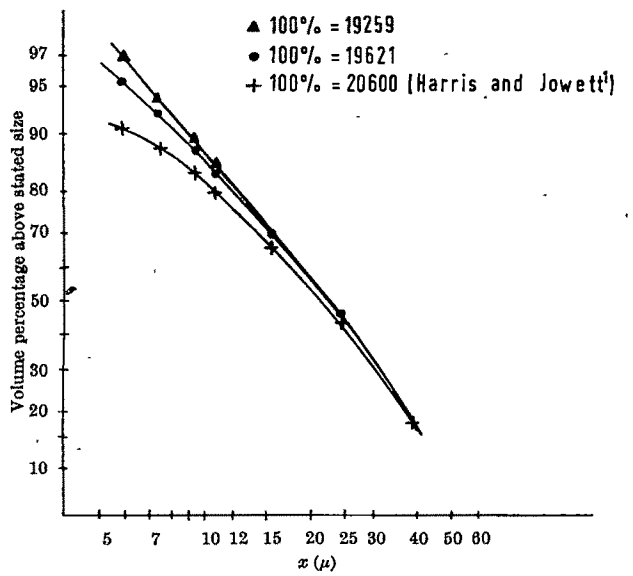


Fig. 2. Cumulative log-probability plots of volume percentage above stated size based on the data of Harris and Jowett (ref. 1)

Table 1. COULTER-COUNTER DATA OF HARRIS AND JOWETT (REF. 1)

x (μ)	Δx	Volume v above stated size (arbitrary units)	Δv	$\frac{\Delta v}{\Delta x}$	Volume percentage above stated size 100% = 20,600	100% = 19,621	100% = 19,259
60·40		0					
	21·72		3,528	162·4			
38·68		3,528			17·13	17·98	18·32
	14·49		5,264	363·3			
24·19		8,792			42 68	44·81	45·65
	8·91		4,731	531·6			
15·28		13,523			65·65	68·92	70·22
	4·60		2,842	617·8			
10·68		16,365			79·44	83·41	84·97
	1·35		818	605·9			
9·33		17,183			83·41	87·57	89·22
	1·93		920	476·7			
7·40		18,103			87·88	92·26	94·00
	1·54		566	367·5			
5 86		18,669			90·63	95·15	96·94
		Volume below detectable size:			1,931	952	590

columns to continue towards the origin in some way, and, if that is true, the 100 per cent value of Harris and Jowett is too large. I therefore suggest that a 100 per cent estimate of either 19,621 or 19,259 should be used instead of 20,600.

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[1] Harris, C. C., and Jowett, A., *Nature*, **208**, 175 (1965).

Distribution of the Derivative of the Likelihood Function

IT is common practice to approximate the distribution of the derivative of the log-likelihood, $\partial L/\partial\theta$, by a normal distribution with mean zero and variance $V = -[E(\partial^2 L/\partial\theta^2)]$. Calculations show that the three-moment χ^2-approximation:

$$(\partial L/\partial\theta) = a + b\chi^2_{(\nu)} \qquad (1)$$

can be a considerable improvement over the normal approximation, where a, b and ν (the degrees of freedom of the χ^2) are determined so that the right-hand side of (1) has its first three moments in common with $(\partial L/\partial\theta)$, that is:

$$b = \tfrac{1}{4}\mu_3/V,\ \nu = \tfrac{1}{2}V/b^2 \text{ and } a = -b\nu \qquad (2)$$

where $\mu_3 = 3(\partial V/\partial\theta) + 2E(\partial^3 L/\partial\theta^3)$.

Table 1. ERROR IN THE LIMITS OF θ: (APPROX. − TRUE)10^2

	Lower 0·01		Lower 0·05		Upper 0·05		Upper 0·01	
x	Normal	χ^2	Normal	χ^2	Normal	χ^2	Normal	χ^2
1	3	−1	2	0	54	6	150	21
2	13	−3	7	−3	45	4	136	17
3	20	−5	10	−2	47	4	128	14
5	30	−6	14	−2	43	4	119	10
10	41	−6	18	−2	39	3	107	8

To illustrate this, we consider a Poisson variate, x, with mean θ so that $\partial L/\partial\theta = (x - \theta)/\theta$. Substituting (2) in (1), we obtain in this case $\chi^2_{(\nu)} = 4(x + \theta)$ and $\nu = 8\theta$. Confidence limits for θ were obtained from Pearson and Hartley's[1] and Harter's[2] tables after introducing the usual continuity corrections, that is, using $x - \frac{1}{2}$ and $x + \frac{1}{2}$ when obtained the lower and upper limits for θ, respectively. The values are given in Table 1 and compared with the normal values. The true values are given by Bartlett[3]. For μ_3 small, that is, ν large, the square or cube root normal approximation of χ^2 can be used.

For several other applications of the χ^2-approximation (2), see Pearson[4] and Tiku[5–7].

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[1] Pearson, E. S., and Hartley, H. O., *Biometrika*, **38**, 112 (1951).
[2] Harter, H. L., *More Tables of Incomplete Gamma-function* (1964).
[3] Bartlett, M. S., *Biometrika*, **40**, 12 (1953).
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[5] Tiku, M. L., *Biometrika*, **53**, 415 (1965).
[6] Tiku, M. L., *Biometrika*, **53**, 630 (1965).
[7] Tiku, M. L., *Austral. J. Statist.*, **7** (in the press).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, May 16

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C 2)—Colloquium on "Main Power Distribution in Industry".

UNIVERSITY OF LONDON (at the Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, London, W.2), at 5 p m —Prof M. Abercrombie: "The Pathology of Cell Movement" (third of a series of four Almroth Wright Lectures).*

UNIVERSITY OF LONDON (in the Botany Theatre, University College, Gower Street, London, W.C.1), at 5 p m.—Prof. David H Brown (Washington University, St. Louis) "Enzyme Reactions in Glycogen Synthesis".*

UNIVERSITY OF LONDON (at Bedford College, Regent's Park, London, N W.1), at 5 30 p.m —Prof. A. Tarski: "What are Logical Notions ?"*

Tuesday, May 17

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place London, W.C.2)—Colloquium on "Laboratory Voltage Reference Standards".

UNIVERSITY OF LONDON (at the Institute of Obstetrics and Gynaecology, Hammersmith Hospital, Du Cane Road, London, W 12), at 3 p.m —Prof. P. E. Polani: "Human Chromosomes and Their Abnormalities (1)".*

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Sir Lawrence Bragg, O B.E., F R.S.. "Famous Experimenters in the Royal Institution" (afternoon lecture for Fourth Form and Sixth Form Boys and Girls from Schools in London and the Home Counties. To be repeated on May 18, 24 and 25)

UNIVERSITY COLLEGE LONDON (in the Anatomy Theatre, Gower Street, London, W.C.1), at 5.30 p.m —Prof Paul Weiss (University of Texas): "The Neuron: a System in Continuous Flow".*

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 5 30 p m.—Prof A. W. Bishop: "Soils and Soft Rocks as Engineering Materials" (Inaugural Lecture) *

UNIVERSITY OF LONDON (at the Royal Dental Hospital of London, Leicester Square, London, W C.2), at 5.30 p.m —Prof. A. S. V. Burgen. "Some Problems in Salivary Gland Physiology" (Warwick James Lecture) *

Tuesday, May 17—Thursday, May 19

BRITISH NUCLEAR ENERGY SOCIETY (at the Institution of Civil Engineers, Great George Street, Westminster, London, S.W 1)—Conference on "Fast Breeder Reactors".

Wednesday, May 18

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W 7), at 5 p.m —Mr J. T. Bartlett: "The Growth of Cloud Droplets by Coalescence", Mr. J. Latham and Mr. C D Stow "The Influence of Impact Velocity and Ice Specimen Geometry on the Charge Transfer Associated with Temperature Gradients in Ice", Mr. D. B Shaw "Computation of Heat Sources and Sinks in the Atmosphere".

INSTITUTE OF MATHEMATICS AND ITS APPLICATIONS (in the Large Physics Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m —Dr I. J. Good: "How to Estimate Probabilities".*

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, W.C 2), at 5.30 p.m.—Mr. A. A. Fulton "The Cruachan Pumped Storage Scheme".

SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMICAL METHODS GROUP (at "The Feathers", Tudor Street, London, E.C 4), at 6 30 p.m.—Discussion Meeting on "The Determination of Fluorine".

Thursday, May 19

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 10.30 a m —Discussion Meeting on "Non-Linear Theory of Wave Propagation in Dispersive Systems", organized by Prof. M J. Lighthill, F.R S.

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W C.2), at 5.30 p m.—Annual General Meeting, followed by a technical film show.

UNIVERSITY OF LONDON (at Bedford College, Regent's Park, London, N.W.1), at 5.30 p m —Prof. A. Tarski. "Truth and Proof".*

UNIVERSITY OF LONDON (at the Middlesex Hospital Medical School, Cleveland Street, London, W.1), at 5 30 p m —Prof J. G. Mulnard (Université Libre de Bruxelles). "Experimental Approach to Mammalian Development".*

INSTITUTE OF SCIENCE TECHNOLOGY, LONDON BRANCH (in the Physiology Theatre, The Medical College of St. Bartholomew's Hospital, Charterhouse Square, London, E C 1), at 6.30 p m.—Mr. D C. Moore "Development of the Volumetric Gas Analysis Apparatus".

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at Manson House, 26 Portland Place, London, W 1), at 7 30 p m.—Prof. D. S McLaren "Present Knowledge of the Role of Vitamin A in Health and Disease".

SOCIETY OF COSMETIC CHEMISTS OF GREAT BRITAIN (at the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 7.30 p m.—Film Evening.

Friday, May 20

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. M. Gell-Mann (California Institute of Technology) "Elementary Particles ?"

Friday, May 20—Saturday, May 21

BIOCHEMICAL SOCIETY (at the School of Pharmacy, Brunswick Square, London, W C.1, and the Royal Free Hospital School of Medicine, Hunter Street, London, W.C.1)—460th Meeting which will include a Colloquium on The Contribution of Enzymes to the Structural Determination of Glycogen and Starch".

Saturday, May 21

NUTRITION SOCIETY (at the Sir John Atkins Laboratories, Queen Elizabeth College, Campden Hill Road, London, W.8), at 10 a.m.—Original Communications.

Monday, May 23

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS GROUP (at Savoy Place, London, W.C.2)—Colloquium on "A New Look at Energy Storage".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS GROUP (joint meeting with the I.E.R.E. Medical and Biological Group, at Savoy Place, London, W.C 2), at 10 a m. and 2.30 p m.—Discussion Meeting on "Electrical Aids to the Handicapped".

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C 1), at 3 p m.—Prof. Raoul H. Bott (Harvard University). "The Riemann-Roch Question in Various Guises" (further lectures on May 24 and 26).*

ROYAL INSTITUTION, LIBRARY CIRCLE (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Mr. W. B. Proudfoot: "Copying Methods: Past and Present".

UNIVERSITY OF LONDON (at the Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, London, W.2), at 5.30 p.m.—Dr. C. E. G. Smith. "The Effects of Civilization on the Viral Zoonoses" (last of a series of four Almroth Wright Lectures) *

SOCIETY OF COSMETIC CHEMISTS OF GREAT BRITAIN (at the Washington Hotel, Curzon Street, London, W 1), at 7 p.m.—Annual General Meeting.

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8 15 p.m —Mr. G. de G. Sieveking. "In the Tracks of Early Man in Thailand".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

SENIOR LECTURER and a LECTURER/ASSISTANT LECTURER (with a good honours degree and industrial/research experience in any aspect of chemical engineering) IN THE DEPARTMENT OF CHEMICAL ENGINEERING—The Staff Officer, The University of Aston in Birmingham, Gosta Green, Birmingham, 4 (May 18).

PRINCIPAL LECTURER and a LECTURER IN THE DEPARTMENT OF PHYSICS to prepare students for honours degree of the University of London, and to assist with postgraduate courses and research—The Principal, West Ham College of Technology, Romford Road, Stratford, London, E 15, quoting Ref. RAW/JA/664/1 (May 20).

ASSISTANT LECTURER (preferably with an interest in some aspects of animal physiology) IN ZOOLOGY—The Registrar, The University, Manchester, 13, quoting Ref 109/66 (May 21).

RESEARCH OFFICER IN THE DEPARTMENT OF PHYSICS to undertake research on aspects of crystal growth—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (May 21).

ASSISTANT LECTURER IN SOCIAL ANTHROPOLOGY—The Secretary, The Queen's University, Belfast, Northern Ireland (May 23).

LECTURER OR ASSISTANT LECTURER IN BIOCHEMISTRY—The Registrar, The University, Leeds (May 23)

LECTURER OR ASSISTANT LECTURER IN SOCIOLOGY IN THE DEPARTMENT OF ADULT EDUCATION AND EXTRA-MURAL STUDIES—The Registrar, The University, Leeds, 2 (May 23)

LECTURER OR ASSISTANT LECTURER IN SOCIOLOGY—The Registrar, University of Kent at Canterbury, Canterbury, Kent (May 23).

LECTURER OR ASSISTANT LECTURER (preferably with an interest in the experimental psychology of perception) IN THE DEPARTMENT OF PSYCHOLOGY —The Registrar, The University, Leeds, 2 (May 23).

RESEARCH DEMONSTRATOR (with a good honours degree in psychology) IN PSYCHOLOGY—Prof. George Westby, Department of Psychology, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (May 23)

SECOND CHAIR OF ELECTRICAL ENGINEERING at Queen Mary College—The Academic Registrar, University of London, Senate House, London, W.C.1 (May 27).

ASSISTANT LECTURER OR DEMONSTRATOR (with medical qualifications registrable in the United Kingdom) IN ANATOMY—The Registrar, The University, Manchester, 13, quoting Ref 118/66 (May 28).

CHAIRS (2) OF PHILOSOPHY—The Assistant Registrar (Establishment), The University of Sussex, Stanmer House, Stanmer, Brighton, Sussex (May 28).

LECTURER IN THE DEPARTMENT OF MEDICAL PHYSICS to take charge of and pursue research in clinical physics work embracing radioisotopes and scanning, radiotherapy and radiodiagnosis—The Secretary, The University, Aberdeen (May 28)

LECTURER OR ASSISTANT LECTURER (preferably able to teach the theory of knowledge, logic or philosophy of science) IN PHILOSOPHY—The Registrar, University College of Wales, Aberystwyth (May 28)

ASSISTANT LECTURER OR LECTURER (with a special interest in X-ray diffraction) IN MINERALOGY—The Registrar, University College of Wales, Aberystwyth (May 31).

LECTURER IN ANATOMY—The Registrar and Secretary, The University, Bristol (May 31).

LECTURER IN PURE MATHEMATICS—The Secretary, The Queen's University, Belfast, Northern Ireland (May 31).

LECTURER (with a good honours degree in physics or mathematics, and some training and research experience, preferably experimental, in meteorology) IN THE DEPARTMENT OF METEOROLOGY—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (May 31).

ASSISTANT LECTURER IN THE DEPARTMENT OF METALLURGY OF THE FACULTY OF SCIENCE—The Registrar, The University, Manchester 13 (June 1).

ASSISTANT LIBRARIAN (with a good honours degree and preferably research experience and/or library qualifications) IN THE UNIVERSITY LIBRARY—The Registrar, The University, Manchester, 13, quoting Ref. 125/66 (June 3).

CHAIR OF ELECTRONIC COMPUTATION IN THE DEPARTMENT OF APPLIED MATHEMATICS—The Registrar, The University, Sheffield (June 4).

PROFESSOR, a SENIOR LECTURER and a LECTURER IN THE DEPARTMENT OF STATISTICS, University of Ibadan, Nigeria—The Inter-University Council, 33 Bedford Place, London, W C.1 (June 5)

RESEARCH ASSISTANT (preferably with interests in the field of fluid mechanics) IN APPLIED MATHEMATICS—The Registrar, University College of North Wales, Bangor, North Wales (June 6).

COURTAULDS CHAIR OF CHEMICAL ENGINEERING at Imperial College—The Academic Registrar, University of London, Senate House, London, W.C.1 (June 7).

SENIOR LECTURER or LECTURER (preferably with interests in physics which can be developed in Barbados—currently in progress are studies on some aspects of geophysics, aeronomy, meteorology and solar energy) IN PHYSICS at the University of the West Indies, Barbados—The Inter-University Council, 33 Bedford Place, London, W.C.1 (June 10).

TUTORS (2) IN PURE MATHEMATICS—The Registrar, University College of Wales, Aberystwyth (June 10)

CHAIR OF MATHEMATICS (Pure) at Chelsea College of Science and Technology—The Academic Registrar, University of London, Senate House, London, W.C.1 (June 15).

ASSISTANT LECTURER or LECTURER IN THE DEPARTMENT OF PHYSICS—The Registrar, The University, Liverpool, 3, quoting Ref. CV/46 (June 18).

RESEARCH ASSISTANT IN THE DEPARTMENT OF ZOOLOGY to participate in work concerned with the effect of antimetabolites on embryos—Dr. F. Billett, Department of Zoology, University of Southampton, Southampton (June 18).

LECTURER IN PHYSICAL CHEMISTRY—The Registrar, The University, Sheffield (June 20).

JUNIOR RESEARCH FELLOW (preferably with an interest in research in the area of laser flash spectroscopy) IN PHYSICAL CHEMISTRY—The Registrar, The University, Sheffield (June 30).

LECTURER (with a postgraduate degree and preferably some experience of teaching biochemistry at university level) IN BIOCHEMISTRY at Lincoln College, University of Canterbury, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, June 30).

MYCOLOGIST, SCIENTIFIC OFFICER GRADE (with a good honours degree) to work on soil-borne diseases of cereals—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (June 30).

SCIENTIFIC OFFICER or SENIOR SCIENTIFIC OFFICER (botanist, with a good honours degree and research experience) to study the physiology of sucrose storage in sugar beet—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (June 30).

LECTURER (graduate in science, with specialization in chemistry or biochemistry; in food science or home science) IN FOOD CHEMISTRY IN THE DEPARTMENT OF NUTRITION in the School of Home Science, University of Otago, Dunedin, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, July 15).

CHURCHILL PROFESSOR OF MATHEMATICS FOR OPERATIONAL RESEARCH—The Registrary, University Registry, The Old Schools, Cambridge, marking envelope "Confidential" (July 16).

LECTURER (with an honours degree in chemical engineering and some industrial experience) IN CHEMICAL ENGINEERING—The Registrar, University of Strathclyde, George Street, Glasgow, C 1.

LECTURER (with an interest in experimental psychology) IN PSYCHOLOGY—The Secretary, Sir John Cass College, Jewry Street, London, E.C.3.

RESEARCH ASSISTANT (preferably with a good honours degree in biochemistry) IN THE DEPARTMENT OF PATHOLOGY to work in the Chemical Pathology Laboratories on biochemical aspects of ocular pathology—The Secretary, Institute of Ophthalmology (University of London), Judd Street, London, W.C.1.

SECOND CHAIR OF PHYSICS—The Academic Registrar, Northampton College of Advanced Technology, St. John Street, London, E.C.1, quoting Ref. P/N.

SENIOR TECHNICIAN and a TECHNICIAN TO WORK WITH AN EXPERIMENTAL IMMUNOLOGY GROUP IN THE DEPARTMENT OF ZOOLOGY—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh.

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NO FRANKS AT M.I.T.

BY an irony that will no doubt give pleasure at All Souls, Oxford is not the only university having to reconcile its special position with educational good sense. A month from now, Dr. Julius Stratton will retire as president of the Massachusetts Institute of Technology after a long and distinguished association with the Institute as student, teacher and chief administrator. His impending departure has brought into prominence again many of the doubts which have plagued the Institute ever since it first became apparent that it is bound to be difficult—and challenging as well—to decide how best to balance the demands of a curriculum concentrated on technology and science and the need that a modern education should equip a person to work in the big wide world. As at Oxford so at M.I.T., the question is how best to make use of the institutional capital of a great university. So far, the Institute has done something to meet criticism about the treatment of undergraduates by grafting on to its strictly technical programme a certain amount of study in the humanities—units of study which range from modern languages to Greek and to what is called 'political science'.

On the face of things, it looks as if the M.I.T. Corporation is hoping for a permanent solution of some of its problems by sleight of hand. For Mr. Howard Johnson, who takes over from Dr. Stratton on July 1, is not a scientist in the accustomed sense but a man whose reputation is based on his outstanding work as dean of the Sloan School of Management at M.I.T. Nobody should hold this against the new president, even though it may be a disappointment that one of the more obvious (and not illiberal) candidates has not got the job. In any case, it is good to know that Mr. Johnson will have Dr. J. Wiesner as Provost. Indeed, there is the strongest possible case for a blending of technological and business studies in the style of Mr. Johnson's school. But it is all too tempting to suppose that the Corporation is expecting that the appointment of a man known as a social scientist may automatically guarantee that M.I.T. moves in the right direction. The inference may be wrong, of course, but so may be the calculation.

One of the troubles at M.I.T. is that, like Oxford, it is an *élite* university. Another is that it is not a university at all in the strict sense, but only a lopsided one. Undergraduates are required to dip into the humanities, but cannot plump for a degree in, say, history if they should find that subject more attractive than, say, physics. Women account for between 5 and 6 per cent of the undergraduate population, although the Institute would no doubt take in more if it could find sufficient able recruits. There are as many graduate students as undergraduates in the student body of 7,000. The ratio of faculty to students is more generous than the University Grants Committee would recommend in Britain, although comparisons are difficult because of the volume of government contract work at M.I.T. The euphemism for all this, which Dr. James Killian is always using, is that M.I.T. is "a university centred on science and technology". Why, then, is it always acting guilty?

The problems of teaching undergraduates properly in a predominantly graduate university are a part of the reason. A decade ago the Corporation was rightly alarmed to find that university teachers at M.I.T. were electing to send their sons (not to mention their daughters) to other universities instead of letting them run the gauntlet of undergraduate life at M.I.T. Now things are a lot better, but difficulties persist. The fast pace makes for competitiveness. Concentration makes things worse. The fact that the faculty is extremely talented does not guarantee that an undergraduate will not feel trapped on an intellectual treadmill from which he can only be released by getting into graduate school after four years of donkey work. Any leavening of the curriculum is obviously desirable, and by all accounts the humanities programme has already brought benefits of morale as well as of breadth. It remains to be seen whether these well-intended innovations are sufficient—and whether they will serve somehow to make less solemn the undergraduates who are still easy sport for B.B.C. television cameras.

Another class of problems stems from the scale and character of undergraduate work at M.I.T. It is natural (and right) that an institution bent on technical training of the highest quality should seek to set its graduates and faculty members real problems for research. One splendid result has been the way in which M.I.T. has spawned successful industries based on science throughout Massachusetts and beyond. The other side of the coin is that the Institute has allowed itself to be overweighted with work that must be a distraction even for a university determined to be useful. In particular, the volume of defence research at M.I.T. may well be too much for comfort. It is always difficult to estimate when a Government research contract is an educative incentive for those engaged on it, and when it becomes an administrative bore, but M.I.T. may have put itself at the disposal of the U.S. Government too readily for its own good and, in the long run, for the Government's. The truth is that M.I.T.—for an *élite* university—is surprisingly lacking in public influence. Killian, Wiesner and Samuelson, to mention only some, have immense personal influence, but the corporate body has been far less influential than its talents could have made it. Yet M.I.T. is well placed to influence decisively the pace and the direction of change in science and technology. Paradoxically, perhaps, that could easily give the undergraduates something more glittering to work for than a chance to work hard at graduate school.

So what should Mr. Johnson do? The first thing to agree is that there are limits to what the humanities can accomplish at M.I.T., at least so long as they remain peripheral. Either Mr. Johnson must go the whole hog, and offer degrees in the humanities, or he must tackle the undergraduate problem more directly. On balance, the second course is preferable. Whatever defects there may be, the present system at M.I.T. has a number of outstanding merits. It has, for example, virtually obliterated the distinction between science and technology, and the new president is lucky that there are already under way important innovations in the self-conscious development of undergraduate curricula. These are important assets on which to build.

A lot can be done within science and technology themselves. Making economics an integral part of the curriculum will be an obvious innovation for Mr. Johnson, but none the less valuable on that account. With its

good supply of able students, the Institute is also well placed to experiment with studies which span conventional boundaries. But it would be still more exciting if it were to attempt to work out ways of giving technology a more purposeful place than it has at present in modern society. Why should not M.I.T. concern itself as much with the problems of urban technology as with those of reaching the Moon? Ideally, Mr. Johnson should set out to give M.I.T. the means of helping society to decide what uses to make of science and technology. His predecessors have accomplished a lot already, but a great deal remains to be done.

MORE BUREAUCRATS

SO far as it goes, the report of the Civil Service Commissioners for 1965 is cheerful about the recruitment of scientists to the British Civil Service. Of the 107 candidates successful in the competition for entry into the Administrative Class—the members of which eventually come to inhabit what are called the corridors of power—no fewer than nine are said to be endowed with a scientific training. The Commissioners say that this was a "marked improvement" on previous years, but plainly there is a long way to go before present methods of recruitment will ensure that the mainstream of the Civil Service is adequately supplied with competent scientists. At the same time, there is continuing anxiety about the recruitment of capable scientists into the Scientific Class. Here the difficulty is not so much an absolute shortage of numbers as a shortage of candidates of really outstanding quality. This does not necessarily mean that the Government research establishments are starved of really able men, for the recruitment of professional scientists to the Civil Service usually entails a period of unestablished service which does not count in the Commissioners' statistics. But evidently there is a lack of really able professionals willing to settle down in Government service. Even though the rapid growth of recent years is clearly part of the cause of the Commissioners' anxiety, things could obviously be better than they are.

Here, as in many other parts of British public administration, the remedy is probably to be found in greater flexibility. Thus the recruitment of scientists to the Administrative Class would be enormously improved if only the Civil Service as a whole would make fuller use of the arrangements which now exist for the transfer of men and women from one class to another. In 1965 there were fewer than a dozen transfers of this kind, yet the Government laboratories are now well supplied with experienced administrators ready and able to spread their wings. Both classes—the Administrative and the Scientific—would be better off if there were a more ready interchange between them. In a very similar way, recruitment to the Scientific Class would be improved if it were easier than it is at present for professional civil servants to retain a sense of kinship with the scientific discipline to which they belong. There needs to be a greater traffic of people between the research establishments and the universities and industry. Sabbatical leave ought to be a necessity, not a luxury. And some of the humdrum customs of public service ought to be abolished. The irony is that British public laboratories have never before been so well stocked with exciting problems to be tackled.

COMMONSENSICAL ENGLISHMEN

The English Paracelsians

By Allen G. Debus. (Oldbourne History of Science Library.) Pp. 222+7 plates. (London: Oldbourne Book Co., Ltd., 1965.) 45*s*. net.

THE sixteenth century was rich in passionate personalities who delighted in being at the storm centre of controversy and whose life and work have aroused either adulation or distaste ever since their own day. Was Cardan, the astrologer-physician, a great mathematician or a fraudulent plagiarist? Was Giordano Bruno a Copernican martyr or a magician and pantheistic heretic, Paracelsus the founder of modern chemistry or an alchemist sunk in mystic and incomprehensible theory? It all depends on one's temperament, and neither Paracelsus nor any of the others have ever lacked defenders. Those who find these mystic and magical aspects of the sixteenth century distasteful and of doubtful value in the history of modern thought can console themselves with the reflexion that the later contemporaries and successors of those followers of the Hermetic tradition (as Frances Yates has recently taught us to consider it) found their books difficult to understand and even nonsensical.

Mr. Debus belongs to the younger generation of historians of science, already in revolt against the rationalist views of his post-War predecessors; he would like to see in Paracelsus a dominant leader in the emergence of modern chemistry. Had he chosen to discuss the Continental Paracelsans of the period before 1650 he would have found, as he himself says, much detailed and solid evidence of Paracelsan influence. But circumstances have led him to write about Paracelsanism in England before 1640, and he is too good a scholar not to recognize that the English even then were too sceptical of high-flown mystic or metaphysical theory to be Paracelsans in any real sense, and, as here emerges, Paracelsan ideas were of negligible importance in Elizabethan and Jacobean England.

It must be remembered that then, as now, Paracelsus was a very inaccessible author. Few Englishmen could (or can) understand the curious mixture of Schweizdeutsch and Latin in which Paracelsus wrote, and most of his works have never been translated into English. (Only two small works were so translated in this period.) In addition, Paracelsus's theory is difficult to understand; he expressed himself obscurely and is often inconsistent. Only two Englishmen ever seem to have been direct followers of Paracelsus: Bostocke and Fludd. (More refer to his theory only to insist they cannot understand it, and a few accepted a diluted version promulgated by later Continental Paracelsans.) Bostocke was an extremely obscure Elizabethan mainly remembered for his comparison of Copernicus and Paracelsus (which Mr. Debus makes less clear because he does not know the Elizabethan usage of 'stars' for 'planets'). Robert Fludd, whom Mr. Debus has studied at some length, was the Jacobean expositor of a 'Mosaic philosophy', a blend of Renaissance science and speculative mysticism which was extensively attacked on the Continent, notably by Mersenne and Gassendi, but was virtually ignored in England. The mystic chemistry to be found in this period belongs to the older alchemical tradition of George Ripley and Thomas Norton; it was not until the 1650's that English mystic chemistry received a new stimulus from the work of Van Helmont. It is to be hoped that Mr. Debus may give us an examination of English Helmontians, who are many and varied.

As *The English Paracelsians* amply demonstrates, English medical men and apothecaries accepted the 'new' chemical remedies and drugs for which Paracelsus had claimed so much without regarding them as necessarily Paracelsan. Partly this is because they recognized that chemical remedies were older than Paracelsus, and partly because they derived them from followers of Paracelsus in whom the pure Paracelsan doctrine was much diluted.

Mr. Debus discusses the influence of Quercetanus (known to his contemporaries exclusively by this Latin form of Duchesne) who wrote in Latin (so that he was accessible to the learned) and whose works were also speedily translated into English. But he does not mention Basil Valentine (some of whose works were available in Latin in this period) nor the text-book of Jean Beguin, well known in England, and does not seem to appreciate the importance of Oswold Croll, very influential for the reliance on chemical drugs which developed in this period. In fact, chemical remedies seem to have crept into the English pharmacopoeia without great opposition; the great battle between the orthodox College of Physicians and the Society of Chemical Physicians belongs to the 1660's. One cannot help wishing that, on all counts, Mr. Debus will choose to write another book on English medical chemistry after 1640; it would surely provide more scope for his scholarship, more important and influential books than those examined here, and some opportunities for dipping into the manuscript sources. This is an effective account of the way in which English medical writers before 1640 accepted medical chemistry while rejecting Paracelsan mystic theory; but the history of common sense cannot be by its nature intellectually exciting.

MARIE BOAS HALL

SOUTH OF THE BORDER

Latin America

An Economic and Social Geography. By Dr. J. P. Cole. Pp. xviii+468. (London: Butterworth and Co. (Publishers), Ltd., 1965.) 57*s*. 6*d*.

A RECENT development in many British universities, both new and old, is the institution of courses or schools in area studies. In particular there is renewed attention to Latin America, where British capital played so large a part in such early developments as railways, ports and trade. There followed a long period, perhaps of disillusion, when Latin America was much neglected, both in commercial circles and in academic studies. The renewed interest recognizes the vast potential of one of the least-developed world regions—14 per cent of the Earth's land surface, but only 7 per cent of world population.

Dr. Cole, at present lecturer in geography in the University of Nottingham, has travelled in most of the Latin American countries—by which he includes all the New World south of the United States border—over a period of 10 years, and in so doing has acquired a good working knowledge of Spanish and a Peruvian wife. He makes the latter his excuse for naïvely apologizing to his readers for a possible over-emphasis on Peru.

Recognizing that there are some excellent general text-books on the geography—notably Preston E. James's *Latin America*, which has been described as one of the best geographical descriptions of any area in the English language—Dr. Cole wisely concentrates on a snapshot picture of the early sixties, in which he relies heavily on the census returns of 1960–61 and the statistical information collected by United Nations agencies. Almost half *Latin America* is devoted to Latin America as a whole—population, physical background and resources, history, present political and economic development, transport, agriculture, mining and manufacturing—the remainder to a treatment by countries. Throughout the book there are certain unusual features. Many of the very large number of simple thematic maps or cartograms ignore the conventional north–south orientation: indeed, Latin America as a whole can be shown with economy of page space if depicted obliquely. However, even the initiated may be forgiven for hesitation in recognizing the U.S.S.R. shown (for comparison) on end. There are no photographs; instead the author's father, Mr. P. Cole, has skilfully translated photographs into effective line drawings.

Dr. Cole recognizes that he is writing not only of a vast and very varied area but also one in a state of rapid evolution. At present no political unit in the world can show the range of conditions exhibited by Brazil—from literally unexplored depths of the Amazonian forest to the ultra-sophisticated life of skyscraper São Paulo, Rio and Belo Horizonte, or the imaginative creation of Brasilia, but daily new motor highways are being pushed across the 3 million square miles of national territory. As the long-discussed Pan American Highway becomes a reality so the Latin American Free Trade Association assumes a major significance. The final chapter is appropriately devoted to foreign trade and economic union. The total value of Latin American foreign trade in 1961 was 17,210 million U.S. dollars with exports worth slightly more than imports, which may be compared with United States foreign trade for the same year of 35,323 million dollars. It comes as a surprise, perhaps, to find that Venezuela leads the nations with 20 per cent of the whole, but this serves to emphasize the huge importance of oil. Oil in fact accounts for 28 per cent of the total exports of all Latin America: it is followed by coffee, 17 per cent.

It is perhaps invidious to pick these few details from a book that is packed with information, well presented, and commendably up to date. L. DUDLEY STAMP

PNEUMATIC CONTROL DESIGN

Components for Pneumatic Control Instruments

The Static and Dynamic Characteristics of Pneumatic Resistances, Capacitances and Transmission Lines. By L. A. Zalmanzon. Translated by R. Hardbottle. Translation edited by F. P. Stainthorp. Pp. xv+321. (London and New York: Pergamon Press, Ltd., 1965.) 110*s*. net.

COMPONENTS for Pneumatic Control Instruments is a misleading title, in that it might lead one to expect detailed description of the wide range of pneumatic components used in process control. The sub-title gives a better clue to the contents and, within the limits indicated by the sub-title, the coverage is comprehensive. There is room for a range of books of this standard under the main title, each covering a limited number of specialized topics.

The process control engineers with their pneumatic controllers were pioneers in automatic control. Because of the early development of the three-term-controller with its wide range of adaptability, there was no urgent need for detailed analysis of design and process, and thus to some extent progress slowed down on pneumatic while hydraulic and electrical systems became the centres of interest. If the pioneer does too well initially he may satisfy immediate demands but fail to develop quickly enough as demands increase.

While the major factors in the development of control systems were, of course, the requirements of the process industries and of the armed services, an important factor is the educational equipment of the designers. Most engineers are well qualified to extend their knowledge in the fields of general mechanical and electrical engineering including hydraulics and electronics; but only a few study aerodynamics, and some of the basic ideas of aerodynamics are essential to the development of pneumatic theory. This book should satisfy the need and is timely since pneumatic control is well into its second stage of development, which includes general applications, transmission of information, optimization and certain logical and computational functions.

The sub-title gives an admirably precise description of the contents. The chapters are headed "Theory fo Restrictions"; "Pneumatic Chambers—Static Characteristics"; "Dynamics of Pneumatic Chambers"; and

"Characteristics of Long Pneumatic Lines". Some useful data from aerodynamics is given in a thirty-page appendix. Restrictions are categorized as turbulent, laminar, and mixed flow types for the purpose of analysis and there is an interesting study of the effect of the eccentricity of the plug in an adjustable restriction. Pneumatic chambers are dealt with as flow and blank chambers in combination with restrictions and in a variety of banking arrangements. This section is rich in design data and gives several examples of design calculations. As for the chambers, the dynamic equations for long pipe-lines are derived and these equations are solved by methods analogous to those used for electrical transmission lines.

The book is written for the designer. While adequate theory is given when required, the plan is rather to state and explain formulae and give references to published work. It is a comprehensive review of knowledge in a limited field and presents this knowledge in a way which makes it readily available for design use. The bibliography has exactly a hundred references (ten to work by Zalmanzon), mostly to publications dated from 1950 and the most recent dated 1960, which is as new as can be expected since the original volume was published in 1961. Many of the references are to Russian papers and the titles of these are helpfully translated. There is no index other than a subject index of some sixty items, but this is not a serious lack since the contents are given in detail.

W. McHUTCHISON

PLANT-GALLS OF EUROPE

Bestimmungstabellen der Gallen (Zoo- und Phytocecidien) an Pflanzen Mittel- und Nordeuropas

Band 1. By Dr. phil. habil. Herbert Buhr. Pp. xvi + 761. (Jena: VEB Gustav Fischer Verlag, 1964.) 139*s.* 9*d.*

IF one were to describe this exhaustive treatise on the plant galls of Central and Northern Europe as a typical example of Teutonic thoroughness, it would be but to pay tribute to the undeniable German genius for this type of encyclopaedic production.

Bestimmungstabellen der Gallen (Zoo- und Phytocecidien) an Pflanzen Mittel- und Nordeuropas is arranged in the way which has been customary in previous systematic cecidological works, that is, strictly alphabetically by host genera; the first volume covers the genera *Abies* to *Myrrhis*. Cryptogamous hosts (algae, fungi, lichens) are also included. The information, though presented in key form, is rendered considerably more accessible and usable than is often the case with an amalgamated key-plus-description presentation by the judicious use of large and small type and other distinctive type-faces, combined with paragraphing and generous spacing. In his preface, the author expresses his appreciation of the co-operation which he experienced from his publishers and printers; and indeed author, publisher and printer alike deserve commendation for the evident care and thought that has been given to lay-out and production. The subsidiary small-type paragraphs under each gall-description include notes on distribution, bionomics, etc., together with copious references to literature.

The keys are preceded by a 64-page introductory section ("Allgemeiner Teil"), in which general aspects and problems of cecidology are discussed. Several pages are devoted to gall aetiology. "Galls arise in the course of mutual action and counteraction between the partners [host and parasite], in a constant opposition between normal and abnormal growth, as a compromise solution. All formative impulses originating from the parasites can only set in motion in the plants processes which fit into the framework of their hereditarily conferred morphogenetic capabilities." There is as yet, however, no agreement between authorities concerning the relative parts played in gall-formation by growth-promoting substances produced by the host-plant itself and by specific substances secreted by the parasite. Attention is directed to peculiar features in the biology of certain galls and their producers, such as the remarkable phenomenon of 'chlorophyll-conservation' in certain poplar-feeding *Stigmella* species (Lepidoptera) where the activity of the leaf-mining larva causes a retardation of the normal process of senescence in the surrounding tissues, which thus remain green until the larva has completed its development. In another section the author discusses the question, What is a gall? He also details methods for the study and preservation of galls and their causative organisms.

The work is illustrated (at the end of Volume 2) by twenty-five plates, containing 443 figures, many of which are taken or re-drawn from Dunzinger's illustrations to the well-known work of Ross and Hedicke (1927), or from the more delicate drawings by Han Alta illustrating Docters van Leeuwen's much more recent (1957) handbook to the plant-galls of The Netherlands.

Prof. Martin Hering, the authority on leaf-miners, in his foreword directs attention to the fact that Dr. Buhr has not only dealt with galls of animal origin, but, unlike all his predecessors in the field, has also included those caused by fungi and other plant-parasites. This gives a unique value to the work. Prof. Hering also tells us that the author has devoted special care to providing the taxonomically and nomenclaturally correct names for the organisms mentioned.

On all counts, one must agree whole-heartedly with the claim of the publishers that this remarkable single-handed production, the crown of a life-work of botanical, pharmacognostic, zoological and phytopathological study, represents a "unique scientific achievement".

H. K. AIRY SHAW

SUGAR CANE RESEARCH

Genetics and Breeding of Sugar Cane

By G. C. Stevenson. (Tropical Science Series.) Pp. xi + 284 + 51 plates. (London: Longmans, Green and Co., Ltd., 1965.) 70*s.* net.

IT is a truism that if a crop is important, the interests which it serves, whether industrial or agricultural, usually spend a great deal of money on research. Such research is often carried out in special research institutes concerned only with one crop; this is the case with sugar cane, sugar beet, rubber, tea, coconuts, etc. This is as it should be, for if a crop has to compete with other crops for funds in an experimental station, it tends to suffer from lack of men, money, and equipment.

Genetics and Breeding of Sugar Cane is the third in the *Tropical Science Series*, the volumes of which serve to present detailed information about tropical crop plants. Such information is widely scattered in a large number of publications often hard to obtain.

The book has eight chapters, dealing respectively with taxonomy; the history of sugar cane improvement; flowering and its control; breeding methods; variation and inheritance; cytology; and breeding systems. There is a final chapter on "The Philosophy of Sugar Cane Breeding" which should be compulsory reading for all students of applied genetics.

The breeding methodology of any plant is dominated by a number of special features. In sugar cane these are: the enormous number of seedlings which can be raised, making it possible to screen adequately for resistance to pathogens, and to establish rigid standards for selection; the existence of several species with wide geographical extension and consequently a wide range of adaptability; and the extensive production of unreduced gametes, making possible the important phenomenon of the addition of genomes (nobilization).

Surveying the sugar cane world from China to Peru, the author has extracted the relevant information about all these aspects from a great number of sources. He has not been content with the mere bald presentation of the material, but has made full use of his thirty years' experience of the crop to comment in detail and to criticize constructively. If there is a fault, it is that the book should have been twice as long and much more information might have been given on how a breeding programme is actually executed. The chapter entitled "Breeding Methods" gives details of techniques rather than the methodology of breeding.

The fact that research on sugar cane is mainly, though not always, paid for by industry has inevitably led to a not unreasonable demand for more or less immediate practical results. These are certainly impressive since, as the author points out, sugar yields in some countries have been more than doubled within the past twenty-five years. At the same time, one gets the impression that although much fundamental work has been carried out, it has often been a by-product of work dictated primarily by economic considerations, and organized for economic results. Thus, apart from the author's own important work, there has been little work on the production of pure lines, a procedure almost essential in a long-term breeding programme. Whether apomixis occurs seems to be still in doubt. If it occurs, even though rarely, it would be of great importance to the breeder. There does not seem to have been any organized attempt to produce haploids—also important in long-term work.

The sugar industry is wealthy and if it could be induced to carry out fundamental research on genetics and physiology without the spectre of profits looming large, the long-term benefits might be unexpectedly great.

This book is essential for all sugar cane workers, and it contains lessons for breeders of other vegetatively reproduced crops. Readers will be grateful for the literary style of the author, which is at once elegant, lucid and clear.

S. C. HARLAND

STARCH: ITS FUNDAMENTALS AND POTENTIALITIES

Starch

Chemistry and Technology. Edited by Roy L. Whistler and Eugene F. Paschall. Vol. 1: Fundamental Aspects. Pp. xviii+579. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 22 dollars.

VOLUME 1 of *Starch: Chemistry and Technology* describes the more fundamental aspects of starch chemistry and technology and sets the scene for a second volume, which will cover industrial aspects. There is much that is described here that has yet to be exploited by industry, most of all on the biological side, the fundamental advances in which have been practically without impact on industry. Two exceptions come to mind; first, the growing 'takeover' by amyloglucosidase from acid as a means of converting starch into glucose; and secondly, the selective breeding of maizes rich in high-amylose or high-amylopectin starch. The full potentialities of biology to the starch industry are very far from realization.

Of all sources of organic raw material, starch offers the major opportunity to the industrialist in search of new products. The demand for starch as the largest single item of diet ensures its continuing supply at steady prices, but the shrinking *per capita* consumption of starch in Europe and North America has meant that its output for food purposes has only been kept from serious shrinking by population increases. In the post-war era, the non-food uses of starch, such as for adhesives and sizing, have met severe competition from other natural or synthetic polymers. The starch industry in the United States has reacted by vigorously pursuing research into new products for food or non-food uses. In the main the attack has been through chemical modification of starch, for example by cross-linking or by oxidation, to impart desirable physical properties. An example of a new product is the use of amylose as an edible film for food packaging. The economics of the process, in the form of the present price of amylose, would seem to represent the only bar to what could be a very large exploitation. Glucose production by enzymatic hydrolysis has already been mentioned, and the real possibility of a cheap enzymatic method for the partial conversion of glucose into the much sweeter fructose, to produce an 'artificial' invert sugar, could well make considerable inroads into the sucrose industry, which has already felt the pinch from glucose alone as a sweetening agent. Given the investment in research, the starch industry need not fear any slackening of demand for its products. Indeed the second chapter in the book under review, by P. L. Farris, predicts "a probable doubling of the market for starch between 1960 and 1980". At the same time, R. L. Whistler, in the introductory chapter, foresees a corn (maize) crop of 300 bushels per acre, against the present United States average of 62·4 bushels. In the rest of the world the present average yield is only 30·2 bushels per acre.

The chapters that follow describe genetic control of starch development; occurrence and development of starch; minor constituents; enzymatic synthesis and hydrolysis; oligosaccharides and *cyclo*amyloses; chemical evidence for structure; crystallinity; gelatinization; organic complexes; fractionation; properties of starch fractions; paste properties; radiation; pyrolysis; non-degradative reactions; and acid and alkaline degradation. The authors, with one exception, are drawn from United States industry and research institutions and are well known and respected.

This and the second volume are intended as successors to the classic compilations by R. W. Kerr. The multiple authorship and the increase in size over Kerr only reflect the greatly increased knowledge of the subject. The coverage of the fundamental aspects is generally well balanced and much previously unreviewed material is gathered, for example on the *cyclo*amyloses (Schardinger dextrins). These particular substances represent a real challenge to industry, for they could be produced very cheaply if required on a large scale. The Schardinger dextrins form inclusion compounds, often crystalline, with every conceivable class of compound, from iodine to bromobenzene, tyrosine and helium. This property must surely find a commercial outlet.

A serious omission from the book is any description of the evidence for starch structure as obtained by the use of enzymes. It is impossible to describe the presently accepted structures of amylose and amylopectin without drawing on the evidence of enzyme action, notably of β-amylase. Indeed, present chemical methods of analysis are, by themselves, inadequate even to elucidate the structures of relatively simple oligosaccharides obtained by degradation of amylopectin, let alone the polymer itself. The Meyer, Staudinger and Haworth formulae for amylopectin have always been presented in a way that takes account of our knowledge of β-amylase action. If one took no account of enzymatic evidence, each of these formulae could be written in a wide variety of ways and many additional formulae could be written that are not excluded by chemical evidence alone. This reservation apart, the book can be recommended as indispensable to anyone with any kind of scientific interest in starch. It reviews the field essentially up to 1963. The documentation is thorough, and the abundant numerical data considerably enhance the value of the book as a work of reference. The senior editor, Prof. R. L. Whistler, has added another notable contribution to his authorship and editorship of works of reference on carbohydrates.

W. J. WHELAN

ON THE INTERPRETATION OF THE LINE SPECTRA OF QUASI-STELLAR OBJECTS

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IN the past 2 years several investigations of the spectra of the quasi-stellar objects have been made so that at present more than forty objects have been observed in sufficient detail for identifications and thus red-shift measurements to be made. In Table 1 we give a compilation of the general properties of the line spectra of these objects, while in Table 2 we give details of the specially rich spectrum of 3*C* 191. So far, attempts have been made to determine the physical conditions in the emitting gas using the line and continuous spectra of 3*C* 48 and 3*C* 273 by Greenstein and Schmidt[1] and by Shklovsky[2]. They have shown that the regions emitting the line spectra in these objects are somewhat similar to the emitting regions in planetary nebulae, that is, electron densities $N_e \approx 3 \times 10^4$–$10^7$ per cm^3, and electron temperatures $T_e \sim 10{,}000°$–$30{,}000°$ K. Now that objects with z up to about 2 have been studied, the main characteristics of the line spectrum of a 'standard' quasi-stellar object in the wave-length range $\sim$1100–7000 Å are available and are contained in Table 1. Estimates of the computed line strengths for different assumed values of N_e and T_e have recently been given by Osterbrock and Parker[3].

Because the spectra of such faint objects can only be obtained with low-dispersion nebular spectrographs, and because the features are frequently weak and diffuse, only very crude estimates of line strengths for the majority of quasi-stellar objects are as yet available.

We shall concentrate here on attempting to account for the widths of the lines and the different stages of ionization which are seen. It should be borne in mind that our standard quasi-stellar object is obviously a composite model, and only extension of the wave-length range of observation will determine to what extent the gaseous envelopes of the individual objects are very similar.

Line Widths

In previous investigations it has been assumed that the great widths of certain of the observed emission lines are due to mass motions in the envelopes, and little attention has been directed to the possibility that the widths are due to electron scattering (in the paper of Greenstein and Schmidt a comment about electron scattering is made in a footnote). However, the discovery that in the case of 3*C* 191 a narrow absorption line spectrum is present[4] shows that at least in the region giving rise to the absorption lines which have widths (corrected for red shift) of $\leqslant 3$ Å the mass motions must be small. Moreover, in this object systematic motions of the emitting region relative to the absorption line region are also shown to be less than a few hundred km/sec. The absence of absorption lines in resonance transitions in the spectra of other quasi-stellar objects can indeed be understood if mass motions are still smaller than is the case in 3*C* 191.

Our suggestion is that the large widths of the emission lines in the quasi-stellar objects arise from electron scattering, and we shall now consider the range of line widths that have to be accounted for and show that, under the physical conditions indicated by the emission lines that are present, electron scattering can provide sufficient broadening.

Because of the graininess of the observed continuum on the photographic plates, the width to be attributed to a line is to some extent a matter of subjective judgment—the estimates of Table 1 are to be interpreted in that sense. In some cases widths in angstroms are given, and in others the symbols w, m, n are used; approximately, w indicates widths of 40–100 Å; m, widths of 20–30 Å; and n, widths of 10–15 Å. From Table 1 we see that in the majority of the quasi-stellar objects the emission lines are broad with widths estimated to lie in the range 30–50 Å. Some of the widest lines are Lyman-α (Ly-α), C IV λ1549, and Mg II λ2798. In some cases the forbidden lines are narrow ($\leqslant$ 10 Å).

In order to decide what width a line should have it is necessary to take account of the nature of the line—whether it is permitted or forbidden—and also to give estimates of the optical depth τ_e against electron scattering and the electron temperature T_e. In the case of permitted resonance lines such as Mg II λ2798 it is not necessary for the optical depth τ_e to be comparable with unity in order that the line be so broadened. For such lines quanta are emitted and reabsorbed in sequence until electron scattering out of the line centre occurs. The opacity to perhaps ± 3 Å from the line centre is large, and not until electron scattering takes the quanta sufficiently off resonance can they escape from the system.

In order to consider the magnitude of the width due to electron scattering we have adopted the following procedure. We take a line to have disappeared into the continuum when its intensity has declined to a third of what it is, not at the centre, but at 3 Å from the centre. We then use the computations of the electron scattering line profiles by Münch[5] and find that a width $\simeq$20 Å is obtained for an electron temperature of 10,000° K, $\sim$30 Å for $T_e \approx$ 30,000° K, and $\sim$50 Å for $T_e \approx$ 100,000° K. The width is not exactly proportional to $T_e^{\frac{1}{2}}$ because of our procedure of comparison with points 3 Å from the line centre; 3 Å is a different fraction of the width in the different cases. For a really strong line this procedure underestimates the width—an intensity decline to about a sixth would then be more appropriate, nearly doubling the width. For the long path lengths which quanta in strong permitted lines such as Mg II λ2798 and Ly-α will traverse before finally escaping, it is thus clear that great widths can be produced by electron scattering at electron temperatures of 10,000°–30,000° K.

In the case of forbidden lines such as [O III] λ5007 the argument is different because an ion which gives rise to a forbidden line does not supply a high opacity near its resonant frequency. To broaden a forbidden line τ_e must

be ~ 1. Again using the results of Münch, we estimate for $\tau_e = 0{\cdot}8$ a width ~ 40 Å at $T_e \approx 30{,}000°$ K, and a width ~60 Å at $T_e = 100{,}000°$ K. In the cases in which narrow forbidden lines are seen, we conclude that τ_e must be small. The general tendency appearing in Table 1 for the lines from permitted ground-level transitions to be wider than the forbidden lines is an argument in favour of the electron scattering hypothesis. We also call attention to 1217 + 02, in which [O III] λλ 4959 and 5007 are much narrower than [O III] λ4363; the first two lines arise from a level of 2·50 eV excitation, and the latter arises from a level of 5·33 eV excitation. This strongly suggests a broadening agent which is a function of depth in the object, the λ4363 line being produced deeper than the λλ 4959, 5007 lines. Electron scattering provides a natural explanation, while mass motions do not.

Finally, we note that in a model for 3*C* 273 based on measurement of the continuum flux, Oke[6] has proposed a very high value of the electron temperature, up to ~160,000° K, in order to obtain the small Balmer discontinuity which is seen. But at such a high temperature, broadening by electron scattering would always be large, particularly for the resonance line Mg II λ2798.

A Model to explain the Different Ionization Stages observed

We propose a three-zone model with the following properties:

(I) Optically thick to quanta with $h\nu > 13.595$ eV.

(II) Optically thick to quanta with $h\nu > 54.403$ eV.

(III) Optically thick to quanta with $h\nu > 24.581$ eV.

It is implied that in zone I the gas is thin to quanta of energies less than the Lyman limit, while in zones II and III it is thin to quanta below the ionization potentials of He^+ and He respectively. (The reason why we have apparently inverted the order of the helium ions will appear at a later stage.) We regard these conditions as being imposed by the hydrogen and helium. Zone I is an H I region. Zone II is an H II, He II region, while zone III is an H II, He I region. In all zones, lines other than those due to hydrogen and helium are produced by excitation of their upper levels by electron collisions. The hydrogen and helium lines are produced by recombination.

Zone I is necessary to explain the presence of the Mg II emission. The ionization potential of Mg^+ is only 15·031 eV and this ion can exist in appreciable abundance only in an H I region. It is an attractive possibility that Mg II λ2798 might be produced by recombination of Mg^{++} in an H II region. The big gap between the ionization potentials of Mg^+ and Mg^{++} (the latter has an ionization potential of 80·12 eV) would lead to a big build-up of Mg^{++}. However, the recombination coefficient of Mg^{++}, which is not known, would need to be greater by a large factor than that of H^+ to overcome the abundance difference and give comparable Ly-α and Mg II λ2798. In zone I the most abundant ionization stages of the common elements are: H, H^+, C^+, N, O, Ne, Mg^+, Si^+, S and Ar. The important lines to be attributed to these ions[7] are H I (Ly-α, Hα, - - -), C II (λλ 1335, 2325), N I (none), O I (none), Ne I (none), Mg II (λλ 2796, 2803), Si II (λλ1190, 1193, 1194, 1197, 1260, 1265, 1304, 1309, 1527, 1534, 1808, 1817, 1818, 2329, 2335, 2345, 2351), S II (λλ 1227, 1233, 1234, 1250, 1254, 1259), Ar I (none). (The notation (none) simply means that in Osterbrock's list[7] of the lines which are expected there is none from the ions in the spectrum range covered.)

The C II and S II lines are seen in absorption in 3*C* 191, as also are the Si II lines shortward of 1500 Å. The lines of C II, S II, Si II are not seen in emission, however, with the exception of C II λ1335 in 3*C* 280·1 and C II λ2325 which is observed weakly in 3*C* 208; Si II λ1817

Table 1*d* Lines seen in Quasi-stellar Objects with estimated Strengths and Widths

Object	m_v	z	C III] 1909	Mg II 2798	[Ar IV] 2854 2869	[Mg V] 2931	[Ne V] 2974	O III 3133	He II 3204	[Ne V] 3426	[O II] 3727	[Ne III] 3869	Hγ 4340	[O III] 4363	Hβ 4861	[O III] 5007
3*C* 273 (*a*)	12·8	0·158		*							ρ		*M*, *w*		*vS*, *w*	*M*, *m*
1217+02	16·5	0·240		*W*, ~20 Å			*W*, 30 Å	*	*	*W*, 20 Å	*W*, 20 Å	*M*, 20 Å	*M*, 30 Å	*M*, 30 Å	*S*, 30 Å	*S*, 10 Å
3*C* 249·1	15·7	0·311		*W*, 40 Å						*vW*, *n*	*vW*, *w*	*W*, 20 Å	*W*, 40 Å		*W*, 40 Å	*M*, < 10 Å
3*C* 277·1	17·9	0·321		*M*, < 10 Å						*W*, < 10 Å	*M*, < 10 Å					
3*C* 48	16·2	0·367		*S*, *w*			*W*, *w*			*M*, 30 Å	> *M*, *n*	*M*, *n*	*S*, *w*		*S*, *vw*	> Hβ
3*C* 351	15·3	0·371								*W*	*W*, *n*	*			> *W*, 25 Å	*M*, *vn*
3*C* 215 (*b*)	18·3	0·411		*S*, 20 Å			*W*, > *m*			*W*, > *m*	*W*, ?*n*	*W*	*	*	*M*, 25 Å	> *M*, *n*
3*C* 47 (*c*)	18·1	0·425		*, 20 Å						*, 20 Å	*W*	*, 20 Å	*	*	*	*, 20 Å
3*C* 270	17·8	0·540		*S*, *w*	*M*, *w*	*W*				*M*, 20 Å	*W*, *w*	*M*, *w*	*			
0*C* 147 (*d*)	19·0	0·615						*		*	*, 100 Å	*				
3*C* 334 (*e*)	16·4	0·555		*S*, 30 Å		*W*	*W*	*W*	*W*	*W*			*W*			
3*C* 275·1	19·0	0·557		*S*, 20 Å				*	*		*W*, *n*			*W*		
3*C* 345 (*f*)	16·5	0·595		*S*, *w*	*W*, *w*	*	*W*		*	*W*		*W*	*			
MSH 03–19	16·2	0·614		*M*, 80 Å						*W*, 80 Å						
3*C* 261	18·2	0·615		*S*, 100 Å							*M*, *n*	*W*, *n*				
3*C* 380 (*g*)	16·8	0·693		*vS*, 30 Å	*M*, 30 Å					*W*, 20 Å	*W*, 20 Å	*W*, 20 Å	*			
4*C* 39·25	(17·3)	0·698	*M*	*M*, 30 Å		*				*W*						
3*C* 254	18·0	0·734	*	*						*	*	*				
3*C* 138 (*h*)	17·9	0·754	*S*, 25 Å	*S*, 40 Å							*M*, 20 Å					
3*C* 286	17·3	0·849	*	*W*, 80 Å						*W*						
1252+11	16·6	0·870	*W*	*S*	*W*, *n*		*									
4*C* 20·33	(17·1)	0·871	*M*, 40 Å	*M*, 40 Å				*								
3*C* 196	17·6	0·872	*W*, 40 Å	*			*			*W*	*M*, 25 Å					
0922+14 (*i*)	18·0	0·898	*vW*, *w*	*M*, 60 Å												
0957+00 (*j*)	17·6	0·905	*S*, 40 Å	*M*, 60 Å						*						
3*C* 336	17·5	0·927	*W*, 60 Å	*W*, 40 Å							*vW*, ?*n*					
MSH 14–121	17·4	0·938	*M*, 60 Å	*M*, 60 Å					W	*M*, *n*						

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Table 1*B*. LINES SEEN IN QUASI-STELLAR OBJECTS WITH ESTIMATED STRENGTHS AND WIDTHS

Object	m_v	z	Ly-α 1216	Si IV; [O IV] 1393, 1402; 1406	C IV 1549	He II 1640	O III] 1663	C III] 1909	Mg II 2798
CTD 141	(17)	1·015						*M*, *m*	*W*, *w*
3*C* 245	17·2	1·029						*	*
CTA 102	17·3	1·037						*	*
3*C* 287	17·7	1·055			*			*	*
3*C* 186 (*k*)	17·6	1·063						*M*, 25 Å	*W*, 40 Å
3*C* 208 (*l*)	17·4	1·110			*M*			*M*	*M*
3*C* 204	18·2	1·112			*W*			*M*	
BSO 1 (*m*)	17·0	1·241			*			*W*	
3*C* 181	18·9	1·382			*M*			*M*	
3*C* 446	18·4	1·402			*S*, *w*			*M*, *m*	
3*C* 298 (*n*)	16·8	1·436			*W*	*W*, *w*		*M*	*M*
0952+18	(17·7)	1·471			*S*, *w*	*W*, *w*	*W*, *w*	*W*, *w*	
3*C* 270·1 (*o*)	18·6	1·519		*W*	*S*, *w*			*M*	
3*C* 280·1 (*p*)	19·4	1·659	*S*, 80 Å	*M*, 40 Å	*vS*, ?	*M*, 20 Å		*W*, 20 Å	
3*C* 454	18·4	1·757	*S*		*S*	*W*	?	*W*	
3*C* 432	18·0	1·805	*S*		*S*			?*vW*	
3*C* 191 (*q*)	18·4	1·946	*S*, *w*		*S*, *w*	*W*		*W*	
PHL 938 (*r*)	17·2	1·93	*S*, *w*	*W*, *w*	*S*, *w*				
3*C* 9	18·2	2 012	*S*, *w*		*S*, *w*				
0106+01	18·4	2·107	*S*, 70 Å	*W*, *w*	*M*, *w*			*W*, *m*	
1116+12 (*s*)	19·2	2·118	*S*, 30 Å	?	*S*, 30 Å	*W*		*vW*	

The spectroscopic material and the red shifts z have been assembled from results given in refs. 1, 4, 8, 9, 11–20, and from unpublished observations of two of us (C. R. L. and M. B.). The apparent visual magnitudes in the second column are mainly from the work of A. R. Sandage *et al.* (and are in general variable).

The nomenclature is as follows: line strengths are given first for each entry, with *W*, weak, *M*, medium, *S*, strong, *v*, very; widths are given second after a comma, with *w*, wide (40–100 Å), *m*, medium (20–30 Å), *n*, narrow (10–15 Å); in many cases actual widths have been estimated, usually from Lynds's spectra. When a line has been measured but no information on its width or strength is available, an asterisk is given.

The table has been divided into two parts to save space, objects with $z<1$ being in Table 1*A* and those with $z>1$ in Table 1*B*. For $z<1$, lines with rest wave-lengths shorter than O III] 1663 are not shifted into the observable range. Similarly, for $z>1$, lines of long wave-lengths are red-shifted beyond the region normally studied. Since the lines [Ne V] λ3346, [Ne III] λ3968, [O III] λ4959 are weaker members of doublets the stronger lines of which are not included in the table, they add no new information and therefore are not included.

FOOTNOTES TO TABLES 1*A* AND 1*B*

(*a*) Balmer lines Hα (*vS*, *w*), Hδ, He also seen.
(*b*) Hγ, [O III] λ4363 blended. Additional lines seen at 2830 Å (He I ?).
(*c*) Hγ, [O III] λ4363 blended.
(*d*) Mg II λ2798 not seen. Magnitude corrected for interstellar absorption.
(*e*) Weak Hδ also seen. Hγ, [O III] λ4363 blended.
(*f*) Mg II λ2798 complex and variable. Affected by He I λ2829 ?
(*g*) Hydrogen lines seen; weak.
(*h*) Magnitude corrected for interstellar absorption.
(*i*) C II] λ2327 possibly present.
(*j*) Mg II λ2798 complex.
(*k*) Very weak [Ne V] λ3426 also seen.
(*l*) C II] λ2327 also measured; weak.
(*m*) Self-absorption in C IV λ1549.
(*n*) Absorption dominates in C IV λ1549.
(*o*) Deep absorption in shortward wing of C IV λ1549.
(*p*) Both SiIV λ1394 and [OIV] λ1407 emission lines seen; emission lines of C II λ1335 and N IV] λ1438 definitely present; Si II emission lines possibly present.
(*q*) Numerous narrow absorption lines seen, C IV 1549 being the strongest. Only emission lines are listed here.
(*r*) Self-absorption in shortward wing of Ly-α and C IV 1549.
(*s*) Absorption in shortward wing of Ly-α. Absorption at 1150 Å, none at 1460 Å. Weak emission from N II λ1085.

Table 2. LINES IDENTIFIED IN THE SPECTRUM OF 3*C* 191

Identification and wave-length (Å)	Absorption or emission
Si II 1190, 1193, 1194, 1197	Abs
Si III 1206	Abs
Ly-α 1216	Abs
Ly-α 1216	Em
S II 1227, 1233, 1234	Abs
N V 1239, 1243	Abs
S II 1250, 1254, 1259	Abs
Si II 1260, 1265	Abs
C II 1335	Abs
Si IV 1394	Abs
Si IV 1403	Abs
C IV 1548, 1551	Abs
C IV 1548, 1551	Em
He II 1640	Em
C III] 1909	Em

may be present weakly in 3*C* 280·1. In some cases the absence of emission lines can be understood in terms of the abundances of the ions and of the electron excitation cross-sections. For example, the probability of exciting the Si II multiplet at λ2329 Å by electron impact is less than that of exciting the Mg II doublet by a factor of more than 50. The critical case is C II λ1335. This line has a large excitation cross-section. Moreover, carbon is expected to be appreciably more abundant than magnesium. How, then, do we explain the fact that C II λ1335 is observed in emission in only one of our list of quasi-stellar objects, whereas the Mg II doublet is often strong? Only, it seems, by taking the electron temperature to be low. The energy difference between 1335 Å and 2798 Å is ~ 4·8 eV, so that the number of electrons capable of exciting Mg II λ2798 exceeds the number capable of exciting C II λ1335 by exp $(4{\cdot}8/kT_e)$ where kT_e is measured in eV. For $kT_e \approx 1$ this is a factor exceeding 100, which is sufficient to exclude C II λ1335. With $T_e \approx 10{,}000°$ K the width of the Mg II doublet should be ~ 20 Å in average cases, and extending upward of 30 Å for strong Mg II emission. How does this compare with the observed widths? From Table 1 it is seen that a width of 20–30 Å for the Mg II doublet agrees with observation for the following cases: 1217 + 02, 3*C* 277·1, 3*C* 215, 3*C* 47, 3*C* 334, 3*C* 275·1, 3*C* 380 and 4*C* 39·25. The seriously discrepant cases are: 3*C* 279, *MSH* 03–19, 3*C* 261, 3*C* 286, 0922 + 14, 0957 + 00, and *MSH* 14–121. These discrepant cases would seem to require the light from the Mg II doublet to pass through a region of higher temperature with $\tau_e \simeq 1$. This would have the effect of broadening the Mg II emission by a further 30 Å or more.

Before leaving zone I we note that the expected ratio of the emission strength of Hβ to Mg II λ2798 for such a zone is about 1 : 5 (ref. 3). It should be noted that in the calculation by Osterbrock and Parker, Mg^+/total Mg is assumed to be 1/5, and a ratio of line strengths Mg II λ2798/Hβ ≈ 1 is obtained. In the present case effectively the whole of the Mg exists as Mg^+ in zone I. Since the strength of Hβ is about twice Mg II λ2798 in both 3*C* 273 and 3*C* 48, it is clear that in at least some quasi-stellar objects most of the emission in the Balmer lines does not come from zone I.

Passing to zone II, we note that the most abundant stages of ionization of the common elements are: H^+ He^+, He^{++}, C^{+3}, N^{+3}, $O^{++} \rightarrow O^{+3}$, Ne^{++}, Mg^{++}, Si^{+4}, S^{+4} Ar^{+3}. If we restrict ourselves to ground-state transitions and to the excitation of levels requiring only moderate energies, the possible emission lines are: hydrogen recombination (Ly-α, Hα and the Balmer series), helium recombination (λλ 1640, 3204, - - -), C IV (λλ 1548, 1551), N IV (none), O III (λλ 4363, 4959, 5007), O IV (λλ 1402, 1405, 1406, 1410, 1413), Ne III (λλ 1794, 1815, 3344, 3869, 3968), Mg III (none), Si V (none), S V (none), Ar IV (λλ 2854, 2869).

All these lines are seen in quasi-stellar objects, except for the weak lines of Ne III at λλ 1794, 1815, 3344. These

are weak because their electronic excitation probabilities are very small[7]. Plainly the gaseous material of quasi-stellar objects is very largely in zone II. This is the region that contributes mainly to the emission lines. Actually the He II λλ 1640, 3204 lines are weak, a fact that caused Osterbrock and Parker[3] some surprise, so that they wondered whether helium might be low in abundance. But they arbitrarily assumed a degree of ionization such that half the helium was He^{++}; in our model there is not much He^{++} because zone II is optically thick for $h\nu > 54{\cdot}403$ eV.

We have given the ionization state of oxygen as $O^{++} \rightarrow O^{+3}$. If all the O^{++} were in the ground state, most of the oxygen would be in this particular ionization stage, since the ionization potential of O^{++} is 54·886 eV, slightly above the ionization potential of He^{+}. However, O^{++} has metastable states several volts above the ground state, so that O^{++} can be excited in two stages, first to a metastable state, then to O^{+3} through the absorption of a quantum with $h\nu$ less than the ionization potential of He^{+}. Provided the metastable states of O^{++} become sufficiently populated most of the oxygen will be converted to O^{+3}. This we believe must be the situation in quasi-stellar objects, otherwise the lines of O^{++} would be somewhat stronger than C IV λλ 1548, 1551 (ref. 3).

The most abundant ionization stages in zone III are: H^{+}, He, He^{+}, $C^{+} \rightarrow C^{++}$, O^{+}, Ne^{+}, Mg^{++}, Si^{++}, S^{++}, Ar^{+}. The corresponding expected emission lines are: H I (Ly-α, Hα - - -), He I (λλ 2830, 2946, 3189, 3447, - - -), C II (λλ 1335, 2325), C III (λ1909), O II (λ3727), Ne II (none), Mg III (none), Si III (λ1206), S III (λλ 1190, 1194, 1201, 1202), Ar II (none). Accepting that the lines of Si III, S III may be blended with the wide Ly-α, the only lines which would be expected to be strong, but which are in fact found only weakly or not at all, are C II (λλ 1335, 2325). These omissions are probably to be explained by the proximity of the ionization potential of C^{+}, 24·376 eV, to that of He, 24·581 eV, and to the fact that C^{+} has a metastable state nearly 5 V above ground-level. With the latter sufficiently populated, C^{+} will be scoured out to C^{++}. The situation here for $C^{+} \rightarrow C^{++}$ is the same as it was for $O^{++} \rightarrow O^{+++}$ in zone II.

The line He I λ2830 may be present in some quasi-stellar objects. An evanescent line at this wave-length has been observed in 3*C* 345 by Burbidge and Burbidge[8]. This line may also be present in 3*C* 215 (ref. 9). The other members of the same series, λλ 2946, 3189, 3447, - - - have not been observed, however.

The zones I, II and III explain the emission lines of quasi-stellar objects quite satisfactorily. A few cases, probably peculiarities, requiring either exceptionally high electron temperatures or an optically thin situation beyond $h\nu = 54{\cdot}403$ eV, remain to be explained, particularly Ne V (λλ 2974, 3346, 3426) in emission, N V λ1240 in absorption in 3*C* 191, and a suspected emission line [Mg V] λ2931 in four objects. Leaving these cases aside, the next step is to decide on the spatial ordering of zones I, II, III.

We suggest the ordering I, II, III with increasing distance from a central object. At first sight we might expect zone I to lie on the outside. But this zone cannot cover zones II and III, otherwise Ly-α could never appear free of strong self-absorption. If zone I lies on the outside its structure must be broken into discrete blobs. The disadvantage of placing I on the outside is that, if Mg II λ2798 arises in it, the great widths shown by that line in some cases would then be hard to explain. With zone I on the inside, light from it must pass through zones II and III. If either zone II or zone III, preferably zone II, has $\tau_e \approx 1$ and $T_e \geqslant 30{,}000°$ K, the Mg II doublet will be substantially widened, to the widths observed in 3*C* 279, *MSH* 03–19, 3*C* 261, 3*C* 286 and *MSH* 14–121. The strong ultra-violet radiation field necessary to produce zone II must then arise outside zone I, by processes discussed elsewhere[10]. If Mg II λ2798 is produced by recombination, zone I is not necessary, and the line must undergo the same electron scattering as Ly-α and C IV λ1549.

We place zone III on the outside because the line [O II] 3727 is emitted most effectively at low densities. Our expectation is that the lowest electron densities are to be found on the outside of the object. Moreover, zone III must contain concentrations of C^{+}, Si^{+}, S^{+} which, while not sufficient to produce an emission line, may be sufficient in some cases to produce absorption, but all the Mg must here be in the form of Mg^{++} or an absorption component in Mg II λ2798 would be produced by Mg^{+}. In the foregoing discussion we have been concerned with the most abundant ionization stage. A not negligible number of ions that differ one stage, up or down, from the most abundant ion are always to be expected. In zone III we expect moderate concentrations of Si^{+} and Si^{+3}. Both these ions are responsible for absorption lines in 3*C* 191. To give a deep absorption it is therefore important that zone III should cover the whole source of the continuum, and this it will do best if it lies outermost from the source of the continuum.

Our picture is shown schematically in Fig. 1. The structure of the system has some similarity to a planetary nebula. The central hole is to be considered as maintained by radiation pressure from a central highly condensed object. The origin of the continuum flux arising in this central region has been discussed elsewhere[10]. Zone II is the main region of emission of lines. In some cases the optical depth τ_e is of order unity in this zone. It is of some interest to calculate the total flux which can be emitted from this region.

Estimates of Flux emitted from a 'Standard' Quasi-stellar Object

The condition that the optical depth for electron scattering $\tau_e \leqslant 1$, which will in general explain the widths of the lines in this model, enables us to compute the total optical emission from a 'standard' quasi-stellar object. Taking $T_e = 30{,}000°$ K in zone II, the number of electron-proton recombinations is $\sim 2 \times 10^{-13} N_e^2$, and the emissivity in Ly-α, Hα, etc., and in the Balmer continuum is $\sim 4 \times 10^{-24} N_e^2$ erg/cm³/sec. Thus the total emission from zone II in Ly-α, Hα, etc., is $\sim 10^{-23} N_e^2 R^3$ erg/sec.

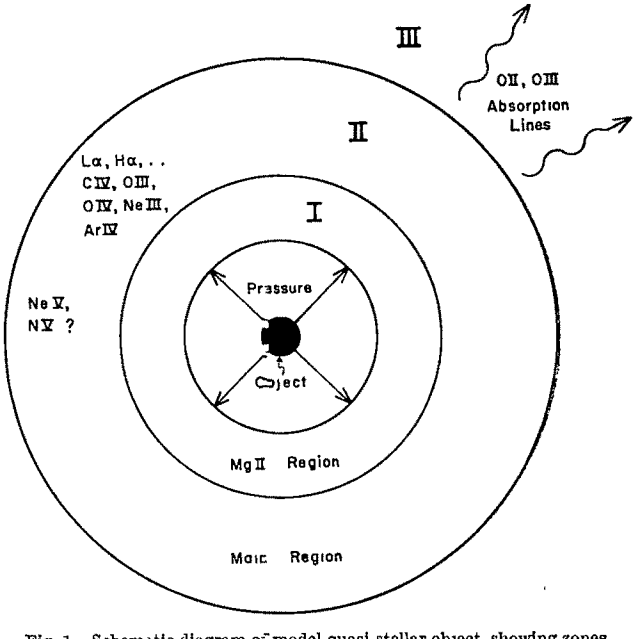


Fig. 1. Schematic diagram of model quasi-stellar object, showing zones I, II, and III

Since the total optical output is about ten times greater than the total hydrogen emission we obtain $\approx 10^{26} R$ ergs/sec for the total output when we set $N_e R \approx 10^{24}/\text{cm}^2$. The size R can be set by the optical and radio fluctuation data which give values of $R < 10^{17}$–10^{18} cm for objects such as 3C 345 and 3C 273. Thus the total energy output can only be of order 10^{43}–10^{44} ergs/sec, and the lower value is close to the luminosities of the Seyfert nuclei.

This result applies to the "standard" model. However, for the case of 3C 273 where there is a measured flux for Hβ it is interesting to derive similar quantities using Hβ alone. For $T_e = 30{,}000°$ K the volume emissivity:

$$E(H\beta) = 10^{-26} N_e^2 \text{ ergs/cm}^3 \quad (1)$$

Equating this to the measured flux, we find that if R is the dimension of the emitting region, $\Delta R = xR$ is the thickness of the shell, and r is the distance of the object:

$$\frac{N_e^2 x R^3}{r^2} = 3{\cdot}4 \times 10^{14} \quad (2)$$

If $N_e x R$ were appreciably greater than $\sim 10^{24}$, the resonance lines would become too broad. Thus we take:

$$N_e x R = 10^{24} \quad (3)$$

Substituting (3) in (2) we find that:

$$\frac{x r^2}{R} \approx 3 \times 10^{33}$$

For the object to be at the distance determined by its red shift, $r \approx 1{\cdot}5 \times 10^{27}$ cm, in which case $R = 7 \times 10^{20} x$. Taking three different values for R, we find:

(*a*) for $R = 10^{17}$ cm, $x = 1/7000$, and $N_e = 7 \times 10^{10}$;

(*b*) for $R = 10^{18}$ cm, $x = 1/700$, and $N_e = 7 \times 10^{8}$;

(*c*) for $R = 10^{19}$ cm, $x = 1/70$, and $N_e = 7 \times 10^{6}$.

These values of N_e are lower limits, since equation (3) is a limit for $\tau_e \leqslant 1$. A value for R as large as 10^{19} cm is probably excluded by the fluctuation data, and the electron densities for (*a*) and (*b*) are orders of magnitude greater than Greenstein and Schmidt's estimates and are incompatible with the observations. Only by making r smaller, apparently, and assuming the object is 'local' can this dilemma be avoided. For example, if we suppose that zone II can be approximated to by a sphere of radius R, and put $N_e R \approx 10^{24}$, then if $r = 5$ Mpc $\approx 1{\cdot}5 \times 10^{25}$ cm, we find: $R = 10^{33} r^2 = 2 \times 10^{17}$ cm, and $N_e = 5 \times 10^6$ cm^{-3}.

The scheme described here is a general one, and clearly the range of widths of emission lines seen requires a range in N_e, T_e and τ_e in the objects. In quasi-stellar objects where [O II] λ3727 is quite strong and wide, N_e may be lower and the line may be produced deeper in the object. Further, expansion motions may occur in some of the objects. We have sought here to reconcile the line fluxes and widths, and the electron densities, and to take account of electron scattering which has hitherto been set aside as unimportant. We have sought also to account for the appearance in some objects of absorption components in resonance lines, and in others not, and to explain why strong absorption in Ly-α and C IV λ1549 is not visible in all quasi-stellar objects and why absorption in the Mg II doublet has not been found in any such objects. In 3C 191, where absorption lines appear, the comparative weakness of absorption in Ly-α relative to absorption in C IV λ1549 can also be accounted for by the fact that H is largely ionized in zone III.

Clearly a range in the physical parameters of the emitting gas must be present in the quasi-stellar objects. One advantage of this model is that the arrangement and maintenance of the zones could be quite sensitive to the size and continuum flux of the central source of energy, about which we have not attempted to say anything here.

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A CONSISTENT NOMENCLATURE FOR THE NUCLEAR STATUS OF FUNGAL CELLS

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WITH the rapid increase in cytological and genetical investigations of fungi, many terms that were originally introduced to describe morphological and physiological aspects of the life cycle have acquired new and often imprecise cytogenetical meanings. Other terms that were originally introduced to describe the cytogenetics of higher organisms have been applied to fungi without sufficient regard to the differences in level of organization[1,2]. The result is unnecessary ambiguity and loss of information. In this article we will examine the situation as it relates to the present-day nomenclature for the nuclear status of fungal cells in an attempt to arrive at a consistent terminology. We will begin with our proposals for a terminology which is essentially an extension of that used by Catcheside[3] and Olive[4].

Categories of Nuclear Status

We can recognize two major categories corresponding with two levels of organizations, namely (i) nuclei within cells and (ii) chromosomes within nuclei, both of which provide a basis of a nomenclature.

Within each of these categories we can recognize differences which are basically numerical (*a*), and differences which are primarily related to gene content (*b*).

(i) *a*. Any cell, spore or mycelium can be classified on the basis of the number of nuclei it contains, for example, uninucleate, binucleate or multinucleate. Equally, a uninucleate cell or spore or a mycelium made up of predominantly uninucleate cells can be referred to as a monokaryon. Similarly, a binucleate cell or spore or a mycelium made up of predominantly binucleate cells is a dikaryon. This

nomenclature can be extended to any number of nuclei per cell, so that for three nuclei, for example, the appropriate terms would be trinucleate and trikaryon. However, with more than two nuclei to a cell the number is usually indeterminate, since it varies from cell to cell in the same mycelium. Above two, therefore, the terms multinucleate, multikaryon (or coenocyte) are as precise as the situation justifies. These terms, of course, imply nothing about the gene content or ploidy of the nuclei themselves.

(i) *b*. In a comparable way, any cell, spore or mycelium can be classified on the gene content of the nuclei it contains. Irrespective of the number of nuclei involved, if they are all genetically identical, the cell, spore or mycelium is a homokaryon. On the other hand, if two or more genetically different types of nuclei are present, the cell, spore or mycelium is a heterokaryon.

(ii) *a*. The numerical status of the chromosomes within a single nucleus provides a basis for classification that is common to all organisms and presents no special difficulties when applied to fungi. Thus haploid, diploid, triploid and polyploid chromosome complements can be recognized as can aneuploid, disomic, trisomic and polysomic complements.

(ii) *b*. At the level of gene content the situation can be simply described in terms of the familiar homozygote and heterozygote.

Essentially, therefore, our terminology distinguishes between the properties of chromosome complements which are associated in the same nucleus and those which are associated in different nuclei within a common cytoplasm. The importance of this distinction is underlined by the results of investigations of the action and interaction of the same genes when associated in these different ways[5,6].

Combination of Categories: Redundancies and Misuses

The four subdivisions which we have recognized as a basis of classification are largely independent since they are describing events at different levels. They can, therefore, be combined in all possible combinations to provide a comprehensive description of any situation that may arise. There are, however, some combinations in which one term is redundant. An example of this is the term homozygous when used in conjunction with haploid (since a haploid nucleus cannot be heterozygous), but this is the only case in which this is invariably so.

A combination that appears at first sight to have redundancies is haploid homokaryotic monokaryon. If this refers to a single cell or a spore, then haploid monokaryon is a sufficient description since all monokaryotic single cells or spores are homokaryotic. On the other hand, not all monokaryons are homokaryotic, and not all homokaryons are monokaryotic. For example, if we are referring to a mycelium, no term in the description haploid homokaryotic monokaryon is necessarily redundant since a haploid monokaryotic mycelium can be heterokaryotic[7] and a haploid homokaryon can be a multikaryon (as, for example, in *Aspergillus* and *Neurospora*).

A common assumption that is implicit in present-day usage is that monokaryon can mean either one nucleus per cell, or one kind of nucleus per cell (homokaryon), or both simultaneously. Dikaryon in the same way can mean either two nuclei per cell, or two kinds of nuclei per cell (heterokaryon), or both simultaneously. A further misuse is to equate dikaryon and heterokaryotic dikaryon[8]. As a consequence, dikaryon cannot be used to describe the binucleate stage which precedes nuclear fusion in the sexual stage of homothallic Ascomycetes and Basidiomycetes even though this stage is clearly evolutionarily equivalent to the morphologically similar stage in heterothallic species[9,10]. Equally confusing is the use of dikaryon and trikaryon for the heterokaryotic association of two and three distinct types of nuclei in the multinucleate, vegetative hyphae of heterothallic Ascomycetes such as *Neurospora crassa* where such associations are confined to strains of the same mating type[11]. These misuses of the term dikaryon are analogous to confining the use of the term diploid to heterozygotes only, but extending its use to any degree of ploidy.

In an attempt to avoid ambiguity some have referred to dikaryons as either homozygous or heterozygous[12,13]. This, however, is also wrong, since it is applying terms to the wrong level of organization. The correct terms are homokaryotic dikaryon and heterokaryotic dikaryon which free the terms homozygous and heterozygous to describe the constitution of the individual nuclei. Even more confusing is the use of haploid and diploid to describe monokaryotic and dikaryotic cells respectively[1]. Now that homozygous and heterozygous diploid nuclei can be produced in fungi by a variety of procedures, strains which are heterozygous diploid homokaryotic dikaryons or homozygous diploid heterokaryotic dikaryons are a possibility. Indeed, in *Coprinus*, haploid and heterozygous diploid nuclei have been associated in a dikaryon to produce a haploid/heterozygous diploid heterokaryotic dikaryon[14]. Only by strict adherence to the terminology proposed here, in which each term is used to specify the correct level of organization, can we hope to describe unambiguously all the situations that have already arisen or that are likely to arise in the future.

Justification and Uses

The proposed terminology clearly satisfies the requirement that it should provide a classification of the nuclear status of fungal spores and cells that brings out the more readily observable similarities and differences. But are these similarities and differences biologically meaningful?

One of the most important proposals in this terminology is that homokaryon and heterokaryon should be used where appropriate in conjunction with monokaryon, dikaryon or multikaryon to describe the vegetative and reproductive cells and the spores of fungi. Heterokaryosis of the type with which we are familiar in imperfect fungi, in homothallic Ascomycetes and within a mating type of heterothallic Ascomycetes is essentially a system of vegetative plasticity[15]. In these fungi, a heterokaryotic association arises solely because of its immediate advantage in vegetative growth over its homokaryotic components and it persists only so long as this advantage is maintained. The longer-term advantages of heterokaryosis, for example, the possibility of outcrossing in homothallic fungi and of parasexual recombination in imperfect and sexual fungi, are only secondary consequences of heterokaryon formation[16]. The success or failure of the new genotypes that may arise in these ways plays no direct part in the success or failure of heterokaryon formation when two homokaryotic strains meet.

Dikaryon formation of the type that is known in heterothallic Ascomycetes and Basidiomycetes is an essential first step in sexual reproduction. And even where, as in many Basidiomycetes, the dikaryotic stage is capable of prolonged and independent vegetative growth before the production of sexual spores, the formation of the heterokaryotic dikaryon does not depend on any immediate advantage in vegetative vigour. On the contrary, stable dikaryons are formed between monokaryons providing that they are sexually compatible even when the heterokaryotic dikaryon has a rate of growth that is inferior to one or both of its component monokaryons[13,17]. Indeed, in some instances, most of the dikaryons produced by random pairing of compatible monokaryons obtained from fruiting bodies growing in the wild may be inferior in rate of growth to one or both component monokaryons. Further investigations of the genetical determination of the vegetative characteristics of dikaryons obtained from the wild suggest that these characters are subjected to

stabilizing selection which favours intermediate performance[17]. Clearly, the formation and persistence of a heterokaryotic dikaryon do not depend on an increase in vigour arising from the heterokaryotic association but rather on the advantages that result from sexual outcrossing of which this association is the first and most important step. Heterokaryotic associations thus appear to be of two markedly different kinds that have evolved to fulfil different and independent roles and as such are subject to different selective forces[18]. We can justify the use of heterokaryon for both only if we are using the term descriptively, implying no evolutionary or functional relationship. Even so, this is preferable to the alternatives at present in use, such as heterozygous dikaryon and the restriction of the term dikaryon to heterothallic species, since these not only imply incorrect relationships and differences but are also incorrect descriptions of the nuclear status.

To give a complete description of the nuclear status of every kind of cell, spore or mycelium using the terminology we have proposed would be tedious and is frequently unnecessary. On the other hand, even such a complete description is considerably briefer, less circuitous and more informative than many of the descriptions that are to be found in the literature. However, we suggest that, in general, our terminology should act merely as a guide to the correct description, and should be fully applied only to an unusual situation or to a formerly unknown nuclear condition (for the particular fungus concerned). Our system will tolerate the use of any term which is unambiguous for the group of organisms to which it applies, and which does not break the rules of our terminology, for example, by applying the term to an incorrect level of organization. Thus, for the filamentous Ascomycetes, the homokaryon–heterokaryotic notation for mycelia will usually be adequate since it is known that the growing points of the mycelia are usually multikaryotic and all the nuclei normally haploid. Similarly, for the heterothallic Basidiomycetes, the monokaryon–dikaryon notation will suffice as a description since the former is usually haploid and homokaryotic and the latter is haploid and heterokaryotic, at least for the incompatibility factors. (Yeasts, on the other hand, could be described as haploid or diploid for most purposes since they usually contain only one nucleus per cell, although care should be taken in referring to the breeding system.)

The purpose of the terminology developed here is clearly not to confuse or to add an unnecessary complication to the notations at present in use. On the contrary, we hope to achieve clarification of the different terms and to develop and generalize the terminology so that it will unambiguously cover all nuclear stages in the fungi.

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RECOLLECTIONS OF LORD RUTHERFORD*

By Academician P. L. KAPITZA

THE only way in which I can satisfy the interest of the members of the Royal Society is to speak of Rutherford the man, of how I remember him during my 13 years' work in the Cavendish Laboratory, of how he worked, how he trained us young scientists and also of his relations with the scientific world. My task is therefore to draw you a portrait of a great scientist and of a great man. Frankly, this is the job of a writer and not of a scientist. If I have now decided to do so, this is mainly for the following reasons. When I look back and see myself as a young man coming to England in 1921 and starting work in the Cavendish Laboratory and, after 13 years, growing into a scientist, I feel that these years of my work were the happiest, and for all my achievements I feel immensely grateful for the attention and kindness which Rutherford showed me, not only as a teacher but as a very kind and sympathetic man for whom I have a sincere affection and with whom I eventually became great friends.

I would like to begin with a small episode which happened in 1930 in the Cavendish Laboratory. At that time a small conference was being held in Cambridge to commemorate the centenary of the birth of Maxwell, the first director of the Cavendish Laboratory. After the official part of the meeting in which some of Maxwell's pupils talked of their reminiscences, Rutherford asked me how I liked the speeches. I answered that they were very interesting, but I was surprised that all the speakers spoke only of the positive side of Maxwell's work and personality and made a "sugary extract" (*sic*) of him, and I said that I would like to see Maxwell presented as a living figure with all his human traits and faults which, of course, every man possesses however great his genius. Rutherford as usual laughed and said that he charged me after his death to tell future generations what he was really like. He was joking and I was laughing too. And now when I try to fulfil his behest and I imagine Rutherford as I have to present him before you, I see that time has absorbed all his minor human imperfections and I can only see a great man with an astounding brain and great human qualities. How well I now understand Maxwell's pupils who spoke about him in Cambridge.

In science, as in history, definite stages of development demand their particular kind of genius. A definite period of development requires men with corresponding mental abilities. In the history of the development of physics, as in any other experimental science, the most interesting periods are those in which we are brought to revise our fundamental scientific conceptions. Then not only deep thinking and intuition are required from the scientist but also a daring imagination.

The peculiar character of Rutherford's thinking could easily be followed when talking to him on scientific topics. He liked being told about new experiments, but you could easily and immediately see by his expression whether he was listening with interest or whether he was

* Based on an address delivered to the Royal Society of London on May 17, 1966.

VOLUME I

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edited by **A. McLaren**

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Institute of Biology Symposia Number 14

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bored. You had to talk only about fundamental facts and ideas without going into the technical details in which Rutherford took no interest. He grasped the basic idea of an experiment extremely quickly.

When I came to Cambridge, Rutherford was no longer doing experimental work by himself; he worked chiefly either with Chadwick or with Ellis. But in both cases he took an active part in experiments. The most attractive thing about Rutherford's experiments was the clear way in which the problem was set out. The simplicity and directness of approach to the solution of the problem were most remarkable.

I did not work with Rutherford, as my investigations were not connected with nuclear physics, and therefore I did not see him working in his laboratory. But I know that up to the very end of his life the main bulk of his time was taken up by his personal scientific research. The detailed guidance of scientific work he left to one of the senior scientific workers, usually Chadwick. But he himself always took an interest in the choice of the problem for experiment and of the experimental approach. He often came to the laboratory, but only for a short time just to make remarks like: "Why don't you get a move on—when are you going to get some results?" When I started working in the Cavendish Laboratory such remarks made a deep impression on me, especially as they were made in a thundering voice and with a severe expression. But eventually I found out that such utterances were automatic, maybe customary for a New Zealand farmer who when going through the fields found it useful to stimulate the workers with a few 'kind' words.

The greatest quality of Rutherford as a teacher was his ability to conduct research work in the right direction, then to encourage the beginner and to give just appraisal of his achievements. What he valued most in a pupil was independent thought and originality in his work. Rutherford did his utmost to develop in his pupil an individuality. I remember how in the first years of my work in the Cavendish I once said to Rutherford: "You know that the work of X is pretty hopeless; don't you think he's wasting his time and apparatus?" Rutherford replied that he too knew that the man was working on a hopeless problem. "But," he said, "it is a problem of his own and even if the work cannot be accomplished it will lead him to another original research problem which will be successful." The future showed that Rutherford was right.

Rutherford was very particular to give credit for the exact authorship of any idea. He always did this in his lectures as well as in his published works. He was also very particular not to give a beginner technically difficult research work. He reckoned that, even if a man was able, he needed some success to begin with, otherwise he might be disappointed in his abilities which could be disastrous for his future. Once, in one of our outspoken talks, he told me that the most important thing a teacher must learn is not to be jealous of the successes of his pupils—which is not so easily done as the teacher gets older! No doubt the greatest quality of a good teacher should be generosity. Rutherford was undoubtedly very generous and I think this is one of the main secrets which explains why so many first-class scientists came from his laboratory. He well understood the importance that his pupils had for him. It was not merely that young research students increased the scientific productivity of the laboratory, but, as he said, "My pupils keep me young". This is very true, as pupils do not permit a teacher to lag behind new achievements in science. Rutherford, with great ease and generosity, always accepted new ideas in physics like wave quantum mechanics, while a number of distinguished scientists of his generation were sceptical of the same ideas. He was very sociable and loved talking to the scientists who came to visit him and the Cavendish Laboratory. I remember one occasion when he brought Prof. Robert Millikan to my room in the laboratory. Rutherford said to me, "Let me introduce you to Millikan; no doubt you know who he is. Show him your installation to produce strong magnetic fields and tell him about your experiments. But I doubt whether he will let you speak as he himself will tell you about his own experiments!" There followed loud laughter in which Millikan joined with rather less enthusiasm. Rutherford then left us, and I soon found out that his prophecy was correct.

Later on, when I became a Fellow of Trinity and used to accompany him home after dinner on Sundays, we often discussed politics. On the first day I started work in the Cavendish I was surprised to hear him saying to me that in no circumstances would he tolerate my making Communist propaganda in his laboratory. At the time this remark came quite unexpectedly. It not only surprised me, but also shocked me and to a certain extent even offended me. Undoubtedly it was a consequence of the current atmosphere of acute political struggle and was connected with the propaganda which existed in those days—only four years after the Russian revolution. Before coming to England, I was so absorbed by my research work in Russia that I was completely unaware of what was happening in Western Europe and could not appreciate the scale of the bitter political controversy which then existed. Later on when my first experimental research was published I presented Rutherford with a reprint and I made an inscription on it that this work was proof that I had come to his laboratory to do scientific work and not to make Communist propaganda. He became extremely angry with this inscription, swore and gave me the reprint back. I had foreseen this and I had another reprint in reserve with an extremely appropriate inscription with which I immediately presented him. Obviously Rutherford appreciated my foresight and the incident closed. Rutherford had a characteristically hot temper but cooled down just as quickly.

Eventually we had many conversations on political questions; we were especially concerned about the growth of fascism in Europe. Rutherford was an optimist and thought that all would soon be over. We now know that this was not the case. Rutherford, like most scientists who work in the exact sciences, had progressive political views. I involved Rutherford in some political activity on two occasions.

The first of these was connected with Langevin. In his younger days, Rutherford had worked with Langevin in the same room at the Cavendish. A deep friendship developed between them. In Paris, my friends, pupils of Langevin, were greatly shocked that Langevin, undoubtedly the best French physicist, had not been elected to the French Academy as a result of his left political views. I told Rutherford of the difficulties Langevin had encountered in France and asked him whether a man who held such leftist views as Langevin could be a foreign member of the Royal Society. Rutherford said something I could not quite follow, then started to tell me what a really good man Langevin was, and then recalled that during the First World War Langevin had been very active in inventing supersonic beams propagated in water by which he had established communications between England and France across the Channel. At this point the conversation ended. I learned later that at the next election in 1928 Langevin was elected a foreign member of the Royal Society and this was much earlier than his election to the French Academy.

The second example occurred much later, when Hitler started to come into power. We were very anxious about the fate of such distinguished physicists as Stern, Franck, Born and a number of others in the conditions of active and increasing anti-semitism in Germany. About this time Szilard came to England and we were faced with the question of how to get these scientists out of Germany without raising suspicion. I spoke to Rutherford and he was very willing to help, writing personal letters to these scientists, and inviting them to come to lecture in Cambridge.

The following will serve to demonstrate Rutherford's skill in handling complicated psychological problems. I think enough time has now passed that I can tell you about this case which involved the then well-known physicist, Paul Ehrenfest.

Ehrenfest was born in Austria. On one of his mountaineering excursions he met a Russian woman scientist and followed her to Russia where he married her. In Russia he published a number of outstanding theoretical works on thermodynamics. Eventually he was invited to Leiden University to take the chair of theoretical physics vacated by the great Lorentz. In Leiden, Ehrenfest and his house became one of the world centres of theoretical physics. Ehrenfest's main quality was his precise critical mind. He was not only a very good teacher of young scientists, who were very fond of him, but his criticisms were regarded as profound and of such high quality that leading theoretical scientists like Einstein and Bohr often came to Ehrenfest to discuss their work. Ehrenfest always noticed even the smallest contradiction or mistake. His critical remarks were made very readily, with great spirit and even sharply, but always very good-naturedly. The quality of his criticism was greatly appreciated.

Ehrenfest's exceptionally strong critical mind evidently acted as a restraint on his creative imagination and he did not succeed in producing scientific work which he himself would have considered of sufficiently high standard. In those days I did not know that in his acute nervous condition Ehrenfest suffered greatly when he could not in his work attain the level of the friends he criticized. In the beginning of 1934 I received a long letter from him in which he described in detail his state of mental depression and spoke of the futility of his achievements. He had come to the conclusion that it was not worth living any more. The only way to save himself, he thought, was to leave Leiden and settle somewhere away from his friends. He asked me to help him to find a chair at some small university in Canada and to ask Rutherford, who doubtless had connexions in Canada, to assist him. I was, of course, very upset by this letter. I handed Rutherford the letter and told him that we were very worried about Ehrenfest's future as, without any doubt, the letter showed that he was mentally unbalanced; perhaps, I said, this state was only temporary and everything possible should be done to help him out of his state of depression. Rutherford said I must not worry and he would handle the case himself. I do not know what Rutherford wrote to Ehrenfest, but shortly afterwards I received a letter telling me that he was once again in a happy frame of mind. He said that Rutherford had explained what a great part he was playing in physics and he added that of course there was now no need for him to go to Canada.

All this happened only a few months before I went to Russia, as usual, to see my mother and my friends and, unexpectedly for me, I was deprived of the possibility of returning to Cambridge. Then for some years I could not participate on the same scale as before in international scientific activities and my correspondence with Ehrenfest ceased. I subsequently learned that a year or two later his state of depression returned and he committed suicide.

Finally, I should like to discuss a question I have come across several times in descriptions of Rutherford's activities. The question is: did Rutherford foresee the great practical consequences which would emerge from his scientific discoveries and investigations into radioactivity?

The immense reserves of energy which are hidden in matter were understood by physicists a long time ago. The question which was not clear at that time was: would it eventually be possible to find technical means of making practical use of these reserves? We know now that the actual possibility of obtaining energy from nuclear collisions was becoming more and more real as nuclear phenomena were better understood. But up to the last moment it was not certain whether it would be technically possible to produce nuclear reactions with a great yield of energy. I remember only rare occasions on which I discussed this question with Rutherford, and in all these conversations he expressed no interest in it. I think that his opinions on the practical applications of nuclear physics had no real value, as they lay outside the scope of his interest and tastes.

The last time I saw Rutherford was in the autumn of 1934 when I went to the U.S.S.R. and was unable to return to Cambridge. For the next three years the only scientist with whom I freely corresponded outside Russia was Rutherford. In his letters he gave me an account of life in Cambridge, spoke about the scientific achievements of himself and his pupils, wrote about himself, made jokes, gave good advice and invariably cheered me up in my difficult position. He understood that the important thing for me was to start my scientific work which had been interrupted for several years. It is no secret that it was only due to his interference and help that I was able to obtain the scientific installation and apparatus of the Mond Laboratory, and in three years' time I was able to renew my work in the domain of low-temperature physics.

I am sure that in the course of time all Rutherford's letters will be published but even so I should like here and now to quote three short extracts which require no comment.

On November 21, 1935, he wrote:

". . . I am inclined to give you a little advice, even though it may not be necessary. I think it will be important for you to get down to work on the installation of the laboratory as soon as possible, and try and train your assistants to be useful. I think you will find many of your troubles will fall from you when you are hard at work again, and I am confident that your relations with the authorities will improve at once when they see that you are working whole-heartedly to get your show going. I would not worry too much about the attitude or opinions of individuals, provided they do not interfere with your work. I daresay you will think I do not understand the situation, but I am sure that chances of your happiness in the future depend on your keeping your nose to the grindstone in the laboratory. Too much introspection is bad for anybody! . . ."

On May 15, 1936, he wrote:

". . . This term I have been busier than I have ever been, but as you know my temper has improved during recent years, and I am not aware that anyone has suffered from it for the last few weeks!

". . . Get down to some research even though it may not be of an epoch-making kind as soon as you can and you will feel happier. The harder the work the less time you will have for other troubles. As you know, a reasonable number of fleas is good for a dog—but I expect you feel you have more than the average number!"

The last letter is dated October 9, 1937. He wrote in great detail about his proposed journey to India. In the last part of the letter he said:

". . . I am glad to say that I am feeling physically pretty fit, but I wish that life was not quite so strenuous in term-time. . . ." Ten days before his death he did not feel that it was so near.

For me the death of Rutherford meant not only the loss of a great teacher and friend; for me, as for a number of scientists, it was also the end of a whole epoch in science.

Obviously we should attribute to those years the beginning of the new period in the history of human culture which is now universally called the scientific-technical revolution. One of the greatest events in this revolution has been the use of atomic energy. In 1921 Rutherford warned me not to make any Communist propaganda in his laboratory, but it now appears that just at that time he himself together with his pupils were laying the foundations for a scientific-technical revolution.

But, nevertheless, the year that Rutherford died there disappeared for ever the happy days of free scientific work which gave us such delight in our youth. Science has lost her freedom. Science has become a productive force. She has become rich but she has become enslaved and part of her is veiled in secrecy.

I do not know whether Rutherford would continue nowadays to joke and laugh as he used to do.

NEWS AND VIEWS

Biochemistry in the University of Liverpool: Prof. R. A. Morton

PROF. R. A. MORTON will retire from the Johnston chair of biochemistry in the University of Liverpool at the end of the present session after 42 years of service on the university staff. Appointed lecturer in chemistry in 1924, he developed a keen interest in spectroscopy under the influence of Prof. E. C. Baly and became a pioneer in the application of absorption spectroscopy to the examination of complex substances of biological origin, particularly the fat-soluble vitamins. His achievement was recognized by the award of the Meldola Medal in 1930, and after a year as visiting professor in Ohio State University, he was appointed special lecturer in spectroscopy at Liverpool in 1931. In collaboration with a number of postgraduate students and visiting research Fellows he discovered vitamin A_2 and demonstrated that the retinal pigment, retinene, was the aldehyde of vitamin A. His experience in characterizing compounds by their absorption spectra was invaluable during the Second World War when he undertook a number of research projects for the Ministries of Food and Supply and participated in the Sheffield experiment organized by the Medical Research Council to determine vitamin A requirements with the aid of human volunteers. He was appointed to the Johnston chair of biochemistry in 1944, and at the end of the War quickly applied the newly developed Beckman photoelectric spectrophotometer to his researches. His valuable contributions to the chemistry and biochemistry of the fat-soluble vitamins, carotenoids and sterols were recognized by his election to a fellowship of the Royal Society in 1950. Although his interests in biological compounds had originally been chemical, he willingly served on a number of committees and panels concerned with the practical aspects of human nutrition. One of his major contributions to biochemistry, however, is evident in the number of former members of his Department who now hold senior appointments in industry and in universities throughout the world. In recent years he and his research group opened up new and widely developing fields of biochemical interests through the discovery of families of biologically active quinones and related compounds by chromatographic and spectrographic methods. These compounds have been found widely distributed in Nature and given the names ubiquinones, ubichromenols and dolichols. Investigations of their functions are still at an interesting but early stage of development.

Prof. Morton has lectured at many academic centres in Europe and the United States. In 1963, the University of Coimbra conferred on him the honorary degree of D.Sc., and recently the Biochemical Society elected him to honorary membership.

Prof. T. W. Goodwin

PROF. T. W. GOODWIN, professor of biochemistry and agricultural biochemistry at Aberystwyth, returns to Liverpool in October at the invitation of the University Council to occupy the Johnston chair of biochemistry, in succession to Prof. R. A. Morton. He was appointed to the chair of agricultural chemistry at the University College of Wales, Aberystwyth, in 1959, and he has developed a flourishing teaching and research school in biochemistry, recently accommodated in new laboratories. He is well known for his work on the carotenoids which developed out of his interest in visible and ultra-violet spectroscopy, and for his collaboration with Prof. R. A. Morton in work on vitamin A. His interests now extend into metabolic studies on all terpenoid materials in plant tissues, particularly in relation to the regulation of their formation in plant cells. He has also made considerable contributions in the field of thiamine and riboflavin biosynthesis. Prof. Goodwin has written a number of books, including *The Comparative Biochemistry of the Carotenoids*, *The Biosynthesis of Vitamins and Related Compounds* and *Recent Advances in Biochemistry*. He has also edited two volumes on *Biological Structure and Function* (with O. Lindberg), *Structure and Activity of Enzymes* (with J. I. Harris and B. S. Hartley), *The Chemistry and Biochemistry of Plant Pigments* and *Aspects of Insect Biochemistry*. Prof. Goodwin has lectured in several European countries and in Egypt, and last session he spent 6 months at the University of California, Davis, as senior foreign scientist Fellow of the National Science Foundation. He was director of a symposium sponsored by the North Atlantic Treaty Organization on the biochemistry of chloroplasts, which was held at Aberystwyth in 1965. He is a committee member of the Biochemical Society and is its symposium organizer and chairman of its Advisory Committee on Publications; he is also vice-president of the Comité International de Photobiologie.

New Directions of Comparative Zoology

ERNST MAYR, director of the Museum of Comparative Zoology and Alexander Agassiz professor of zoology at Harvard University, emphasized the 'gratifying rejuvenation' that systematics and evolutionary biology, the two branches most actively pursued at the Museum of Comparative Zoology, have undergone in the past few years. While the basic quest is still the same—"to investigate the diversity of the living world in order to understand the causes of this diversity"—Prof. Mayr said that field work to study the living organism in relation to its environment has become a necessary part of research. In addition to his traditional task of describing and naming new organisms, the taxonomist "increasingly studies behavior, distribution, niche occupation, population structure, environmental physiology, and all aspects of evolution". To facilitate this new emphasis on living organisms, the Museum is in the process of acquiring a field research station in Concord, Massachusetts, and is planning a new wing, largely devoted to the study of living animals.

Increasing teaching commitments of the scientific staff are also changing the Museum's traditional position as a research body. To meet the demand, new teaching facilities are being installed in the Museum and will be ready for the academic year 1966–67. Staff members for the first time are offering specialized seminars under Harvard's Freshman Seminar Programme. In addition, members are giving fifteen courses in the biology and geology departments.

Prospects for Biology

THE content and organization of degree courses in the biological sciences are undergoing considerable reappraisal.

Two significant, though not exclusive, lines of development are worthy of comment. First, degree courses in biology *per se* are being established in contrast to the traditional study of botany and zoology as separate disciplines. Such an approach places emphasis on the integration of plant and animal studies, on unitary concepts in biology and on a fuller appreciation of plant–animal interactions. Secondly, microbiology is coming to be recognized as a degree subject in its own right, no longer to be equated with medical bacteriology or other narrow specialist treatments. These two approaches have much in common, among the most important being their attempts to integrate and rationalize biology and to require its firm basis in the physical sciences.

Although the output of microbiology graduates from British universities and colleges is small, the present demands from industry and research laboratories are considerable and ever expanding. On his appointment to the newly created chair of microbiology in University College, Cardiff, Prof. D. E. Hughes has directed timely attention to this widening scope and importance of microbiology (*Microbes in Action*. An Inaugural Lecture delivered at University College, Cardiff, April 30, 1965. By Prof. D. E. Hughes. Pp. 21. Cardiff: University of Wales Press, 1965. 3*s*. 6*d*.). Apart from the more academic aims and achievements of contemporary microbiology, reference is made to the extension of industrial processes, based on the activities of micro-organisms, and to the technological exigencies imposed by such operations. Prof. Hughes's inaugural address presents a broad survey of microbiology and gives heed to its further advance, exploitation and some possible consequences of its neglect. It is to be hoped that the training of graduates in this field will keep pace with the requirements of industry and elsewhere and that disparity in present supply and demand will be redressed. The time is opportune for a careful assessment of the types of biologist Britain needs and will continue to need in the approaching decade; the recent misjudgment of the number of medically qualified graduates required is a cogent argument for such an enquiry.

Royal Society/Royal Geographical Society Expedition to Mid-West Brazil

THE Royal Society and the Royal Geographical Society are considering jointly sponsoring scientific investigations in the little-known interior of Mato Grosso in mid-west Brazil. A reconnaissance party of three is now on its way for a two-month visit to discuss with Brazilian scientists and officials plans for a larger expedition from Britain, possibly next year.

The leader of the reconnaissance party is Mr. A. F. MacKenzie, a tropical plant ecologist, and he will be accompanied by Mr. I. R. Bishop, a zoologist from the University of Leicester, and Mr. D. R. Hunt, a botanist from the Royal Botanic Gardens, Kew. Mr. MacKenzie sailed for Rio de Janeiro in mid-April, with the reconnaissance party's supplies, and will be followed shortly by Messrs. Bishop and Hunt.

Lasers for Teaching

THE laser is now an accepted new light source for use both in teaching laboratories and in scientific and technological research. Though specifically related to the demountable continuous gas laser produced by Scientifica and Cook Electronics, Ltd., the manual entitled *The Gas Laser and its Experimental Applications*, by R. J. Horton and P. D. Cook, is a valuable and easily understood guide to various experiments which can be used for teaching and demonstration purposes using a helium–neon gas laser (Pp. 31. London: Scientifica and Cook Electronics, Ltd., 1965. 12*s*. 6*d*.). Detailed instructions are given for alignment and operation of the laser, in addition to the precautions necessary while handling the source. A clear introductory statement describes the theory of the laser, and the various cavity arrangements and the dependence of action of the laser on them. The experiments chosen as examples consist of the demonstration of lens aberrations, Fraunhofer diffraction and interference patterns, Fourier transforms, the Michelson interferometer, and the Abbé theory of image formation.

Root Rot of Teak

DR. B. K. BAKSHI, of the Forest Research Institute, Dehra Dun, India, has written to the Editor reporting an outbreak of root rot diseases of teak (*Tectona grandis* Linn. f.). The outbreak occurred in a plantation at the demonstration forest attached to the Institute (the plantation was established in 1928 and coppiced during 1945): "Two pathogens were encountered, namely, *Polyporus zonalis* Berk., and a species of *Peniophora*. The decay in the roots has resulted in wind throw of trees. So far as I can ascertain, *Peniophora* has not previously been recorded in teak. It colonizes old coppiced stumps and spreads freely through the soil by means of yellow rhizomorphs which infect healthy roots, in which a yellow spongy or yellow laminated decay develops. Sporophores are common. The fungus has not been encountered before".

Geological Society of London

AT the annual general meeting of the Geological Society of London on April 27 the officers and Council for the ensuing year were elected as follows: *President*, Prof. K. C. Dunham, University of Durham; *Secretaries*, W. B. Harland, University of Cambridge; Prof. J. Sutton, Imperial College of Science and Technology; *Foreign Secretary*, Prof. O. M. B. Bulman, University of Cambridge; *Treasurer*, Dr. W. Bullerwell, Geological Survey and Museum.

U.S. National Academy of Sciences

DR. H. BROWN, professor of geochemistry at the California Institute of Technology, has been elected to a second four-year term as foreign secretary of the U.S. National Academy of Sciences. The following were also elected members of the Council of the Academy for a three-year period: PROF. H. E. CARTER, head of the Department of Chemistry, University of Illinois; PROF. J. L. GREENSTEIN, head of the Department of Astrophysics and chairman of Faculty, Mount Wilson and Palomar Observatories, California Institute of Technology; PROF. W. O. FENN, professor of physiology, School of Medicine and Dentistry, University of Rochester; PROF. ESAU, emeritus professor of botany, University of California.

Zoological Society of London

THE Zoological Society of London has made the following awards: *Silver Medal*, Mr. D. Attenborough of Richmond, Surrey; *Scientific Medal*, Dr. E. H. Ashton, the Medical School, University of Birmingham; Mr. B. B. Boycott, University College, London; Dr. R. M. Laws, Nuffield Unit of Tropical Animal Ecology, Queen Elizabeth National Park, Uganda; *Stamford Raffles Award*, Dr. E. G. Neal, Taunton School, Somerset; *Thomas Henry Huxley Award*, Dr. J. S. Gray, University of Leeds; *Prince Philip Prize*, M. E. Greenhalgh, formerly of Kirkham Grammar School.

The Australian Academy of Science: New Officers

AT the annual election of officers of the Australian Academy of Science on April 28, the following were elected: *President*, Sir Macfarlane Burnet; *Secretary* (physical sciences), Dr. A. L. G. Rees; *Secretary* (biological sciences), Prof. R. J. Walsh; *Treasurer*, Sir Hugh Ennor.

New Fellows

ALSO at the annual general meeting of the Australian Academy of Science, LORD CASEY, the Governor-General, was elected a Fellow of the Academy by special election because of his service to the cause of science. The following were elected to fellowships by ordinary election: PROF. J. M. BLATT, professor of applied mathematics, University of New South Wales; DR. S. V. BOYDEN, professorial Fellow, Australian National University, Canberra; PROF. S. C. B. GASCOIGNE, assistant director, Mount Stromlo Observatory, Australian National University, Canberra; DR. S. D. HAMANN, chief of the Division of Physical Chemistry, C.S.I.R.O. Chemical Research Laboratories, Melbourne; PROF. A. E. RINGWOOD, Department of Geophysics, Australian National University, Canberra; PROF R. F. WHELAN, professor of physiology, University of Adelaide.

The Leopoldina German Academy of Sciences: Elections

THE Deutsche Akademie der Naturforscher Leopoldina, Halle, recently elected the following new members in the sections indicated: *Physics*, Prof. P. P. Ewald (New Milford), Prof. S. L. Mandelštam (Moscow) and Prof. E. Ruska (Berlin); *Meteorology*, Prof. H. Flohn (Bonn); *Chemistry*, Prof. G. Schwarzenbach (Zurich); *Mineralogy*, Prof. E. H. Jagodzinski (Munich); *Geology*, Prof. R. Hohl (Halle), Prof. H. Kölbel (Berlin), Prof. P. E. Kuenen (Groningen) and Prof. W. P. de Roever (Amsterdam); *Paediatrics*, Prof. J. H. P. Jonxis (Groningen); *Surgery*, Prof. K. Boshamer (Wuppertal-Barmen) and Prof. J. Kudász (Budapest); *Dermatology*, Prof. O. Braun-Falco (Marburg).

University News: London

PROF. J. R. D. FRANCIS, professor of municipal engineering in the Institute of Science and Technology, University of Manchester, has been appointed to the chair of hydraulics tenable at the Imperial College of Science and Technology.

Swansea

DR. R. H. TREDGOLD, reader in physics in the University College of North Wales, has been appointed to the chair of physics and headship of the Department of Physics in the University College of Swansea, on the resignation of Prof. F. Llewellyn-Jones, who has been appointed principal of the College.

Appointments

PROF. W. F. POUNDS has been appointed dean of the Alfred P. Sloan School of Management at the Massachusetts Institute of Technology as from April 1. Dr. Pounds is a specialist in operations management and quantitative managerial analysis, and has had extensive experience both in industry and in management education.

DR. L. COHEN has been appointed secretary to the Institute of Physics and the Physical Society. He has had wide experience in industrial research and has recently been research manager at Pyrotenax, Ltd. He has been honorary secretary of the Manchester Literary and Philosophical Society for 8 years.

Announcements

IN the Programme for 1965–66, Unesco, in co-operation with the International Brain Research Organization, is providing fellowships for research and training in the basic sciences concerned with the brain, together with funds to supplement offers of fellowships in brain research made by member States. Further information can be obtained from IBRO–UNESCO, 2 Place de Fontenoy, Paris 7*e*.

DR. N. D. ZINDER, professor of genetics and microbiology in the Rockefeller University, has received the United States Steel Foundation Award for distinguished work in molecular biology. Dr. Zinder and the group working with him were the first to show that virus replication in the RNA bacteriophage was not dependent on DNA; that, in fact, the RNA phage acts both as its own genetic material and as a template for directing protein synthesis.

IT has been agreed by the University of Reading and by the Fruit and Vegetable Preservation Research Association that the Research Association should become associated with the University. The Association, which is a non-profit-making company supported by the food industries, with grants from the Ministry of Technology, will not change its present constitution or objectives. The agreement will provide for certain lines of co-operation between the Association and appropriate University departments, such as those dealing with agriculture, and food science and technology.

THE forty-ninth annual conference of the Chemical Institute of Canada will be held in the University of Saskatchewan during June 6–8. Further information can be obtained from the Chemical Institute of Canada, 48 Rideau Street, Ottawa 2, Ontario.

THE 1966 San Diego Symposium for Biomedical Engineering on "From Diagnosis to Therapy" will be held in San Diego during June 6–9. Further information can be obtained from L. T. Gregg, 2063 Cardinal Drive, San Diego, California.

A SYMPOSIUM on "Energy Conversion by the Photosynthetic Apparatus' will be held at Brookhaven National Laboratory during June 6–9. Further information can be obtained from Dr. J. M. Olson, Biology Department, Brookhaven National Laboratory, Upton, New York.

A CONFERENCE on "Materials in Biomedical Engineering", sponsored by the New York Academy of Sciences, will be held in New York during June 9–11. Further information can be obtained from Dr. S. N. Levine, Department of Material Sciences, State University of New York, Stony Brook, Long Island, New York.

THE second international conference of the European Atomic Energy Agency on "Methods of Preparing and Storing Labelled Compounds" will be held in Brussels during June 6–11. Further information can be obtained from the Labelled Compounds Division, European Atomic Energy Agency, 51–53 Rue Belliard, Brussels.

A SERIES of summer courses in marine science will be held by the University of Texas Institute of Marine Science during June 3–August 6. The programme will include courses on marine microbiology, marine geology, ecology of fishes, marine chemistry, adaptive mechanisms in marine animals and estuarine ecology. Further information can be obtained from the Director, Institute of Marine Science, Port Aransas, Texas.

THE following summer courses will be held at the Enrico Fermi International School of Physics, Varenna: theory of magnetism in transition metals (June 6–25); interaction of high-energy particles with nuclei (June 27–July 9); plasma astrophysics (July 11–30). Further information can be obtained from Dr. G. G. Low, Atomic Energy Research Establishment, Harwell, near Didcot, Berkshire (course 1); Dr. T. E. O. Ericson, CERN, 1211 Geneva 23 (course 2); Dr. P. A. G. Scheuer, Cavendish Laboratory, Cambridge (course 3).

ERRATUM. The heading of the article entitled "Large Amounts of Deoxyphenylalanine in the Hydrolysate of Melanosomes from Harding-Passey Mouse Melanoma", by Dr. H. Takahashi and Prof. T. B. Fitzpatrick, which appeared on p. 888 of the February 26, 1966, issue of *Nature*, should read "Large Amounts of Dioxyphenylalanine . . .".

STABILITY OF EPIDEMIC PROCESSES

By WILLIAM GOFFMAN
Western Reserve University, Cleveland, Ohio

THE notion of stability of a physical process is closely associated with the theory of differential equations. In fact this problem has been studied extensively as a part of this theory, most notably by the Russian mathematician A. M. Lyapunov[1]. The methods of Lyapunov should thus be applicable in the investigation of any physical process which can be characterized in terms of a system of differential equations. It is the purpose of this article to show that the stability of an epidemic process is equivalent to stability in the sense of Lyapunov.

Epidemic Processes. In general an epidemic process E can be described in terms of a set N (a population) and a set of internal states σ (susceptible, infected and removed) which effects a partition of N at a given point in time, where state transitions are the result of exposure to some phenomenon (infectious material). The process E can itself be in any one of a set of three states at a point in time, namely: (1) Increasing state; the change in the rate at which the number of infectives accrues with respect to time is positive. (2) Decreasing state; the change in the rate at which the number of infectives accrues with respect to time is negative. (3) Stable state; the change in the rate at which the number of infectives accrues with respect to time is zero.

In its simplest form an epidemic process can be represented by the system (1) of differential equations[2].

$$\begin{aligned} \frac{dS}{dt} &= -\beta SI \\ \frac{dI}{dt} &= \beta SI - \gamma I \\ \frac{dR}{dt} &= \gamma I \end{aligned} \qquad (1)$$

This model assumes that there is a susceptible population of size S, an infective population of size I and a removed population R where removal constitutes an absorbing state which can be reached only by passing through a state of infection. It is further assumed that infection is spread by direct contact between susceptibles and infectives with β equal to the rate of infection and γ equal to the rate of removal. The total population $N = S + I + R$ remains constant with respect to time, there is homogeneous mixing among the members of N and the latency period equals the incubation period equals zero. That is, infections occur continuously in time and a susceptible becomes an infective as well as a manifest case at the time that effective contact occurs.

A necessary condition for the process of equations (1) to enter an increasing state is that

$$\frac{dI}{dt} = \beta SI - \gamma I > 0$$

Thus $S > \gamma/\beta = \rho$ constitutes a threshold density of susceptibles, that is, an epidemic can develop from time t_0 only if the number of susceptibles S_0 at that time exceeds the threshold ρ.

The process will attain a peak at the point in time at which the rate of change of susceptibles and infectives is maximum, that is, when:

$$\frac{d^2(S+I)}{dt^2} = -\gamma \frac{dI}{dt} = -\gamma[\beta SI - \gamma I] = 0$$

and $S = \frac{\gamma}{\beta} = \rho$. Therefore the point at which the process will make a transition from an increasing to a decreasing state is that point in time when the number of susceptibles reaches the threshold ρ.

According to the Kermack–McKendrick theorem[3] for processes of type (1), the initial number of susceptibles will be reduced over the course of the epidemic by a value as far below the threshold as it was originally above it. Hence if:

$$S_0 = \rho + \varepsilon$$

then the point at which the process re-enters a stable state is the point at which:

$$S = \rho - \varepsilon$$

Since N remains constant throughout the course of development of the process, that is, N is a closed population, the process of equations (1) must always return to a stable state after having entered an increasing state. This is, in fact, one of the principles underlying the effectiveness of quarantine as a control. Thus an epidemic process in a closed population is in a sense a stable process.

Let us now consider an epidemic process in an open population, that is, in a population N which does not remain constant with time. In such a process new supplies of susceptibles and infectives are introduced into the population as the process proceeds on its course of development. We again consider a population N consisting of S susceptibles, I infectives and R removals in which infectious material is transmitted by direct contact between members of I and members of S. β is the rate of infection, $\delta(\gamma)$ the rate of removal of susceptibles (infectives) and $\mu(\nu)$ is the rate at which new susceptibles (infectives) are introduced in N. If the assumptions concerning mixing, latency and incubation are the same as before, this process can be represented by the system of equations (2):

$$\begin{aligned} \frac{dS}{dt} &= -\beta SI - \delta S + u \\ \frac{dI}{dt} &= \beta SI - \gamma I + \nu \\ \frac{dR}{dt} &= \delta S + \gamma I \end{aligned} \qquad (2)$$

A threshold density of susceptibles is obtained as before. Thus, if a single infective is introduced into a population of S_0 susceptibles at time t_0, then:

$$S_0 > \frac{\gamma - \nu}{\beta} = \rho$$

is a necessary condition for the process in equations (2) to enter an increasing state, that is, for an epidemic to develop. Since N is open, that is, increasing with time, there is no guarantee that if the process (2) enters an increasing state that it will reach a maximum, make a transition to a decreasing state and subsequently to a stable state as is the case when N is closed. Necessary and sufficient conditions for a process of type (2) to stabilize itself have been given[4]. It was shown that such a process will be stable if and only if $dR/dt = \delta S + \gamma I$ is constant.

Stability. Consider a physical process which can be described by a system of differential equations of the form:

$$\frac{dx_i}{dt} = f_i(x_1, x_2, \ldots x_n) \quad i = 1,2, \ldots n \qquad (3)$$

where the x_i's represent the variables which characterize the process. These variables are called the phase coordinates of the process and are usually functions of time t. Since the behaviour of equations (3) is described by the values of the n real numbers $x_1, x_2, \ldots x_n$, that is, by the

vector $\bar{x} = (x_1, x_2, \ldots x_n)$, the vector space X of vectors $\bar{x}$ constitutes a phase space for the system (3). Thus equation (3) can be written in vector form:

$$\frac{d\bar{x}}{dt} = f(\bar{x}) \quad \bar{x} = (x_1, x_2, \ldots x_n) \tag{4}$$

A system of differential equations always has an infinite number of solutions. A definite solution is obtained by specifying a set of initial values which correspond to the initial conditions of the process that the equations are intended to describe. By the uniqueness theorem[5] a solution or trajectory passing through a given point is unique. Suppose that the phase co-ordinates of equation (4) have reached a fixed point $\bar{a} = (a_1, a_2, \ldots, a_n)$. Then $f(\bar{a}) = 0$ and $\bar{x} = \bar{a}$ represents a state of equilibrium for the process characterized by (4). The problem of stability is concerned with how trajectories g^* which pass near a point of equilibrium $\bar{a}$ behave with respect to the trajectory g passing through $\bar{a}$. If the g^* remain close to g then the point $\bar{a}$ is said to be stable.

It is convenient to consider $\bar{a}$ as the initial point of the process, and hence as the origin of the phase space X. This is easily accomplished geometrically by the transformation of co-ordinates so that we shall be dealing with a system of equations for which $f(0) = 0$. The question of stability can now be stated in terms of the origin of the process. Thus the origin $\bar{a}$ is said to be stable in the sense of Lyapunov if there exists an n-dimensional spherical region $S(H)$ of radius H surrounding $\bar{a}$ such that for $h \leqq H$ a trajectory g^* emanating from a point $\bar{x}_0$ in the interior of the region $S(h)$ of radius h will remain within the region $S(H)$. In other words, if the trajectory g starting from $\bar{a}$ is thought of as being encased by a tubing then a trajectory starting near $\bar{a}$ which penetrates this tubing must remain within a larger encasement of tubing. On the other hand, the origin is said to be unstable if for any h, no matter how small, a trajectory emanating from the interior of $S(h)$ reaches the border of $S(H)$.

The method of Lyapunov[6] reduces these considerations to the properties of a special type of function V. A function $V(x_i)$ is called a Lyapunov function if (1) $V(x_i)$ together with its first partial derivatives are continuous in a certain region Ω about the origin, (2) $V(0) = 0$, (3) $V(x_i)$ is positive everywhere except at the origin, (4) $\frac{dV}{dt} \leqq 0$. Lyapunov's stability theorem states that the origin is stable if there exists a Lyapunov function in some neighbourhood of the origin. That is, a disturbed motion near the origin is stable in the sense of Lyapunov if it is possible to find a function which is sign-definite and whose derivative is either sign-opposite to it or vanishes immediately.

The epidemic process of type (2) can be represented by a system of equations of the form:

$$\begin{aligned} \frac{dS}{dt} &= f_1(S, I, R) \\ \frac{dI}{dt} &= f_2(S, I, R) \\ \frac{dR}{dt} &= f_3(S, I, R) \end{aligned} \tag{5}$$

where f_1, f_2 and f_3 are single-valued functions, continuous in the domain of their arguments S, I, R which are unknown functions of the real variable t. The system of equations (5) can be treated as a 3-dimensional vector space E which constitutes a phase space with phase co-ordinates S, I and R for the epidemic process. It was shown that a process of type (2) will be stable if and only if $\frac{dR}{dt} = k$ where k is a constant. It can be seen that if this is the case then $\frac{dR}{dt} = V(S, I)$ possesses the properties required of a Lyapunov function, hence its existence in the neighbourhood of the origin guarantees its stability. For let $\bar{a} = (S_0, 1, 0)$ be the initial state of the process. By translation of co-ordinates, $\bar{a}$ can be taken as the origin of the phase space E. Clearly (*a*) $V(S, I)$ together with its first partial derivatives are continuous in the neighbourhood of the origin; (*b*) $V(0) = 0$; (*c*) $V(S, I)$ is positive everywhere except at the origin; (*d*) $\frac{dV}{dt} = 0$ since $\frac{dR}{dt} = k$.

Since $V(S, I)$ is positive everywhere except at the origin, the surface $y = V(S, I)$ has the general shape of a cup emanating from the origin and $V(S, I) = k$ represents loops around the origin for different values of k.

Given a spherical region $S(H)$ surrounding the origin, there exists a loop K_H, determined by some k_H which lies just within the sphere $S(H)$. There also exists an $h \leqq H$ such that the sphere $S(h)$ lies just within the loop K_H. Consider a point $\bar{x}_0$ in the interior of $S(h)$ and a trajectory g^* starting at $\bar{x}_0$. Since $V(S, I) < k_H$ at the point $\bar{x}_0$ and since V is non-increasing, it follows that g^* will never reach the loop K_H. Hence g^* will always remain within $S(H)$. Thus any trajectory emanating from the region $S(h)$ surrounding the origin will remain within the region $S(H)$, hence the origin is stable.

This work was supported by the U.S. Air Force Office of Scientific Research.

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PROBLEMS OF HEAT TRANSFER IN LAKE VANDA, A DENSITY STRATIFIED ANTARCTIC LAKE

By R. A. HOARE

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LAKE Vanda (77° 35′ S., 161° 39′ E.) is a permanently ice-covered lake which occupies a depression in the Wright Valley, Victoria Land, Antarctica. It is about 5 miles long and 1 mile wide, is fed by melt-water from glaciers, has no outlet, and all water loss is by evaporation. It is of unusual interest because of its relatively high bottom temperature of 25° C in a region the mean annual air temperature of which is about −20° C, and its strong density stratification caused by a salt concentration which increases with depth from zero at the top up to 10 per cent by weight at the bottom. Several expeditions have measured temperatures in the lake[1–4] and established that the isothermal surfaces are horizontal, over most of the area of the lake, and that the temperature increases downwards, reaching a maximum of 25° C at the bottom. The decrease in density to be expected from the increase in temperature is more than compensated by the increasing salt content.

Wilson and Wellman[3] showed that the shape of the temperature profile below 160 ft. is consistent with the supply of heat to the water by the absorption of solar radiation, with conduction as the only other form of heat transfer, and that the necessary amount of energy is capable of penetrating the surface ice and water to this depth. They found that from 160 ft. to 125 ft. the temperature gradient is much less than that below 160 ft., so that much less heat can be conducted upwards above the 160 ft. level than below it. This implies that either the temperature at the 160-ft. level is rapidly increasing with time, or the heat transfer above this level is not solely by conduction. As the density appeared to increase downwards, it seemed unlikely that convection could be responsible; this article reports the investigations which I undertook to resolve the problem.

Wilson and Wellman reported that there were no changes in temperature, density or concentration from 125 ft. to 55 ft., which is consistent with strong convection in this region; however, they considered the region from 55 ft. up to 20 ft. to be another anomalous region. Ragotzkie and Likens[4] obtained temperature measurements similar to those of Wilson and Wellman and showed by radioactive tracer methods that convection occurred in the region from 45 ft. to 70 ft. They postulated a set of four convection cells extending over most of the area of the lake, and ranging in thickness from 5 ft. to 65 ft. The present article provides additional evidence for solar heating, and shows that the anomalous regions consist of a series of conductive and convective layers.

In early January 1964 I undertook detailed *in situ* measurements of temperature, density and electrical conductivity of the lake waters. Temperature was measured to $\pm 0{\cdot}05°$ C at all depths using a copper-constantan thermocouple with a portable potentiometer, and to $\pm 0{\cdot}01°$ C to a depth of 65 ft. using a multiple thermocouple which consisted of six thermocouples in series.

Density was measured by weighing a 'Perspex' cylinder of volume 568 c.c. suspended on light nylon monofilament line, which was wound on a reel hooked on to the balance arm. A simple balance with an accuracy of about ± 10 mg was used, mounted in its case directly over a hole in the ice. Changes in the density of the water displaced at different depths resulted in changes in the 'apparent' weight of the 'Perspex' cylinder. A correction was made for the change in apparent weight of the suspending line when it was run off the reel into the water. The main sources of error result from changes in the volume of the 'Perspex' cylinder due to changes in temperature and pressure. The effect due to pressure is negligible at the present degree of accuracy, and that due to temperature is found to be sufficiently small that general conclusions will not be altered. (There was a small apparent difference in density of the top water between two measurements, one taken at the start of a run, and the other after pulling the 'Perspex' cylinder up quickly from the warm bottom waters). The 'Perspex' cylinder was 8 in. (22 cm) long, so that detailed structure of density was not clear.

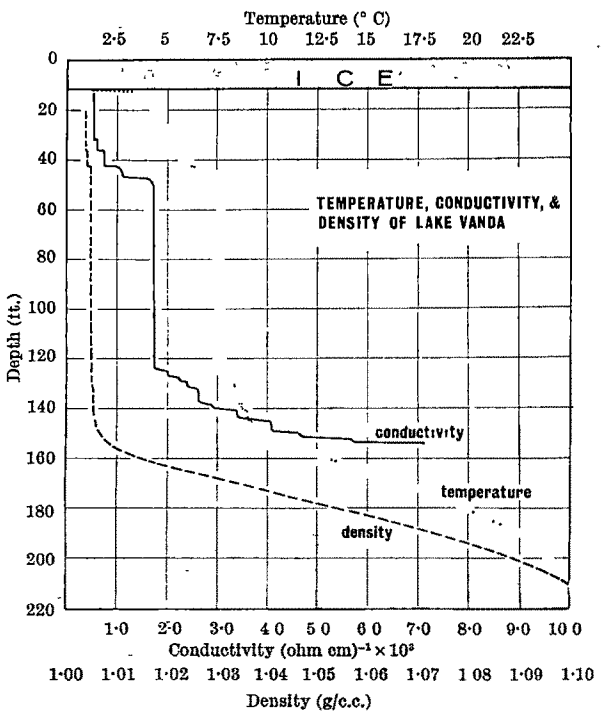


Fig. 1

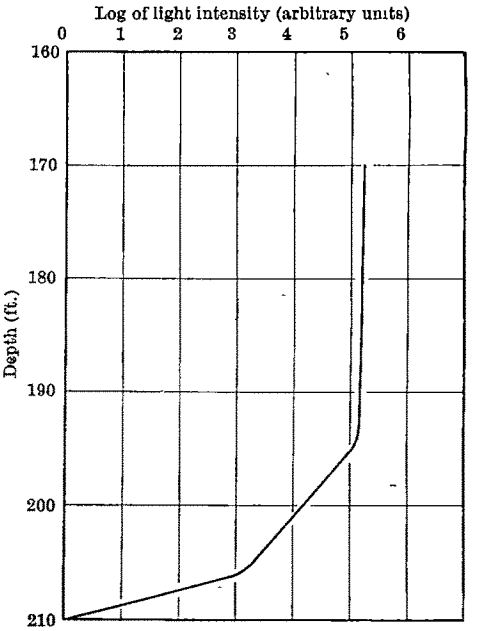


Fig. 2

Electrical conductivity was measured to a depth of 155 ft. with a Whitney underwater conductance meter (Whitney Underwater Instruments, Ltd., San Luis Obispo, California).

Measurements were taken at intervals which depended on the rate of change of the quantity being measured, and were sometimes as close as 3 in. ($7\frac{1}{2}$ cm). Temperature measurements were made in two holes, about a mile (1·6 km) apart, while the other measurements were made in only one of these. Depths were measured with respect to water-level, which was 9·0 ft. below a bench mark established by my expedition near the middle of the south side of the lake (the bench mark consists of a cairn of rocks, with a reference line and the January 1964 height above water-level painted on it).

Heat transfer below 160 *ft.* Fig. 1 shows the temperature profile which I observed in early January 1964 through the second hole, at a position near Wilson and Wellman's second hole, and about 400 m south-west of Ragotzkie and Likens's 2,000-m station. The profile has the same shape as that reported by Wilson and Wellman, between 160 ft. and 200 ft., but about 0·5° C higher, so that, for the reasons given by them, heat transfer is by conduction and radiation. (Both sets of measurements were performed with the same copper constantan thermocouple and with respect to an ice-water cold junction.) At the 200-ft. level the temperature gradient decreases more abruptly than that reported by Wilson and Wellman, in a similar manner to Ragotzkie and Likens's results for late December 1962. The temperature gradient increases again at 205 ft., which is inconsistent with steady-state heat transfer by

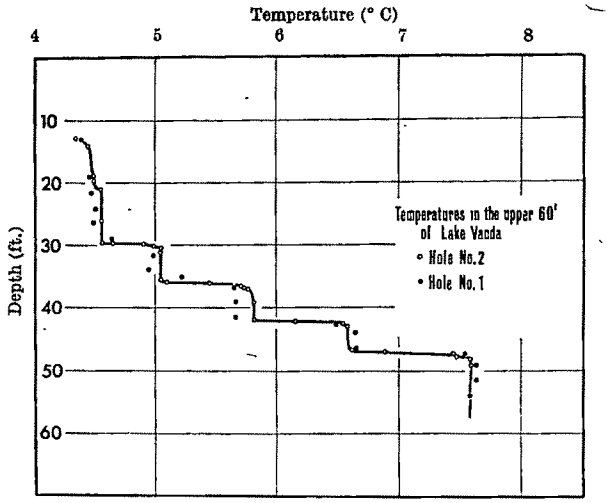


Fig. 3

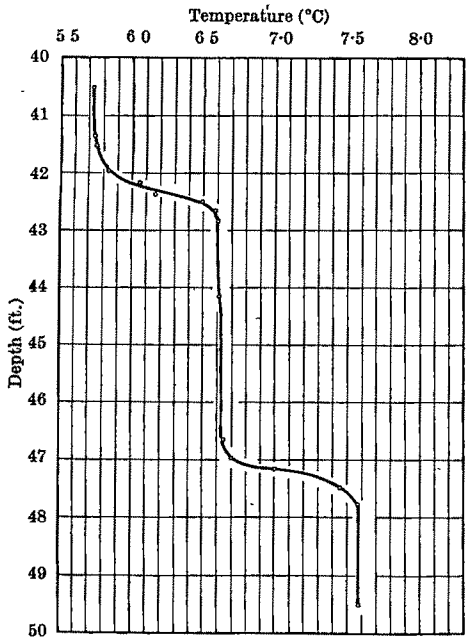


Fig. 4

conduction alone. This region of the lake has a high optical density (extinction coefficient), as shown by measurements of light intensity made with a selenium photo-electric cell and potentiometer (see Fig. 2). The difference between Wilson and Wellman's measurements and subsequent ones may indicate that their bottom coring operations disturbed the lake bottom and resulted in the discoloration of the water (personal communication), and that the present temperatures are not steady-state temperatures because of consequent alterations in the radiated heat.

Heat transfer above 160 *ft.* The electrical conductivity measurements through the second hole are shown in Fig. 1 to a depth of 155 ft., below which the concentration was too high to allow reliable correction for polarization. Hole 1 was about a mile (1·6 km) to the east of the hole 2, and the temperatures in the upper region of the lake at the two holes are compared in Fig. 3. It can be seen that the temperatures, although slightly different at the two holes, change at the same depths at both holes.

These regions of constant temperature and conductivity can only be explained by supposing that they are strongly convecting. The foregoing measurements show that the reason for the anomalously low value for the average temperature gradient in the region between the top and 160 ft. is that twelve convecting regions transfer heat upwards with a very small temperature gradient, the boundaries between these regions being horizontal and extending across the whole lake. Within each convecting region the temperature, salinity and density are constant—within the limit of accuracy of these measurements. At the boundaries of these regions temperature and salinity rise smoothly, as in the lower, density stratified region. This is illustrated by the results shown in Fig. 4, which are temperatures taken between 40 ft. and 50 ft. with the multiple junction thermocouple.

If the boundary regions are assumed to be non-convective, it is possible to calculate the heat flow through them and compare it with that expected on the basis of solar heating.

The two boundaries shown have maximum temperature gradients of about 1·3 ± 0·2° C/ft. At a depth of 160 ft., the gradient is 0·4° C/ft., so if the thermal conductivity of the water at 50 ft. is the same as that at 160 ft., about four and a half times as much heat is conducting upwards at the higher level than at the lower one.

I was able to detect, by means of a thermopile-type bolometer, that solar radiation halved in intensity every 50 ft. This means that a quarter of the light reaching 50 ft. will reach 150 ft. If the heat flowing upwards is derived from solar radiation which is being absorbed in the water, then the heat flow would be expected to be four times greater at 50 ft. than at 150 ft.—which is in agreement with the foregoing measurements of temperature gradient.

The observed temperatures agree well with the theory that the heat in the lake comes from absorbed solar radiation. In the lower waters of Lake Vanda heat transfer upwards is by conduction through the density stratified water. Above this region, convecting layers ranging in thickness from a few feet to 70 ft. alternate with conducting layers a few inches thick. The limited data available (see also ref. 4) indicate that the boundaries are horizontal and extend across the entire lake.

These convecting layers are among the most stable, large, natural convective systems in the world. The ice cover of the lake provides a stable platform from which instruments can be lowered to any point within the convective cells.

Apart from their interest to studies of convective systems in general, there is the challenging problem of the origin of the convecting regions. One possible mechanism is that at some time the lake was continuously, but weakly, density stratified. The heat conducted upwards would have produced temperatures which increased with depth, which would have caused the density to decrease with depth by thermal expansion. If the heat flow were sufficiently high, the thermal expansion might have overcome the salt-produced density stratification, and enabled convection to commence. The vertical dimension of the convection cell would have been limited by the gradients of salt concentration and temperature, and would have depended on the variation of thermal expansion of water with temperature and salt content. Further work is in progress to elucidate details of this problem.

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SUBMILLIMETRE ABSORPTION AND DISPERSION OF LIQUID WATER

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THE absorption and dispersion of liquid water in the radio-frequency and far-infra-red regions have been the subjects of numerous investigations over the past 20 years. The position in 1948 was reviewed by Magat[1], who was of the opinion that the dispersion could be divided into two parts; the first, occurring at wave-lengths greater than 1 mm, was associated with the non-resonant viscously damped reorientation of dipoles (the Debye process), and the second, occurring at wave-lengths less than 1 mm, was due to resonant absorption by the intramolecular modes of the liquid lattice. However, experimental observations in the region from 10 cm^{-1} to 100 cm^{-1} were few and of unknown reliability. Since much higher power sources are now available[2] measurements of greater accuracy can be made and there is an incentive to have these results in that they are needed in making improved calculations of atmospheric attenuation.

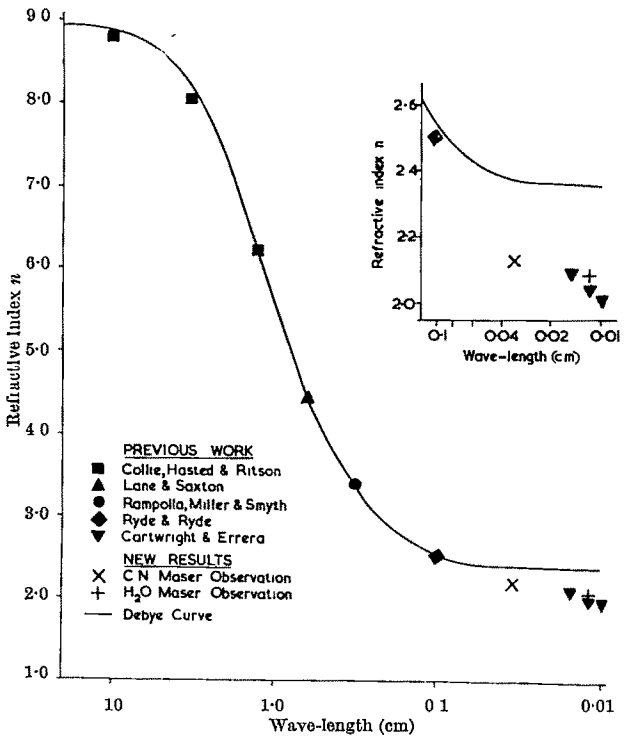


Fig. 1. Dispersion of liquid water at 20° C. The Debye curve is calculated from the parameters given in ref. 3 The inset shows an enlarged section of the lower portion of the graph

Refractive Index

Measurements have been made with a CN maser (frequency 29·7 cm^{-1}) and with a water vapour maser (frequency 84·6 cm^{-1}) using the method of determination of the absolute reflectivity at normal incidence. The results for water at 20° C are $n^2 = 4{\cdot}55 \pm 0{\cdot}08$ and $4{\cdot}30 \pm 0{\cdot}08$, respectively; and for heavy water at 20° C $n^2 = 4{\cdot}07 \pm 0{\cdot}09$ at 29·7 cm^{-1}. The data for water are plotted in Fig. 1 together with earlier microwave and far-infra-red values[4–7]. In Fig. 1 is also shown a Debye curve determined from the microwave points of Collie, Hasted and Ritson[3]. The increasing departure from Debye behaviour is clearly seen at high frequencies. If the parameter ε_∞ which Collie, Hasted and Ritson determine from their microwave data is chosen to agree with n^2 at 84·6 cm^{-1} then poor agreement is obtained at the intermediate frequencies. In fact, a good fit to the microwave and submillimetre data cannot be obtained with a Debye function.

The temperature dependence of the refractive index at 29·7 cm^{-1} has been measured over the range from 20° C to 85° C. The results are shown in Fig. 2 and it will be seen that the index rises by about 8 per cent for a 50° C increase in temperature.

Absorption Coefficient

The absorption coefficient α for liquid H_2O at 29·7 cm^{-1} and 20° C is found to be 200 ± 10 nepers/cm. The absorption due to the Debye process should reach an asymptotic value at high frequencies given by:

$$\alpha_\infty = \frac{(\varepsilon_0 - \varepsilon_\infty)\, 2\pi}{\sqrt{\varepsilon_\infty}\, \lambda_c}$$

where ε_0, ε_∞ are the dielectric constants at zero and very high frequency, respectively, and λ_c is the critical wave-length. For water, with the parameters of Collie, Hasted and Ritson this asymptotic value should be 111 nepers/cm, that is about half the observed value. We have determined the absorption spectrum of liquid water from 10 cm^{-1} to 100 cm^{-1} using an interferometer. Very thin samples had to be used (approximately 50μ thick) and the thickness of these was determined by measuring the attenuation at 29·7 cm^{-1} using the CN maser. Our results are shown in Fig. 3 together with those of other workers[8,9]. The steeply rising absorption has been found[9] to reach a maximum at 193 cm^{-1} as a shoulder on a still stronger band the central frequency of which is 685 cm^{-1}. Both these features are to be attributed to the vibrations

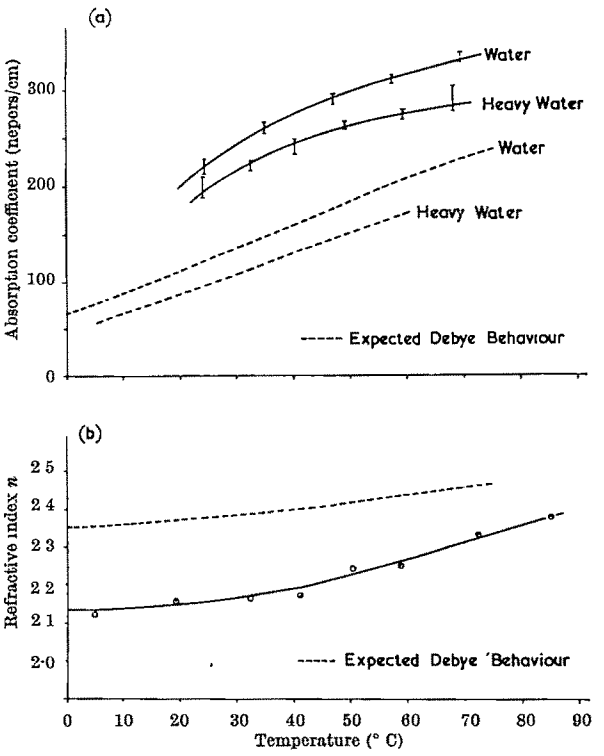


Fig 2. *a*, Variation with temperature of the absorption coefficients of water and heavy water at 337μ; *b*, variation with temperature of the refractive index of water at 337μ. In both *a* and *b* the expected Debye behaviour is calculated from the parameters given in ref 3

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of the hydrogen bonded liquid lattice[9,10]. Magat[1] has suggested an additional absorption band at 50 cm^{-1} but no distinct feature corresponding to this could be observed. Similar measurements for deuterium oxide are shown in Fig. 4.

The temperature dependence of the absorption coefficient has also been measured at 29·7 cm^{-1} using the CN maser. The results are shown in Fig. 2. The results show a considerable increase of absorption with temperature. If it is assumed that the Debye relaxation mechanism makes a distinguishable contribution to α at 29·7 cm^{-1}, the amount of this may be calculated and this is also shown in Fig 2. It will be seen that nearly all the temperature variation of the absorption can be attributed to the Debye process. Similar behaviour is shown by deuterium oxide.

Conclusions

The division of the liquid water dispersion into two regions has been confirmed by our work. At wave-lengths below 1 mm the Debye process is dominant but at shorter wave-lengths the "liquid lattice" modes[11] become more and more important so that at 29·7 cm^{-1} they already account for half the total absorption. This is also reflected in the behaviour of the refractive index where the departures from the Debye type of behaviour become marked at the higher frequencies. The temperature dependence of n is more rapid than would be expected from the Debye functions and is probably to be ascribed to the shift of the lattice bands to lower frequencies on heating.

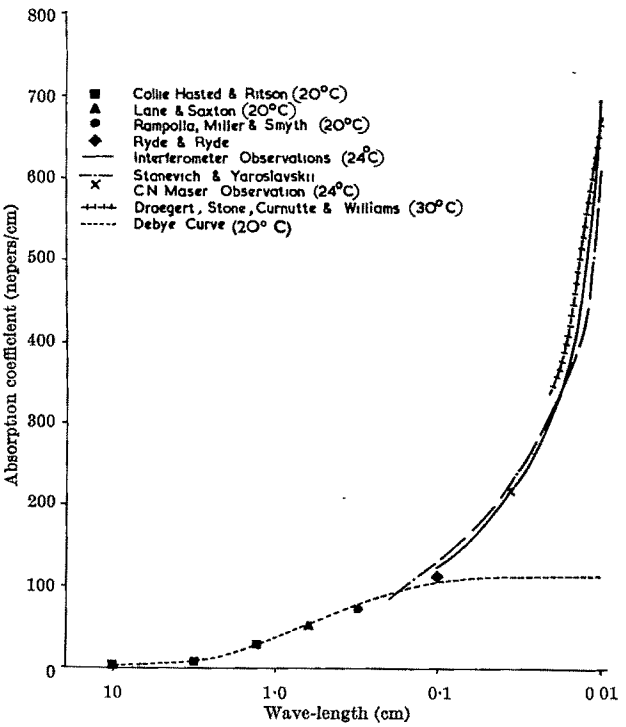


Fig. 3. Dielectric absorption of liquid water The Debye curve is calculated from the parameters given by Collie, Hasted and Ritson (ref. 3) for 20° C

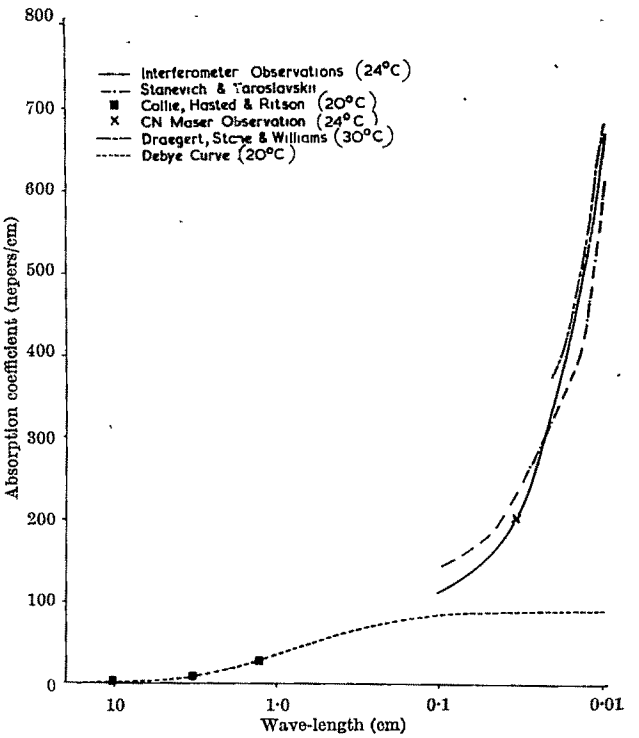


Fig 4. Dielectric absorption of liquid heavy water. The Debye curve is calculated from the parameters given by Collie, Hasted and Ritson (ref. 3) for 20° C

In spite of the reported peaks in Raman scattering[10] at 60 cm^{-1} and the well-known peak observed in slow neutron scattering at 56 cm^{-1}, no distinct peak in the infrared absorption can be detected below 193 cm^{-1}. This discrepancy indicates the presence of a significant group of low-frequency oscillations with surprisingly little infrared activity.

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CALCIUM METABOLISM IN A FRESHWATER MOLLUSC: QUANTITATIVE IMPORTANCE OF WATER AND FOOD AS SUPPLY FOR CALCIUM DURING GROWTH

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ATTENTION has repeatedly been directed to molluscs as indicator organisms for radiocontamination in fresh-water for strontium-90 (refs. 1–3), radium-226 (ref. 4), carbon-14 (ref. 5) and other radionuclides[6], as well as in sea-water for zinc-65 (refs. 7, 8), cobalt-60 and other radioisotopes[9,10].

Since only a small isotopic dilution is possible in fresh water, higher concentration ratios $\frac{(^{90}\text{Sr/g body-weight})}{^{90}\text{Sr/g water}}$ are observed for ^{90}Sr in freshwater than in marine molluscs: values of 1 or 2 in the latter animals[11,12], as compared with

60–5,000 in the former[1,6]. This higher concentration ratio makes these freshwater animals particularly useful as indicator-organisms, since measurements on these animals are relatively more accurate and simpler to perform.

Nothing is yet known about the quantitative relations in freshwater molluscs between the amount of calcium taken up directly from the water[3,13] and that extracted from the food. These data would also be of importance for the interpretation of radiocontamination results.

We therefore set up a long-term experiment, keeping young freshwater gastropods in water with a trace of ^{45}Ca, while their food (lettuce) contained no tracer. The proportion of the calcium intake which the animals took up from the water would thus be reflected in the proportion of ^{45}Ca in the body. Thus, the ratio of the specific activity of the increase of calcium in the animals during the experiment to the specific activity of calcium in the water will give the percentage of the calcium taken up from the water.

Lymnaea stagnalis (L), from a laboratory culture kept in tap-water, were used. This water contains 35 mg/l. $Ca^{\cdot\cdot}$, 8 mg/l. $Na^{\cdot}$, 4 mg/l. $K^{\cdot}$, 4 mg/l. $Mg^{\cdot\cdot}$, 25 mg/l. $C^{\cdot\cdot}$ and 12 mg/l. HCO'_3, and allows a normal development. It approximates fairly closely to the composition of the natural habitat of these animals in the neighbourhood of the laboratory. The animals used for the present investigations had a shell-length of 1·88 ± 0·04 cm, at the start of the experiment. This corresponds to about 4 mg organic nitrogen as determined by a micro-Kjeldahl method.

They were placed in groups of ten in four high-pressure polyethylene beakers filled with 1 l. tap-water, and containing 3·6 μc. ^{45}Ca/l. at a specific activity of 0·08 μc./mg calcium. In a fifth beaker, snails were kept in the same conditions but without radioisotope, in order to determine eventual effects of irradiation on the growth of the animals. Corrections for evaporation were determined in a sixth beaker without animals or food.

The lettuce is renewed daily and fed *ad lib.* This food has no influence on the concentration or specific activity of calcium in the water in the experimental conditions used.

Excrements were evacuated weekly and prepared for determination of total calcium and calcium-45. The water is changed every week to maintain its total concentration of calcium above 10 mg/l., because below this level the direct uptake of calcium could be reduced[3].

The concentrations of calcium were measured in a spectrophotometer with an acetylene-air flame (Unicam '*SP*-900'). The calcium-45 was determined by liquid scintillation counting (Packard 'Tri-Carb'). The calcium content at the beginning of the experiment was determined by analysing three groups of ten animals each of the same size as the experimental animals. Afterwards, the animals were ashed (500° C), the ash dissolved in hydrochloric acid and the mixture diluted to about 15 mg calcium/l. The experiments were stopped after 74 days. The calcium content and radioactivity of each group of ten animals from each beaker were measured as described previously.

The statistical limits indicated throughout this paper are the fiducial limits of the mean at a probability of 95 per cent[14] ($=\bar{x} \pm ts_{\bar{x}}$).

At the start of the experiment, the animals contained 16·1 ± 0·7 mg calcium each; they reached 53·1 ± 3·2 mg at the end. The mean gain was thus 36·6 ± 3·8 mg calcium per animal.

The specific activity of this gain of calcium was = 106·700 ± 7·200 c.p.m./mg (c.p.m. = counts per minute) and of the calcium in the water = 129·000 ± 6·400 c.p.m./mg. Mean ratio of these two specific activities is 0·82 ± 0·05. This indicates that about 80 per cent of the calcium originated from the calcium in the water: about 30 mg was thus supplied directly by the water, and 7 mg came from the food.

The radioactivity had no measurable effect on the uptake. The gain of calcium in the non-radioactive beaker was 38·7 mg/animal, while the highest value observed in the radioactive animals was 39·7 mg/animal.

No bacterial or algal growth that could serve as an intermediate between water and snails for calcium fixation was found. When a colloidal dye such as Evans blue was added to the water, no pigmentation in the intestine was seen and drinking by the snails can therefore be neglected.

Estimation of intestinal efficiency for absorption of calcium.

We can estimate the efficiency of the intestinal absorption of calcium using these results, the specific activity and the total calcium content in the excrements. Indeed, if we put:

c = total calcium content of the excrements,

g = non-radioactive calcium not absorbed from the food by the gut, and remaining in the excrements,

e^* = (calcium + ^{45}Ca), excreted by the gut (Intestinal excretion of calcium is real, as shown by the fact that ^{45}Ca-loaded animals which are kept in non-radioactive solutions and fed non-radioactive food excrete radioactivity in the excrements),

a^* = (Ca + ^{45}Ca) from the water and contaminating the excrements by adsorption or exchange,

then

$c = g + e^* + a^*$, or

$g = c - e^* - a^*$.

If F = calcium extracted from the food during the whole experiment, as estimated by the ratio of specific activities, then the efficiency (E) of intestinal absorption (as percentage of calcium extracted from the food) will be

$$E = 1 - \frac{F + g}{g}$$

The following results are obtained:

Total calcium content of the excrements = 4·6 ± 0·6 mg/beaker (added over a period of 10·5 weeks).

^{45}Ca-content of the same excrements: 138·000 ± 14·000 c.p.m.

Specific activity of the excrements: 29·000 ± 2·500 c.p.m./mg.

Specific activity of the water: 129·000 (= 5·170·000 c.p.m. for 44 mg/l).

The value of 138·000 c.p.m. in all the excrements of the ten snails in one beaker therefore represents $44 \times \frac{138}{5 \cdot 170}$ = 1·1 mg (calcium + ^{45}Ca) coming: (*a*) from the water by intestinal excretion of ^{45}Ca that was previously directly absorbed from the water by the animals (= e^*) and (*b*) from radiocontamination of the excrements by adsorption (= a^*).

The residue of non-radioactive calcium not absorbed from the food by the intestine and thus left in the excrements ($g = c - e^* - a^*$) amounts to 4·8 mg − 1·1 mg = 3·7 mg.

Since the ten animals in one beaker extracted about 70 mg calcium from the food, the efficiency will be given by $1 - \frac{3 \cdot 7}{70 + 3 \cdot 7} = 0 \cdot 95$, which indicates that nearly all the calcium obtainable from the food was extracted by the animals.

This estimation could possibly be affected by two errors which act in opposite direction:

(*a*) diffusion of calcium from the excrements to the water, and (*b*) intestinal excretion of non-radioactive calcium coming from the part of the shell and other calcium reserves formed before the experiment began. However, adding radioactive excrements to non-radioactive water shows only a small and slow loss of calcium from the excrements which are included in mucus.

The approximation made here is therefore only the best obtainable for the time being, since estimations based on consumption of food and its calcium content are difficult, as a result of the variations in the content of calcium and water from leaf to leaf or even within the same leaf[15].

Transport over the food chain is usually considered responsible for radiocontamination in non-planktonic aquatic animals[16–18], although direct uptake of fission products has also been observed in different animals[18,20].

In molluscs living in sea-water, such direct uptake of soluble or particulate calcium and probably also of strontium occurs[21–24]. Diffusion of calcium ions from the water to the animals could explain this uptake[23,25]. In freshwater molluscs, on the other hand, the diffusible calcium in the animal is 5 to 10 times higher than in the surrounding medium[31] and diffusion alone could thus not lead to a significant uptake of calcium ions from the water. Fromming[26] denies that direct uptake of calcium from the water is possible here, but the observations of De Vorges[27], Schoffeniels[13] and Van der Borght[3,28] have shown direct uptake of calcium from the water, occurring against an electrochemical potential gradient in *L. stagnalis*[29].

The present results confirm the quantitative importance of this uptake of calcium. Even when food is offered *ad libitum* and when the period of observation is relatively long (2·5 months versus the life-cycle of 4 months from egg to egg in laboratory conditions), about 80 per cent of the calcium fixed by the animals is derived directly from the water. As pointed out previously[3,29], an analogy exists between the uptake of calcium and that of strontium in *L. stagnalis*. It may be thus supposed that the radiocontamination by ^{90}Sr in these animals also reflects the soluble radiocontamination of the water in which they live. This may also show that food is not a necessary link in this chain. Since the whole shell does not exchange calcium or strontium with the water to an appreciable extent, assimilation of these soluble radionuclides from the water is an integrative process for a period of about one year. Absorption of soluble calcium from the gut after drinking is not a likely pathway, as is shown by the experiment with the colloidal dye. Furthermore, drinking in general is limited in freshwater animals, since they have rather to excrete water than to take it up[30].

The estimated high efficiency of calcium extraction from the food is in good agreement with the data given by Wagge[15] for the land snails *Helix aspersa*, and indicates that the strontium content of the food can also contribute to the total radiocontamination of the animals, particularly when the food has a high strontium content and is more radiocontaminated than the water.

The importance of absorption of ions such as Ca^{++} emphasizes the role of ionic regulations for radiocontamination of aquatic organisms.

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INCORPORATION OF INORGANIC PHOSPHATE INTO SUGAR PHOSPHATES DURING CARBON DIOXIDE FIXATION BY ILLUMINATED CHLOROPLASTS

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PHOTOPHOSPHORYLATION has been widely studied since its discovery in 1954[1,2]. When supplied with inorganic phosphate and a co-factor such as phenazine methosulphate (PMS), illuminated chloroplasts will carry out 'cyclic' photophosphorylation (reaction 1) of exogenous adenosine diphosphate (ADP) at rates as high as 2,000 μmoles phosphate esterified/mg chlorophyll/h.

$$\text{ADP} + \text{inorganic phosphate} \xrightarrow{\text{light}} \text{adenosine triphosphate (ATP)} \quad (1)$$

If the co-factor is omitted and the illuminated chloroplasts are supplied with nicotinamide adenine dinucleotide phosphate (NADP) as well as ADP and inorganic phosphate, 'non-cyclic' photophosphorylation[3] occurs (reaction 2).

$$\text{NADP} + \text{H}_2\text{O} + \text{ADP} + \text{inorganic phosphate} \xrightarrow{\text{light}} \text{NADPH}_2 + \text{ATP} + \tfrac{1}{2}\text{O}_2 \quad (2)$$

Ferredoxin, a non-haem iron-containing protein, which is readily lost from chloroplast fragments, has been shown to participate in reaction 2 and must be added back to 'broken' chloroplasts to obtain the best rates of phosphate esterification.

The discovery of photophosphorylation was of the utmost importance because it showed that there was a mechanism within the chloroplast which could produce ATP in a reaction which was driven by light rather than by the consumption of a food material. Considerable attention was therefore given to the actual rates. According to present views[4], nine molecules of ATP and six molecules of $NADPH_2$ are required in photosynthesis for every three molecules of carbon dioxide converted to triose phosphate. The intact leaf can reduce carbon dioxide to sugar at rates as high as 180–200 μmoles/mg chlorophyll/h (ref. 5). This would require a photophosphorylation rate of about 600 μmoles/mg chlorophyll/h. When rates of this magnitude were actually demonstrated, it became clear that the chloroplast might be self-sufficient in the sense that it could be induced to form ATP at a rate which was more than ample for whole-cell photosynthesis.

In 1954, Arnon, Allen and Whatley[1] also showed that in addition to their ability to catalyse photophosphorylation, isolated chloroplasts could reduce carbon dioxide to carbohydrate. Since this first demonstration, the actual rates of carbon dioxide fixation have been improved by a factor of ten but the best[6–8] are still only 10–20 per cent of those attained by the intact leaf. There has been some tendency to argue that carbon dioxide fixation is unlikely to be limited by photophosphorylation since the actual phosphorylation mechanism must clearly retain its ability to function effectively in the isolated chloroplast. As Gibbs[9] has pointed out, such arguments should be regarded with caution and the fact that the highest rates of photophosphorylation have been obtained in the presence of added ADP and 'non-physiological' co-factors, such as PMS and pyocyanine, must not be overlooked. Also, maximal rates are only obtained with disrupted chloroplasts and there are some grounds for supposing that the chloroplast with an intact envelope may be impermeable to ADP and therefore incapable of supporting photophosphorylation of exogenous ADP[7].

For these and other reasons it would be of value if endogenous photosynthetic phosphorylation could be studied in chloroplasts which still retain some ability to fix carbon dioxide. This article reports a first attempt to measure such light-induced incorporation of inorganic phosphate into sugar phosphates by intact chloroplasts. Reactions were carried out in the absence of added ADP, NADP, ferredoxin or co-factors of cyclic photophosphorylation. The method used was one in which chloroplasts were incubated with inorganic phosphate containing phosphorus-32. After illumination in saturating red light at 20° C the inorganic phosphate was precipitated by one of two methods[10,11] and the radioactivity in organic phosphates remaining in solution measured by placing samples on planchettes in a Geiger–Müller counter. Other samples were chromatographed in two dimensions in butanol–acetic acid and water saturated phenol on paper previously washed with oxalic acid[12].

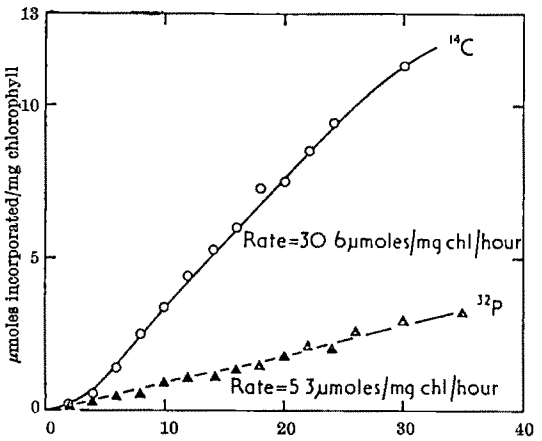


Fig. 1

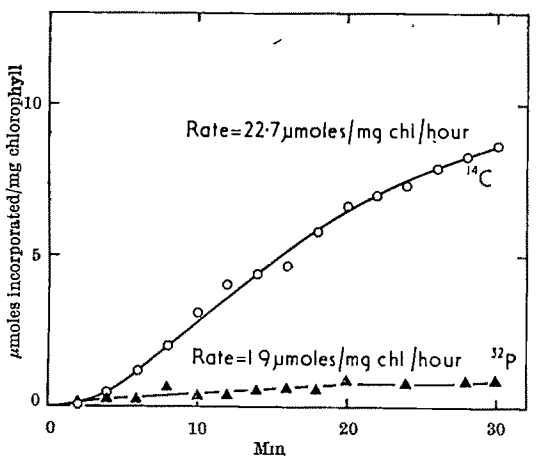


Fig. 2

Figs. 1 and 2. Simultaneous measurements of carbon dioxide fixation and phosphate incorporation by intact chloroplasts. In Fig. 1 ribose-5-phosphate was added to both reaction mixtures to promote carbon-dioxide fixation and in Fig. 2 this was replaced by a mixture of fructose-1, 6-diphosphate and dihydroxyacetone phosphate (see text). In Fig. 1 each reaction mixture contained 0·05 mg chlorophyll and in Fig. 2, 0·55 mg

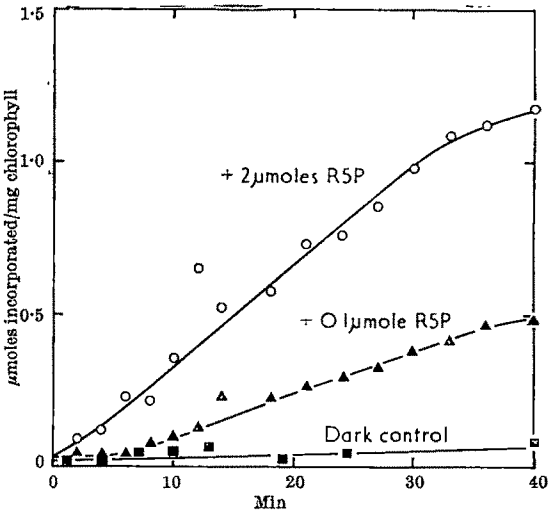


Fig. 3. Incorporation of inorganic phosphate as a light-induced reaction. The middle curve shows the effect of lowering the level of added sugar phosphate to a 'catalytic' level. Chlorophyll, 0·096 mg

Chloroplasts were isolated from 14-day-old pea seedlings in 0·33 M sucrose, 0·1 M phosphate (*p*H 6·8) containing small amounts of magnesium chloride, sodium chloride and sodium isoascorbate[6,7,13] and resuspended in a similar medium from which the phosphate had been omitted and the sucrose increased to 0·45 M. Normally, between 50 per cent and 80 per cent of the chloroplasts in such preparations have intact envelopes[13]. These retain their stroma and in suitable reaction mixtures will fix carbon dioxide at rates between 20 and 40 μmoles/mg chlorophyll/h[13]. Chloroplasts (0·1 ml.) in suspending medium were illuminated in reaction mixtures (0·3 ml.) containing (in addition to the sugar phosphates noted elsewhere in the text) reduced glutathione, 1·0 μmole; manganese chloride, 0·5 μmoles; magnesium chloride, 0·25 μmoles; EDTA, 0·5 μmoles; inorganic phosphate, 0·5 μmoles; tricine[14] buffer, 7·5 μmoles, *p*H 7·5; carbonate–bicarbonate, 2·95 μmoles; sucrose, 30·0 μmoles. Reaction mixtures used to follow carbon dioxide fixation contained a proportion of the carbonate as carbon-14 (50 μc.) and those for phosphate determinations phosphorus-32 (30 μc.). In the former, any $^{14}CO_2$ not fixed after illumination was driven off when samples were acidified with four parts of 0·2 N hydrochloric acid. In the latter, sampling was followed by neutralization with ammonium hydroxide and the addition of 5·8 parts of a mixture of magnesium chloride, ammonium chloride and ammonium hydroxide to precipitate inorganic phosphate[10]. Precipitation was allowed to proceed overnight and a small additional quantity of cold inorganic phosphate was then added to give a suitably bulky precipitate. Alternatively[11], samples were acidified with four volumes of 1 N perchloric acid followed by 8 vols of 0·1 M ammonium molybdate and 2 vols of 0·1 M triethylamine HCl (containing a trace of bromine). Finally, the precipitates were compacted by centrifugation at 2,000*g* for 10 min and samples of the supernatant (the organic phosphate fraction) withdrawn with a micro-syringe.

Figs. 1 and 2 show the results of experiments in which carbon dioxide fixation and organic phosphate were measured simultaneously in similar reaction mixtures.

In Fig. 1 carbon dioxide fixation was promoted[6,13] by the addition of 2 μmoles of ribose-5-phosphate, and in Fig. 2 this was replaced by a mixture of fructose-1,6-diphosphate (2 μmoles) and dihydroxyacetone phosphate (0·1 μmole). These sugar phosphates were added in amounts sufficient to give maximal rates of carbon dioxide fixation though it has been demonstrated that such fixation will occur at slower rates in the presence of catalytic quantities of sugar phosphates and even in their complete absence[8,13]. In the experiments illustrated in Figs. 1 and 2 it was not possible to determine the organic phosphate level sufficiently accurately to be certain that the characteristic initial lag in carbon dioxide fixation[13]

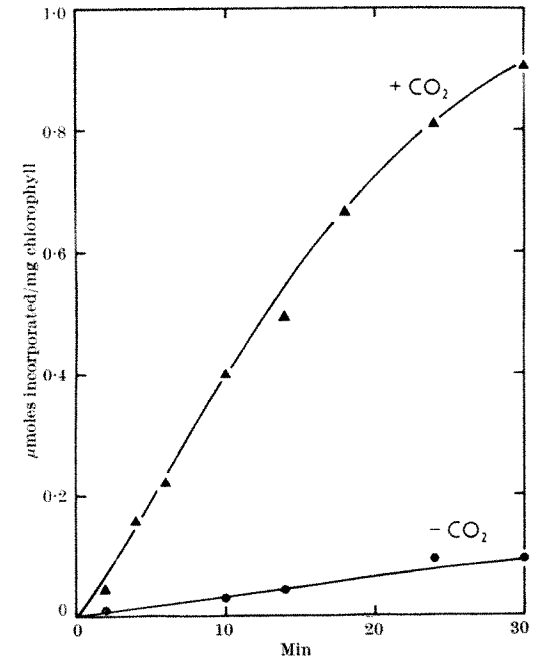


Fig. 4

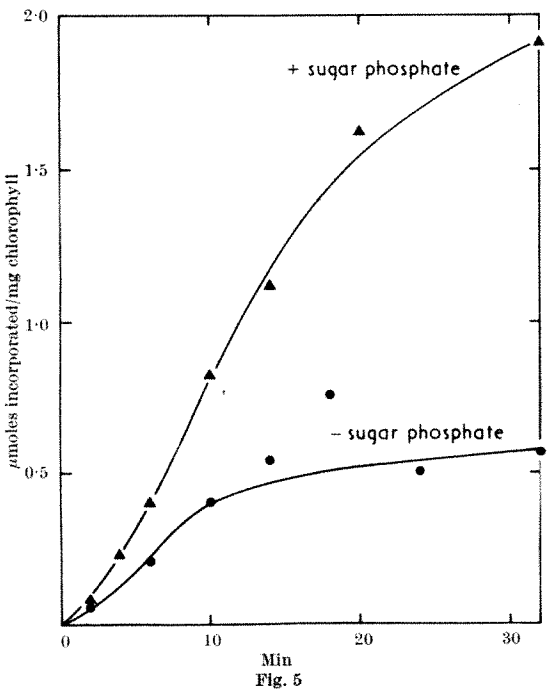


Fig. 5

Figs. 4 and 5. Dependence of phosphorus-32 incorporation on carbon dioxide fixation. In Fig. 4 the lower curve shows the effect of omitting carbon dioxide from the reaction mixture. In Fig. 5 the lower curve shows the effect of omitting the sugar phosphate normally added to promote carbon dioxide fixation. Chlorophyll, 0·176 mg (Fig. 4); 0·091 mg (Fig. 5)

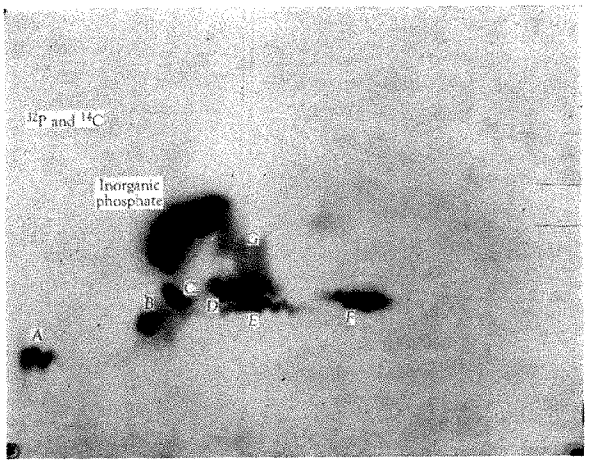


Fig. 6

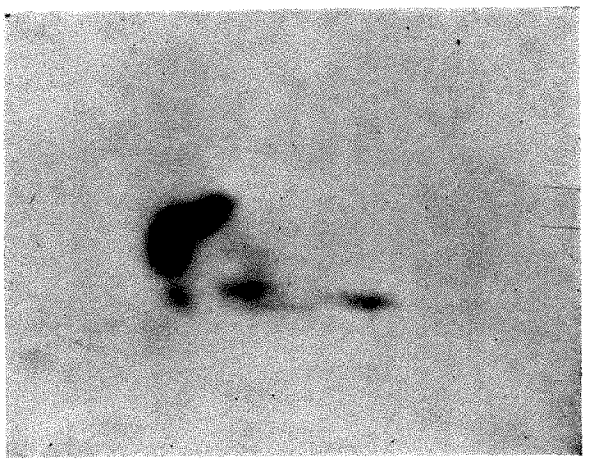


Fig. 7

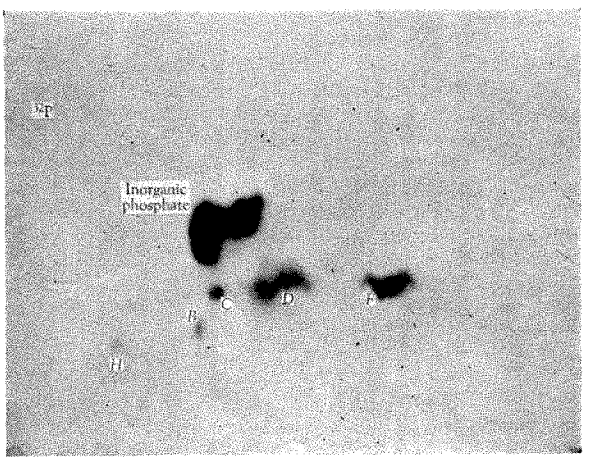


Fig. 8

Figs. 6, 7 and 8. Incorporation of phosphorus-32 and carbon-14 into sugar phosphates by illuminated chloroplasts. Fig. 6 shows an autoradiograph obtained after co-chromatography of samples from two reaction mixtures containing carbon-14 and phosphorus-32, respectively (from which the inorganic phosphate had not been precipitated). Fig. 7 is an autoradiograph of the same chromatogram in which a sheet of cardboard was interposed between the chromatogram and the film so that the latter was largely exposed by phosphorus-32 rather than carbon-14. Fig. 8 is an autoradiograph prepared after chromatography of a similar reaction mixture which contained phosphorus-32 but not carbon-14. In this experiment, added ribose-5-phosphate was replaced by a mixture of fructose-1,6-diphosphate and dihydroxy-acetone phosphate (see text) and though the products were much the same there was rather less radioactivity in *A*. The major product, *F*, is dihydroxyacetone phosphate, *A* is thought to be largely ribulose diphosphate and *B*, *C*, *D* and *E* mostly hexose and pentose monophosphates. Solvents (→) water-saturated phenol followed by (↑) butanol (222)-acetic acid (57)-water (150)

was paralleled by a similar lag in organic phosphate formation, though in subsequent experiments an initial lag in esterification became more evident (Figs. 3 and 5). However, the overall increase of phosphorus-32 in the organic fraction, though small, was quite unambiguous and occurred only in the light (Fig. 3). Radioactivity was detected in the organic fraction in the dark but did not increase with time and is thought to be largely a consequence of incomplete precipitation of inorganic phosphate. After 40-min illumination, the radioactivity in the organic fraction was often as much as 10–20 times that of reaction mixtures kept in the dark.

The extent to which phosphorylation and carbon dioxide fixation were linked is also shown in Figs. 4 and 5. When carbon dioxide was omitted (Fig. 4) there was a marked decrease in the amount of phosphorus-32 incorporated into the organic fraction. Similarly, omission of the sugar phosphate normally added to promote carbon dioxide fixation[13] caused a corresponding decrease in esterification (Fig. 5). When sugar phosphate was added in a catalytic quantity[13] the rate of esterification was diminished (Fig. 3).

Chromatography showed that carbon-14 and phosphorus-32 were largely incorporated into the same products. Fig. 6 shows an autoradiograph of a two-dimensional chromatogram which was prepared by co-chromatography of two reaction mixtures, one containing carbon-14 and the other phosphorus-32. Fig. 7 shows an autoradiograph of the same chromatogram except that a sheet of cardboard was interposed between the chromatogram and the film so that the latter has been largely exposed by phosphorus-32. Fig. 8 shows a chromatogram from a similar experiment in which only phosphorus-32 was used (and in which, incidentally, fructose-1,6-diphosphate rather than ribose-5-phosphate was added to promote carbon dioxide fixation). These results are consistent with our previous experience[6,7,13] that the products of carbon dioxide fixation under these conditions are largely sugar phosphates. The major product, *F*, has been identified (by its chromatographic behaviour in a number of different solvents) as dihydroxyacetone phosphate. Product *A* is believed to be largely ribulose diphosphate and most of the radioactivity in *B*, *C*, *D* and *E* (which also contain both carbon and phosphorus) is present in compounds whose chromatographic characteristics are similar to those of pentose and hexose monophosphates.

In the photosynthetic carbon dioxide fixation cycle there are two reactions in which ATP, produced by photosynthetic phosphorylation, is thought to be consumed[4]. In one, 1,3-diphosphoglycerate is formed from 3-phosphoglycerate, but this is immediately followed by the reduction to glyceraldehyde-3-phosphate in which the newly introduced phosphate group is released as inorganic phosphate. The only step leading to an incorporation of phosphate is therefore the reaction in which ribulose-5-phosphate is converted to ribulose-1,5-diphosphate prior to the carboxylation leading to 3-phosphoglycerate. In addition, in the complete cycle, there are two reactions in which sugar diphosphates are hydrolysed to the corresponding monophosphates[4]. These also contribute to the release of newly introduced phosphate so that for every nine molecules of ATP consumed only one molecule of phosphate is incorporated into triose phosphate according to the overall equation:

$$3CO_2 + 3H_2O + H_3PO_4 \rightarrow \text{triose phosphate} + 3O_2 + H_2O \quad (3)$$

If the photosynthetic cycle occurs in chloroplasts in the presence of phosphorus-32 the radioactivity of the sugar phosphate fraction should increase until it eventually reaches a steady-state level which should be the same as that of the inorganic phosphate. The specific activity of the latter should decrease as it is diluted by non-radioactive phosphate released by hydrolysis of non-radioactive sugar phosphates added to the reaction mixture, or already present in the chloroplasts.

In the experiments carried out so far (in which ribose-5-phosphate was added to reaction mixtures to promote carbon dioxide fixation), the overall rate of phosphate esterification was about one-sixth of the rate of carbon dioxide fixation (see, for example, Fig. 1). Such a rate is consistent with the appearance of phosphorus-32 in triose phosphate according to equation 3 and the subsequent conversion of the triose phosphate to other compounds (for example, hexose and pentose monophosphates) in which the ratio of carbon : phosphorus is less than 3 : 1. With added fructose-1,6-diphosphate the ratio was nearer 11 : 1 (see, for example, Fig. 2) and this may in part be attributable to dilution of the labelled inorganic phosphate by enzymatic hydrolysis of the added diphosphate. It should be noted, however, that in the experiments in which ribose-5-phosphate was added to the reaction mixtures in substrate amounts (for example, Figs. 1 and 4) it still did not apparently act as a phosphate acceptor at a rate which was unrelated to that of carbon dioxide fixation.

The rate of endogenous ATP formation must of course be related to the rate at which the limited quantity of ADP within the chloroplast is again made available for further esterification and this in turn is likely to be dependent on the rate at which ATP is utilized in carbon dioxide fixation (Fig. 9).

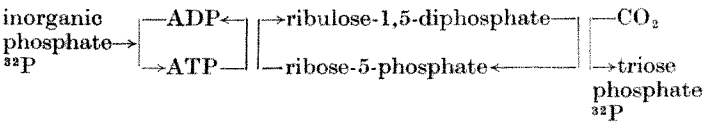


Fig. 9

In such a situation, enhanced carbon dioxide fixation could lead to increased phosphate esterification and increased esterification could in turn bring about enhanced fixation. This could contribute to the autocatalytic acceleration of carbon dioxide uptake in isolated chloroplasts[8,13]. It would also pose the important question of what constitutes the rate-controlling mechanism and to what extent this, rather than any artefact of extraction, limits the rate of fixation by isolated chloroplasts. The present experiments provide no answer to this question but they do show that it is possible to measure the light-induced incorporation of phosphorus-32 into sugar phosphates by isolated chloroplasts and that the rate is affected by factors which promote carbon dioxide fixation. A fuller clarification of this system would undoubtedly enhance our knowledge of regulatory mechanisms in photosynthesis.

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METHYLATION OF NUCLEIC ACIDS IN KREBS II ASCITES TUMOUR CELLS

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THE discovery of methylated bases in both ribonucleic acid and deoxyribonucleic acid[1–4] has stimulated the study of nucleic acid methylation at both the enzymatic and cellular levels. Enzymatic methylation of soluble RNA (*s*RNA)[5–8] has been shown to occur at the polynucleotide level with *S*-adenosyl-L-methionine as the methyl group donor in bacterial and animal cell-free systems. In addition, enzymes have been isolated from *Escherichia coli* capable of methylating bases in ribosomal RNA[9] and in DNA[10].

The *in vivo* methylation of ribosomal and soluble RNA, and in some cases DNA, has been investigated using *E. coli*[11,12], sea-urchin embryos[13], *Blastocladiella emersonii*[14] and recently HeLa cells[15]. These investigations were based on the assumption that the methylation of both RNA and DNA bases occurs after synthesis of the primary structure and that the incorporation of label from ^{14}C-[methyl]-L-methionine would be a specific indication of such a process.

The work reported here is an attempt, on similar lines, to investigate the *in vivo* methylation of nucleic acids occurring in Krebs II ascites tumour cells of the mouse and was prompted by the observation of Bergquist and Matthews[16] that *s*RNA from certain tumour cells contains the highest levels of methylated bases in *s*RNA ever recorded, and also that methylation in nucleated cells has been shown to occur primarily in the nucleolus rather than in the chromatin[17,18].

The method described by Martin *et al.*[19] was used for the cultivation of Krebs II ascites tumour cells. After they had been collected, the cells were washed in phosphate-buffered saline and suspended in Hanks's medium supplemented with horse serum as described by McNamara[20]. The cells in this medium were incubated at 37° C at a concentration of 10^7 cells per ml. Such a procedure was found to be necessary by McNamara[20] and by me to maintain RNA synthesis in these cells.

After incubation with appropriate additions the cell suspension was made 1 per cent with respect to sodium dodecyl sulphate (SDS) and 0·5 ml./2·5 per cent bentonite was added. After allowing to stand at room temperature for 5 min the suspension was extracted with 80 per cent phenol with vigorous shaking for 10 min at room temperature. The resulting emulsion was separated by centrifugation at 10,000*g*, the aqueous phase removed, re-extracted with phenol and then with ether to remove traces of phenol. After the removal of ether with a stream of nitrogen, the addition of two volumes of ethanol precipitated the cell DNA and RNA. This preparation of total cell nucleic acids, after further ethanol precipitation, was, in some experiments, submitted to chromatography on methylated albumin kieselguhr (MAK) as described by Comb *et al.*[21].

From this nucleic acid preparation, DNA could be isolated by the method of Marmur[22], after RNase treatment (50 μg/ml. for 30 min at 25° C), and also RNA, by treatment of another portion with DNase (10 μg/ml.) for 5 min at 37° C in the presence of 5 mM magnesium chloride and 3 mg/ml. bentonite, followed by phenol extraction and repeated ethanol precipitation as already outlined.

When the total cell nucleic acids were isolated after incubating Krebs II tumour cells with 2 μc./ml. ^{14}C-[methyl]-L-methionine for 2 h and submitted to chromatography on MAK columns a separation of *s*RNA, DNA and ribosomal RNA was achieved, similar to that described by Comb *et al.*[21]. One disadvantage of the technique when applied to the nucleic acids of mammalian cells is that a good separation of the two ribosomal RNA components cannot be accomplished. However, by measuring the optical density of each fraction and the radioactivity of the precipitate produced by adding trichloroacetic acid (TCA), it was possible to calculate the degree of methylation of each nucleic acid species. The results of this experiment are shown in Table 1, from which it is clear that in Krebs II tumour cells the degree of methylation of the *s*RNA is exceedingly high when compared with the ribosomal RNA.

Table 1. SPECIFIC RADIOACTIVITIES OF DNA, RIBOSOMAL AND SOLUBLE RNA OF KREBS II ASCITES TUMOUR CELLS AFTER INCUBATION OF THESE CELLS WITH ^{14}C-[METHYL]-METHIONINE FOR 2 H

Fraction	c p m /μg nucleic acid
*s*RNA	996
DNA	38
Ribosomal RNA	62

Separation of these fractions was achieved by MAK chromatography as described in the text. Each fraction was assayed for optical density at 260 mμ and TCA-insoluble radioactivity as described in the legend to Fig. 1 for the assay of fractions collected after sucrose-density gradient experiments.

If the DNA labelled in such experiment is isolated and hydrolysed to its component bases with formic acid under the conditions of Vischer and Chargaff[23] and the resulting hydrolysate submitted to paper chromatographic analysis a single radioactive component was found which behaved as 5-methyl cytosine.

Similarly, when RNA isolated from the same experiment was hydrolysed with perchloric acid under the conditions of Marshak and Vogel[24], and following the procedure outlined by Brown and Attardi[15], it was found that, if the black insoluble material formed by the polymerization of ribose moieties during the hydrolysis was assayed for radioactivity, it accounted for 6–7 per cent of the total RNA radioactivity. Thus it would appear that about 93–94 per cent of the methyl groups in Krebs II cell RNA are present as methyl groups on the RNA bases and the remainder as methyl groups on the ribose moieties. Indeed, Brown and Attardi[15] found that up to 80 per cent of the methyl groups in isolated ribosomal RNA of HeLa cells are present as methyl groups on the ribose moieties and they suggest that these methyl groups would be present as 2-*O*-methyl ribose. Paper chromatographic analysis of the bases liberated from the RNA after both formic acid and perchloric acid hydrolysis indicated that no radioactivity derived from the methyl group of L-methionine could be found in any of the four RNA bases. Identification of the methylated bases labelled in these experiments is difficult owing to the low specific

radioactivities involved, but so far the following labelled bases have been tentatively identified: 6-methyl amino purine, 2-methyl adenine, 1-methyl guanine and thymine. However, at least three more methylated bases remain to be identified and work is in progress to achieve this.

In another 2-h labelling experiment with ^{14}C-methionine the RNA was isolated and submitted to sucrose density gradient centrifugation (Fig. 1*A*). Three peaks of optical density at 260 mμ were observed corresponding to 30*S*, 19*S* and 4*S* RNAs[25], and TCA-insoluble radioactivity was detected in positions corresponding to 30*S*, 19*S* and 4*S*. Once again the high specific radioactivity of the *s*RNA (4*S*) was observed. Moreover, the two ribosomal RNA components (30*S* and 19*S*) were not equally methylated. The 19*S* RNA contained about 1·2 times more methyl groups than the 30*S* component. A similar phenomenon was also observed by Brown and Attardi[15] in their studies on HeLa cells. However, in addition to these peaks of radioactivity there is a further peak of activity sedimenting faster than the 30*S* RNA in a position corresponding to about 35*S* as well as some, less well defined, of even higher *S* value (about 45–50*S*). In a similar experiment carried out in the presence of actinomycin *D* (2 μg/ml.), no significant labelling could be detected in the 35*S* and 30*S* regions (Fig. 1*B*), although a very small amount could be detected in the 19*S* region and slightly more in the 4*S* region. This observation that actinomycin inhibits the labelling of the high-molecular-weight RNA indicates that the labelling occurring in the absence of actinomycin is not simply due to a protein contamination of the RNA preparation, since actinomycin *D* does not affect the incorporation of amino-acids into protein by these cells under these conditions.

In order to be certain that the high degree of labelling encountered in the 4*S* region (see Fig. 1*A*) is due to the incorporation of methyl groups into the bases (or ribose moieties) of *s*RNA, the fractions in the 4*S* region were pooled and tested for alkaline stability. An incorporation of methyl groups into some of the bases of *s*RNA would be expected to give a product with the same alkaline stability of ordinary RNA. On the other hand, the ester bond of methionyl-*s*RNA would be expected to show the alkaline lability reported for other types of amino-acyl RNA[8]. The radioactivity occurring in the region of 4*S* RNA isolated from Krebs II cells after incubation with

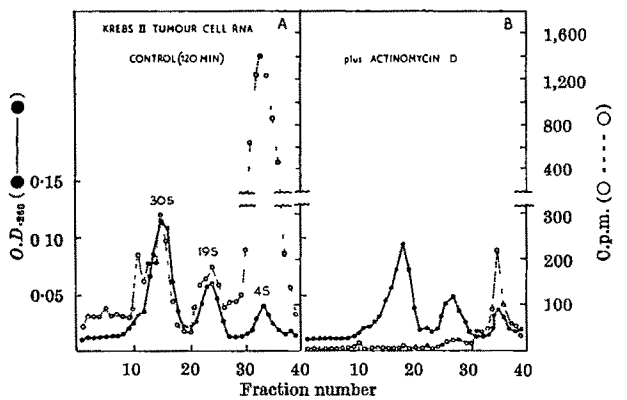


Fig. 1. Sucrose density gradient analysis of RNA from Krebs II ascites tumour cells labelled with ^{14}C-[methyl]-methionine (2 μc /ml.) over a period of 2 h in the presence (*B*) and absence (*A*) of actinomycin *D* (2 μg/ml.)
0·1 ml. of RNA in 0·02 M *tris*-hydrochloric acid buffer, *p*H 7·1, was layered on to 4·6 ml. linear sucrose gradients of from 5 per cent to 20 per cent concentration in 0·02 M *tris*-hydrochloric acid, *p*H 7·1–0·1 mM magnesium chloride and 0·01 per cent with respect to SDS and then centrifuged at 39,000 r.p.m. in the *SW* 39 rotor of the Spinco model *L* ultracentrifuge for 3 h. Fractions were then collected, after puncturing the tube, into 2 ml. portions of 0·02 M *tris*-hydrochloric acid, *p*H 7·2, 0·01 per cent with respect to SDS. Each fraction was first assayed for optical density at 260 mμ and then for acid-insoluble radioactivity by collection of 5 per cent TCA-insoluble material on a 'Millipore' filter and counting these filters, after drying, in 5 ml. of scintillator fluid (5 per cent 2,5 diphenyloxazole plus 0·3 per cent 1,4-*bis*-2-(5-phenyloxazolyl)-benzene in toluene) using a Nuclear Chicago scintillation spectrometer

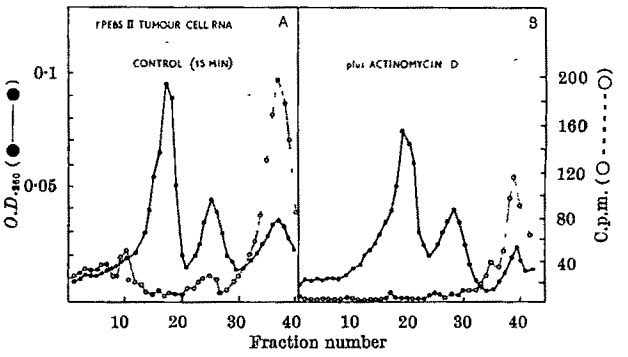


Fig. 2. Sucrose density gradient analysis of RNA from Krebs II ascites tumour cells labelled with ^{14}C-[methyl]-L-methionine (2 μc /ml.) over a period of 15 min in the presence (*B*) and absence (*A*) of actinomycin *D* (2 μg /ml). Remainder of conditions as for Fig. 1

^{14}C-methionine for 2 h was completely resistant to treatment at 37° C at *p*H 8 (or 9) for 30 min under the conditions described by Svensson *et al.*[8], whereas under the same conditions the half-life of methionyl-RNA is about 19 min (ref. 8). Incubation of the Krebs II 4*S* ^{14}C-methyl labelled RNA, on the other hand, at *p*H 13 for 1 h, or with 50 μg/ml. RNase for 30 min at 25° C, renders the radioactivity completely acid-soluble.

If, instead of incubating the Krebs II cells with ^{14}C-methionine for 2 h, a shorter incubation period of 15 min is used, then the pattern of RNA labelling is somewhat different (Fig. 2*A*). Practically no acid-insoluble radioactivity occurs in the 30*S* region although there is already some in the 19*S* region. As was the case with the 2-h incubation period, the 4*S* region is extensively labelled, and again there is a significant amount of activity in the 35*S* region. If the experiment is carried out in the presence of actinomycin *D* no significant label appears in any fraction other than the 4*S* region. Labelling of the 30*S* fraction does not occur until after 1 h of incubation with ^{14}C-methionine.

Although these experiments do not suggest a biological role for the process of methylation in tumour cells, they indicate that the DNA, as well as ribosomal and soluble RNA of Krebs II tumour cells, are methylated *in vivo* with L-methionine as donor of methyl groups presumably by way of *S*-adenosyl-L-methionine as intermediate. The high degree of methylation of tumour *s*RNA first encountered by Bergquist and Matthews[16] is amply demonstrated. Furthermore, because many investigations have been undertaken to determine the function of rapidly labelled high-molecular-weight RNA in rapidly growing, undifferentiated mammalian cells, such as Krebs II ascites tumour[20], HeLa[26] and *FL* cells[27], the methylation of the 35*S* component noted in these experiments is of considerable interest. It has been suggested[20,26,28] that this component together with a 45*S* component are precursors of ribosomal RNA. The experiments reported here suggest that it is the 35*S* component which is methylated at an early stage, possibly in the nucleolus[17,18] after the formation of the high-molecular-weight RNA species on a DNA template. The absence of an extensively methylated RNA component in the 45*S* region, the first type of RNA to be synthesized in HeLa cells[26] and *FL* cells[27], might be reasonable in view of the evidence of Yoshikawa-Fukada *et al.*[27], which suggests that this high-molecular-weight component is a 'messenger' type RNA.

Treatment of the tumour cells with actinomycin *D* considerably depresses the methylation of all RNA components and suggests that RNA methylation is a process which proceeds only in the presence of a continual supply of nascent RNA. The methylation of the 4*S* component occurring even in the presence of actinomycin *D* may only be due to the fact that actinomycin *D* does not completely suppress the synthesis of *s*RNA[25,29].

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RELATIVE ORIENTATION OF GENE, MESSENGER AND POLYPEPTIDE CHAIN

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IN studies on the colinearity between the *Escherichia coli* tryptophan synthetase *A*-gene and *A*-protein, two cases were observed in which different amino-acid substitutions occurred at the same position in the *A*-protein[1]. In strains *A*23 and *A*46 a single glycine residue (Gly I) in tryptic peptide TP3 was replaced by arginine and glutamic acid respectively, and in strains *A*58 and *A*78 another glycine residue (Gly II), in the adjacent peptide TP6, was replaced by aspartic acid and cysteine respectively. As a result of subsequent investigations of the amino-acid substitutions associated with primary site reversions at these sites, two families of proteins have been recognized, in which the substituent amino-acids are related to the parent glycine residues by a series of single-step mutational events[2–4]; these are shown in Fig. 1. The probable DNA codons for the different amino-acids are also indicated[5,6]. (Oligonucleotide sequences are expressed according to the accepted convention starting with the 5′-terminus on the left, unless indicated otherwise. Throughout this article codons of the DNA strand possessing the same sequence of nucleotides as the messenger are used.)

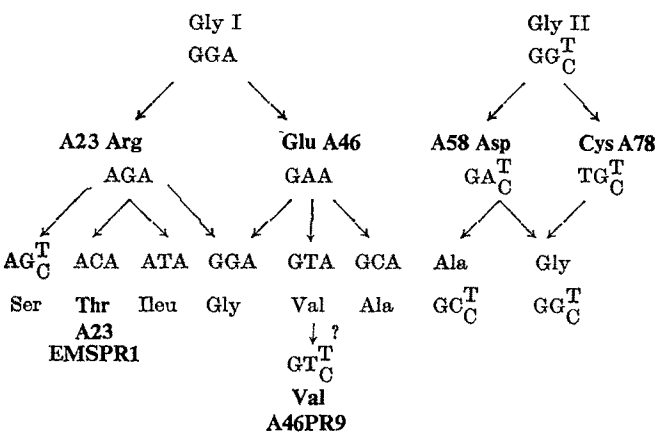


Fig. 1. The amino-acid substitutions observed at the critical sites in TP3(I) and TP6(II) of the tryptophan synthetase *A*-protein. The associated nucleotide sequences represent those of the DNA strand which has the same sequence as the *m*RNA. The strains shown in bold-face type have been used in investigations on intra-codon recombination. DNA codon assignments (refs 5 and 6) are based on the RNA codons determined by *in vitro* investigations (refs. 7 and 8)

The existence of several pairs of mutant strains differing in the composition of only one codon has permitted an investigation of amino-acid substitutions associated with intra-codon recombination[2,4,9]. The purpose of this report is two-fold: (*a*) to summarize the information obtained from intra-codon recombination investigations, and (*b*) to present the results of three-point genetic tests which orient the *A*-gene, its map, the messenger-RNA for the tryptophan operon, and the polypeptide chain of the *A*-protein, relative to one another.

Intra-codon Recombination

Reciprocal crosses were performed by transduction with phage *P1kc* lysates prepared on each donor strain, and a *cys*⁻ derivative of each recipient strain; the details of the method, the precautions taken, and calculation of recombination frequencies have been described previously[4,9]. The results of six crosses between strains having different amino-acids at the same sites are shown in Table 1. Tryptophan independent recombinants were obtained in four of the crosses: Glu × Arg, Val × Arg, Glu × Thr and Cys × Asp. According to the general rules which have been recognized for intra-codon recombination[10], this indicates that the corresponding pairs of codons differ in nucleotide composition at two or three positions within the triplet. Two of the crosses failed to produce tryptophan-independent recombinants (namely, Glu × Val and Arg × Thr) and this can be interpreted as being due to the presence of different nucleotides at only one position in the respective codon. These predictions are readily explained in terms of the ancestry of the strains as illustrated in Fig. 1. The Arg and Glu, and Asp and Cys pairs of mutants are derived from the same parent, presumably by single mutational events; the observed recombination and assigned DNA codons (Fig. 1) indicate that, in each member of a pair, a different nucleotide position in the parent codon was affected. The Val × Arg and Glu × Thr strains are separated by at least three mutational events and, consequently, different nucleotides could occupy one, two or three positions in their respective altered codons. In the Glu × Val, Arg × Thr pairs, one

partner is presumed to be the immediate parent of the other, allowing only a single nucleotide difference between the two codons. Recombination to tryptophan independence was not found here, even though it now appears that the *A46PR9* (Val) strain is separated from its parent *A46* (Glu) by more than one mutational event (see later).

The observed frequencies of intra-codon recombination (0·001–0·003 per cent) are very low. Nevertheless, they are of the order anticipated from calculations based on either (*a*) the length of the *A*-gene (4 per cent, four recombination units)[1], the number of amino-acid residues in the *A*-protein (280), and three nucleotides per codon, which give a value of 0·002 per cent between nucleotide pairs, or (*b*) the average experimental value for the ratio of genetic map distance per amino-acid residue in the *A*-protein of about 0·02[1], which gives a somewhat higher figure, 0·007 per cent per nucleotide pair.

The *A*-proteins of selected recombinants were examined in primary structure investigations. In the cross between mutants *A23* × *A46* only glycine was found at the critical position. Recombination should give a second codon in addition to that of the original wild-type strain. In the *A23* × *A46* cross, this codon would correspond to the amino-acid lysine. It is unlikely that a recombinant with this amino-acid at the *A23–A46* position could be detected, since the protein with the similar amino-acid, arginine, at this position, is inactive. This argument is strengthened by the fact that *A46* never reverts to a strain in which lysine replaces the *A46* glutamic acid[6], although this replacement would involve only a single nucleotide change and therefore would be permissible. Lysine is also the amino-acid that corresponds to the second recombinant codon expected in the *A46* × *A23* *EMSPR1* cross, and the same arguments can be offered for the absence of such recombinants. Analyses of the *A*-protein of recombinants from this cross revealed the presence of alanine at the critical site in the *A*-proteins of three strains. A fourth isolate, bearing a glycine residue, is not compatible with recombination and it has therefore been attributed to a reversion event. In terms of the likely codons (Fig. 1), the results of the cross between *A23* × *A46PR9* (Arg × Val) are also somewhat unusual. The only Val codon that can be derived from GAA (Glu) by a single mutational event is GTA. Crossing AGA × GTA should produce GGA (Gly) and ATA (Ileu) recombinants. Since strains with isoleucine at this position are known to have the partial revertant phenotype, and since the plating medium contained 5-methyl tryptophan which restricts recoverable types to strains with glycine, alanine or serine, only the GGA (Gly) recombinant type was expected. The simplest explanation for the anomalous appearance of recombinants containing Ser as well as the expected recombinants with Gly is that the strain used in the cross, *A46PR9*, is the product of two mutational events GAA (Glu)→GTA (Val) → GT^T_C (Val) (see Fig. 1). The second event, from GTA (Val) → GT^T (Val), could have occurred subsequent to the isolation and characterization of the original revertant strain. The other new codons that would be derived from the *A23* × *A46PR9* cross (see Table 1) corresponding to isoleucine and valine would not be expected to be represented in recombinants because of the 5-methyl tryptophan supplement. The *A46* × *A46PR9* cross could yield Val and Asp codons, but the recombinants with these codons would not be expected to grow on the selective medium used. In the *A78* × *A58* cross, the new codons that are produced correspond to Gly and Tyr. The fact that Gly but no Tyr recombinants were found is understandable as it is unlikely that the *A*-protein with Tyr at the *A58–A78* position is active. If it were active, Tyr revertants of *A78*

Table 1. RECOMBINATION WITHIN A CODING UNIT

Cross*	Amino-acids at same position in strains crossed	Probable corresponding DNA codons†	Possible recombinant codons	Number of recombinants	Recombination frequency (approx. per cent)	Amino-acid at relevant position of recombinant proteins‡
A46 × *A23*	Glu × Arg	GAA × AGA	GGA(Gly); AAA (Lys)	32	0·001	5 Gly (GGA)
A23 × *A23EMSPR1*	Arg × Thr	AGA × ACA	None	0	<0·0002	—
A46 × *A23EMSPR1*	Glu × Thr	GAA × ACA	GCA(Ala); AAA(Lys)	4	0·003	3 Ala (GCA) + 1 Gly§
A23 × *A46PR9*	Arg × Val	AGA × GT^T_C	$\overset{A}{\underset{C}{GGT}}$(Gly); AG^T_C (Ser); $\overset{A}{\underset{C}{ATT}}$(Ileu); GTA(Val)	5	0·002	3 Gly (GGA)¶ + 2 Ser (AG^T_C)
A46 × *A46PR9*	Glu × Val	GAA × GT^T_C	GA^T_C (Asp); GTA(Val)	0	<0·0002	—
A78 × *A58* (*A90*, *A94*)	Cys × Asp	TG^T_C × GA^T_C	GG^T_C (Gly); TA^T_C (Tyr)	76	0·001	4 Gly (GG^T_C)

* Selfings were performed with all strains but no prototrophs were recovered. *Cys*⁻ recipients were used to reduce interference from reversion and 5-methyltryptophan was included in the plating medium in all crosses involving partial revertants (PR).
† For simplicity, the nucleotide sequences indicated are those of the DNA strand which corresponds to the *m*RNA.
‡ Indicates the number of times the amino-acid shown has been found in a recombinant protein, and the probable corresponding DNA codon (in parentheses).
§ Origin unknown, probably a revertant and not included in the calculation of recombination frequency.
¶ GGA preferred because a double cross-over within the codon would be required to give GGT or GGC.

Table 2. OUTSIDE MARKER DISTRIBUTION IN INTRA-CODON RECOMBINATION

Cross: Donor	Recipient	Non-selective marker distribution* *anth*⁻	*anth*⁺	Suggested order
(1) *cys*⁺ *anth*⁻ *A46*	→ cys⁻ *anth*⁺ *A23*	14	3	*anth-A23-A46*
(2) *cys*⁺ *anth*⁻ *A23*	→ cys⁻ *anth*⁺ *A46*†	4	4	inconclusive
(3) *cys*⁺ *anth*⁻ *A58*	→ *cys*⁻ *anth*⁺ *A78*	8	6	inconclusive
(4) *cys*⁺ *anth*⁻ *A78*	→ *cys*⁻ *anth*⁺ *A58*	4	13	*anth-A78-A58*

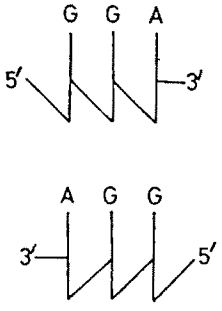
* The sum of recombinants from at least three crosses of each type. Selection was for *cys*⁺*A*⁺ recombinants.
† Because the cross performed in this direction yields very few recombinants, lysates were exposed to ultra-violet light to increase recombination frequency.

should have been detected in the extensive reversion investigations with this mutant[4,6].

Orientation of Codons Relative to the Gene and the Polypeptide Chain

Although codons have been designated for the members of the two families of related amino-acids, the orientation of each codon within the gene and relative to the polypeptide chain of the *A*-protein has not been established. For example, in the case of the Gly I residue, situated in the *A*-protein as shown below, the question posed is, which of the following triplets represents the correct orientation of the nucleotides in the GGA codon in the gene or messenger ?

H_2N–Met····· Gly·····Ser–COOH



The investigations on colinearity with the tryptophan synthetase system have established the relative orientation of the *A*-protein and its genetic map. Because it has been shown that the *A*23–*A*46 and *A*58–*A*78 mutational events affect different nucleotide positions within the corresponding glycine codons, an answer to the question posed was sought in an investigation of the relative order of the positions of nucleotide changes within these codons. The standard procedure for intra-codon transductional crosses was adopted except that a closely linked marker, resulting in an anthranilic acid requirement, was introduced into the donor strains as the non-selective marker. The distribution of this non-selective marker in the recombinants obtained in reciprocal crosses is shown in Table 2. Convincing results were obtained in two of the crosses (1 and 4) and these indicate that the relative orders of the altered sites are: *anth* . . . *A*23–*A*46 and *anth* . . . *A*78–*A*58. A comparison of the parent glycine codons with those for the mutant codons (Fig. 1) shows that the *A*46 and *A*58 mutational events lead to substitution of the guanine at the mid-position in the GGA and GG^{T}_{C} codons, whereas the *A*23 and *A*78 mutations effect the replacement of the guanine in the 5′-terminal position. Arranging the codons such that the positions of the *A*23 and *A*78 nucleotide alterations are to the left of the positions of the *A*46 and *A*58 alterations, in accordance with the order derived experimentally, is consistent with only one orientation of the codons within the gene. The details of the two meaningful crosses and the orientation of the codons relative to the order of the mutant sites are illustrated in Fig. 2. Now by definition the DNA codons used here have the same polarity as the functional RNA codons of the messenger, so this strand of DNA and the *m*RNA are analogous. Furthermore, because the relative orientation of the gene and polypeptide chain have been established in previous investigations (as well as the relative positions of the *A*23–*A*46 and *A*58–*A*78 sites)[1], it is now possible to indicate the orientations of the genetic map, gene, messenger and polypeptide chain with respect to each other (Fig. 3). It follows that the 5′- and 3′-termini of the DNA strand with the same nucleotide sequence as the *m*RNA correspond to the amino-terminal and carboxyl-terminal ends of the gene product respectively. In view of the relative orientation of gene and protein and the fact that the process of polypeptide elongation commences at the amino terminus[11], the corresponding *m*RNA must be read in the 5′ → 3′ direction. Similar conclusions regarding the polarity of the genetic message have been reached by Thach *et al.*[12] and Salas *et al.*[13] in recent investigations correlating the structure of the peptides synthesized *in vitro* in response to synthetic *m*RNA having specific terminal sequences. Observations (*in vivo*) on the amino-acid substitutions associated with a reading frame shift involving the codons of five amino-acids in the phage *T*2 lysozyme gene also support these conclusions[14]. However, earlier experiments[15,16] on the effects of specific exonuclease digestion

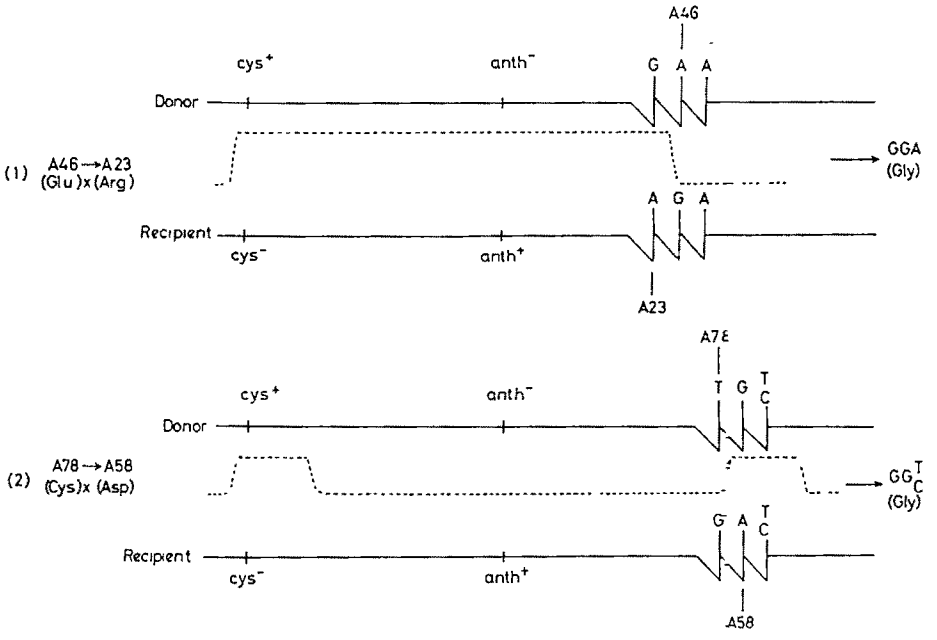


Fig. 2. The ordering of the positions of nucleotide changes within codons, relative to an outside marker

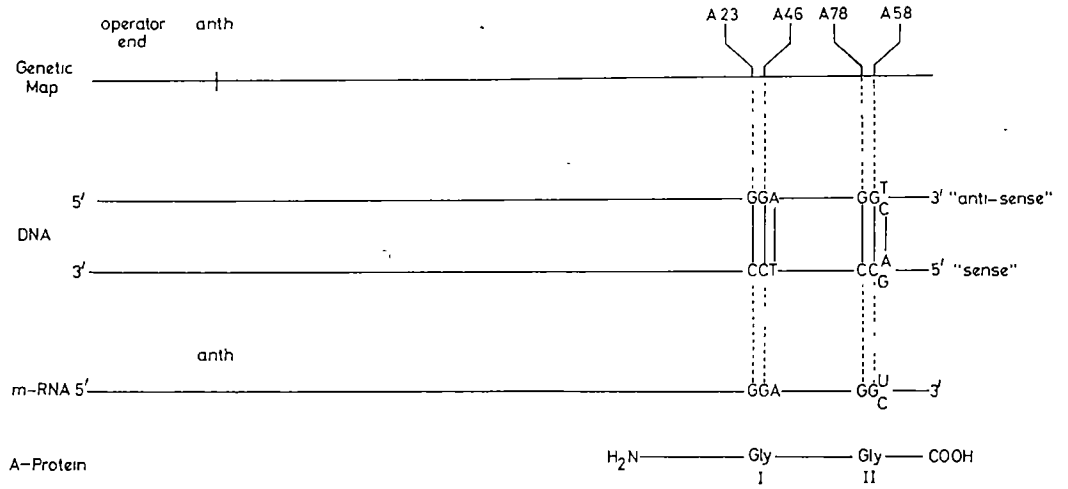


Fig. 3. A diagrammatic representation of the relative orientation of the genetic map of the tryptophan operon, related DNA and RNA nucleotide sequences, and the amino-acid sequence of the *A*-protein. The operator region is believed to be at the anthranilate synthetase end (ref. 21), and transcription appears to start at this end (ref 22)

of reticulocyte polyribosomes led to the opposite conclusion—that is, that the 3′ end of the haemoglobin *m*RNA corresponds to the amino-terminus of the protein.

Further information can be derived from the results presented here, when they are considered in conjunction with recent advances in our understanding of the transcription process. Evidence from both *in vivo*[17] and *in vitro*[18,19] investigations have indicated that during the synthesis of *m*RNA by DNA-dependent RNA polymerase the direction of elongation of the poly-ribonucleotide is from the 5′ to the 3′ end. Accordingly, the polymerase would be expected to transcribe in the 3′ → 5′ direction along the complementary strand of DNA. Therefore, by establishing the orientation of the gene relative to the *m*RNA and the polypeptide chain it can be concluded that, of the two polydeoxynucleotide strands of any operon (Fig. 3), the strand polarized 3′ → 5′ (with respect to the operator) is the 'sense'-strand (that is, the one carrying the genetic information) and the complementary strand with the opposite polarity (relative to the operator), 5′ → 3′, is the 'anti-sense' strand. It also follows that both transcription and translation mechanisms exhibit identical polarities and more important, in both cases, progression along the operon starts at the operator region, thereby confirming present theories on the mechanism of genetic control of protein synthesis[20].

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CELLULAR LOCATION OF THE GENES CONTROLLING PENICILLINASE PRODUCTION AND RESISTANCE TO STREPTOMYCIN AND TETRACYCLINE IN A STRAIN OF *Staphylococcus aureus*

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THE gene controlling high-level resistance to streptomycin in *Staphylococcus aureus* has been shown to be chromosomally located and, since its phenotypic expression is delayed until segregation is complete[1], it seems to be a recessive gene. Studies of transduction of the capacity to produce penicillinase in strains of *S. aureus* have been described[2–4]. In the absence of direct evidence for association of this gene with the chromosome, these workers have described it as a plasmid. Evidence for this included the finding that this gene can sometimes, though not always, be eliminated using acriflavines[3–6]. A high natural mutation rate of loss of penicillinase production[3,7] and the absence of a detectable reverse mutation rate have been reported[8] and have been taken as a further indication of cytoplasmic location for this gene. Furthermore, Novick[3] has reported that the penicillinase marker exhibits no phenotypic lag and that ultra-violet irradiation of the transducing phage lysate fails to enhance the rate of transduction. Ultra-violet irradiation of transducing phage has been shown to enhance transduction of some staphylococcal markers including streptomycin resistance[9], and in *Escherichia coli* was found to increase the frequency

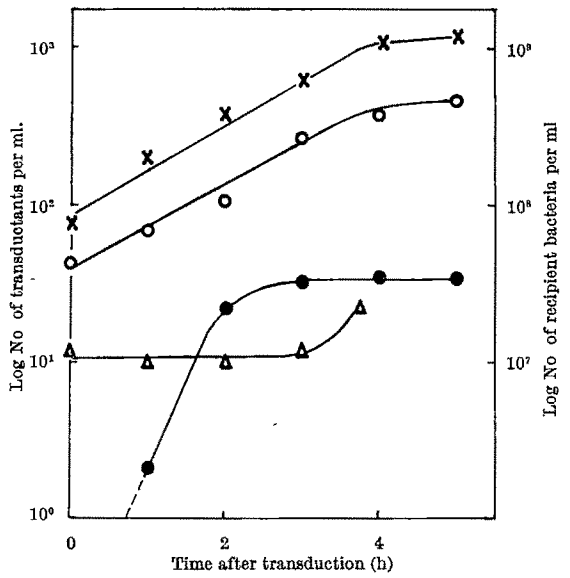


Fig 1. The phenotypic expression of markers. The log of the number of transductants and recipient bacteria per ml is plotted against the time of incubation in broth before selection. Recipient strain *S26 P⁻ SS TS* was treated with phage obtained by ultra-violet induction of donor strain *S26 P⁺ SR TR*. Transductants were selected on antibiotic-containing solid media at intervals of time after transduction. △, Penicillinase-producing transductants (*P⁺*); ●, streptomycin-resistant transductants (*SR*), ○, tetracycline-resistant transductants (*TR*); ×, recipient *S26 P⁻ SS TS*

of transduction of chromosomal markers whereas the rate of episomal transfer was not affected[10]. This increase in the number of stable chromosomal transductants is probably due to increased recombination between the transduced marker and host DNA[10].

In this work the kinetic development of phenotypic expression and the effect of ultra-violet irradiation on the frequency of transduction were used as criteria to judge the location of markers responsible for high-level resistance to streptomycin, resistance to tetracycline and production of penicillinase. All recipient and donor strains were derivatives of a penicillinase-producing strain of *S. aureus S26 P⁺*, that is, N.C.T.C. 8511, lysogenized with phage 53 (N.C.T.C. 8406) kindly supplied by Dr. M. L. Morse. The use of this type of system, with the homologous lysogenic strain as the recipient, has been reported[9,11] and has the advantage of removing the possibility of loss of transductants due to lysis. The recipient *S26 P⁻* was a spontaneously occurring penicillinase-negative mutant of *S26 P⁺* which was obtained when 2×10^4 cells were screened on cellulose acetate membranes by the method of Knox and Smith[12]. No penicillinase was detected in *S26 P⁻* using a modification of the hydroxylamine assay method[13] nor with a modification of the iodometric assay method[3]. Donor phage was obtained by ultra-violet induction of a streptomycin-resistant, tetracycline-resistant mutant of *S26 P⁺*. This strain (*S26 P⁺SRTR*) was prepared from *S26 P⁺* by selection of a naturally occurring streptomycin-resistant mutant on 500 μg/ml. streptomycin agar, followed by transduction to tetracycline resistance using phage 53 propagated on a tetracycline-resistant strain of *S. aureus* 13136 (ref. 14). In transduction experiments, cocci were grown in brain heart infusion broth to a count of about 3×10^8 per ml., concentrated two-fold in nutrient broth (Difco) and mixed with an equal volume of transducing phage at a multiplicity of infection of 0·5–1·5 with a final concentration of 400 μg/ml. calcium chloride: after 45 min at 37° C when about 95 per cent phage was adsorbed, the culture was spun down and resuspended in ten times the original volume of brain heart infusion broth. Throughout this work this time was regarded as time zero. Transductants for tetracycline resistance were selected on 5 μg/ml. tetracycline agar, for penicillinase production on 0·06 μg/ml. benzylpenicillin agar and for streptomycin resistance on 500 μg/ml. streptomycin agar. The kinetics of phenotypic expression were followed by removing aliquots at timed intervals, sampling on to the selective media and making viable counts on non-selective media. Ultra-violet-irradiated transducing phage was obtained by exposing 5-ml. amounts of undiluted phage preparation to a source of ultra-violet light emitting $5{\cdot}4 \times 10^4$ ergs/cm²/min; 0·2 ml. samples were removed at timed intervals, 0·1 ml. assayed for surviving phage and 0·1 ml. diluted 10^{-1} in Difco nutrient broth, stored overnight at 4° C and then used for transduction. Phage assay plates were kept away from bright light in order to avoid photoreactivation[15] of adsorbed phage. A control sample was treated in the same way before irradiation was commenced. Transductants were identified after three to four single colony isolations by lysogenic typing using the supernatant fluid of a broth culture and by antibiotic sensitivity pattern using Oxoid multodisks (11–14*C*) on digest agar. *CY* agar[3] was used for selection of transductants and viable counts. Digest agar (Southern Group Laboratories) containing 400 μg/ml. calcium chloride was used for phage assay plates.

Different patterns of phenotypic expression were shown by the three markers (Fig. 1). Streptomycin-resistant transductants exhibited a typical phenotypic expression lag. Between 1 and 2 h the number of these transductants showed a ten-fold increase, while the total recipient population only increased two-fold. This seems to represent the outcome of the expression of a recessive gene rather than growth. Using hetero-diploids of *E. coli* which carried streptomycin-resistant and streptomycin-sensitive alleles, Lederberg[16] found that such bacteria were streptomycin-sensitive and therefore concluded that streptomycin resistance is recessive. The penicillinase marker showed no lag in phenotypic expression but the number of transductants failed to increase over the next 3 h although the recipient itself multiplied exponentially in the same period. These

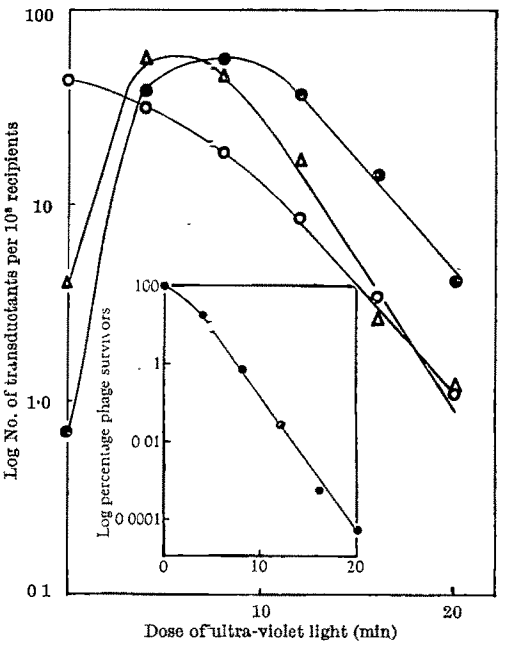


Fig. 2. Effect of ultra-violet irradiation of phage on transduction frequency and phage survival (plaque formation) A donor phage preparation from strain *S26 P⁺ SR TR* was treated with various doses of ultra-violet irradiation and tested for transduction of the recipient strain *S26 P⁻ SS TS*. Transductants were selected on antibiotic-containing solid media immediately after transduction for the *P⁺* and *TR* markers and after 2-h incubation for the *SR* marker. Transduction frequencies are given as the number of transductant colonies per 10^8 recipients, and plotted against the dose of ultra-violet light (minutes). The inset shows the percentage of phage particles surviving (plaque formation) after ultra-violet treatment. △, *P⁺*; ●, *SR*; ○, *TR*; •, donor phage (inset)

findings probably reflect segregation of a dominant gene. In the case of tetracycline-resistant transductants, both phenotypic lag and segregation lag were absent and the transductants grew at a rate similar to that of the recipient culture. The absence of any lag can be explained by the replication of the gene immediately on entering the cell, before the onset of cell division, so that all progeny received the determinant controlling tetracycline resistance. This type of behaviour occurs in non-chromosomally located genes (for example, episomes and plasmids).

Reconstruction experiments have shown that cells carrying any one of these three markers are able to grow at a rate similar to that of the antibiotic-sensitive recipient culture when present at a ratio of 1 in 10^6 and thus the foregoing findings must reflect the establishment of the markers into the recipient genome.

The effect of using ultra-violet-irradiated transducing phage on the frequency of transduction was studied and the results shown in Fig. 2. The frequency of transduction of streptomycin resistance and penicillinase production after 6 min of ultra-violet treatment of the phage was increased by a factor of 69 and 14, respectively, at a phage inactivation level greater than 90 per cent. However, under identical conditions, the frequency of tetracycline transductants showed no increase but fell with increasing doses of ultra-violet irradiation. These results add support to the chromosomal location of the genes for streptomycin resistance and penicillinase production as indicated by the phenotypic delay and segregation lag already described. The characteristics of tetracycline-resistant transductants, that is the absence of phenotypic or segregation lag and absence of increased transductional frequency using ultra-violet-irradiated phage, seem to point to a cytoplasmic location for this gene.

The results described here for phenotypic expression of the streptomycin marker agree with the findings of Morse[1] and those described for the effect of ultra-violet on transduction of streptomycin resistance with those of Korman and Berman[9]. On the other hand, the finding that the penicillinase gene seems to have a chromosomal location in this strain does not agree with Novick's results[3] which, using another strain, suggested a non-chromosomal (plasmid) location. This does not imply that either interpretation is wrong but suggests that the penicillinase gene can exist in either state depending on the strain. Many other genes have been shown to exhibit this behaviour.

I thank Dr. J. T. Smith for his help.

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CHROMOSOMAL LOCATION OF THE GENETIC ELEMENTS CONTROLLING PENICILLINASE PRODUCTION IN A STRAIN OF *Staphylococcus aureus*

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EVIDENCE that the genetic elements controlling penicillinase production (and thus penicillin-resistance) in *Staphylococcus aureus* may be located on extra-chromosomal fragments or plasmids was presented by Novick and Richmond[1] and may summarized as follows. (1) The ability of a strain to produce penicillinase is lost spontaneously at a relatively high rate. This loss is apparently irreversible and may be accompanied by loss of other markers such as resistance to mercury salts[2] and in certain strains resistance to macrolide antibiotics[3]. (2) The ability to produce penicillinase can be transduced into suitable recipient strains but the rate of transduction is decreased if the transducing lysate is irradiated with ultra-violet light[4]. (3) Loss of the ability to produce penicillinase can be accelerated by agents not generally regarded as mutagenic—for example, elevated temperatures (43°–44° C)[5–7] or acridine dyes[3,8] and other substances (see ref. 1).

This article describes results obtained with three different strains of *S. aureus*. In one, the genes controlling penicillinase production would seem to be extra-chromosomal, in the second to be chromosomal, and in the third to have characteristics of both chromosomal and extra-chromosomal elements. These three strains and their derivatives are as follows.

(1) *PS* 80, the propagating strain for typing phage 80, is a naturally occurring penicillinase-producing strain, resistant to mercuric chloride. The ability to produce penicillinase is a stable character in this strain; it is not lost on storage or during growth at 43°–44° C (ref. 7). Resistance to mercury salts, however, is lost, both on storage on agar slopes at room temperature and as the result of growth at 43°–44° C. The difference in stability of these two markers suggested that they were not linked. Tetracycline-resistance was transduced into this strain from strain 2 (ref. 7) and this transductant (*PS* 80 P^rT^r) was used as donor of resistance to penicillin and to tetracycline in transduction experiments.

(2) Strain 2 P^rT^r (see ref. 7), a naturally occurring penicillinase-producing strain, resistant both to tetracycline and mercury salts. All three characters are unstable, in that they show a high spontaneous rate of loss which can be increased by growth at 43°–44° C. Resistance to penicillin and to mercury salts are nearly always lost together, indicating a close linkage between these two markers, while tetracycline resistance is lost independently. Strain 2 P^rT^r was used as donor of resistance to penicillin and to tetracycline in transduction experiments. A mutant of strain 2, sensitive to all three drugs, and designated strain 2/*S* was isolated after growth at 43°–44° C. It was used as recipient in transduction experiments where the donors were *PS* 80 P^rT^r and strain 2 P^rT^r.

(3) Strain 8325-18, described by Novick[4], produces penicillinase and is resistant to mercury salts. These two markers show a high spontaneous rate of loss which, however, is not increased by growth at 43°–44° C. They are linked in this strain in that they are lost together and are co-transduced. Strain 8325, the penicillin-sensitive, mercury-sensitive strain from which strain 8325-18 was

derived, was used in transduction experiments as recipient of these two markers from 8325-18.

Typing phage 80 was used as transducing phage in all experiments. It was propagated in lytic cycle on the three different donor strains, and preparations of phage are designated according to the strains on which they were propagated (phage 80/80 PrT^r, phage 80/2 PrT^r and phage 80/8325-18). Transducing lysates, diluted in nutrient broth, were irradiated for various lengths of time, using a 15-W germicidal lamp at a distance of 22 cm. After irradiation, samples were titrated for survivors and used in transduction experiments.

Transduction experiments were carried out as follows. A log-phase culture of the recipient strain containing 1–2 × 10^8 viable units/ml. was mixed with sufficient phage to give a multiplicity of infection of from 0·3 to 0·7. Sufficient calcium chloride was added to give a final concentration of 200 μg/ml. The mixtures were held at 37° C for 30 min to allow phage adsorption. They were then centrifuged, the supernatant fluid was discarded, and the bacteria were resuspended in broth containing 0·02 M sodium citrate to prevent further phage adsorption. Incubation was continued for a further 2 h. The cultures were then centrifuged, and the bacteria resuspended in a few ml. of broth. The whole of the suspension was examined for transductants by mixing 0·5 ml. volumes with 2·0 ml. of molten nutrient agar containing the appropriate antibiotic in the following concentration: penicillin—0·1 μg of benzyl penicillin/ml. : tetracycline—5·0 μg (= 5 u.) of tetracycline/ml. This was overlaid on agar plates containing the same concentration of antibiotic. Plates were incubated at 37° C, and transductants counted after 24 h and 48 h. Transduced clones were purified by streaking on antibiotic-containing medium. They were tested for resistance to mercury salts by a disk test[9]. The identity of a reasonable number was confirmed by phage-typing.

Figs. 1, 2 and 3 show the results of transduction experiments with three different phage lysates which had been exposed to various doses of ultra-violet irradiation. The frequency of transduction is plotted as a function of the dose of ultra-violet light given to the phage. Transduction frequencies were calculated as the ratio of the number of transductants to the number of plaque-forming particles in the unirradiated lysate.

In Fig. 1 the donor strain was 8325-18, the recipient strain was 8325 and the marker transduced was penicillin resistance. The rate of transduction decreased exponentially with the dose of ultra-violet light, confirming the

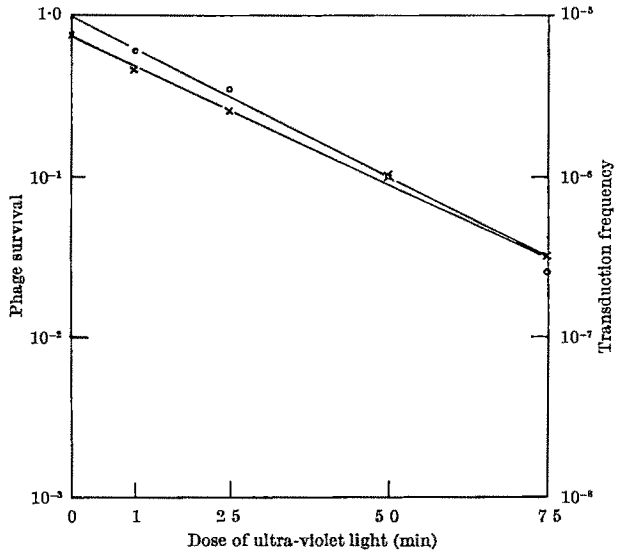


Fig. 1. Frequency of transduction of penicillinase production as a function of ultra-violet irradiation of transducing phage. Donor = 8325-18. Recipient = 8325. ○, Phage survival curve, ×, transductants

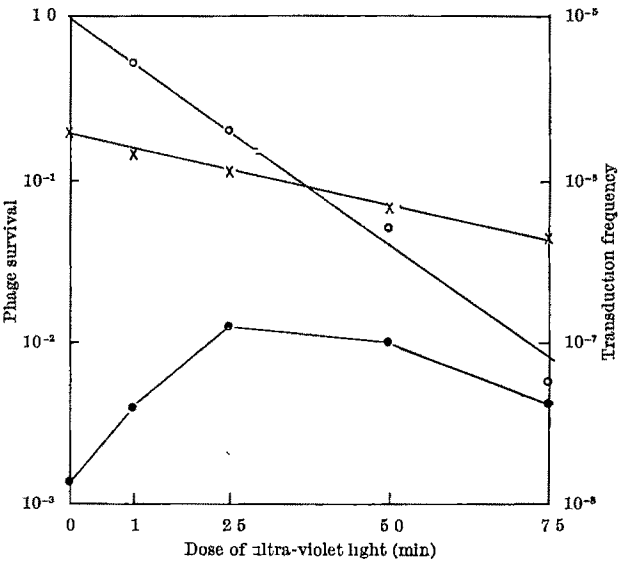


Fig. 2. Transduction frequency of resistance to penicillin and tetracycline as a function of ultra-violet irradiation of transducing phage. Donor = *PS* 80 PrT^r. Recipient = strain 2/*S*. ○, Phage survival curve; ×, tetracycline-resistant transduction curve, ●, penicillin-resistant transduction curve

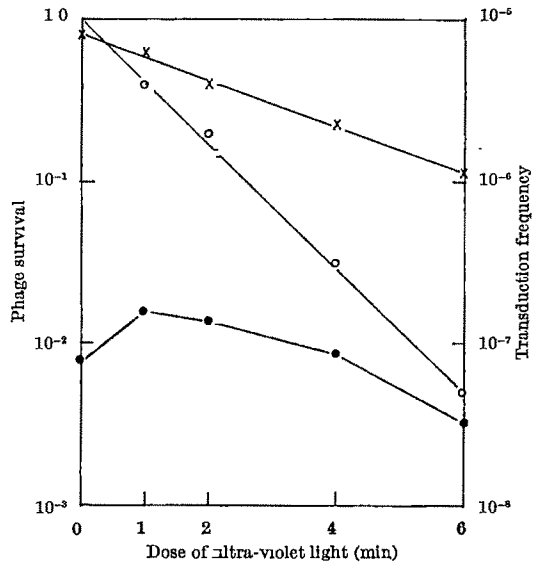


Fig. 3. Transduction frequency of resistance to penicillin and tetracycline as a function of ultra-violet irradiation of transducing phage. Donor = strain 2 PrT^r. Recipient = strain 2/*S*. ○, Phage survival curve; ×, tetracycline-resistant transduction curve; ●, penicillin-resistant transduction curve

results obtained by Novick[4]. Twenty of the transduced clones were tested for resistance to mercury salts, and all were found to have received this marker together with penicillin resistance.

Fig. 2 shows the results obtained when *PS* 80 PrT^r was the donor and strain 2/*S* the recipient. Transduction of resistance both to penicillin and to tetracycline was tested for in the same experiment by dividing the phage-treated bacterial suspension into two equal portions and screening each for one of the markers. The frequency of transduction of tetracycline resistance decreased exponentially with increasing doses of ultra-violet irradiation, but at a slower rate than that observed for penicillin resistance in Fig. 1, suggesting that the genes controlling tetracycline resistance are less sensitive to ultra-violet light than those controlling penicillin resistance. The curve for transduction of tetracycline resistance is consistent with the hypothesis that this marker is carried on a plasmid. The frequency of transduction of penicillin resistance, however, showed a ten-fold increase when the phage was given a dose of ultra-violet light sufficient to reduce the proportion

of survivors to 0·2. Penicillin-resistant transductants failed to show any co-transduction of mercury resistance, confirming that, in this strain, these two markers are not linked.

Fig. 3 shows the frequency of transduction of penicillin and tetracycline resistance when the donor was strain 2 P^rT^r and the recipient strain 2/*S*. The results obtained for the transduction of tetracycline resistance were similar to those in Fig. 2. The frequency of transduction of penicillin resistance showed a two-fold increase with a dose of ultra-violet which reduced the proportion of active phage to 0·4. This result was unexpected, since penicillin resistance in strain 2 P^rT^r is unstable and was, therefore, believed to be carried extra-chromosomally. The experiment was repeated a number of times, and this small increase in frequency occurred on each occasion.

Arber[10] investigated the effect of ultra-violet irradiation on transducing lysates of phage *P*1. He found that irradiation increased the frequency of transduction when the transduced marker was chromosomal, but decreased it when the marker was extra-chromosomal. The frequency of transduction of extra-chromosomal markers was approximately 100 times higher than for chromosomal markers. He attributed this difference to the fact that, when the transduced marker was chromosomal, transfer of the genetic determinants for the marker was not sufficient to produce a stable transductant. Recombination was necessary for the transduced marker to become stably integrated into the chromosome of the recipient. Ultra-violet irradiation of the transducing phage might increase the frequency of transduction of chromosomal markers by stimulating recombination.

The results obtained for transduction of penicillin resistance with phage 80/8325-18 are consistent with the hypothesis that, in this strain, the genetic elements controlling penicillinase production are carried on a plasmid which also carries those responsible for resistance to mercury salts. The frequency of transduction was high ($8·3 \times 10^{-6}$), but decreased when the transducing lysate was irradiated.

When the donor strain was *PS* 80 P^rT^r, however, the results were quite different. The frequency of transduction was low ($1·4 \times 10^{-8}$) but increased to $1·3 \times 10^{-7}$ when the transducing phage was irradiated. Both facts favour the hypothesis that the genes controlling this marker are on the chromosome in this strain. It might be argued that the phage preparation used was a poor transducing one, but the same preparation transduced tetracycline resistance with a high frequency ($2·1 \times 10^{-6}$). Consistent failure of attempts to isolate penicillin-sensitive mutants from this strain, both from old stored cultures and after growth at elevated temperatures, is further evidence in favour of a chromosomal location for the genes controlling penicillinase production.

It is more difficult to interpret the results obtained with strain 2 P^rT^r. It shows a fairly high rate of spontaneous loss of penicillin resistance, which can be increased by growth of the strain at 43°–44° C. This suggests an extra-chromosomal location for the genetic elements controlling penicillin resistance. When this marker was transduced to a sensitive recipient, however, the frequency of transduction was low ($8·3 \times 10^{-8}$) and showed a two-fold increase after a small dose of ultra-violet irradiation. These inconsistencies would be explained if it were assumed that the relevant genes are episomic, and have a chromosomal location in some cells and an extra-chromosomal location in others. If this were so it would follow, in view of the low frequency of transduction, that in the majority of cells the genes are located on the chromosome.

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DELAYED UPTAKE BY TUMOURS OF TRITIUM FROM THYMIDINE

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IN most work on cell population kinetics in which tritium-labelled thymidine is used, the assumption is made that thymidine injected into an animal is rapidly metabolized and that after an 'availability time' of about half an hour no further uptake occurs[1,2]. The main reason for questioning this assumption is the possibility of the re-utilization of tritium-labelled DNA breakdown products from cells which were labelled during the initial availability time but which later die in the normal course of cellular renewal. There have been a number of claims[3,4] that this phenomenon has been observed autoradiographically. In general, the degree of re-utilization demonstrated has been slight except when tritium-labelled DNA has been transferred to non-radioactive animals[5], a situation which does not immediately indicate the extent to which re-utilization could arise as an artefact.

It was on the assumption that systemic re-utilization did not occur to a significant extent that an experiment was designed to detect cell loss from tumours. The rate of cell loss is an important parameter in any attempt to relate cell proliferation in tumours to their overall rates of growth, and no quantitative investigations have been made of it so far as I am aware. The experiment consisted in giving tritium-labelled thymidine to tumour-bearing animals and later following the total tritium content of the tumour. The initial experiment was made on a transplantable tumour carried subcutaneously in the August female rat; it arose as a mammary tumour in this strain in the laboratory of Dr. Goldfeder (United States), from whom it was obtained in 1952. It has now undergone many hundreds of transplants, is undifferentiated and has an initial volume doubling time of about 30 h. This is not, therefore, a tumour in which considerable cell loss would be expected, but it served to establish the method.

The total tritium uptake by the tumour was linearly related to tumour weight at injection, up to a size of 1 g, and therefore for this type of experiment the tumour size at injection must be accurately controlled. Each animal was selected on the morning of the day on which its tumour reached 7 mm. diameter (including skin thickness). It was injected intraperitoneally with 50 μc. of tritium-labelled thymidine (Radiochemical Centre, Amersham; 12·6 c./mmole) and then randomized into one of five groups, to be killed at intervals from 6 h to 7 days. The tumours were weighed fresh, then sliced and fixed in neutral

10 per cent formol saline. They were then desiccated, homogenized by grinding and assayed by liquid scintillation counter. It was anticipated that during the growth of a tumour the tritium content thus observed would stay constant or at least fall slightly with time. The results, shown in Fig. 1, show that there was, on the contrary, a four-fold increase of total tritium content during the week following injection; this has since been confirmed by a separate experiment. The growth-curve over the period of the experiment is also shown. Tumour weight increased by a factor of 25; most tumours at the 7-day interval showed fairly extensive necrosis and further protraction of the experiment was prevented by the larger tumours reaching a prohibitive size (the experiment cannot be started on smaller tumours on account of the difficulty of accurately establishing tumour size at injection).

There are two possible processes by which the acquisition of tritium by a tumour could occur: systemic re-utilization, and a net migration of labelled cells into the tumour. The second of these could scarcely explain an effect of the magnitude observed, for even if the migratory cells had a labelling index as high as the tumour itself (25 per cent), by 7 days more than 10 per cent of the cells present in the tumour mass would have to come by immigration. That this does not occur has been confirmed by autoradiography of tumours implanted into tritium-labelled animals.

The experiment as originally performed did not indicate whether it was the DNA of the tumour cells which incorporated the re-utilized activity. In a repeat of the original experiment, DNA extractions were therefore performed using a modified Schmidt–Tannhauser method and the total DNA tritium of the tumour was determined. These results, also shown in Fig. 1, confirm the acquisition of tritium by the DNA of the tumour. The rise in DNA tritium between 6 h and 1 day is difficult to understand. If it is a real effect it suggests that the metabolism of thymidine into the DNA of this tumour is not complete within 6 h.

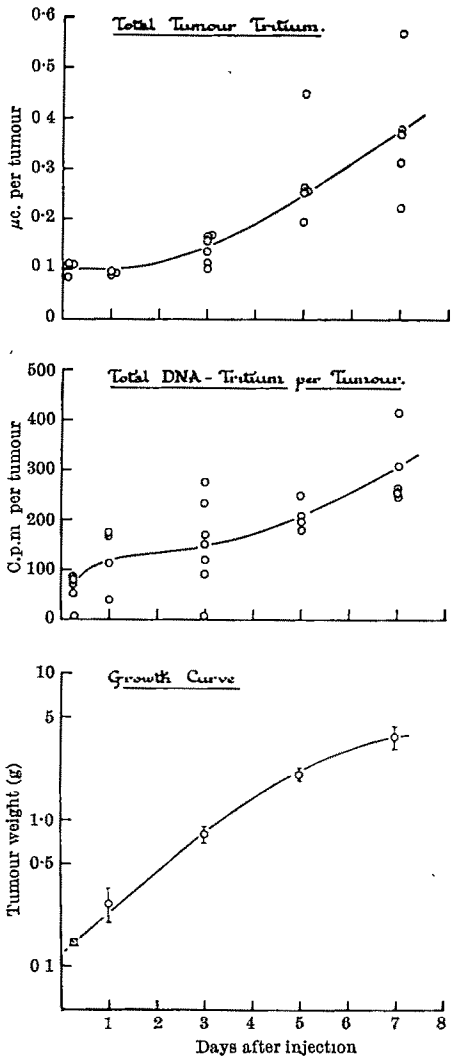


Fig. 1 Results on the August transplanted tumour

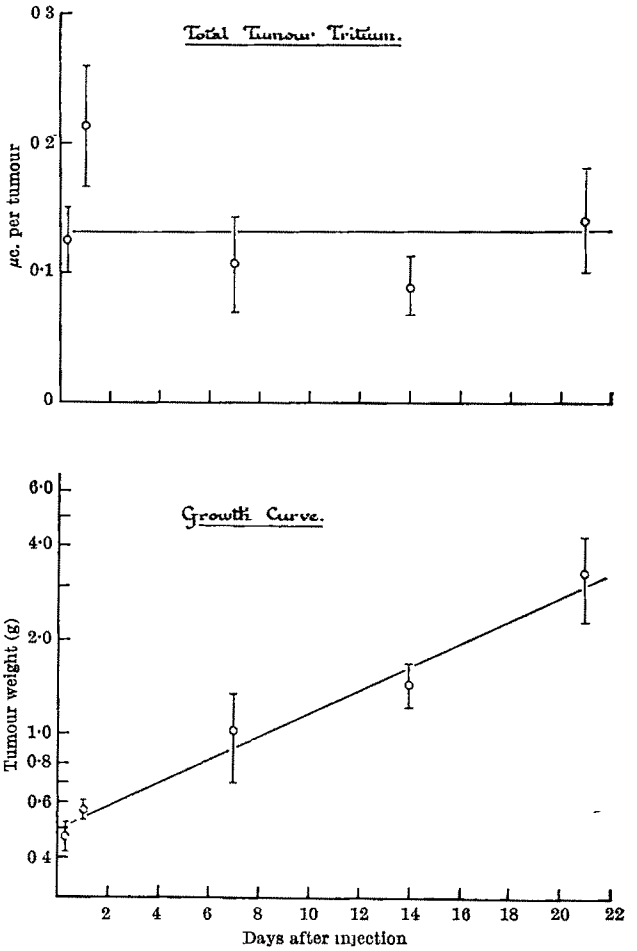


Fig. 2 Results on the spontaneous C^{+} mammary tumour

The immediate conclusion from these experiments is that cell loss from tumours cannot reliably be investigated by following total tumour tritium from thymidine. The underlying mechanism of the re-utilization process presents two main problems: first, the source of the re-utilized tritium; secondly, the molecular form in which it is re-utilized. The latter problem is now the subject of a considerable amount of investigation[6]. There is little doubt about the source of the re-utilized tritium. The work of Steel and Lamerton[7] on the retention of tritium from thymidine in eight renewal tissues of the rat showed that while there is little loss of incorporated tritium during the first 2 days following injection, considerable amounts of tritium leave the renewal tissues during the next 3 days. This is especially true of the small intestine which, within this 3-day period, discharges 5 per cent of the injected dose to the whole animal. The results shown in Fig. 1 fit into this picture remarkably well. There is no uptake by the tumour in at least the first day following injection, and the excess tritium acquired by 5 days amounts to 0·3 per cent of the injected dose. It is not possible to say how much of the activity which is made available following

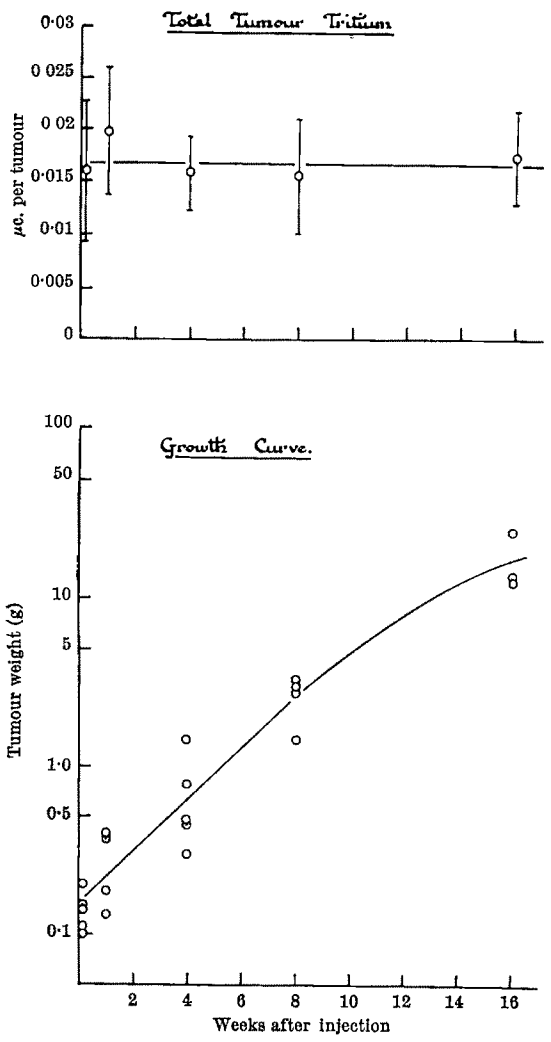


Fig. 3. Results on the transplanted Marshall fibrosarcoma

the death of a tritium-labelled cell is normally re-utilized within the animal as a whole, nor is it possible to say whether tumour tissue is more proficient than normal tissues in re-incorporating it. It is clear, however, that DNA tritium is released in amounts which are much greater than are found to be taken up by the tumour.

The question of whether tumour tissue preferentially utilizes the DNA breakdown products from dead cells is an interesting one, and it could have important implications. It is necessary first to establish whether re-utilization is demonstrable in a wide variety of tumours, and in particular whether the growth-rate of the tumour is a vital factor. Experiments were therefore carried out as before on two rather different tumours. The first was a spontaneous mammary tumour arising in the C^{+} mouse. In this case the tumours were selected for injection at a diameter of 10 mm, and because of the lower growth-rate the intervals were spread out to 21 days. The results are shown in Fig. 2. The second tumour was a fibrosarcoma which arose spontaneously in the Marshall rat and which has now been transplanted twenty-five times. This is a slow-growing tumour, and the intervals were therefore spread out to 4 months (Fig. 3). In each case there was no detectable re-utilization and, furthermore, no evidence of significant cell loss over the whole growth period. The most probable inference is that the change from a tumour doubling time of 30 h (August tumour) to 7·8 days for the spontaneous tumour and 14 days for the fibrosarcoma is sufficient to reduce the total gain of tritium by the tumour to an undetectable level. Presumably the tumour must have a reasonably high growth-rate during the period of high availability of tritium-labelled breakdown products, this period being 2–7 days following injection[7]. This requirement has received clear confirmation during the preparation of this report in the work of Bryant[8], who has grown ascites tumour cells in thymidine-labelled mice and shown that within this period the uptake of tritium by the tumour cells can be demonstrated autoradiographically. The present work supplements that of Bryant in showing that in tumours of high growth-rate it is a simple matter to demonstrate re-utilization of DNA tritium. It is clear that both in normal tissues[7] and in tumours the possibility of re-utilization artefacts in tritiated thymidine studies is of significant importance.

I thank Prof. L. F. Lamerton for his support and advice. I also thank Miss Kay Adams and Miss Sylvia Clack for their technical assistance and Dr. G. Threlfall for the DNA extractions.

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SYNTHESIS OF ENZYMES AND DNA IN SYNCHRONOUS CULTURES OF *Schizosaccharomyces pombe*

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THERE has been considerable interest in recent years in the production of enzymes by synchronous cell cultures[1–13]. The long-term aim of this work is to understand the mechanisms which regulate the rate of synthesis of specific proteins during the cell cycle. We have assayed a number of enzymes in synchronous cultures of the fission yeast, *Schizosaccharomyces pombe*. We have also used these cultures to determine the pattern of DNA synthesis. This has previously been an intractable problem because of the difficulty of selectively labelling the DNA[14].

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Synchronous cultures of 200–700 ml. of *S. pombe* (N.C.Y.C. 132) in Edinburgh minimal medium[15] ($1 - 4 \times 10^{6}$ cells/ml.) were prepared by the method of Mitchison and Vincent[16]. Glucose gradients were used instead of sucrose gradients in order to avoid perturbing the culture by changing the carbon source. In some cases, an asynchronous control culture was prepared from the remaining cells in the gradient after the top layer had been removed to make the synchronous culture. The cultures were grown at 32° C and samples removed at intervals for enzyme assay (washed and freeze-dried), DNA assay, scoring of cell plates and, sometimes, cell numbers. Aspartate transcarbamylase (ATCase) was assayed by

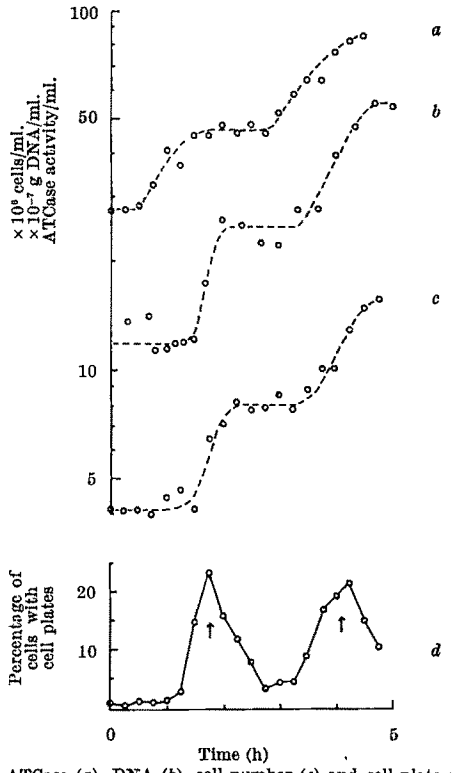


Fig. 1. ATCase (*a*), DNA (*b*), cell number (*c*) and cell plate index (*d*) in a synchronous culture

resuspending freeze-dried samples in 0·1 ml. 1 M glycine (*p*H 9·5) and 0·1 ml. 0·25 M L-aspartic acid (*p*H 9·5) in an ice bath and then adding 0·1 ml. carbamyl phosphate (4 mg/ml.) to complete the reaction mixture. The reaction was carried out at 28° C and the carbamyl aspartic acid formed estimated by the method of Gerhart and Pardee[17]. Ornithine transcarbamylase (OTCase) was assayed in exactly the same way, except that 0·03 M L-ornithine was substituted for the aspartic acid. The citrulline formed was estimated by the same method as was used for carbamyl aspartic acid. Alkaline phosphatase was assayed by the method of Torriani[18]. Sucrase (invertase) was assayed by the method of Masters *et al.*[2]. A similar method was used for maltase, with maltose as the substrate. DNA was measured by a modified version of the fluorometric technique of Kissane and Robins[19]. This is up to a hundred times more sensitive than the diphenylamine reaction[20]. Comparative tests with the two methods gave very similar DNA values with this material. The cell plate index (percentage of cells showing the transverse cell plate which precedes division) was measured on dried smears which were negatively stained with 10 per cent waterproof black drawing ink and then stained with 0·25 per cent crystal violet. This index is analogous to a mitotic index and is constant at about 10 per cent in a normal culture growing asynchronously. Cell numbers were determined by counting in a haemocytometer.

In the synchronous culture shown in Fig. 1, measurements were made of ATCase, DNA, cell numbers and cell plate index. There were two synchronous divisions and, slightly before them, two peaks in the cell plate index. DNA synthesis was sharply periodic with a doubling in total amount at about the time that the cell numbers doubled. In a series of similar experiments, the mean time of DNA doubling was a little earlier (about 15 min) than the doubling in cell numbers. This relation is shown in Fig. 3*c*. The single nucleus of *S. pombe* divides shortly before the appearance of the cell plate[21]. It seems, then, that DNA synthesis, at least in these synchronous cultures, has the following pattern. At the completion of nuclear division, the DNA synthetic period (*S*) commences almost immediately. There is therefore little or no presynthetic (*G*1) period between nuclear division and the *S* period. It is difficult to determine the exact length of the *S* period but in a number of experiments (including that in Fig. 1) the rate of increase of DNA during the *S* period was the same as that of the cell numbers. This would imply a very fast replication process. Finally, there is a long postsynthetic period (*G*2) before the next nuclear division. A restricted period of DNA synthesis during the cell cycle is the usual pattern of higher cells and it also occurs in budding yeast[22,23]. It is less common for *G*2 to occupy most of the cycle, though two other organisms in which this is known to happen are the slime mould *Physarum*[24] and sea-urchin eggs[25]. DNA synthesis in *S. pombe* is at present being explored by one of us (C. J. B.).

Fig. 1 also shows that ATCase activity rose in steps. Assuming that the enzyme is stable, it is synthesized only during a restricted period of the cell cycle. The length of this period would seem to be rather longer than that for DNA synthesis. The time of initiation of the burst of synthesis is the middle of the cycle in each of the two cycles. A similar result with another synchronous culture is shown in Fig. 2. In this experiment, a control asynchronous culture was set up from the same gradient and it showed an exponential increase in ATCase activity without any steps. The periodic nature of enzyme synthesis is therefore a property of the cell cycle and not simply the consequence of re-inoculating the cells from the gradient into fresh medium. The initiation times from five experiments are shown on a cell cycle map[2,4,8] in Fig. 3*a*.

OTCase activity followed a very similar pattern except that the steps were less well defined and somewhat more variable in their timing in the cycle (Figs. 2 and 3*b*). The average initiation times for the ATCase and OTCase steps are plotted on a cell cycle map in Fig. 3*c*, together with the mean cell plate peak and the mean mid-point of DNA synthesis. Although there is a difference between the ATCase and OTCase means, it is not statistically significant.

We have also measured the activity of three other enzymes—sucrase, maltase and alkaline phosphatase. The first two are at basal level in Edinburgh minimal medium (where the carbon source is glucose) but can be induced by the appropriate carbon source. Alkaline phosphatase appears to be constitutive since its activity per cell cannot be altered either by raising or by lowering the phosphate content of the medium. This enzyme has also

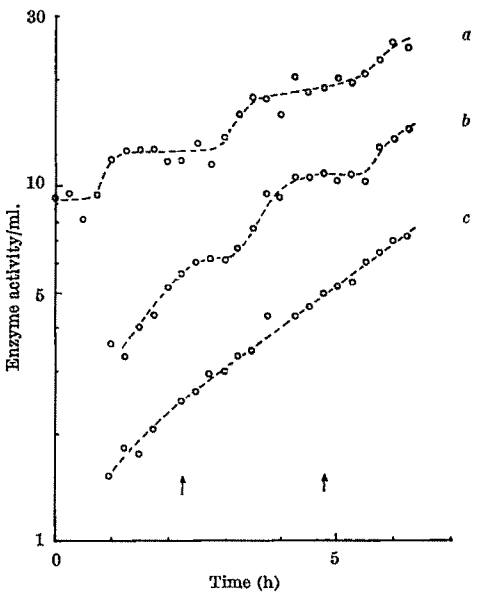


Fig. 2. OTCase (*a*) and ATCase (*b*) in a synchronous culture, and ATCase in a control (*c*) asynchronous culture from the same gradient. The arrows indicate the cell plate peaks in the synchronous culture, as in Fig. 1

been shown to be constitutive in another yeast[26]. With all three enzymes there was a continuous increase in activity through the cell cycle in synchronous cultures. Three experiments with synchronous and control cultures are shown in Fig. 4. There was an initial fluctuation in the maltase activity which occurred in both synchronous and control cultures and was probably a result of the synchronizing procedure. These enzymes, unlike ATCase and OTCase, appear to be synthesized continuously and smooth curves have been drawn provisionally through the points on the logarithmic plot in Fig. 4, but there may in fact be changes in the rate of synthesis similar to those found with repressed enzymes in synchronous bacterial cultures (alkaline phosphatase in *Bacillus subtilis*[5], alkaline phosphatase and β-galactosidase in *Escherichia coli*[3]). This point is being investigated by one of us (J. M. M.).

One purpose of this work was to discover whether all enzyme synthesis during the cell cycle was periodic, as reported for *Saccharomyces cerevisiae*[8,9], or whether it was periodic for some enzymes and continuous for others, as in bacteria[3,5,6]. The results reported here indicate that both periodic and continuous syntheses of enzymes occur. Furthermore, it was found that basal synthesis of the sucrase and maltase was continuous, while there was periodic synthesis of ATCase and OTCase which may be controlled autogenously[3]. In this respect, therefore, the results obtained with *S. pombe* are identical to those obtained with *B. subtilis* and *E. coli*[2-6]. The constitutive synthesis of alkaline phosphatase was also continuous. An opposite result to this has been found in budding yeast where there are two steps in this enzyme in each cycle[6]. This may be due to differences in the degree of repression in the two systems since alkaline phosphatase activity in synchronized *B. subtilis* is continuous when fully repressed[5] and stepwise when derepressed[6]. The continuous synthesis of this enzyme and of the sucrase and maltase is in accordance with the hypothesis that periodic enzyme synthesis results from the operation of a feed-back of end-product on enzyme synthesis[3,4,6,27,28]. It is also possible to explain continuous synthesis in terms of periodic transcription through the cycle of a number of genes in different positions on the genome[9]. Without very accurate assays, it is impossible to distinguish a curve showing continuous rise in enzyme activity from one with more than two or three steps. This explanation would not, however, account easily for the doublings in rate of synthesis at a particular point in each cycle in the bacterial systems[2,5].

Another object of this work was to determine whether the initiation of periods of enzyme synthesis was closely connected with periods of gene replication. The results show particularly clearly that initiation of enzyme steps can occur during periods when there is no net DNA synthesis. This confirms for *S. pombe* the conclusion reached for *B. subtilis*[6].

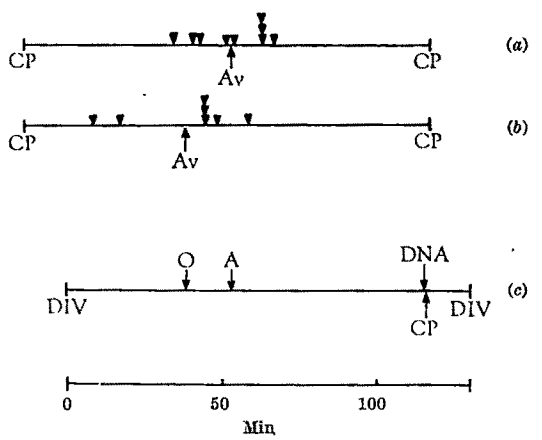


Fig. 3. Cell cycle maps of enzyme steps. (*a*) ATCase from five synchronous cultures. Each triangle is the initiation time. CP is the cell plate peak. (*b*) OTCase from four synchronous cultures. (*c*) Overall map for ATCase (A) and OTCase (O). DIV is cell division (mid-point of rise in cell numbers). DNA is the mid-point of rise in DNA

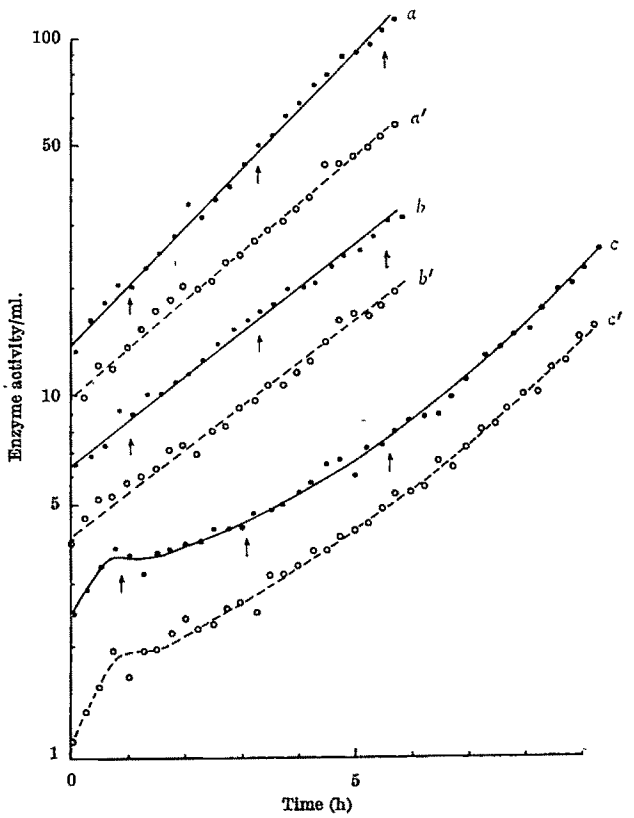


Fig. 4. Alkaline phosphatase (*a*), sucrase (*b*) and maltase (*c*) in synchronous cultures and in control (*a'*, *b'* and *c'* respectively) asynchronous cultures from the same gradients. The arrows indicate the cell plate peaks in the synchronous cultures, as in Fig. 1

These results therefore extend our knowledge of the cell cycle of fission yeast and indicate that similar modes of enzyme synthesis are found in both prokaryotic and eukaryotic cells.

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GENETICS OF STARCH-GEL ELECTROPHORETIC VARIANTS OF HUMAN 6-PHOSPHOGLUCONIC DEHYDROGENASE: POPULATION AND FAMILY STUDIES IN THE UNITED STATES AND IN MEXICO

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FILDES and Parr[1] described a horizontal starch-gel electrophoretic technique to characterize human erythrocytic 6-phosphogluconic dehydrogenase (6-PGD). The enzyme migrated most commonly as a single fast (*A*) band or less frequently as two fast/slow (*A–B*) bands. In a population study in Great Britain, these investigators found the *A–B*, 6 PGD phenotype in the erythrocytes of ten out of 150 adults; the remainder had an *A* phenotype. Examination of the families of several *A–B*, 6-PGD subjects suggested that the *A–B* individuals were probably heterozygous for a gene pair with co-dominant autosomal transmission. Based on a prevalence of one *A–B* subject in fifteen, it was predicted that one person in 900 would be homozygous for the *B* variant of 6-PGD. A subsequent report by the same investigators introduced a modified genetic mechanism[2]. The more common form of 6-PGD still appeared as a single fast *A* band; the *A–B* variant, however, showed a band in the position of *A*, a slower band, *B*, and a third, very faint, *C*, the slowest of all. The *A* and *B* bands of this variant stained with equal intensity; both bands were less dense than the *A* pattern. These authors[2] then postulated a second genetic mechanism which produces sub-units of 6-PGD, two of which combine to form the whole enzyme. They further suggested that a pair of wild-type genes, $\alpha\alpha$, would synthesize the *A* enzyme, and that an individual heterozygous for the α and β alleles, producing equal amounts of α and β sub-units, would form 25 per cent *aa* (*A*), 50 per cent $\alpha\beta$ (*B*) and 25 per cent $\beta\beta$ (*C*); another variant was found[2] characterized by three evenly spaced bands; the intensity of the middle band was twice that of the faster and slower bands. A third allelic gene, γ, was postulated. The slowest band of the new variant was interpreted as $\alpha\alpha$, the middle band $\alpha\gamma$, and the fastest band $\gamma\gamma$.

In a population survey of variants of 6-PGD in Caucasians of European origin and of Negroes in the United States, a hitherto undescribed variant of 6-PGD, which will be described later, was first found in the erythrocytes of the mother of an *A–B*, 6-PGD subject of European origin. A similar variant was then found in a Turkish family; subsequently, several examples of the same variant were discovered in population and family studies of Lacandón Indians in Chiapas, México. Later, during the course of the United States survey, this new variant was also found in a Caucasian male of European origin. The 6-PGD electrophoretic variants and the results of population investigations in the United States and in Yucatán, México, will be presented first. Family studies in the United States and in the Lacandón Indians of Chiapas will then be summarized. These family studies give results compatible with the original hypothesis of Fildes and Parr[1]. Their explanation[2], however, of the minor bands of 6-PGD is either in need of modification, or the techniques of electrophoresis described in this report and that of Fildes and Parr are comparable only for the major 6-PGD electrophoretic bands.

The subjects in the United States consisted of blood donors of the University of Chicago Hospitals and Clinics, healthy employees of the University of Chicago and their unrelated spouses, as well as healthy volunteers at Stateville Prison, Joliet, Illinois. In this group, totalling 1,016, there were 600 Caucasians of European origin and 416 Negroes. Blood was drawn in ACD solution. Techniques for vertical starch-gel electrophoresis were modified from those previously reported for electrophoresis of haptoglobins, glucose-6-phosphate dehydrogenase and other proteins[3]. The starch-gel apparatus of Boyer and Hiner[4] was used, but 'Lucite' slot-formers (constructed by H. R. Smith) were substituted for the metal slot-formers. The stock buffer consisted of 0·9 M *tris*, 0·3 M boric acid and 2×10^{-2} M ethylene diamine tetraacetic acid (EDTA, free acid), *p*H 8·6. A 1/20 dilution of this buffer was prepared for the gel. Starch was obtained from Connaught Laboratories, Toronto, Canada. The amount of starch varied with the lot number. If, say, a starch concentration of 10·3 g/100 ml. was listed on the starch container as optimum for the boric acid–sodium hydroxide buffer of Smithies[3], the buffer already described required a starch concentration of 11·4 g/100 ml. The gel was prepared according to Smithies[3] and nicotinamide adenine dinucleotide phosphate (NADP) was added to a final concentration of $1{\cdot}6 \times 10^{-5}$ M in the hot gel just before degassing. The anodic compartment contained a 1/5 dilution of the stock buffer; the cathodic compartment contained a 1/7 dilution of the same buffer and a final concentration of NADP of $3{\cdot}2 \times 10^{-4}$ M.

Haemolysates for electrophoresis were prepared in the following manner: The erythrocytes were packed by centrifugation at 1,100 g at 4° C for 30 min; the plasma was removed and the red cells were washed three times by suspension in 0·154 M sodium chloride and centrifugation. After the last wash, the supernatant was removed, and the cells were lysed by rapid freezing and thawing three times. The haemolysate was then diluted 1 : 3 with *tris*-hydrochloric acid buffer (0·05 M, *p*H 8·6) and centrifuged at 4° C at 20,000*g* for 1 h in a Sorvall '*RC*-2' refrigerated centrifuge. The ghost-free haemolysate was then adjusted to a haemoglobin concentration of 3·5 g/100 ml. for electrophoresis. Electrophoresis was for 16 h, 6 V/cm at 4° C. The gels were stained for 3–4 h in the dark at 25° C in a solution of 150 ml. 0·5 M *tris*-hydrochloric acid, *p*H 8·6, to which was added 10 ml. 0·01 M magnesium chloride, 250 mg 6-phosphogluconate, hexylammonium salt, 10 mg nitro-blue-tetrazolium, 6 mg phenazine methosulphate, 40 mg NADP, and EDTA (disodium salt) to a concentration of 10^{-2} M.

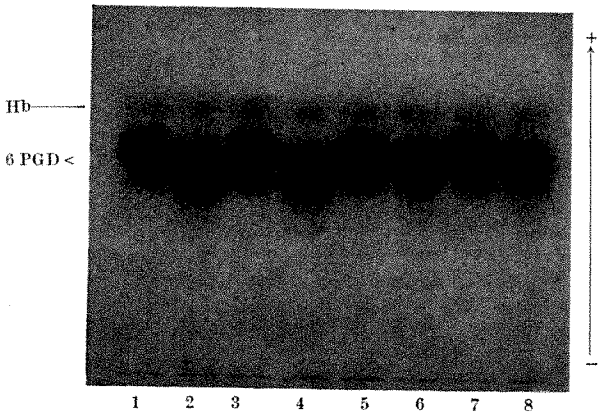


Fig. 1. Starch-gel electrophoresis of variants of 6-PGD. The blood samples were electrophoresed in an eight-slot gel and are from eight different subjects. In this figure, and subsequently in the text, 6-PGD phenotypes will be referred to as Pd *A*, Pd *A–B* and Pd *B*. Pd *B* is the new 6-PGD electrophoretic variant. The corresponding genotypes will be termed: Pd^A/Pd^A, Pd^A/Pd^B, and Pd^B/Pd^B. This terminology is analogous to the nomenclature of glucose-6-phosphate dehydrogenase variants recently introduced by Boyer and Graham (ref. 5). Slots 1, 3, 5 and 7 contain Pd *A*, slots 6 and 8, Pd *A-B*, and Pd *B* is in slots 2 and 4

In this buffer system, the 6-PGD bands migrated behind haemoglobin A (Fig. 1). Evidence for designating the new variant as representative of homozygous B 6-PGD will be presented later. A diagram of the 6-PGD patterns is shown in Fig. 2. Henceforth in this presentation the 6-PGD nomenclature outlined in Fig. 1 will be used. The erythrocytes of the first subject discovered to have the new 6-PGD variant were re-tested on nine occasions within a 10-month period without change in patterns. Samples of erythrocytes representing the three 6-PGD forms were stored at 4° C for at least 4 weeks and tested several times during this period; the 6-PGD patterns were not altered. Blood samples were also glycerolized and frozen at −20° C for 8 months without alteration of the 6-PGD bands. The buffer system of Boyer *et al.*[3] was initially used for electrophoresis; however, the Pd *A* band migrated in the position of haemoglobin A; consequently, haemoglobin A obscured the Pd *A* band, and the detection of fast minor bands was impossible. The three forms of 6-PGD were also distinguishable by electrophoresis with a different buffer system at a *p*H of 8·0. This analysis was performed by Dr. George J. Brewer, Department of Human Genetics, University of Michigan Medical School[6].

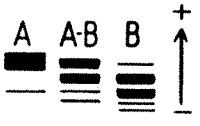


Fig. 2. Diagrammatic representation of 6-PGD phenotypes on starch-gel electrophoresis. The new variant is labelled *B*

Minor bands were also observed. A faint minor band (Figs. 1 and 2) was consistently noted behind the Pd *A* pattern. If results of the electrophoretic system used by Fildes and Parr[1] are comparable with those of this report, a comparison which may not be justified, the minor band in the Pd *A* phenotype is incompatible with their second hypothesis of the mechanism and formation of 6-PGD electrophoretic patterns. The intensity of staining of the two major bands of the Pd *A–B* phenotype was comparable to the description reported for this pattern[1]; additionally, however, at least two minor bands were observed behind the *B* band of Pd *A–B* (Figs. 1 and 2). One of these bands had the position and the intensity of the *B* band of the Pd *A–B* phenotype; the other band was comparable in position, but not in intensity, to the minor bands of the Pd *A* and Pd *A–B* phenotypes. Furthermore, at least three other minor bands were found in the new variants: two of these migrated behind the slower of the major bands; the other minor band was in the position of the major band of the Pd *A* phenotype. Gels were incubated without substrate; no staining in the position of the major or minor 6-PGD bands was observed. When gels were prepared without NADP or with nicotinamide adenine dinucleotide phosphate (NAD) alone, the 6-PGD patterns were diffuse.

During the course of these investigations, several hypotheses to account for the electrophoretic patterns of 6-PGD variant were considered. The first two families in which the new 6-PGD variant was found, however, provided the major clue to the working hypothesis of this study. Late in the United States survey, another very important family was discovered through a PD-*B* propositus. The pedigrees of these three families are illustrated in Fig. 3.

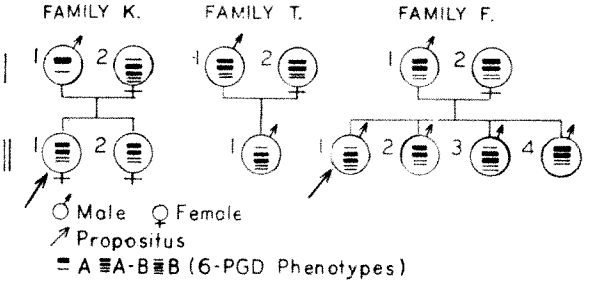


Fig. 3. Pedigrees of three key families showing that the new variant is the homozygous form of B 6-PGD. In Family *K.*, the mating Pd *A* × Pd *B* = Pd *A–B*, only; in Family *T.*, the mating Pd *A–B* × Pd *A–B* = Pd *B*. The mating of Family *F.* is similar to Family *T.*; however, Family *F.* is more informative: Pd *A–B* × Pd *A–B* = Pd *A–B*, Pd *B*, Pd *A* would also be predicted from this mating. Blood from Family *T.* was referred for electrophoresis of glucose-6-phosphate dehydrogenase by Dr. Izzet Berkel, Ankara University Hacettepe Medical Center, Hacettepe Faculty of Medicine, Ankara, Turkey

The nature of the minor or multiple bands of 6-PGD will rest on further studies. This report is concerned only with the genetics of these 6-PGD variants. The slower major band of the new 6-PGD variant might be thought to represent the gene product of a third allele at the 6-PGD locus. However, Family *T.* and Family *F.*, Fig. 3, disprove this hypothesis. Population and additional family studies of 6-PGD electrophoretic phenotypes indicated that the new variant represents the homozygous *B* phenotype and not the product of a third allele at the 6-PGD locus.

The results of population studies in the United States are recorded in Table 1. A pattern comparable to the γ allele[2] was not found. In the Caucasian population of European origin (Table 1), the observed and expected numbers of Pd *A*, Pd *A–B* and Pd *B* were such that chi-square calculations were unnecessary. It was also

Table 1. POPULATION STUDIES OF STARCH-GEL ELECTROPHORETIC PATTERNS OF 6-PHOSPHOGLUCONIC DEHYDROGENASE IN THE UNITED STATES

6-PGD phenotypes	Caucasians of European origin			Negroes		
	Males	Females	Total	Males	Females	Total
Pd *A*	514	40	554	379	6	385
Pd *A–B*	40	5	45	31	0	31
Pd *B*	1	0	1	0	0	0
Total	555	45	600	410	6	416

Estimated gene frequencies*

	Caucasians of European origin				Negroes		
	Pd *A*	Pd *A–B*	Pd *B*	Total	Pd *A*	Pd *A–B*	Total
Observed:	554	45	1	600	385	31	416
Expected:	553·9	45·2	0·9		$2\,p\text{–}2p^2 = 0{\cdot}0745$		
	$Pd^A = 0{\cdot}9608$				$Pd^A = 0{\cdot}9613$		
	$Pd^B = 0{\cdot}0392$				$Pd^B = 0{\cdot}0387$		
	1·0000				1·0000		

(Expected frequency of Pd^B/Pd^B in each population = 0·0015.)

* Pd^A and Pd^B gene frequencies in Caucasians of European origin were estimated by the gene counting method; in Negroes, these gene frequencies were estimated from the prevalence of heterozygotes, Pd A-B.

apparent that there were no significant differences among the frequencies of the 6-PGD phenotypes in Caucasians of European origin and Negroes of the United States and the British population. The frequency of the *A–B* phenotype in the British study of 150 study subjects[1] was 0·0714; in the present United States survey, the frequency of this phenotype in Caucasians of European origin was 0·0750, and in Negroes, 0·0745.

A survey of 85 unrelated males and females in Yucatán, México (Mestizos), revealed that all had the Pd *A* phenotype. The Pd *A–B* phenotype was not found. Although the sample was small, this suggests that the frequency of the Pd^B allele in this population is lower than in the Negro and the Caucasian population of European origin studied in the United States and than in the population reported from Great Britain.

Additional family studies in the United States and in the Lacandón Indians of Chiapas, México, supported the concept that the first mechanism of inheritance of 6-PGD electrophoretic variants proposed by Fildes and Parr[1] is valid, and that the new variant described in this report is the homozygous *B* pattern. The results of family studies in the United States and in México are listed in Table 2 and are classified according to a two-allele system. This scheme, of course, does not exclude additional alleles at the 6-PGD locus. The variant[2] described by Fildes and Parr may be an example of a heterozygous form of a third allele, as these authors suggest.

Table 2. FAMILY STUDIES OF STARCH-GEL ELECTROPHORETIC VARIANTS OF 6-PHOSPHOGLUCONIC DEHYDROGENASE

Matings*	No. of families	No. of children	Phenotypes Pd *A*	Pd *A–B*	Pd *B*
(1) $Pd^A/Pd^A \times Pd^A/Pd^A$	21	54	54	0	0
(2) $Pd^A/Pd^A \times Pd^A/Pd^B$	6	18	10	8	0
(3) $Pd^A/Pd^A \times Pd^B/Pd^B$	2	3	0	3	0
(4)† $Pd^A/Pd^B \times Pd^A/Pd^B$	2	5	0	2	3

* Matings: $Pd^A/Pd^B \times Pd^B/Pd^B$ and $Pd^B/Pd^B \times Pd^B/Pd^B$ were not observed in this survey.

† In a Lacandón family, the mother was Pd *A–B*; one child was Pd *A*, one child was Pd *A–B* and two children were Pd *B*. Blood from the father was not available; however, according to this hypothesis, he could only be Pd *A–B*.

The survey of the Lacandones, a markedly inbred Mayan Indian population, provided direct as well as indirect support for the view that the new variant of this report is homozygous 6-PGD *B*. These Indians number approximately 200 and live in scattered small family and clan groups in the jungles of Chiapas; 150 members were available for study. The Lacandón Indians now form two separate breeding groups: (1) the populations of Lacanjá and San Quintín, and (2) the groups of Najá, Metzabok, Monte Líbano, Arroyo Mendez and Santo Domingo. These two populations are separated by distance and dense jungle and have had no contact for many years. The Lacandones in Santo Domingo were not examined. Pd *A*, only, was found in 62 family members from Lacanjá and San Quintín. In the Najá, Metzabok, Monte Libano and Arroyo Mendez groups, Pd *A–B* was found. The probability of finding subjects who are homozygous for the 6-PGD *B* variant would be higher in this inbred population than in the random mating populations investigated in the United States; conversely the probability of finding a rarer third allele in these Indians would be small. Thus, in the Lacandón Indians a variant other than Pd *A* or Pd *A–B* would in all likelihood be Pd *B*. In the samples of subjects of Najá, Metzabok, Monte Libano and Arroyo Mendez, four had the new variant, PD *B*. From estimates of the frequency of the Pd^B/Pd^B homozygote in Caucasians of European origin and in Negroes in the United States (Table 1), a sample size of slightly in excess of 2,600 Caucasians of European origin or Negroes would be necessary to obtain the number of Pd *B* subjects found in these 88 Lacandones.

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[6] Brewer, G. J. (personal communication to Bowman, J. E.); the technique used was that described by Shows, jun., T. B., Tashian, R. R., and Brewer, G. J., *Science*, **145**, 1056 (1964), with the exception that 6-phosphogluconate was substituted for glucose-6-phosphate in the staining procedure.

ELECTROPHORETIC DEMONSTRATION OF STROMAL EFFECTS ON HAEMOLYSATE GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND 6-PHOSPHOGLUCONIC DEHYDROGENASE

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THE present electrophoretic demonstration of stromal effects on haemolysate enzymes stems from two sources: (1) previously reported changes[1–6] in enzymatic activities of haemolysates of normal human erythrocytes induced during incubation of the haemolysates with their stromata, and (2) the genetics of human 6-phosphogluconic dehydrogenase (6-P-GD) as revealed by the starch-gel electrophoresis of haemolysates described by

Bowman *et al.*[7]. The induced changes are (1) inactivation of glucose-6-phosphate dehydrogenase (G-6-PD), (2) apparent activation of glutathione reductase (GSSR-R), and (3) partial inactivation of 6-P-GD.

The inactivation of G-6-PD is caused by stromal NADP-ase which removes bound NADP necessary for stability of the enzyme[2,8,9]. The mechanism for the apparent activation of GSSG-R is unknown; however, the activation does not occur during incubation of haemolysates of G-6-PD deficient erythrocytes which have intrinsically elevated GSSG-R activity[6,10,11]. The inactivation of G-6-PD during these incubations can be prevented by addition of protective NADP and it was in this situation that the third change, partial inactivation of 6-P-GD, was found[6].

The data for these changes were obtained only in terms of determinable activity by enzymatic assay of haemolysates. The observations presented by Bowman *et al.*[7] stimulated our interest in the electrophoretic patterns with respect to these changes. Unlike the X-linked G-6-PD which migrates as a single band in haemolysates of blood from hemizygous males and homozygous females, 6-P-GD migrates in multiple bands in three genetically determined forms, Pd *A*, Pd *A–B* and Pd *B* (ref. 7). The question, therefore, with respect to 6-P-GD, was whether or not the partial inactivation of 6-P-GD would be distinguishable electrophoretically as well as by decreased activity. If so, would the effect also be distinguishable among the three forms, hopefully, by specific removal of one or another of the major or minor bands.

Packed erythrocytes prepared by repeated centrifugation and washing (three times) with 0·162 M sodium chloride from fresh heparinized blood (drawn from healthy individuals) were haemolysed by rapid freezing and thawing. After dilution with 0·162 M sodium chloride to a final haemoglobin concentration of 5–7 g/100 ml., aliquots were prepared for incubation as follows: (I) stroma-free haemolysate. Stromata were removed by centrifuging at 30,000*g* (in a 'Lourdes' refrigerated centrifuge), (II) whole haemolysate (stromata not removed), (III) whole haemolysate as in (II) but NADP (P-L Biochemicals, Inc.) to a final concentration of 10^{-4} M was added at the beginning of incubation, (IV) stroma-free haemolysate as in (I), but also with NADP (10^{-4} M) added at the beginning of the incubation.

Incubation was performed at 45° C so that the effects could be demonstrated in 1 or 2 h; lower temperatures require longer incubation. Immediately after incubation stromata were removed from haemolysates II and III by centrifuging at 30,000*g*. Aliquots of the incubated haemolysates and of the control non-incubated stroma-free haemolysate were taken for starch-gel electrophoresis using conditions identical to those described by Bowman *et al.*[7]. The gels were then sliced horizontally and one half stained for G-6-PD, the other half for 6-P-GD. The remainder of the haemolysates were diluted for assay of G-6-PD and 6-P-GD by the method of Glock and McLean[12] using the conditions reported in ref. 6. GSSG-R was assayed by the method of Long and Carson[13] (except that the final *p*H was 6·7–6·9 instead of 7·6). For the assays a 'Beckman *DU*' spectrophotometer fitted with a 'Gilford' multiple sample absorbance recording system was used.

1-h incubations performed during this series gave activity changes entirely similar to those previously reported[6]. Therefore, for this report we show in Table 1 the results of the 2-h incubations which were used to enhance the 6-P-GD effect found with haemolysate III. The results are tabulated according to the electrophoretic type of 6-P-GD for enzyme activities, 6-P-GD, G-6-PD and GSSG-R. The chief difference from the results for 1-h incubations is the increased loss of G-6-PD activity by heat denaturation especially in haemolysate I. The increased activity of GSSG-R in haemolysates II and III, compared with haemolysates I and IV, is similar to the results for 1 h.

Table 1. RESULTS OF ENZYME ASSAYS OF HAEMOLYSATES I, II, III, IV PREPARED AS DESCRIBED IN THE TEXT, AFTER 2-H, INCUBATION AT 45° C

Values are percentage of the initial values of the stroma-free, non-incubated haemolysate. All individuals gave normal initial values

Enzyme	6-P-GD Type	Haemolysate I	II	III	IV
6-P-GD	Pd *A**	97	82	55	96
	Pd *A–B*†	77	63	37	57
	Pd *B*‡	65	56	30	51
G-6-PD	Pd *A**	21	5	72	86
	Pd *A–B*†	18	5	83	94
	Pd *B*‡	11	2	80	89
GSSG-R	Pd *A**	107	136	136	111
	Pd *A–B*†	107	138	137	112
	Pd B§	118	138	148	118

* Average of two experiments with blood from two individuals.

† Average of two experiments with blood drawn at two different times from the same individual.

‡ Average of three experiments with blood drawn at three different times from the same individual.

§ One experiment.

The data for 6-P-GD activities reveal the principal loss of activity to be in the incubated haemolysate III, that is, both stromata and NADP were required for this loss of activity. The results for the Pd *A* type both at 1 and 2 h are entirely consistent with the previous series; there is negligible loss of activity in haemolysates I and IV and less than 20 per cent loss in haemolysate II. However, all the haemolysates, I, II, III and IV, for the Pd *A–B* and Pd *B* types showed greater loss of activity than the Pd *A* type not only after 2 h incubation but also after 1 h. This suggests that the 6-P-GD of the Pd *A–B* and Pd *B* types may be more labile than the 6-P-GD of the Pd *A* type and is consistent with the recent communication of Parr and Parr[14].

The electrophoretic patterns for G-6-PD after 1 h incubation of haemolysates prepared from a single blood

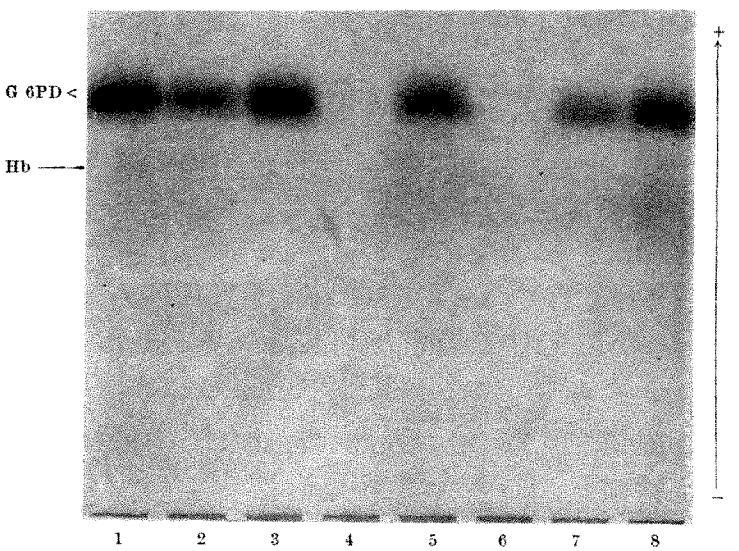


Fig. 1. Starch-gel electrophoresis of haemolysate G-6-PD, 1 h incubation of haemolysates prepared as described and numbered in the text. Aliquots were placed in the slots as follows: (1) control, no incubation, (2) haemolysate I, (3) haemolysate IV, (4) haemolysate II, (5) haemolysate III, (6) haemolysate I treated with 'Norit' prior to incubation, (7) same as (6) with NADP added after removal of 'Norit', (8) duplicate of (5)

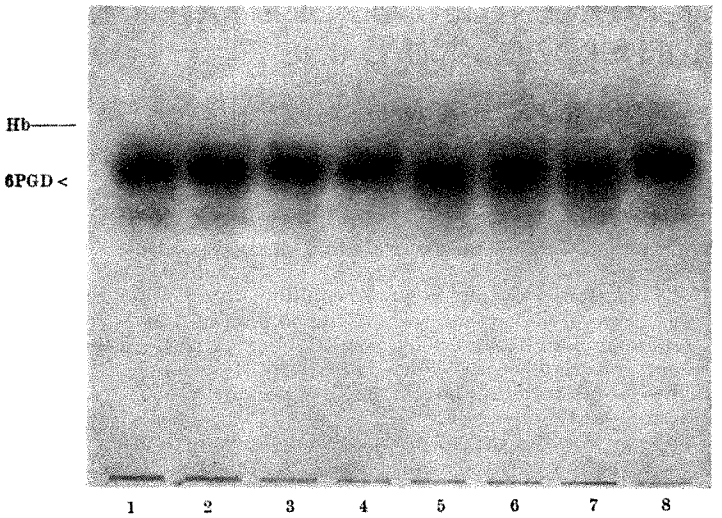


Fig. 2. Starch-gel electrophoresis of haemolysate 6-P-GD, Pd *A* types, 2 h incubation (except for slot 6) of haemolysates prepared as described and numbered in the text. Aliquots were placed in the slots as follows: (1) Control, no incubation, (2) haemolysate I, (3) haemolysate IV, (4) haemolysate II, (5) haemolysate III, (6) haemolysate III after 1 h instead of 2 h incubation, (7) haemolysate III treated with 'Norit' after incubation, (8) duplicate of (4)

sample are shown in Fig. 1. Slot 1 is the non-incubated control. Slot 2 shows the partial loss of activity by heat denaturation of the stroma-free haemolysate and slot 3 the protection by added NADP. Slot 4 demonstrates the loss of G-6-PD when incubated with stromata and slot 5 the protection by NADP. (Slot 8 is a duplicate of slot 5.)

In Fig. 2 are shown the results for 6-P-GD of the Pd *A* type. All haemolysates except haemolysate III retained their configuration; haemolysate III is distinguishable as a diffuse spot without clear bands occupying a larger area both after 1 and 2 h of incubation (slots 6, 5). Entirely similar results have been obtained in the bloods from the Pd *A–B* and the Pd *B* types. That is, the usually distinguishable bands have merged, there is no specific removal of any band, and the effect is essentially that of smearing. Although the Pd *A–B* and Pd *B* types of 6-P-GD were more labile than the Pd *A* type in this study, the electrophoretic smearing occurred only in haemolysate III.

Elucidation of this effect will require extensive investigation, especially after purification of both the enzyme and of the membranes. However, the following additional experiments have been performed.

(1) Dialysis of the haemolysates by gel filtration (through 'Sephadex *G*-25') after incubation removes free coenzymes, reduced and oxidized glutathione (GSH and GSSG), but not bound coenzymes. This procedure did not restore the 6-P-GD pattern or activity in haemolysate III, nor alter the patterns of the control haemolysate or of haemolysates I, II or IV.

(2) Acid-washed 'Norit' (45 mg/ml.) added to stroma-free haemolysate, prior to incubation, shaken and then removed by filtration, removes GSH, GSSG, free and bound coenzymes. Removal of the bound NADP results in a very labile G-6-PD and this activity, as with the stromal effects, is lost on incubation unless prevented by addition of NADP[2]. Pontremoli and co-workers made use of this effect of 'Norit' to remove G-6-PD in their procedure for crystallization of 6-P-GD from *Candida utilis*[15]. In Fig. 1 the loss of G-6-PD after treatment with 'Norit' is shown in slot 6 and the protection by added NADP in slot 7. However, no loss of 6-P-GD activity or smearing of the electrophoretic pattern is observed after treatment of stroma-free haemolysates with acid-washed 'Norit' prior to incubation with added NADP. Neither does the treatment with 'Norit' cause increased activity of GSSG-R. For both of these changes stromata are required. In addition, treatment of haemolysate III with 'Norit' after incubation does not restore either 6-P-GD activity or the electrophoretic pattern (slot 7, Fig. 2). (Experiment performed with the Pd *A* and Pd *A–B* type but not with the Pd *B* type.)

(3) Substitution of NAD for NADP in haemolysates III and IV fails to protect G-6-PD and also fails to induce the 6-P-GD effect in haemolysate III, that is, activity is not decreased and the electrophoretic pattern does not change or smear.

(4) However, alteration in the electrophoretic pattern of 6-P-GD has been obtained by modifying the preparation of the starch-gel. If NADP is not added to the gel, or if NAD instead of NADP is added to the gel, the 6-P-GD bands in all the haemolysates no longer appear separately, the spots are diffuse, and haemolysate III can no longer be distinguished from the others (either before or after incubation). Similarly, the Pd *A*, Pd *A–B* and Pd *B* types also cannot be distinguished unless NADP is added to the gel.

The decrease in 6-P-GD activity of haemolysates induced by incubation with stromata and NADP may be associated with an actual alteration of enzyme protein. Unlike the inactivation of G-6-PD and apparent activation of GSSG-R by stromata, NADP is also necessary to effect this change. The effect is not reversed by dialysis or treatment with 'Norit', nor can NAD be substituted for NADP to induce the effect. On the other hand, similar electrophoretic effects are observed by failing to add NADP to the starch gel; again, NAD cannot substitute for NADP. Thus, although NADP does not seem to be directly necessary for protection of the catalytic activity of 6-P-GD as it is for G-6-PD, this compound may affect the structural state of 6-P-GD, a fact not apparent in our previous investigations.

Whether these stromal effects in haemolysates are of physiological or pathophysiological significance is not known. The erythrocyte membrane does appear to have the capability of altering the metabolism of the erythrocyte by effecting changes in the cytoplasmic enzymes.

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DETERMINATION OF TRACES OF ETHYLENEGLYCOL DINITRATE (AND NITROGLYCERINE) IN BLOOD AND URINE

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THE physiological action of ethyleneglycol dinitrate (EGDN) and nitroglycerine (NG) is of considerable interest to medical personnel associated with the manufacture of explosives[1-3]. Up to the present time, workers in this field have determined EGDN and NG in body fluids by using colorimetric methods to estimate nitrate or nitrite ions[1]. We have found that such methods are not only insufficiently sensitive but they suffer from the serious disadvantage that they do not readily distinguish between organic and inorganic nitrates. It seemed to us that the electron capture detector used in conjunction with gas chromatography might form the basis for a reliable method for the quantitative determination of EGDN and NG because it was already known that the electron capture detector was extremely sensitive towards certain organic chlorinated and nitrocompounds[4]. We have devised a method which can readily determine less than 0·04 ng (= 0·04 × 10^{-9} g) of EGDN, and preliminary experiments have shown that a lower limit of detection of less than 1 ng could be reached for NG.

EGDN is considerably more volatile than NG and readily penetrates a wide range of materials[5]. It is therefore likely to be found in the blood and urine of explosives workers. By the application of this method we have confirmed the presence of EGDN in such samples.

The determination of EGDN in a sample of heparinized blood or in urine was made in the following way. A measured volume (usually 0·5 ml.) of the sample was transferred to a stoppered glass tube (10 ml.) followed by the addition of 1 ml. of water and 0·3 g of sodium chloride, which was dissolved by shaking the tube. The solution so obtained was thoroughly shaken with 2 ml. *n*-hexane and the organic extract was then used directly for chromatography after centrifuging. The gas chromatographic separation was carried out using 10 per cent '*E*301' silicone elastomer (Imperial Chemical Industries, Ltd.) as stationary phase on 'Embacel' (May and Baker, Ltd.) utilizing a metal column 2 ft. long and of 0·25 in. diameter. The column was heated in an oven at 140° C with a sample preheater and vaporizer[6] at 160° C, the electron capture detector being connected to the outlet of the column at the top of the oven. Nitrogen (oxygen free), previously dried with molecular sieves, was used as carrier gas at a flow-rate of 150 ml. per minute. It was necessary to pay careful attention to the design and geometry and conditioning of the electron capture detector for it was found that even two apparently identical detectors could behave markedly differently. The detector finally chosen for this work was made in the laboratory workshops; the body, consisting of a 'Teflon' cylinder (4 cm long, 2·5 cm external diameter and 1 cm internal diameter), was closed with brass end pieces which served as the electrodes and enclosed a gas volume of about 1·5 c.c. The cathode was hollowed out to accommodate a standard tritium source consisting of a strip of titanium-plated copper foil (supplied by the Radiochemical Centre, Amersham), 3 cm × 1 cm, activity 300 mc. The greatest sensitivity was obtained when the foil was positioned so that it protruded a distance of about 1 mm beyond the end of the cathode. Under the conditions used, a potential of about 30 V applied to the detector gave the most satisfactory background current (1–2 × 10^{-9} amp using a Perkin–Elmer amplifier). Before a definite response could be obtained for EGDN, it was necessary to preheat the column at 180° C for about an hour followed by conditioning of the column at its working temperature with 1-μl. amounts of hexane solution containing 1 μg NG per μl., injected at about hourly intervals.

For the actual analysis of a hexane solution derived from a blood or urine sample, a volume of 10–40 μl. was transferred to the column and the peak due to EGDN was compared with standards. The standards were prepared under the same conditions as the test by extracting dilute aqueous solutions containing known amounts of EGDN with hexane. Fig. 1 shows a series of chromatograms derived from 10-μl. amounts of hexane obtained in the manner described earlier from samples of blood containing amounts of EGDN equal to zero, 0·02, 0·04, 0·08, 0·12 p.p.m. (that is, zero, 0·05, 0·10, 0·20, 0·30 ng).

As the rate of disappearance of EGDN in human blood *in vitro* is not known, this was determined by adding small amounts of EGDN to a series of blood samples and following the rate of fall in EGDN concentration over a period of time. Fig. 2 shows the results obtained from studying blood samples which originally contained between 0·03–0·18 p.p.m. of EGDN. It is quite clear that the rate of fall of EGDN is sufficiently slow to allow for a delay between the taking of a blood sample and its subsequent analysis. However, as the fall is appreciably more rapid at the lower concentrations of EGDN, estimation is ideally performed as soon as possible.

Because of the great sensitivity of the analytical method and the likelihood of contamination during the collection of blood and urine samples, the degree of persistence of EGDN on the skin was studied. About 5 mg of EGDN was spread over part of the palm of the hand with the aid of a glass rod, so that it covered an area of 4 sq. cm. After an interval of 3 min the hand was washed with hot soapy water, rinsed and dried. Using a scalpel, about 5 mg of skin was removed from the area to which the EGDN had been applied and extracted with a suitable volume of hexane and EGDN determined by the method

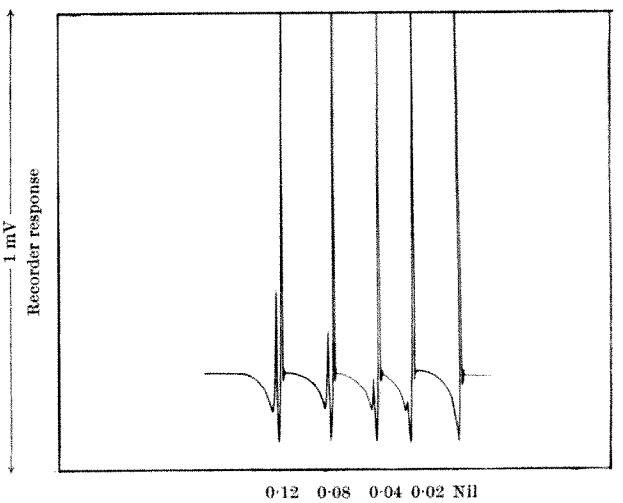


Fig. 1. Chromatograms for EGDN (p.p.m.)

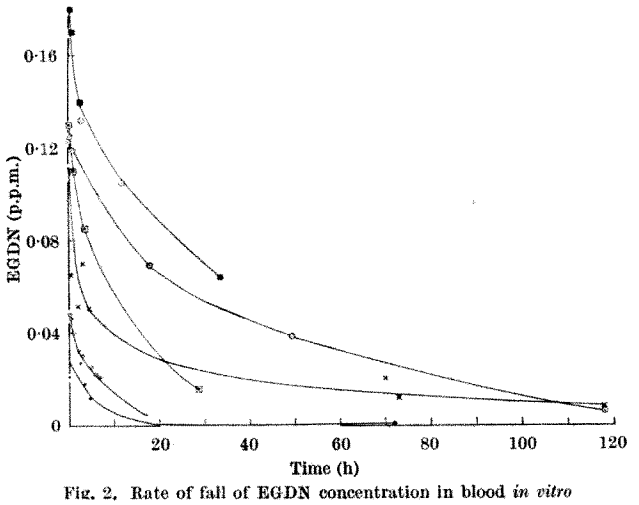


Fig. 2. Rate of fall of EGDN concentration in blood *in vitro*

described. An amount equivalent to 30 ng/mg skin was found. After a further period of 1 h (without further washing of the hand) this fell to 3·5 ng/mg skin; 30 min later the hand was washed again in hot soapy water and a final sample of skin yielded 2 ng EGDN/mg skin. These results showed that EGDN, when applied to human skin, may persist after ordinary methods of cleansing. The method subsequently used for cleansing, before taking a blood sample, was to wash the skin with 'Saroul' followed by absolute alcohol.

The blood and urine of a number of plant operatives were examined for EGDN. The analyses were made on blood samples taken from five different operatives working on a section of an explosives plant where EGDN contamination was likely to be greater than at other parts of the plant. The samples of blood were obtained from a peripheral vein within a few minutes of the operator leaving his work, and all the samples were examined within a period of 30 min of being taken. The blood samples for the five operatives contained 0·04, 0·01, 0·07, 0·06 and 0·05 p.p.m. EGDN respectively. The corresponding urine samples contained 0·02, 0·03, 0·02, 0·04 and 0·08 p.p.m. EGDN. The blood and urine of two control personnel gave negative tests. When the same operatives were re-examined after a period of 16 h away from explosives manufacture, EGDN was no longer detectable in either their blood or their urine.

Work on the fate of EGDN in the human circulation is in progress and is being extended to the study of the pharmacological action of NG.

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ROLE OF LONG EXTRACELLULAR CHANNELS IN FLUID TRANSPORT ACROSS EPITHELIA

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THIS article presents the results of correlated physiological and anatomical experiments to identify the route by which water is transported across epithelial cells such as those of the intestine, gall-bladder, and kidney. Such epithelia produce specific secretions or absorbates by active transport of some solute, with water movement probably a secondary result of local osmotic gradients set up within the tissue by active solute transport[1]. The main problem is to explain how local osmotic equilibration can be so complete that water follows solute in virtually isotonic proportions. Calculation shows that the measured passive permeability of many epithelia to water would be much too low to account for the observed isotonicity of transported fluid without some special geometrical arrangement. A hypothesis for the route of water transport must therefore answer this question—What structural device prevents actively transported solute from diffusing away before sufficient water has followed to achieve osmotic equilibrium?

The epithelium selected for these experiments was the gall-bladder of the rabbit. This tissue offers the advantage of a relatively simple anatomy and physiology, and is able to maintain a high rate of water transport *in vitro*[2]. To concentrate bile, the gall-bladder reabsorbs sodium and chloride actively, with water following the salt in isotonic proportions[3–7].

Water transport in isolated, cannulated rabbit gall-bladders was measured under various physiological conditions by means of a gravimetric method already described[2,3]. At the end of each experiment the gall-bladders were fixed by buffered osmium tetroxide and embedded in 'Epon 812'. Sections 2μ thick were examined in a phase contrast microscope, employing Zeiss 'Neofluar' optics. In some cases thin sections from the same specimens were also examined in an electron microscope. The electron microscope findings in general confirmed those obtained with the light microscope, and will be presented in detail elsewhere.

The organ consists of a sac, with a single layer of epithelial cells supported by an outer layer of connective tissue. As shown in diagrammatic form in Fig. 1, the epithelium possesses several salient features found under all the experimental conditions to be reported: (1) The cells are tall and slender. (2) The apical surface of the cell (that is, the surface facing the lumen of the gall-bladder) contains an array of microvilli, similar in structure to those found, for example, in the brush border of intestinal and kidney epithelia. (3) The cells are joined together at their luminal borders by a continuous system of terminal bars (tight junctions)[8]; by this structural device, the luminal contents of the gall-bladder are sealed off from direct access to the lateral intercellular spaces. (4) The area of the lateral cell surface is greatly increased by numerous cytoplasmic projections into the lateral intercellular space. These projections bear a superficial resemblance in size and shape to microvilli, but otherwise

lack certain distinctive morphological features of the latter. The width of the intercellular space separating the lateral borders of adjacent cells is quite variable, depending on the experimental conditions. Under conditions where this width is minimal, the cytoplasmic projections from each cell form complex mutual interdigitations with those of neighbouring cells. (5) The basal cell surface is without membrane infoldings and rests on a typical basement membrane. (6) The only prominent cytoplasmic organelles are the abundant mitochondria, which are absent only in the terminal web immediately beneath the apical microvilli. Elsewhere they are distributed fairly uniformly throughout the cell, although at a somewhat higher concentration towards the apical pole. (7) Vesicles occur but are quite few in number. The other usual cytoplasmic systems and organelles (such as endoplasmic reticulum, Golgi apparatus) are unimpressively represented.

In preliminary experiments, gall-bladders were placed in fixative within 50–100 sec after interruption of the blood supply. The epithelial cells were found to be separated from one another by lateral spaces whose width was variable but averaged approximately 0·4μ. The extent to which these freshly excised gall-bladders had completed concentrating bile and hence to which they were still absorbing water is unknown and was probably variable.

In order to obtain preparations known to be transporting water at maximal rates, gall-bladders were cannulated and incubated at 37° C in a Ringer's solution containing 110 mM sodium chloride and 25 mM sodium bicarbonate. After transport rates of 228–592 μl./h had been measured for 1 h, the gall-bladders were fixed and examined as described above. These organs differed from freshly excised preparations in that the lateral intercellular spaces were quite dilated (see Fig. 2*A*). The measured width of these spaces in five gall-bladders was found to average 0·9μ (standard error = 0·1μ, $n = 58$). (These and all subsequent measurements of the lateral spaces were made half-way between the basal and apical surfaces of the cells, using a ×100 Zeiss 'Neofluar' oil immersion lens, $N.A. = 1{\cdot}3$. The smallest separation measurable in this manner was approximately 0·2–0·3μ,

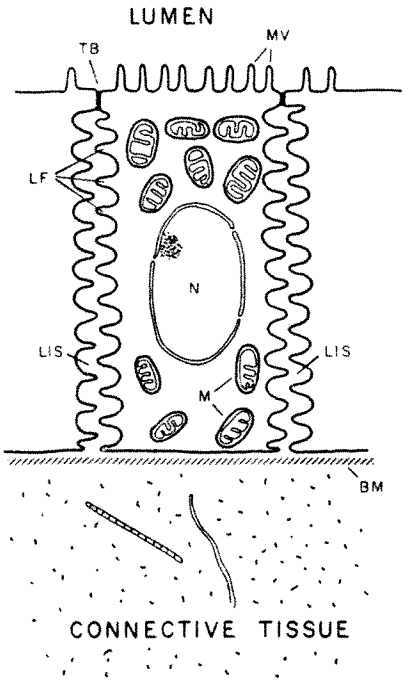


Fig. 1. Schematic representation of the structure of an epithelial cell from rabbit gall-bladder (not to scale). The height of the cells relative to their width is actually more than twice that depicted here. An unbroken sheet of such cells faces the lumen of the gall-bladder. *TB*, terminal bar; *MV*, microvilli; *LF*, lateral folds; *LIS*, lateral intercellular spaces; *M*, mitochondria; *N*, nucleus; *BM*, basement membrane

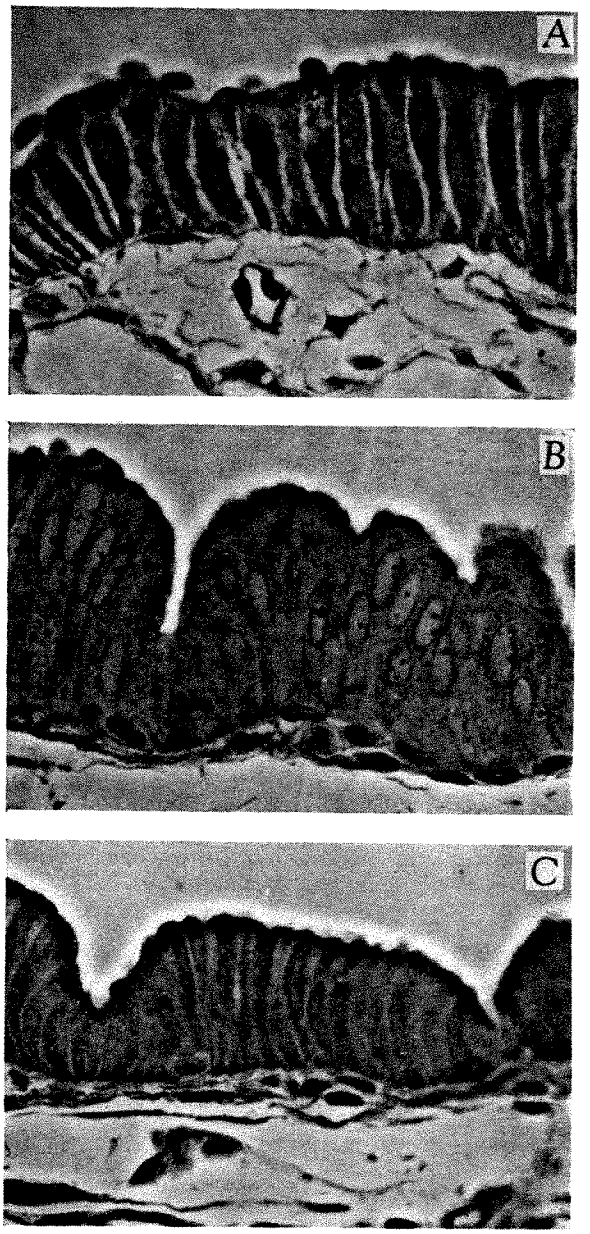


Fig. 2. Phase contrast micrographs of rabbit gall-bladder epithelium *in vitro* under three different experimental conditions. All three photographs are at identical magnifications of ×800. (*A*) Actively transporting preparation in sodium chloride Ringer's solution at 37° C. Note the clear spaces, approximately 1μ wide, between adjacent cells. (*B*) Transport stopped by 10^{-3} M ouabain. There are no intercellular spaces visible so that it is difficult to see precisely where one cell steps and the next begins. The cells are also substantially taller than in the actively transporting preparation. (*C*) Transport stopped by substitution of sucrose for sodium chloride in the luminal bathing solution. As in 2*B*, there are no spaces evident between the cells. The cells are also substantially smaller than in the actively transporting preparation

that is, at the limit of resolution of the light microscope.) While the spaces were generally dilated for most of their length, adjacent cells always remained tightly attached to one another at the terminal bar. Similar observations have recently been reported[9].

To determine whether this dilation of the spaces was correlated with fluid transport, we next examined gall-bladders in which water transport had been stopped by four different sets of conditions. In each experiment the rate of water movement was followed gravimetrically for 30–60 min before fixation to make sure that water transport had ceased. The four conditions used to stop transport, and their effects, were as follows:

(1) *Low temperature.* Gall-bladders were maintained in Ringer's solutions containing 110 mM sodium chloride

and 25 mM sodium bicarbonate, but at 4° C. The rate of water transport dropped to between 4 and 10 μl./h. The principal difference in the appearance of these cells from those of transporting preparations was that the lateral spaces were nearly closed. No space was visible between 75 per cent of adjacent cell pairs with the × 100 objective, and spaces between the remaining cells were no more than 0·4μ wide.

(2) *Ouabain.* This cardiac glycoside inhibits active transport in the gall-bladder[3,7] as well as in many other tissues. In the present experiments 10^{-3} M ouabain reduced fluid absorption at 37° C to an average level of 20 μl./h. The morphological findings (Fig. 2*B*) were that almost all the lateral spaces were completely closed, so that the borders between adjacent cells became difficult to recognize. In addition, the cells appeared substantially taller than transporting cells.

(3) *Isotonic luminal sucrose.* When the solution in the lumen of the gall-bladder is replaced by an isotonic sucrose Ringer's solution, there is no longer any luminal salt that can be actively transported, and two phases of salt and water movement across the gall-bladder wall follow. First, there is a brief transient during which fluid continues to pass outwards (that is, into the serosal bathing solution). This movement has been shown to be the result of the epithelial cells pumping some of their own salt and water content out across their serosal surfaces, with consequent cell shrinkage[3]. It is noteworthy that this salt does not leave the cells by diffusing down the sodium chloride concentration gradient from the cells to the salt-free solution in the lumen, but rather is pumped in the opposite direction against the concentration gradient. This has been interpreted as evidence for the location of a salt pump at the lateral and/or basal cell surface. Secondly, at the end of the initial transient a steady state is reached in which fluid slowly moves from the serosal solution into the lumen of the gall-bladder. This is due to water accompanying the slow passive diffusion of salt back down its concentration gradient into the lumen[2,3,7].

In the present experiments a small reversed movement of fluid into the lumen at 12–20 μl./h was observed in the steady state. As expected, the cells appeared considerably shrunken in comparison with transporting cells. Despite this shrinkage, about 50 per cent of the cells showed no separation at all in the phase-contrast microscope (Fig. 2*C*) and the remainder were separated by an average of 0·4μ.

(4) *Hypertonic luminal solutions.* The gall-bladder can transport water against osmotic gradients between the luminal and serosal bathing solutions[2,5–7], until these external gradients exert a force exceeding that of the local osmotic gradients developed within the tissue by active solute transport. Hence gall-bladders were investigated in Ringer's solutions at 37° C containing 110 mM sodium chloride and 25 mM sodium bicarbonate, but with the luminal solution made hypertonic by addition of 120–200 mM sucrose. The rate of fluid transport dropped below 28 μl./h, and either no net flow or small reversed flows were observed in most cases. The principal morphological change, as compared with normally transporting preparations, was that the lateral spaces decreased in width to an average of 0·4μ.

It therefore appears that the lateral spaces are dilated when fluid transport is occurring, and closed or nearly closed under four different sets of conditions which prevent fluid transport. The simplest explanation for the dilation of the lateral spaces during water flow would be that the route of transport proceeds from the cells by means of the lateral spaces out through the basement membrane. However, two other possible explanations must be considered:

(1) If water transport caused consistent changes in cell size, the changes in the lateral spaces could be secondary to changes in cell geometry, for example, because of cells shrinking and pulling apart. To check this possibility, cell dimensions were measured in transporting gall-bladders and under the four sets of conditions used to stop transport. For each type of experiment a total of 20–50 cells from two or more gall-bladders was measured. The average height and width of cells from transporting gall-bladders were 33μ and 5μ, respectively. Cells from gall-bladders maintained at 4° C were appreciably larger (41μ × 4·5μ), and cells from gall-bladders poisoned with ouabain were larger still (44μ × 5μ). Hypertonic luminal solutions caused little if any, cell shrinkage (30μ × 5μ) compared to conditions of maximal transport. Isotonic luminal sucrose caused considerable shrinkage (26μ × 4μ). Since non-transporting cells may thus be either swollen or shrunken with respect to transporting cells, transport does not produce consistent changes in cell geometry that might expand lateral spaces secondarily.

(2) When blood circulation is intact, absorbed fluid is removed by way of capillaries present near the basement membrane. However, *in vitro*, absorbed fluid must traverse the connective tissue layer beyond the basement membrane. If sufficient pressure were built up within the connective tissue, this back pressure might force the cells apart without providing any clue to the route of water transport through the cells. To determine whether physiologically reasonable back pressures are sufficient to dilate the lateral spaces to the degree observed during transport, we everted gall-bladders so that the epithelial cell layer faced outwards. The cannula was then connected to the inside fluid compartment (facing the connective-tissue layer). At 4° C, back pressures from the connective tissue towards the cells were maintained by filling the cannula to various heights for 10 min, after which time the gall-bladder was fixed. When back pressures of 2·0, 2·1, 4·3, or 4·9 cm of water were applied, the lateral spaces were not found to be significantly wider than those in non-transporting gall bladders. A pressure of 9·7 cm of water damaged the preparation irreversibly, as judged by the sudden onset of leakiness, and morphologically there was not only dilation of some lateral spaces but also rupture of the cell layer at several points. It is also noteworthy that Diezschy found that back pressures as small as 1–5 cm of water already suffice to impair the transport function irreversibly. The pressures of 2·0–4·9 cm of water employed in our experiments must therefore be greater than those present in a transporting gall-bladder, but they nevertheless fail to cause a significant increase in width of the lateral spaces. Hence back pressures generated by water flow through connective tissue during transport are unlikely to be the explanation for the observed dilation of the lateral spaces.

Because changes in cell geometry and back pressures do not account for the dilation of lateral spaces associated with transport, the correct explanation is probably the simplest—that water flow goes through these spaces. Fig. 3 shows the route of water transport deduced from these experiments. Presumably, sodium chloride is being actively transported out of the cells into the spaces, and this is where the local osmotic gradient responsible for water transport is built up. (*A priori*, salt could also have been transported into these spaces across the terminal bar without entering the cells. However, this is rendered unlikely by the previously cited observation that after removal of luminal salt, the cells can pump out more salt and water and thus shrink themselves.) The solution near the luminal end of the spaces would be hypertonic, and the resultant water flow into the spaces would carry out the salt towards the basement membrane. With the entire length of the space available for osmotic equilibration, salt could gradually become diluted to an isotonic concentration by the time it is swept out of the basal end of the space.

In most other epithelia carrying out isotonic transport, one also observes long extracellular channels which could serve the same purpose as the lateral spaces of rabbit gall-bladder—to permit complete osmotic equilibration

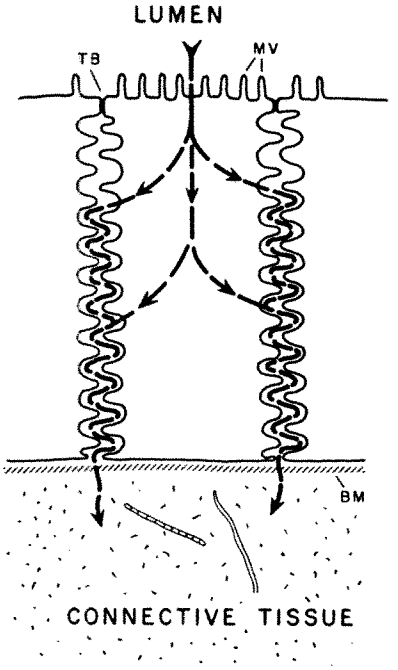


Fig. 3. Route of water transport across the epithelial cells of rabbit gall-bladder deduced from the present experiments. Water enters the cells at the surface facing the lumen, then goes out across the lateral cell membrane into the lateral intercellular spaces. These spaces are presumably hypertonic because of active transport of sodium chloride. The fluid passes down these long spaces and has equilibrated to isotonicity by the time it reaches the basal end. Finally, fluid crosses the underlying basement membrane and leaves the epithelium

of actively transported solute. Familiar examples are the basal membrane infoldings of kidney proximal tubule cells, lateral spaces of intestinal absorptive cells, and secretory canaliculi of parietal cells. The significance that has generally been attached to these membrane elaborations is that of providing greater membrane area and more transport sites. The present work, however, suggests that such structures may also possess a geometrical significance. If solute is actively transported into the closed end of a long channel bounded by cell membrane, osmotic equilibration can go on continuously as the solute moves down the channel. In a long, narrow channel, high linear velocities of water flow would be generated, and the sweeping effect of the osmotic water flow caused by solute transport would probably be more important than diffusion in carrying solute out of the channel. One would therefore have a self-regulating arrangement to ensure equilibration of actively transported solute to isotonicity, regardless of the transport rate. This geometrical arrangement might also explain why values reported for the passive osmotic permeability of epithelia appear to be too low to account for the water flow during solute transport[1,10]. Osmotically active solutes added to the external bathing solution might not measure the water permeability of the long spaces, since water flow out of the dilated spaces might bar them to external solutes during transport and since the spaces collapse in the absence of water flow.

In summary, we have observed that the lateral spaces between epithelial cells of the gall-bladder are dilated during solute-linked water transport and are closed when water transport is stopped by any one of four sets of conditions. The probable explanation for these changes is that water transport goes by way of the lateral spaces, as pictured in Fig. 3. Structures with similar geometry in other epithelia (for example, basal infoldings and secretory canaliculi) may also serve the function of preventing actively transported solute from diffusing away until water can follow in isotonic proportions.

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SIGNIFICANCE OF THE HISTOCHEMICAL DEMONSTRATION OF ATPASE IN EPITHELIA NOTED FOR ACTIVE TRANSPORT

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THE histochemical localization of ATPase has been described in a variety of epithelia noted for active transport of electrolytes. The enzyme has been demonstrated by both light and electron microscopy in such systems as kidney tubules[1,2], frog skin[3], toad bladder[4,5], gall-bladder[6], and ciliary epithelium[7–9]. Activity is often found associated with those very subcellular structures which are believed on other grounds to play a major part in transcellular transport. The question is: What physiological conclusions, if any, can be derived from such histochemical results?

The principal justification for relating ATPase activity to ion pumps, and hence to transcellular active transport, comes from a growing body of biochemical investigations which point to the conclusion that Na^+ is transported by a mechanism very intimately associated with a specific ATPase[10–12]. This specific enzyme is biochemically distinguished from other forms of ATPase by the following criteria: (1) Sodium and magnesium ions are absolutely necessary for its activation. (2) In many cases, potassium ions are also necessary for its activation. In no case, however, is the enzyme activated by potassium in the absence of sodium. (3) It is inhibited by calcium and low concentrations of cardiac glycosides, such as ouabain. Hence, before a role in ion pumping can be assigned with some confidence to a histochemically demonstrated ATPase, it must be shown to be activated and inhibited in accordance with these criteria.

Unfortunately, most histochemical investigations have not included rigorous attempts to establish the nature of the ATPase described. A notable exception is an investigation by Novikoff *et al.*[13], who used the conventional Wachstein–Meisel ATPase medium and were unable to find any evidence of sodium-pump ATPase.

Bonting *et al.*[14] have stated that the Wachstein–Meisel medium and similar histochemical procedures based on

the Gomori technique cannot be used to demonstrate sodium-pump ATPase. This conclusion is based on unpublished experiments in which they found that the addition of 0·01 M Pb^{++} to mixtures used for the biochemical assay of sodium-pump ATPase caused almost 100 per cent inhibition of this enzyme. Nevertheless, Berg[15] has recently shown that the chemical mechanisms involved in histochemical stains for triphosphatases are more complicated than those proposed by Gomori. Most of the lead in ATPase media seems to be present not as the free plumbous ion but in the form of chelation complexes with other components of these mixtures. In the present state of incomplete knowledge about the chemistry of ATPase staining procedures, it remains conceivable that media might be developed which contain lead in chemical states such that sodium-pump ATPase would not be completely inhibited.

It is of great interest, therefore, that both Cole[7] and McClurkin[16] have recently published reports of successful attempts at histochemically localizing sodium-pump ATPase by using substantial modifications of the Wachstein–Meisel medium.

Recognizing the importance of demonstrating specific sodium-pump ATPase, this laboratory has undertaken a careful histochemical study of the ciliary epithelium of the rabbit eye. These cells secrete the aqueous humour, apparently by means of a sodium pump. Biochemical investigations have shown that about 40 per cent of the total ATPase present in the ciliary epithelium is typical sodium-pump ATPase[12,17].

Ciliary bodies from the eyes of adult albino rabbits were frozen in isopentane at −70° C. Frozen sections about 6μ thick were cut in a cryostat. The sections were either used unfixed or first fixed in ice-cold solutions of either 6 per cent calcium–formol, 2·5–10 per cent α-hydroxyadipaldehyde, or 3 per cent glutaraldehyde. The last two fixatives were buffered at *p*H 7·3 by 0·1 M sodium cacodylate. Fixation times varied from 5 to 60 min. Sections were washed in *tris*-maleate or *tris* buffer for 5–30 min before being incubated at 37° C in the media of either Wachstein and Meisel[18], Cole[7], or McGlurkin[16], as described later.

Using any of the above fixatives for times longer than 10 min, and using the conventional Wachstein–Meisel medium, ATPase activity was found to be localized principally in the 'non-pigmented' epithelial layer of the ciliary epithelium. Most of the ATPase activity was found in large patches adjoining the peripheries of the individual cells (cf. Fig. 1*A*). This corresponds to the location of the elaborate membranous interdigitations that are the most striking feature of these cells as seen in the electron microscope[19]. Similar localization has been reported by others[9], and the reaction product has been shown by electron microscopy to lie within the interdigitations[8]. Such localization is most intriguing, since there is reason to believe that these interdigitations play a major part in electrolyte secretion. The enzyme is also found to a lesser extent in the neighbourhood of the basal infoldings of the 'pigmented' cell layer and at the junction between the 'non-pigmented' and 'pigmented' epithelial layers.

(1) *Effect of ouabain.* This cardiac glycoside inhibits the sodium-pump ATPase of the ciliary epithelium, as determined *in vitro* by both physiological[20] and biochemical[12,17] methods. When ouabain concentrations of the order of 5×10^{-5} M are employed, inhibition is virtually complete within a few minutes. In our experiments, 10^{-4} M ouabain was added to either the Wachstein–Meisel medium or the Cole medium. Several lots of the drug, from various sources, were tried. In some instances the sections were soaked in cold buffer containing the drug for up to four hours before incubation. The incubation time was varied over a range from 5 min to 3 h. Serial sections were incubated alternately in media with and without ouabain. Later, individual ciliary processes were followed from section to section and the reaction was compared in the same cells. In some cases the ouabain-treated processes were less intensely stained than the controls, but the reverse was found with equal frequency. Thus we were unable to find any evidence that ATPase activity was inhibited by ouabain during total periods of exposure to the drug of up to seven hours.

(2) *Effects of sodium and potassium ions.* Using the media recently described by McClurkin, an attempt was made to demonstrate sensitivity of the histochemical reaction to sodium and potassium. Unfixed serial sections were incubated alternately in three different media. The 'control' medium contained buffer, magnesium, and sodium-free ATP (prepared by treatment of the barium salt of ATP with 'Dowex 50*W*' ion exchange resin). The 'Na-K' medium was identical to the control but contained in addition 100 mM sodium and 30 mM potassium, which is close to the optimal concentration of these two ions for activating sodium-pump ATPase. The 'K' medium was similar but contained 130 mM potassium only.

Sections incubated in the 'Na-K' medium exhibited much greater activity than sections incubated in the 'control' medium. However, the 'K' medium consistently yielded staining effects indistinguishable from those obtained with the 'Na-K' medium. Theoretically, if the difference in results between McClurkin's 'control' and 'Na-K' media was due to sodium-pump ATPase, the 'K' medium should have produced results identical to those obtained with 'control'. That this was not the case

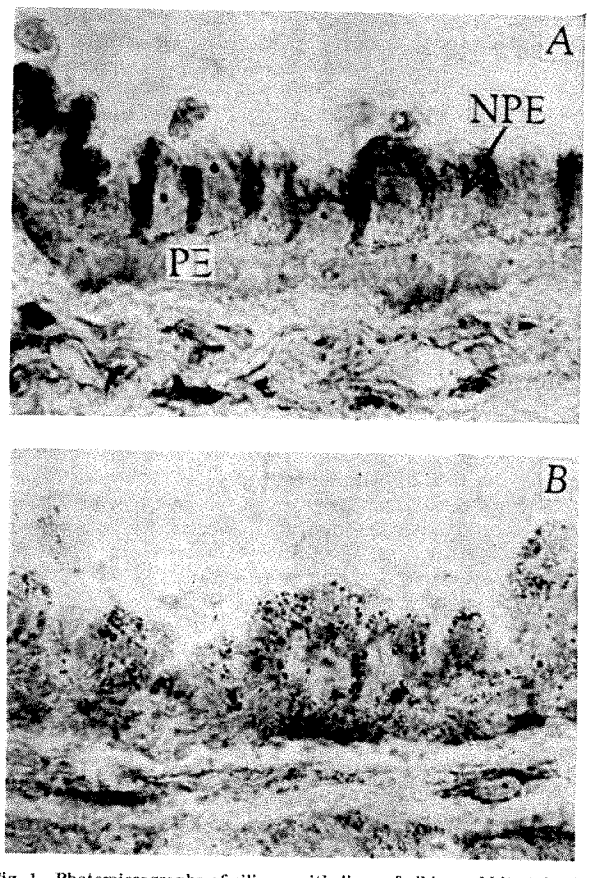


Fig. 1. Photomicrographs of ciliary epithelium of albino rabbit stained with Wachstein–Meisel ATPase medium (× 1,100). *A*, fixed 15 min with 3 per cent glutaraldehyde, shows membrane-associated ATPase activity. Most of the reaction product is found in the 'non-pigmented' epithelial layer (*NPE*), where it is localized adjacent to lateral cell borders and, to a lesser extent, near the free cell surfaces. This distribution corresponds to the distribution of plasma membrane interdigitations (infoldings), as seen by electron microscopy. Very small amounts of reaction product are also found in the basal portion of the 'pigmented' epithelium (*PE*) and between the two epithelial layers. *B*, Fixed 5 min with 2·5 per cent hydroxyadipaldehyde, shows punctate reaction product corresponding to distribution of mitochondria through the cytoplasm of both cell layers. A considerably weaker, diffuse staining is associated with the interdigitations

indicates that the different results with 'Na-K' and 'control' media were due not to specific enzyme activation but to a non-specific effect of the total ionic strength of the medium. Other experiments were carried out in which sodium and potassium ions were added to the conventional Wachstein–Meisel medium, and no differences were observed.

(3) *Effects of magnesium and calcium ions.* Both mitochondrial ATPase and sodium-pump ATPase are specifically activated by magnesium and inhibited by calcium. It was therefore of interest to compare the effects of these two cations on the ATPase activity associated with mitochondria and that associated with interdigitations. When the sections were fixed for 5 min in 2·5 per cent α-hydroxyadipaldehyde, both forms of activity were well preserved and localized, with mitochondrial activity evident as discrete, short rods distributed throughout the cytoplasm of both cell layers of the epithelium (Fig. 1*B*). Serial sections fixed in this way were incubated alternately in three media. The 'Mg' medium was identical to the one originally described by Wachstein–Meisel, containing 0·01 M magnesium ions. The 'Ca' medium was the same, except for the replacement of magnesium with an equimolar concentration of calcium. The 'control' medium contained neither cation.

In the 'Mg' medium the mitochondrial reaction was quite pronounced, whereas the interdigitations showed relatively moderate activity. In the 'Ca' medium the mitochondrial activity was extremely weak, but the reaction of the interdigitations gained considerably in strength. Virtually no activity was present after incubation in the 'control' medium. This result argues directly against the view that the activity associated with interdigitations is related to the sodium pump. Not only is it not inhibited by calcium, but it seems to be more strongly activated by it than by an equal concentration of magnesium. Mitochondrial ATPase, on the other hand, demonstrates the same specificity to divalent cations as predicted from biochemical experiments. The results with mitochondria help to confirm the reliability of the histochemical methods employed here; if the sensitivity of mitochondrial ATPase to various cations can be clearly demonstrated in tissue sections by standard histochemical techniques, there seems to be little room for believing that the specificities of other types of ATPase should not be equally well demonstrated by the same techniques.

The only reasonable conclusion from the foregoing experiments is that sodium-pump ATPase is not demonstrated by either the Wachstein–Meisel medium or the variant methods proposed by Cole and by McClurkin. Since about 40 per cent of the biochemically demonstrated ATPase activity of the ciliary epithelium is sodium-pump ATPase, and since a wide variety of conditions of fixation were employed, it seems unlikely that failure to demonstrate the enzyme can be attributed to a low concentration or to sensitivity to fixation. The *p*H of the incubating media employed here was 7·2–7·3, which is close to the optimum for sodium-pump ATPase. The most likely explanation, in keeping with the observations of Bonting[14], is that the lead which is a necessary component of these media has inhibited the enzyme to the point that it is virtually undetectable.

In spite of the present lack of methods for demonstrating clearly sodium-pump ATPase, it is still a remarkable coincidence that widely differing transporting tissues reveal striking localization of ATPase at those very membranes across which active transport is thought to occur. An absence of membrane-associated ATPase activity, furthermore, has been reported in the case of human epidermis, where no active transport is believed to occur[22]. On the basis of comparative observation of a variety of epithelia, both transporting and non-transporting, might one be justified in concluding that histochemical ATPase activity is related to active transport, although

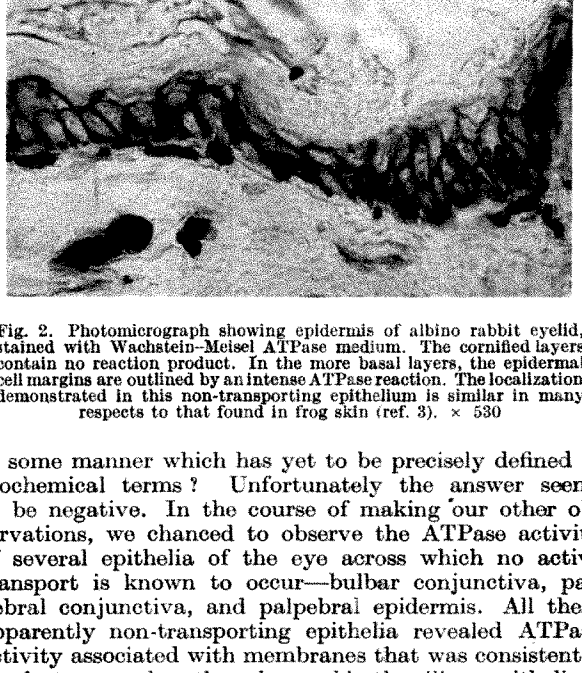


Fig. 2. Photomicrograph showing epidermis of albino rabbit eyelid, stained with Wachstein–Meisel ATPase medium. The cornified layers contain no reaction product. In the more basal layers, the epidermal cell margins are outlined by an intense ATPase reaction. The localization demonstrated in this non-transporting epithelium is similar in many respects to that found in frog skin (ref. 3). × 530

in some manner which has yet to be precisely defined in biochemical terms? Unfortunately the answer seems to be negative. In the course of making our other observations, we chanced to observe the ATPase activity of several epithelia of the eye across which no active transport is known to occur—bulbar conjunctiva, palpebral conjunctiva, and palpebral epidermis. All these apparently non-transporting epithelia revealed ATPase activity associated with membranes that was consistently much stronger than that observed in the ciliary epithelium (Fig. 2). Moreover, an absence of histochemically demonstrable ATPase activity has been reported in the case of the secretory epithelium of the avian salt gland[23] in spite of the fact that such glands are notably engaged in electrolyte transport and contain high concentrations of sodium-pump ATPase[24]. Clearly, then, there is no necessary relationship between histochemically demonstrated ATPase and transcellular transport.

The answer to the question posed at the beginning seems clear. There is at the present time no warrant for drawing physiological conclusions from the histochemical localization of ATPase in active transport systems.

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RESTORATION OF THE RESPONSE OF THE ISOLATED ATRIA OF GUINEA-PIGS TO TYRAMINE AND POSSIBLE SIGNIFICANCE OF β-HYDROXYLATION

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AFTER pretreatment with reserpine, an organ usually loses its responsiveness to tyramine or other substances which act through the release of catecholamine from the postganglionic sympathetic nerve endings[1]. However, when such an organ is exposed to, or perfused with, norepinephrine, its responsiveness to tyramine is restored. This is usually interpreted as a 'refilling' by exogenous norepinephrine of the previously depleted stores and subsequent release by tyramine of the stored norepinephrine. However, this phenomenon is not restricted to norepinephrine; several of its precursors, such as dopamine, have been found quite effective in this regard[2,3]. However, the significance of β-hydroxylation, which is a final step in the synthesis of norepinephrine, in the restoration by these precursors of the response to tyramine has yet to be determined. The experiments described in this article were undertaken to obtain information on this point. Disulfiram, which is a potent inhibitor of dopamine β-oxidase[4], was used as a means of inhibiting β-oxidase in this investigation. The effect of inhibition of dopamine β-oxidase by disulfiram on the restoration by α-methyl dopa and α-methyl dopamine of the response to tyramine was also investigated.

Isolated atrial preparation from guinea-pigs. Guinea-pigs of 400–500 g weight were killed by a blow at the base of the neck, decapitated and the heart rapidly removed. The atria were freed of ventricular muscle, connective tissue, fat and blood vessels, then placed in a modified Tyrode solution maintained at 33° ± 1° C and containing $2{\cdot}9 \times 10^{-6}$ M atropine sulphate. A mixture of 95 per cent oxygen and 5 per cent carbon dioxide was bubbled through the bathing fluid through a sintered glass plate at the bottom of the bath.

The spontaneous beat of the preparation was recorded by connecting the atria to a strain gauge transducer and recording the beat on the 'Sanborn' model 321 by direct writing lever. A counter-weight of 0·5 g was used. The atria were allowed to equilibrate until their spontaneous resting rate did not change by more than 5 beats/min during a 10-min period of observation. This took about 1–1·5 h. During this time, the bathing solution was repeatedly changed.

The procedure of the experiment was the same as described by Trendelenburg and Crout[5]. After a steady rate of spontaneous beat was attained, 10 μg/ml. of tyramine hydrochloride was introduced into the bath and left for 5–6 min. Later, the atria were repeatedly washed and then incubated with other drugs for 15–20 min. Whenever disulfiram was present during the period of exposure to amines it was added 30 min before the addition of amine and removed together with it. After thorough washing for 30 min the atria were again tested with tyramine. The restoration of the response to tyramine was calculated as the difference between the initial and final response to tyramine.

In experiments with male Sprague–Dawley rats, weighing 180–220 g, disulfiram was administered 1 h before intravenous administration of 20 μc. of norepinephrine-7-^{3}H (13 mc./mg, New England Nuclear Corp., Boston). Tyramine (20 mg/kg) was administered intramuscularly at 15 min after the labelled catecholamine. 1 h later, the animals were killed by a blow at the base of the neck and the heart removed, dissected free of atrial tissue and homogenized quickly in 10 ml. of ice-cold 0·4 N perchloric acid. After centrifugation the catecholamines in the clear supernatant were absorbed on alumina, eluted with 0·04 N perchloric acid, and assayed for tritiated norepinephrine[6].

The following substances were used: levarterenol (*l*-norepinephrine) bitartrate, *l*-dopa, dopamine hydrochloride, *l*-alpha methyl dopa hydrochloride, alpha methyl dopamine, *l*-alpha methyl norepinephrine hydrochloride, reserpine ('Serpasil', Ciba Chemicals, Ltd.), tyramine hydrochloride, tetraethylthiuram disulphide (antebuse, disulfiram) and atropine sulphate. The doses of norepinephrine, dopa and reserpine are given in terms of free base. Unless otherwise indicated, doses of other substances are expressed in terms of the salt used. Details of doses are given in a later part of this article. Student's *t*-test[7] was used for all statistical evaluations.

Effect of disulfiram on the depletion of ^{3}H-norepinephrine by tyramine. Since disulfiram was to be used as a pharmacological tool to inhibit the β-oxidase, it was considered necessary to determine its effect on uptake of norepinephrine or the release of norepinephrine by tyramine. Results presented in Table 1 show that disulfiram did not influence significantly the uptake of norepinephrine-^{3}H by the rat heart. About 40 per cent of the myocardial norepinephrine-^{3}H taken up by the rat heart was released by tyramine, whether or not the animals had been pretreated with disulfiram.

Table 1. EFFECT OF DISULFIRAM ON RELEASE OF NOREPINEPHRINE

Drug	None	Disulfiram
None	265 ± 15	268 ± 13
Tyramine	147 ± 9	151 ± 11

Rats were given disulfiram (400 mg/kg, intraperitoneally) 1 h before intravenous administration of 20 μc. *d,l*-norepinephrine-^{3}H. These and other animals which received only the labelled norepinephrine were given tyramine (20 mg/kg) intramuscularly 15 min after the norepinephrine. 1 h later, hearts were removed and assayed for norepinephrine-^{3}H. Results are expressed as mμc. norepinephrine-^{3}H per g tissue ± *S.E.M.* for groups of six animals.

Response of the isolated atria from reserpinized guinea-pig to tyramine before and after 'repletion'. Atria from the guinea-pigs pretreated with reserpine (1 mg/kg, 24 h before the experiment) failed to respond to 10 μg/ml. of tyramine. However, when these atria were exposed to 5 μg/ml. of *l*-norepinephrine for 15–20 min, response to tyramine (determined 20 min after exposure) was partly restored. Likewise, other sympathomimetic amines were effective in restoring the response to tyramine. The results are summarized in Table 2.

Effect of disulfiram on restoration of the response to tyramine. In this series of experiments disulfiram was introduced in the bath 30 min before the atria were exposed to various sympathomimetic amines. The response to 10 μg/ml. of tyramine hydrochloride was determined 20 min before the addition of disulfiram and 30 min after the exposure of the atria to various amines. The results are summarized in Table 2. Disulfiram did not affect the normal response of the atrial pacemaker to tyramine. Restoration of the response of the atrial pacemaker to tyramine by norepinephrine and α-methyl norepinephrine was unaltered by disulfiram, but disulfiram did antagonize the restoration by the precursors of these amines.

Table 2. EFFECT OF INHIBITION OF β-HYDROXYLATION BY DISULFIRAM ON RESTORATION BY VARIOUS SYMPATHOMIMETIC AMINES OF THE RESPONSE OF THE ATRIAL PACEMAKER TO 10 μg/ml. OF TYRAMINE

Treatment	Concentration (μg/ml.)	Mean increase (beat/min) ± S.E.		P
			Disulfiram pretreatment	
Atria from normal guinea-pigs				
None	—	62 ± 3·6 (16)	63 ± 3·2 (6)	*N.S.*
Atria from reserpinized guinea-pigs				
None	—	1·4 ± 0·8 (40)	—	
Dopa	50	21 ± 2·7 (5)	3·7 ± 2·7 (6)	< 0·001
Dopamine	50	30 ± 3·1 (16)	5·1 ± 2·1 (5)	< 0·001
Norepinephrine	5	45 ± 3·8 (16)	42 ± 4·6 (6)	*N.S.*
α-Methyl dopa	50	39 ± 3·0 (12)	5·6 ± 3·2 (6)	< 0·001
α-Methyl dopamine	20	32 ± 4·3 (6)	7·4 ± 3·7 (6)	< 0·001
α-Methyl norepinephrine	10	48 ± 4·2 (12)	46 ± 4·5 (6)	*N.S.*

Isolated atria were obtained from guinea-pigs pretreated with 1 mg/kg of reserpine 24 h before the experiment. They were first tested with tyramine, then exposed to the amine, washed repeatedly for 30 min and tested again with tyramine. The values are the mean difference between the first and second response of the pacemaker to tyramine. Disulfiram (20 μg/ml.) was added in bath 30 min before atria were exposed to various amines. *P*, value for difference between the means. *N.S.*, no significant difference.

Tyramine owes its sympathomimetic effect, at least in part, to a release of catecholamine from its storage site(s) in postganglionic sympathetic nerve endings. Depletion of the norepinephrine stores by pretreatment with reserpine greatly reduces the response to tyramine. However, the response to tyramine is partly restored after treatment with norepinephrine. Muscholl[8] presented evidence that reserpine pretreatment almost abolishes the uptake of norepinephrine by various tissues. However, the recent study by Trendelenburg and Crout[3] has shown that isolated atria of reserpine-pretreated guinea-pigs take up from the bath small but measurable amounts of norepinephrine. These findings suggest that there are some stores of norepinephrine which are still functional in the reserpinized animals.

In the present investigation, dopamine, like norepinephrine, was able to restore the response of the atrial pacemaker to tyramine. The restored response after dopamine could be due either to a release of retained dopamine itself or of norepinephrine synthesized by β-hydroxylation of this substance in the atrium. In reserpinized animals the heart was able to synthesize norepinephrine from its precursor dopamine[9]. The restored response after *l*-dopa is in all likelihood due to a release of either dopamine or norepinephrine or both, synthesized by this substance in the atrium and then retained, since *l*-dopa itself has no sympathomimetic activity.

The importance of β-hydroxylation in the restoration by dopamine of the response to tyramine is demonstrated by its inhibition. Following pretreatment with disulfiram, a potent inhibitor of dopamine β-oxidase[4,10], incubation with dopamine or dopa failed to restore the response of the atrial pacemaker to tyramine (Table 2). Disulfiram did not interfere with response of the atrial pacemaker to tyramine (Table 2) or with restoration by norepinephrine (Table 2) or with release of norepinephrine from the heart by tyramine (Table 1). It could be concluded, therefore, that precursors of norepinephrine themselves cannot restore the response of the reserpinized preparation to tyramine but must be β-hydroxylated to form norepinephrine in order to restore the response to tyramine.

The effect of α-methyl dopa in restoring response of the atrial pacemaker to tyramine closely resembles the effect of dopa in restoring the response to tyramine. α-Methyl dopa, like dopa, is a substrate for dopa decarboxylase, thus being converted to α-methyl dopamine and dopamine respectively. α-Methyl dopamine can be further β-hydroxylated to form α-methyl norepinephrine[11,12]. It is difficult to explain the restoration of the responses of the atrial pacemaker by α-methyl dopa, α-methyl dopamine or α-methyl norepinephrine by assuming that norepinephrine is formed; there are no published results indicating that α-methyl groups can be removed. While, in the presence of disulfiram, α-methyl norepinephrine restored the response of the atrial pacemaker to tyramine, α-methyl dopa and α-methyl dopamine must be β-hydroxylated to form α-methyl norepinephrine, and α-methyl norepinephrine can serve in place of norepinephrine to mediate response to tyramine.

To sum up, isolated atria obtained from guinea-pigs pretreated with reserpine (1 mg/kg given 24 h before the experiment) were exposed to norepinephrine and its precursors dopa and dopamine. 30 min after the removal of these amines from the bath the response of the atrial pacemaker to tyramine was partly restored. α-Methyl dopa, α-methyl dopamine and α-methyl norepinephrine were also able to restore the response to tyramine.

Disulfiram antagonized the restoration of the response to tyramine when it was present during the period of exposure to dopa, dopamine, α-methyl dopa or α-methyl dopamine. Since disulfiram did not influence the restoration of response by norepinephrine and α-methyl norepinephrine or the release of catecholamine by tyramine, these results suggest that β-hydroxylation of amines is important to restoration of the response to tyramine.

It is concluded that precursors cannot restore the action of tyramine themselves—they must first be β-hydroxylated in order to be effective.

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EFFECTS OF LIGHT ON 3,4-BENZPYRENE CARCINOGENESIS

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THE problem of the conflicting data from *in vivo* experiments on the influence of light on the carcinogenic processes evoked by tars or polycyclic hydrocarbons has been approached by our group. We have assumed that the accelerations of cancer induction by light so far reported[1–4] are dependent on a definite optimum concentration of photodynamic substance and intensity of radiation[5,6]. The inhibition of cancer induction by light[7–9] may be due to a higher than optimum level of these factors. At this level, these factors destroy susceptible cellular components and thereby reduce the total number of potential malignant cells. The lack of action by ultraviolet and visible light on tar and polycyclic hydrocarbon carcinogenesis[10–12] would then be a result of a level of

experimental conditions filling in between those already mentioned.

We report here the preliminary data of three experiments carried out on 955 mice given 3,4-benzpyrene (3,4-BP) and irradiated with Wood light or kept in normal daylight. The purpose of the research was to test the aforementioned theoretical assumptions.

Swiss albino mice raised in the laboratories of the Institute of General Pathology, University of Milan, and of the Institute of Cancer Research, Naples, were used. The mice were painted (one drop) twice weekly with 3,4-benzpyrene (0·5 or 0·25 per cent in acetone or in benzene) in the interscapular region. All animals were kept in dark rooms. The irradiation was carried out using a high-pressure Wood 125-W Philips bulb '57236 *E*/70' at 30 cm. This source has its maximum output at 365 mμ in the long ultra-violet, and is capable of exciting 3,4-BP. The energy delivered by one bulb was about $1·5 \times 10^5$ ergs/cm²/sec at 30 cm according to measurements by an 'Eppley' calibrated thermopile.

The general procedure consisted in keeping control groups in dark rooms under thick black curtains, and in exposing the experimental groups twice weekly soon after painting in the same room to irradiation for different periods of time ranging from 30 min to 3 h.

Three experiments are reported here, two of which were carried out in the Institute of General Pathology of the University of Milan and one in the Institute of Cancer Research in Naples. In the latter experiment, a group of mice given 3,4-benzpyrene was kept in a room illuminated by daylight through conventional windows.

Experiment 1. An experiment was set up using 300 male mice, 8–12 weeks old. The mice were divided into six groups, treated respectively only with Wood light twice weekly, only with 3,4-BP (one drop of acetone solution = 100 μg) twice weekly, and with 3,4-BP plus Wood light twice weekly for 0·5, 1, 2 and 3 h. The irradiation was carried out for 28 weeks. The experiment lasted 32 weeks. The total light dosages were 12×10^{10}, 8×10^{10}, 4×10^{10} and 2×10^{10} ergs/cm². The irradiation was carried out on groups of twenty mice in plastic cages 35 × 48 cm with high walls and a metallic net at the bottom. A fan set underneath the cages regulated air circulation and temperature. The 3,4-BP was given by dropping at random within a clipped area of about 1·5 cm² interscapularly. In this experiment attention was paid only to the onset of malignant growths. The principal results of this experiment were as follows: (*a*) very low tumour incidence (4 per cent) in the group of mice treated with 3,4-BP and kept in the dark; (*b*) highest tumour incidence (58 per cent) in the group treated with 3,4-BP plus Wood light at a dosage of 8×10^{10} ergs/cm². This was not the highest dose used.

Experiment 2. It was decided to repeat the foregoing experiments in the Institute for Cancer Research at Naples. 480 Swiss mice of both sexes 7–8 weeks old were used. The animals were divided into five groups treated, respectively, only with Wood light, only with 3,4-BP (one drop of a benzene solution = 100 μg) twice weekly, with 3,4-BP plus Wood light twice weekly for 2 and 3 h and with 3,4-BP plus normal daylight. The latter experimental condition was achieved by keeping the animals in metallic cages covered by a net in a room illuminated by conventional windows. The 3,4-BP was given by dropping in a clipped interscapular area of about 1 cm², when skin was in resting phase. The total Wood light dosages were about $10 \times$ and 6×10^{10} ergs/cm². The duration of the experiment was 24 weeks. Wood light irradiation was carried out keeping each mouse in sections of special containers 3 cm high covered by a loose net. A fan system directed at the light beam regulated temperature at about 24° C at the level of the mice. In this experiment attention was also paid only to the onset of a carcinoma. The results showed that 3,4-BP, in the presence of Wood light, produces carcinomata earlier (at the eleventh week) than in the dark (at the seventeenth week). However, on continued irradiation the effect of light on BP-induced carcinoma formation is inhibitory (at twenty-fourth week: 8 per cent in Wood light, 27 per cent in the dark). The daylight, within the seasonal (autumn, winter and spring) and environmental variations, also induces an acceleration of carcinoma onset, which, however, leads later to an increase of carcinoma with respect to the dark (at the twenty-first and twenty-fourth weeks, respectively: 35 per cent and 47 per cent in daylight, 5 per cent and 27 per cent in dark).

Experiment 3. Another experiment was set up in the laboratory at Milan using 175 mice, 8–12 weeks old, of both sexes. The animals were divided into four groups treated, respectively, only with Wood light twice weekly, only with 3,4-BP (one drop of an acetone solution = 50 μg) twice weekly, and with 3,4-BP plus Wood light twice weekly for 1 and 2 h. The total light dosages were $8·6 \times 10^{10}$ and $4·3 \times 10^{10}$ ergs/cm². The irradiation was carried out on groups of twelve mice kept in 'Plexiglass' containers, 4 cm high, divided into four sections, and covered by a loose set. A fan system regulated air circulation and temperature at about 24° C during irradiation. The 3,4-BP was given by dropping in a 1 cm² clipped area in the interscapular region. In this experiment attention was paid to the onset of both papilloma and carcinoma. The results showed that Wood light brings about a definite increase of the neoplastic processes as follows: papilloma formation was up to 60 per cent at the twenty-second week in the irradiated group, and up to 60 per cent at the twenty-eighth week in the control group (dark); the carcinoma incidence was up to 100 per cent at the thirtieth week in the irradiated group, and only 3 per cent at the same week in the control group. Detailed results of histological observations will be reported in a subsequent publication.

On the whole, the results demonstrate that (*a*) 3,4-BP is carcinogenic independently of light, and (*b*) the 3,4-BP carcinogenicity can be influenced by Wood light as well as by daylight with phenomena either of acceleration or of inhibition. The acceleration by light is interpreted as a process due to an excitation of 3,4-BP which is still compatible with cellular life. The inhibition by light is interpreted as the consequence of a photodynamic process leading to a severe damage of cellular components rather than to the alteration of the carcinogen due to a photosensitized oxidation, thus decreasing its effective dose[13,14]. This is supported by histofluorescence and histological observations, still in progress. The results are consistent with the theoretical approach.

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LETTERS TO THE EDITOR

GEOPHYSICS

Backscattering from the Upper Atmosphere (75–160 km) detected by Optical Radar

A COMMUNICATION with the above title was recently published by McCormick *et al.*[1]. This work gave, for October 29, 1965, a light return of about 10 counts per pulse at heights from 75 km to 125 km and, for October 31, about 1·5 counts per pulse at heights from 75 km to 100 km. If one takes the system parameters given and assumes 3 per cent photomultiplier quantum efficiency, 30 per cent receiving system transmission and 80 per cent atmospheric transmission, then the 10 counts per pulse corresponds to a return 150 times greater than that which would be given by Rayleigh scattering from molecules at 75 km, and 10^5 greater at 125 km.

We carried out observations with an optical radar of similar performance during ten nights between October 10 and November 14, 1965; this work is being fully reported elsewhere[2]. We calibrated the sensitivity of our equipment on the Rayleigh scattering from molecules observed below 60 km and found it to be about half that calculated above for McCormick's equipment. However, our estimates of transmission through their receiving system and atmosphere may be much too high, in view of measurements on our own system. Although our background count is much lower, we have never detected any light from above 80 km, and in the region 70–80 km our return was not more than a factor of three greater than Rayleigh scattering from molecules. To achieve these results, a rotating shutter had to be fitted to prevent the escape of fluorescent light after the transmission of the main *Q*-switched pulse; previously a large apparent return from high levels had been obtained.

These results indicate a very large difference in the constitution of the upper atmosphere above Maryland and South-east England at this time; data from their observations on other nights, which were not reported, would show if this was exceptional. Alternatively, McCormick *et al.* may not have succeeded in removing the effects of fluorescent light from their results, despite their measurement of the background count.

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Slough.

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The Satellite Geoid may have a Westward Drift

SOME time ago in a communication, I presented a model which speculated on the origin of the D″ layer between the mantle and the core of the Earth[1]. This layer is believed to be predominantly a molten sludge of mantle material but is richer in silica and lighter in density than the mantle because some iron has been lost to the core. At the core–mantle boundary, the electric currents generate Peltier heating; mantle material will be melted, gradually become a molten sludge and produce undulations at the boundary.

If the process of Peltier melting is slightly dominant over the process of Peltier freezing, the molten sludge will gradually accumulate. By integrating the equation of state at the core–mantle boundary, we note that Peltier heating is aided by ohmic heating while, of course, Peltier cooling is suppressed; since the electrical resistivity of mantle is much larger than the resistivity of the core, the ohmic heating is quite significant within the mantle. Thus we speculate that the electric current loops which give rise to non-dipole magnetic secular variations give rise also to a molten sludge and some undulations at the core–mantle boundary.

The molten sludge with lower density than mantle gives rise to a negative gravity anomaly, while the molten iron of the core with respect to the sludge yields a positive anomaly.

If the gravity anomalies are due to the sludge and the molten iron, then a westward drift of the gravity anomaly is also expected. Vogel[2] and Egyed[3] have also speculated on the possible relationship between magnetic secular variations and the gravity anomalies. The model which I introduced[1] attempted to describe the relationship between the magnetic and the gravity anomaly and predicted a similar westward drift for the gravity anomalies.

The total gravity anomalies on the equatorial belt have a maximum amplitude of ±20 mgals over some 10,000 km distance. If the westward drift is uniform and about 0·5 degree per year, this leads to a 0·2 mgal change per year which cannot be easily distinguished with the present gravity meters. The mean free air anomalies calculated by Jeffreys[4] for gravity data recorded before 1950, when compared with the satellite geoids calculated by Izsak[5] and Kaula[6] for 1960, show a distinct westward drift of 12–15 degrees (Fig. 1). Kaula's data are based on observations made during 1960, while those of Jeffreys probably extend over a much longer period. The westward drift on the non-dipole magnetic anomalies according to Runcorn[7] is 0·2–0·8 degrees per year. If the longitudinal location of the anomalies calculated by Jeffreys, Izsak and Kaula are reliable, then the estimated westward drift of gravity anomalies is of the same order of magnitude as the westerly drift of the non-dipole magnetic anomalies.

Some of my colleagues believe that if the satellite geoid had a westerly drift, such a phenomenon would

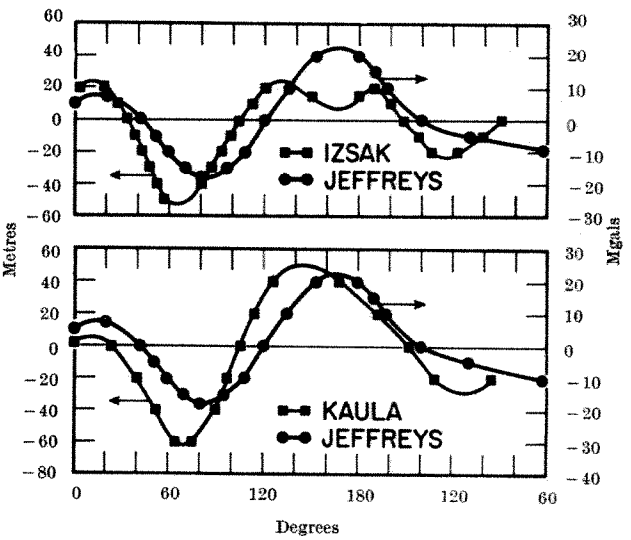


Fig. 1

have been reflected on tidal measurements at various stations; on the other hand, others indicate that the density of data available for Jeffreys was not adequate to form a reliable comparison. In spite of all these arguments, it seems to me a remarkable coincidence that: (1) the drift is in a westward direction; (2) the drift is not of a local nature, but covers half-way across the globe; (3) the magnitude of the drift is of the same order of magnitude as that of non-dipole magnetic field.

Woolard *et al.*[8] have observed some systematic increase of 0·4–1·5 mgals over a period of a few years for the gravity measurements in India. Although Woolard assigns this systematic change to the eccentricity of the screw of the gravity meter, it can also be interpreted as a westward drift of the gravity anomaly. In the same communication Woolard indicates that the value of the gravity at Dehra Dun increased about 0·8 mgals at a rate of 0·1 mgal per year. If the westerly drift of the free air gravity anomalies exist, for the next few decades one would expect a gradual drop in gravity field in the Middle East, Sudan and Egypt, and conversely a gradual increase in South-east Asia.

For an unequivocal answer regarding the westward drift of gravity field, either we have to design and construct gravity meters with extremely small drift or wait another 10 years and compare the present satellite geoids with those of the 1970's.

I am at present investigating the possibility of designing and building such a sensitive gravity meter, which will make use of super-conductivity and modern cryogenic techniques.

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Non-Eckersley-law Whistlers observed at Equatorial Latitudes with Satellite *Injun* 3

THIS communication reports two new whistlers discovered in the very-low-frequency radio noise data from *Injun* 3 satellite. This satellite carried a very-low-frequency receiver with a magnetic loop antenna oriented so as to detect the component of the wave magnetic field which is propagating perpendicular to the geomagnetic field. The pass band of the very-low-frequency receiver was about 0·5–7 kc/s. Altitudes ranging from 237 km to 2,785 km are covered by the *Injun* 3 very-low-frequency data.

Frequency-time spectrograms of the two new whistlers are shown in Figs. 1 and 2. A unique feature of these whistlers is that over the observed portion of the frequency spectrum the delay time increases with increasing frequency, thus deviating markedly from the Eckersley law for whistlers (time delay inversely proportional to the square root of frequency). Because of their appearance on a frequency-time spectrogram, we call the whistlers in Figs. 1 and 2 the 'riser' and the 'check whistler', respectively.

Both the riser and the check whistler have been received simultaneously from two different satellite tracking stations; therefore we are certain that these signals are not caused by interference in the data transmission. We also note that the whistler signals show strong attenuation above 7 kc/s, corresponding to the upper cut-off frequency of the *Injun* 3 very-low-frequency receiver[1]. Thus we feel certain that these signals were detected by the loop antenna.

We present the following evidence to substantiate our claim that these events are whistlers. As will be shown, all these events occurred in the vicinity of the magnetic equator at altitudes above 900 km. In the *Injun* 3 data, Eckersley-law whistlers are quite regularly observed in this region whereas commonly known types of very-low-frequency emissions are virtually never observed here. Also, a common feature of very-low-frequency emissions is repetition at short intervals[2]. No such repetition is found with the events on which we report. In fact, often only one or two events are found in a 20-min satellite pass.

From the analysis of 20 h of very-low-frequency data a total of seventy risers has been found. All risers observed thus far were received within 23 degrees (magnetic latitude) of the magnetic equator and above 900 km altitude. The majority (80 per cent) of these whistlers

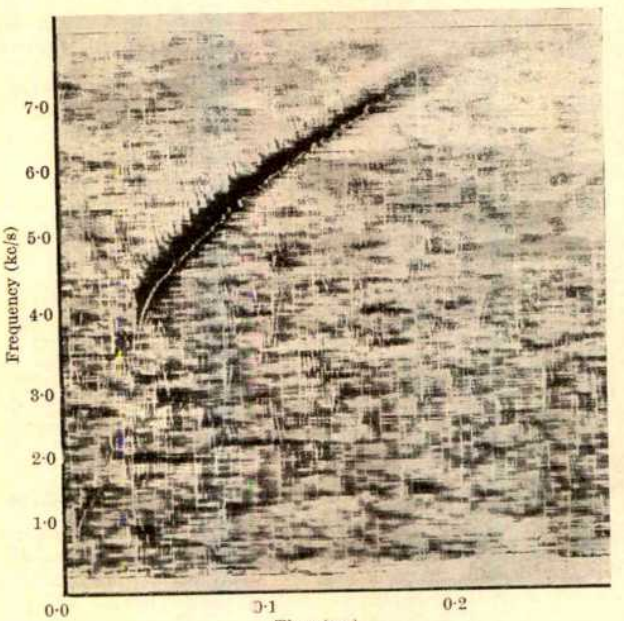


Fig. 1

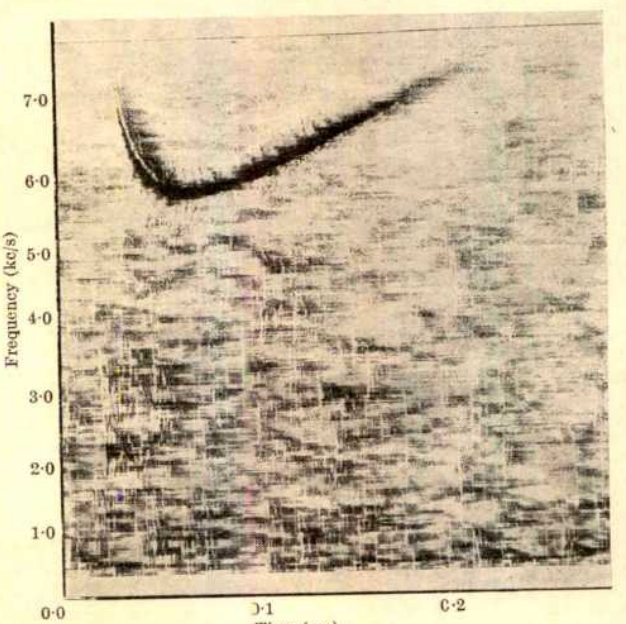


Fig. 2

are found within 15 degrees of the magnetic equator and above 2,000 km altitude.

The equatorial very-low-frequency data used for the riser study has yielded six occurrences of the check whistler. All six were received within 23 degrees of the magnetic equator and above 2,000 km altitude.

In considering possible explanations for the riser and check whistler we have eliminated the possibility that these whistlers are nose whistlers[3] because the nose frequencies at these invariant latitudes are at least ten times the frequencies at which we observe these new whistlers. Because waves from equatorial lightning sources received in the ionosphere near the equator are likely to be propagating nearly perpendicular to the geomagnetic field, we believe that the unusual dispersion of the riser and check whistler arises because of the lower hybrid resonance[4]. The lower hybrid resonance frequency can range between 3 and 8 kc/s in the region of the ionosphere at which we observe these whistlers.

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GEOCHEMISTRY

Major Element Composition of Georgia Tektites

Clarke and Carron[1] reported the first major element analysis of a Georgia tektite (*USNM* 1396) and discussed its composition in relation to analyses of moldavites and the tektite from Martha's Vineyard, Massachusetts. King[2] published an analysis of a second Georgia tektite (*DGA*-1), and compared it with the previous analysis of *USNM* 1396. Clarke and Henderson[3] and King[4,5] have discussed the chemistry of Georgia tektites in relation to tektites from other occurrences and possible parent materials. Zähringer[6] has established identical potassium–argon ages of 34 ± 1 million years for all the North American tektites.

A new analysis of a Georgia tektite (*DGA*-2) is presented in Table 1 together with the previously available major element data. *DGA*-2 has been described and illustrated by King[4].

Table 1. CHEMICAL ANALYSES OF GEORGIA TEKTITES (PERCENTAGE)

Oxide or element	*USNM* 1396 (*a*)	*DGA*-1 (*b*)	*DGA*-2 (*c*)	Range
SiO_2	80·54	81·84	80·08	80·08–81·84
Al_2O_3	11·21	10·40	11·64	10·40–11·64
Fe_2O_3	0·33	0·00	0·10	0·00–0·33
FeO	2·40	2·30	2·52	2·30–2·52
CaO	0·61	0·58	0·56	0·56–0·61
MgO	0·65	0·67	0·66	0·65–0·67
MnO	0·05	0·03	0·06	0·03–0·06
Na_2O	1·16	0·97	1·08	0·97–1·16
K_2O	2·38	2·15	2·23	2·15–2·38
H_2O-	0·00	0·22	0·17	0·00–0·22
H_2O+	0·02	0·32	0·29	0·02–0·32
TiO_2	0·43	0·41	0·57	0·41–0·57
P_2O_5	nd.	Trace	0·01	Trace–0·01
C	nd.	nd.	0·00	—
H	nd.	nd.	0·00	—
Totals	99·78	99·89	99·97	

(*a*) From Clarke and Carron (ref. 1); analysts, M. K. Carron and W. W. Brannock.
(*b*) From King (ref. 2); analyst, Jun Ito; weight of analysed fragment—4·442 g.
(*c*) Analyst, Jun Ito; weight of analysed fragment—2·152 g. Analysis reported June 1, 1964.

The Georgia tektites are characterized by high silica, high alumina, very low ferric to ferrous iron ratio, and an excess of potash over soda—as are almost all tektite analyses. The silica values are among the highest reported for any tektites, and are exceeded or equalled only by a few of the moldavites and bediasites, and the single specimen reported from Martha's Vineyard, Massachusetts, by Kaye *et al.*[7]. The inverse variations of alumina (Fig. 1) and ferrous iron (Fig. 2) with the silica content are apparent even though the three analyses have a small range of silica values. The analyses also suggest an inverse variation of titania with silica as observed in the bediasites by Chao[8]. The major element composition of the Georgia tektites is almost identical to the silica-rich bediasites. Clarke and Carron[1] and Chao[8] noted the compositional similarity of the Martha's Vineyard tektite, silica-rich bediasites and *USNM* 1396, the only Georgia specimen then analysed. The very low lime and magnesia contents observed in the Georgia specimens are typical of North American tektites (Chao[8], King[9]). The total iron values of Georgia tektites are in the extreme low portion of the range reported in bediasites (Barnes[10,11]; Chao[8]). The $(FeO + MgO + CaO)/(K_2O + Na_2O)$ values of the Georgia tektites range from 1·06 to 1·14, within the range reported

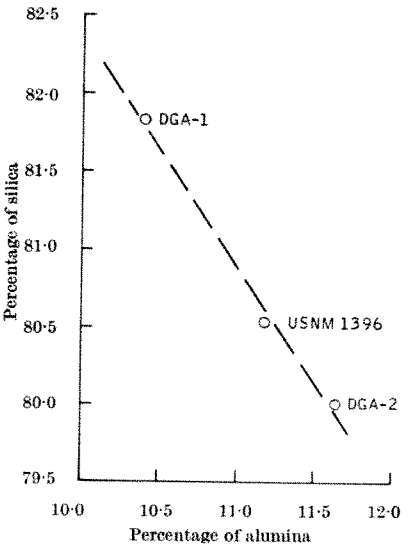


Fig. 1. Variation of alumina with silica in three Georgia tektites

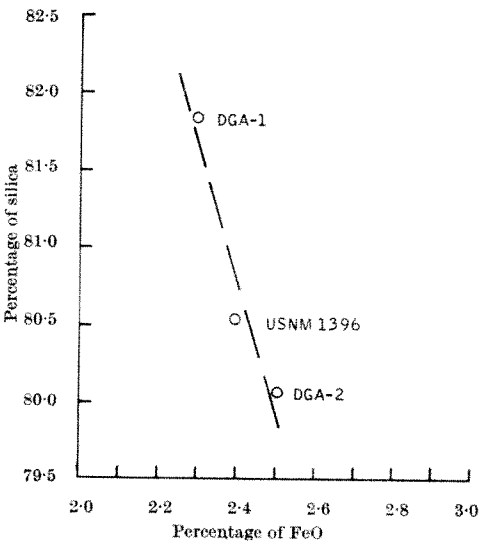


Fig. 2. Variation of FeO with silica in three Georgia tektites

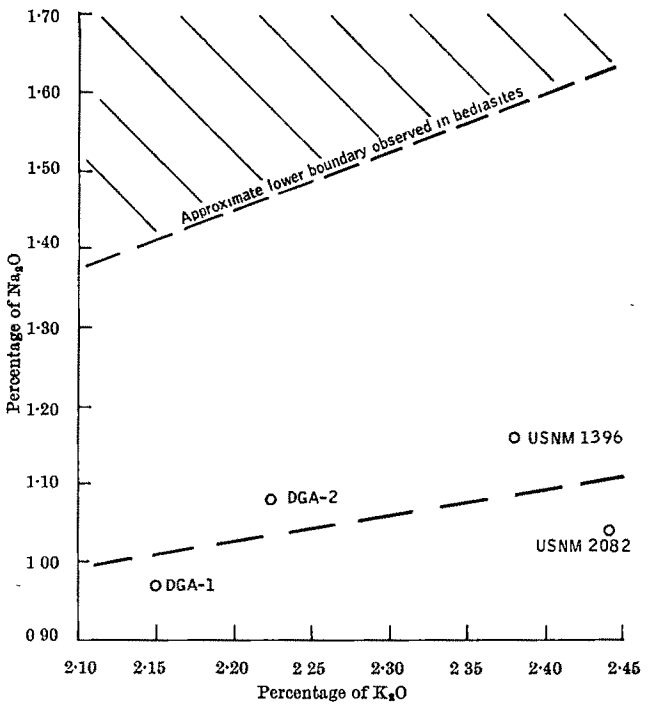


Fig. 3 Variations of K_2O with Na_2O in the Martha's Vineyard and Georgia tektites as compared with the range of values reported in bediasites

for silica-rich bediasites (Chao[8]). The 'essential' water values reported for *DGA*-1 and *DGA*-2 are higher than the water contents reported in tektites by Friedman[12,13] and may be in error. Potash plus soda in the Georgia tektites is well within the range observed in bediasites, but the Georgia specimens are significantly lower in their soda to potash ratio than the bediasites (Table 2). The Martha's Vineyard tektite shows the same low soda to potash value. A plot of K_2O with Na_2O (Fig. 3) shows a trend that is roughly parallel to but lower in soda than that given by Chao[8] for the bediasites. The difference in the soda to potash ratios between the bediasites and other Northern American tektites probably resulted from a difference of the ratios in the parent materials.

Table 2. SODA TO POTASH RATIOS IN NORTH AMERICAN TEKTITES*

Specimen	Locality	Na_2O/K_2O
DGA-1	Dodge Co., Georgia	0·45
DGA-2	Dodge Co., Georgia	0·48
USNM 1396	Dodge Co., Georgia	0 49
USNM 2082	Martha's Vineyard, Mass.	0·43
BM-1	Fayette Co., Texas	0·65
BM	Fayette Co , Texas	0·69
BM-14	Fayette Co., Texas	0 69
30775-77	Grimes Co. (?), Texas	0 68
Average Bediasite Texas		0·74 ±

* Values for bediasites calculated from analyses reported by Chao (ref. 8); *USNM* 1396 from Clarke and Carron (ref. 1); *USNM* 2082 from Kaye *et al.* (ref. 7), *DGA*-1 from King (ref. 2).

The systematic relation of physical properties and composition in tektites has been demonstrated by Barnes[10], Chao[8] and others. Relatively few precise determinations of Georgia tektite specific gravities are now available, but it would seem that the range is only about 0·06. This is about half the range of specific gravities observed in bediasites. Although the few Georgia tektites now known may not be entirely representative of the total group, the variations in physical properties are less than would be obtained in an equal number of randomly selected bediasites. It is probable that the Georgia tektites have an even more restricted range of composition than the bediasites. Analyses of Georgia tektites covering a wider range of physical properties and precise measurements of physical properties on additional specimens will be necessary to establish the exact range of composition.

On the basis of the Georgia tektite analyses now available, the Georgia tektites are very similar in their major element composition to silica-rich bediasites. The Georgia tektites show decreasing contents of alumina, ferrous iron, and possibly titania with increasing silica. The variation of soda with potash defines a trend that does not overlap values observed in any bediasite yet analysed. In view of the identical potassium–argon ages, close chemical similarity, and general geographic association, the North American tektites are probably the product of a single event. However, the Georgia tektites and the specimen from Martha's Vineyard can be classed as a distinct sub-group characterized by low soda : potash ratios.

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Possible Rubidium–Iron Covariance in Potassium Feldspars

THE existence of Fe^{3+} in potassium feldspars is well known and it has been assumed that the iron is substituting for aluminium[1]. We have been investigating the geochemical characteristics of the Enchanted Rock batholith, Llano Uplift, Texas[2,3], and have noted the rubidium–iron covariance reported here (see Fig. 1).

The batholith is a Precambrian granitic intrusion which exhibits a gradual trend from a more mafic interior to more felsic outer zones[4,5]. The samples were taken along

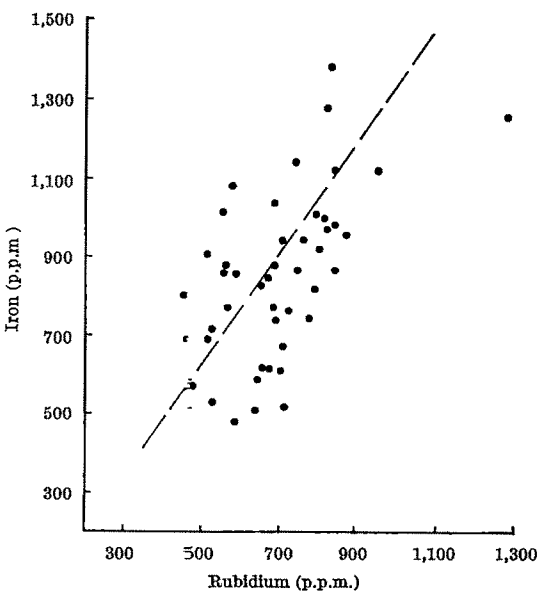


Fig. 1. Rubidium–iron covariance

four profiles radiating from the centre of the batholith to the outer margin. The potassium feldspars were separated by standard heavy liquid methods and checked for purity both petrographically and by X-ray diffraction. Rubidium and iron were determined by atomic absorption. The analytical techniques and precision tests (Rb = ± 1·5 per cent; Fe = ± 2·5 per cent) have been reported elsewhere[6].

The data presented in Fig. 1 suggest a covariance between rubidium and iron in the potassium feldspars. The correlation coefficient is +0·561 which is significant at the 0·9995 level. Since the analytical method reports total iron, Fe^{2+} is included. If the following hypothesis is correct, a correlation of rubidium and only Fe^{3+} would be even more significant.

We would like to submit a possible explanation of the rubidium–iron correlation. The Rb^{1+} ion is larger than the K^{1+} ion and may expand the feldspar lattice sufficiently to allow (require?) replacement of an Al^{3+} ion ($r = 0{\cdot}57$ Å) by Fe^{3+} ($r = 0{\cdot}67$ Å). This is indirectly supported by the fact that the mean concentration of rubidium (675 p.p.m.) is similar to that of iron (807 p.p.m.). In addition, Rb^{1+} has a slightly lower ionization potential than K^{1+} which results in a more easily polarized oxygen ion, which, in turn, benefits Fe^{3+} substitution since it is a more polarizing ion than Al^{3+} ($Fe^{3+}_I = 30{\cdot}6$; $Al^{3+}_I = 28{\cdot}44$).

Rosenquist[7] and Divljan[8] have proposed that the large ionic radius of K^{1+} relative to Na^{1+} or Ca^{2+} allows substitution of Fe^{3+} for Al^{3+}. If this simple relationship is true, rubidium-iron covariance might be expected because of the increase in lattice size resulting from substitution of rubidium for potassium.

A possible test of the hypothesis proposed here would be to examine the spatial relationship of rubidium and iron in potassium feldspars by electron probe microanalysis. Assuming that rubidium is not uniformly distributed throughout the feldspar crystal, indirect support of the hypothesis would be given if rubidium and iron covaried spatially.

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GEOLOGY

Olivine–Liquid Reaction and the Depth of Crystallization of the East Aberdeenshire Gabbros

RECENTLY, Weedon[1] has argued that the presence of corona structures surrounding olivines in the Aberdeenshire gabbros implies a low pressure of crystallization, equivalent to a depth of burial of less than 9 km, and comments that this is inconsistent with published views on the time of emplacement and depth of burial of the gabbro masses. Weedon's argument rests on two assumptions: that the suppression of an olivine–liquid reaction relationship in the system $MgO–SiO_2$ by increasing pressure is an accurate indicator of the behaviour of the olivine–liquid relationship in basic magma, and that the corona structures described from the gabbros indicate crystal–liquid reaction, not solid–solid reaction after consolidation.

Boyd *et al.*[2] have shown that the olivine–liquid reaction relationship in the system $MgO–SiO_2$ is suppressed by a pressure not greater than 5·4 kbars (~20 km depth of burial) and suggest that it might be suppressed by pressures as low as 2·3 kbars (~9 km). Increasing pressure may consequently be expected to suppress the related reaction relationships between olivine and liquid to form pyroxenes observed in the systems $MgO–FeO–SiO_2$ (ref. 3), $CaO–MgO–FeO–SiO_2$ (ref. 4), $CaO–MgO–SiO_2$ (ref. 5) and $CaO–MgO–Al_2O_3–SiO_2$ (refs. 6 and 7). Pressures of 20 kbars[8] and 10 kbars[9], respectively, are known to be adequate for the suppression of the reaction relationships in the two last-mentioned systems. The experimental evidence does not at present support any stronger statement than that increasing pressure is likely to suppress the olivine–liquid reaction relationship in dry tholeiitic silica-saturated magmas at some pressure less than 10 kbars (~35 km).

However, these are not the only olivine–liquid reaction relationships known. Reaction between olivine and liquids initially undersaturated in silica to yield diopsidic pyroxene is reported at atmospheric pressure from the systems nepheline–diopside plus forsterite, silica, wollastonite or akermanite[10,11], while reaction between olivine and silica saturated and undersaturated liquids to yield pyroxene plus magnesio-ferrite is reported during crystallization at constant $pp O_2$ at atmospheric pressure in the system $MgO–FeO–Fe_2O_3–SiO_2$ (ref. 12).

There is no evidence to suggest that the latter relationship at least is suppressed by increase of pressure. The suppression of the reaction relationship in nepheline-diopside bearing systems may occur with increasing pressure but is clearly independent of relationships in the $MgO–SiO_2$ system.

In water-bearing systems, moreover, there is abundant evidence of reaction relationships between olivine and a variety of liquids to yield, among other products, amphiboles and pyroxenes. In the system $CaO–MgO–Al_2O_3–SiO_2–H_2O$ this relationship persists to above 10 kbars[13] and similarly in the systems olivine tholeiite–water, high alumina basalt–water and alkali olivine basalt–water[14], reaction relationships between olivine and liquid are observed at pressures up to and presumably beyond 10 kbars.

It appears, therefore, that olivine–liquid reaction relationships of four characters exist, and that two at least of these (those due to changes in oxygen content or the presence of water in the magma) are likely to persist to pressures at least as great as those resulting from a postulated depth of burial of about 30 km[15]. The presence of olivine–liquid reaction relationships in the Aberdeenshire gabbros cannot, therefore, be taken as evidence of high-level emplacement until the character of the reaction relationship has been established, and even in the interpretation most favourable to Weedon[1] they would at present only indicate pressures less than 10 kbars (~35 km).

The interpretation of textures is notoriously difficult, and the described reaction textures quoted by Weedon from the literature might alternatively be post-consolidation features produced by reaction in the presence of volatiles derived either from the magma itself, or from the later granites, or may be an incipient metamorphic event consequent on a change of mineral facies as the gabbro cooled at relatively great depth. Such alternative origins were clearly envisaged by Stewart[16] and Sadashivaiah[17], and a subsolidus origin appears likely for those described by Read[18] being developed between olivine and plagioclase. Those described by Read[19] and Read *et al.*[20] are clearly of the oxidation type (magnetite–hypersthene rims about olivine) or involve phases (amphibole, biotite) implying the presence of water in the system. In these circumstances, the suppression of the reaction relationship in $MgO–SiO_2$ is irrelevant to the problem under discussion,

and the depth of emplacement of the Aberdeenshire gabbros is in no way restricted by available evidence on olivine–liquid reaction relationships.

We have suggested that the experimental work cannot be used to indicate a high-level intrusion of these bodies. Other factors suggest a relatively deep level of intrusion. The Huntly complex, for example, lies close to the boundary of the kyanite and andalusite zones of metamorphism of the Dalradian rocks. It is probable that the main metamorphism and the intrusion of the gabbros both occurred between the F_2 and F_3 fold movements. Although these events may not have been synchronous, the temperatures of this part of the region probably remained high until and during the F_3 movements as there is no evidence of widespread retrograde metamorphism attributable to these[21]. Other features which may relate to deep-seated consolidation are the relatively highly aluminous character of some of the orthopyroxenes[22,23], and the unusually widespread effects of contamination and remelting near the margins of some of the Aberdeenshire intrusions (see, for example, refs. 19 and 24).

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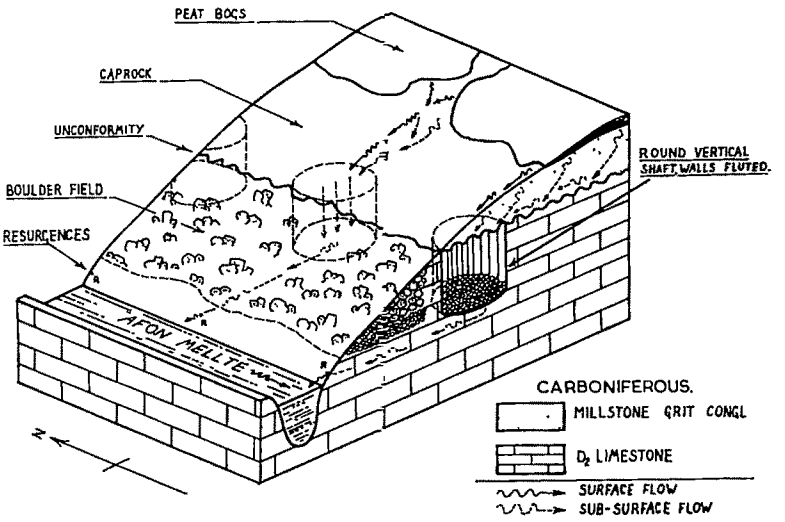


Fig 1. Block diagram of the E bank of the Afon (River) Mellte showing relationships between vertical shafts and geological structure. The shaft illustrated in section lies midway in the mature stage of development, and hydrological connexions from peat bogs via shafts to the river are shown

A New Mechanism for the Formation of Vertical Shafts in Carboniferous Limestone

In the course of study of a newly discovered series of caverns in Carboniferous Limestone at Ystradfellte, Breconshire (National Grid Refs. *SN* 91 : 926·115 to 929·112), we have observed a process of spelaeogenesis apparently unrecorded hitherto.

In this area the limestone series is about 1,000 ft. thick and is covered unconformably (the post-Avonian Unconformity) by a comparatively thin layer of the Basal Quartz Conglomerate of the Millstone Grit, both rocks dipping 10° south in the vicinity of the caverns.

The outstanding feature of these caverns is the presence of incised vertical grooving, that is, fluting, which is due to the process of formation by drip. Water leaving a series of peat-bogs at the top of the hillside (see Fig. 1) runs both over and within the grit, eventually reaching the limestone. The *p*H is on the acid side, gradually becoming neutral as it traverses the limestone. Figures recorded are: (i) before reaching the limestone, 3·8–4·2; (ii) within the caverns, 5·2–5·6; (iii) water of the presumed resurgence, 5·6–7·4. Subsequent to the formation of a small initial solution cavity on the plane of unconformity, water drips on the limestone from the overlying Grit caprock, dissolving a series of drip-pockets. These are vertically orientated cavities of circular cross-section and with a concave base. Examples were found from 2·5 to 10 cm diameter and from 2·5 to 17 cm depth respectively. Penetration by dripping water beyond the base of a drip-pocket extends it mainly vertically rather than laterally. Recent work by one of us (A. R. B.) indicates that vertical growth proceeds four times as fast as lateral growth during juvenile stages. Lateral growth is responsible for the eventual coalescence of neighbouring juvenile tubes. As a result of this coalescence, slender columns of limestone remain. These eventually collapse, forming debris at the base of the shaft.

The mature shaft of greater diameter thus formed eventually causes collapse of the roof due to lack of support. At this stage, the shaft can be termed senile. When the ratio of shaft-diameter to caprock thickness is adequate, collapse extends to the surface and a doline of collapse results. This can be shallow- or steep-sided, or vertical, depending on the angle of grading of the limestone walls by collision or frost-action. Collapse of the roof causes a simultaneous advance of the surface-junction between limestone and grit and hence also of the area of maximum drip. The process of formation then continues to enlarge headward, and less mature, cavities further up the valley side, leaving lower abandoned caverns to grading, forming an inclined boulder-field.

Fig. 2 shows diagrammatically the stages assumed by any one cavern over time, superimposed on calculated curves of vertical and lateral growth over time.

We have concluded that:

(1) In the aforementioned caverns, the roofs are flat because they coincide with the plane of unconformity. Somewhat similar shafts have been studied in the United States[1], but they have dome-shaped roofs and they originate in the limestone at a lower level, developing upwards till caprock is reached, at which stage the roofs become flat.

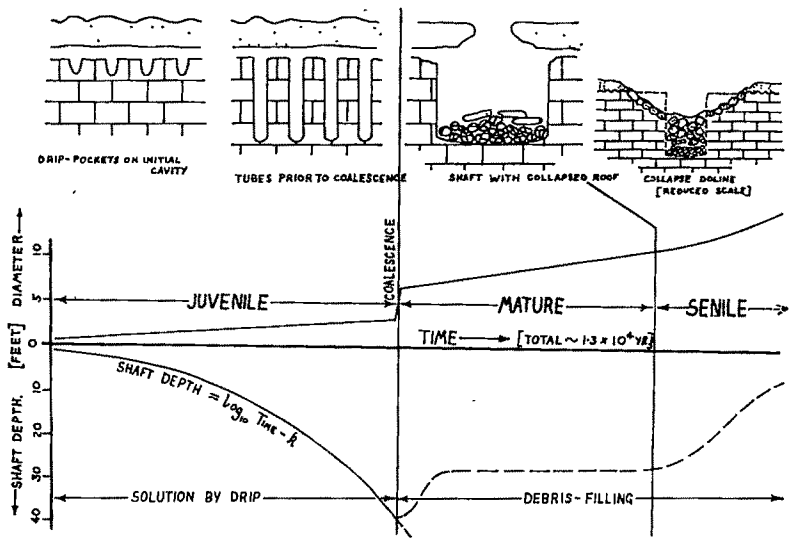


Fig. 2. Showing the relationships between shafts in various stages of development, superimposed on calculated depth and diameter versus time curves

(2) The United States workers propose abrasion by dripping and the effects of trickling water as being responsible for shaft formation. They make no mention of solutional effects. In contrast to this, the caverns described here are considered to be formed mainly by the solutional effect of water of an acid *p*H. It has been found that carbonic and organic acids are present in the incoming water. Titrations using EDTA reveal an increasing amount of calcium carbonate in solution, with a range of 0–105 p.p.m. (Corresponding *p*H readings have been given previously: (i). (iii).) No abrasive materials from either the grit or the limestone have been found in the pocket bases, but the abrasion due to friction of dripping water on limestone is yet to be estimated. It is considered that solutional effects, rather than physical ones, predominate.

(3) The transition from the juvenile to the mature stage has been observed to be due to lateral coalescence. This does not appear to have been mentioned in the American literature.

(4) Similar features have been located in other caverns of the same nature within the area (for example, Coeden Prop and Pulpit Hole).

(5) The formation of collapse dolines at senility illuminates the views of Thomas[2] and is correlated to the distributions which he illustrates, that is, that they are concentrated on the Millstone Grit.

Full results will be published elsewhere.

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Salt Weathering and Inselbergs

RECENTLY, Wellman and Wilson[1] have directed attention to 'salt weathering', that is, 'the fragmentation of rocks by the crystallization of salts', as a geomorphic process of appreciable efficiency, especially in arid regions and along the sea coasts, but also at other places. They have shown that the formation of 'rock meal' and the development of pitted and cavernous rock surfaces are the most obvious symptoms of this type of rock decay.

During my field work, particularly in the Alps of Central Switzerland, I have here and there come across rock meal linings as described by these authors, for example, under overhanging rock ledges and at cave entrances, and in some cases the presence of 'salts' was obvious, either visible in form of efflorescences or verifiable by the taste of the rock meal. Once, I could even watch a mouse licking eagerly the salty rock meal. Such observations certainly lend support to the authors' findings.

Wellman and Wilson, however, also suggest that salt weathering has been, and is, operative in the formation of "some kinds of tors, and at least some hills that have been described as inselbergs". While I do not intend to question their observations of salt weathering and its influence on such land forms, I feel it should be emphasized in this context that salt weathering cannot—in the overall picture—be considered the principal, or even a major, process leading to the development of inselbergs and related forms. During geomorphic studies made over several years in West Africa[2], I have become convinced that these land forms are essentially developing under semi-arid climatic conditions as a late stage in the sequence (or cycle) of pediplanation (scarp retreat and pedimentation)[3]. An assessment of the late Quaternary progress of pediplanation in Ghana has revealed that the scarp retreat associated with pedimentation is an astonishingly fast process and must—apart from erosive removal of debris—be due principally to vigorous physical breakdown ('exfoliation' in the widest sense, that is to say, the result of release of internal stress in the bedrock at or near its surface, ascribed either solely to removal of load by erosion, or to temperature variations, or differential hydration, or a combination of these and other causes[4]). While rounded exfoliation forms are preponderant on inselbergs consisting of granite or similar massive bedrock, sharply sculptured surfaces usually occur on the inselbergs of less massive bedrock. I do not remember having seen the symptoms of salt weathering on West African inselbergs and am inclined, therefore, to assume that this agent can only have very minor, if any, significance in that region.

It is now well established that the Quaternary climatic changes have left their traces not only in the higher but also in the lower latitudes of the Earth, and that, in the latter regions, they consisted essentially in variations of rainfall, or humidity. Thus it is understandable that inselberg landscapes, although developing typically in areas of moderate rainfall, are frequently found outside the present-day semi-arid zones in both humid and arid climatic regions. While with increased humidity they undergo modifications chiefly by chemical weathering under soil and vegetation cover (see, for example, the special cases described by Twidale[5]), an arid environment would obviously favour modifications by salt weathering as described by Wellman and Wilson.

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PHYSICS

Electrical Simulation of Optics of Moving Media

THE early experiments of Fresnel-Fizeau on the velocity of light in a moving medium can be described in modern terminology as the development of an optical delay line with controlled dwell time. Similar changes in a light beam can be generated by present-day versions of an optical delay line (of which there are a number), where controlled dwell time is achieved by the use of an electro-optic medium[1,2].

It should be mentioned that the explanation of the Fresnel-Fizeau experiment is in terms of the Doppler effect of the virtual Huygens sources. The description of Fizeau's experiment rests on the following fact[3]: If a refracting medium of index n is moving away from a stationary observer with a velocity v and if ν is the frequency of the light relative to an observer moving with the medium, then the Lorentz transformation requires the frequency to assume the value

$$\nu' = \nu\beta\,[1 + n(v/c)] \qquad (1)$$

relative to a stationary observer. Thus, to a first approximation, the velocity of propagation of light in the moving medium relative to the stationary observer is

$$u' = \lambda'\nu'/n = (c/n)\,[1 + n(v/c)\,(1 - 1/n^2)] \qquad (2)$$

(the primes being used to denote quantities relative to the stationary observer). To the stationary observer, the velocity of light in the moving medium may be increased or decreased as compared to the value c/n determined by an observer moving with the refracting medium.

As would be expected, the value for ν' is that measured by an observer for a source moving relative to the observer with a velocity v, that is, the virtual Huygens sources act as sources moving with a velocity v.

In Fresnel's terminology, the effect considered so far may be called the convection Doppler effect. This is not directly observable. What is observable is only the overall result, the controlled dwell time of an optical delay line. More direct is the possibility of simulating the Doppler effect resulting from reflection at a moving mirror. This is based on the characteristics of a symmetrical side band generator. It is well known and can be shown from the representation of the electromagnetic field in terms of Hertz vectors that side bands occur when the electric susceptibility of the medium varies harmonically with time[4].

The equations defining these vectors are[5]:

$$\nabla^2\Pi_e - \ddot{\Pi}_e/c^2 = -4\pi P$$

$$\nabla^2\Pi_m - \ddot{\Pi}_m/c^2 = 0 \qquad (3)$$

if $M = 0$.

In terms of these vectors,

$$B = H = \mathrm{curl}\,(\dot{\Pi}_e/c + \mathrm{curl}\,\Pi_m)$$

$$D = E + 4\pi P = \mathrm{curl}\,(-\dot{\Pi}_m/c + \mathrm{curl}\,\Pi_e) \qquad (4)$$

In a refracting medium, the polarization P varies directly as the electric field E, that is,

$$P = \eta E, \text{ where } \eta \text{ is the electric susceptibility} \qquad (5)$$

The case of interest for the operation of the simulator has the following characteristics: The medium through which the light beam is propagated is isotropic and the susceptibility η is specified by

$$\eta = f(\nu)e^{2\pi i\nu_P t} + \ldots + \text{constant term} \qquad (6)$$

where $\nu_P \neq \nu$ is a frequency which determines the harmonic variation of ν with time, the constant term is proportional to $n^2 - 1$, and $f(\nu)$ is independent of the spatial co-ordinates. It follows from equations (5) and (6) that the strength of the polarization generated by the light beam depends on its frequency.

In the case $f(\nu) \equiv 0$, the Hertz equations lead to the familiar result $\lambda\nu = c/n$. Independently of the form of $f(\nu)$, both side bands, $\nu \pm \nu_P$, are present. Depending on the dimensions of the medium, its optical characteristics, and the Poynting flux of the incident radiation, the energy in the incident light beam will eventually be concentrated in the side bands. In general these side bands will consist not only of the two bands $\nu \pm \nu_P$ adjacent to the frequency of the incident light but also of the extended spectrum of side bands $\nu \pm m\nu_P$ where $m > 1$ is an integer. When the radiation is coherent, it would simplify simulation if conditions could be formulated under which only one side band is generated. In a highly dispersive medium, the side bands may be markedly deflected from the direction of the light beam of frequency ν, depending on the uniformity of the susceptibility of the medium.

Consider the special case where in certain definite regions the function $f(\nu)$ decreases steeply with ν, such regions of abrupt descent being separated by less sharply reached maxima or, possibly, by plateaux. The light in the side bands is channelled into these frequency ranges of steep descent. Let ν_M be the frequency at the edge of a region in which $f(\nu)$ tends rapidly towards zero; the radiation in the side bands will tend to concentrate near $\nu = \nu_M$, and on the side of the maximum where the susceptibility decreases sharply with ν. Such an asymmetrical distribution of radiant energy with frequency relative to that of the incident light beam would correspond roughly to the pattern expected for a Doppler shift.

The structure required for achieving a time-dependent polarization with a dependence on ν consisting of steep descent and shallow rises would occur in a medium with a sharp absorption (resonance) line in the neighbourhood of ν, the frequency of the incident radiation, provided the strength of the absorption line could be made to vary harmonically with time. This condition could be achieved if the concentration in the lower level of the radiative (absorption) transfer were made to vary harmonically with time, for example by optical pumping with high intensity coherent radiation varying harmonically with time.

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Fresh Fall-out in Israel from the Second Chinese Nuclear Detonation

A NUCLEAR detonation was reported to have occurred on the Chinese mainland on May 14, 1965. The test was apparently conducted in the atmosphere and involved a uranium-235 fission device[1]. Fall-out attributable to this detonation was first detected in Israel on air filters on May 28, 1965. About 3 weeks later, iodine-131 was detected in milk samples and in animal thyroids. Monitoring of iodine-131 in these samples was continued for several months.

Surface air activity. About 200 m³ of air were sampled daily, using a 'Victoreen' positive displacement pump. The activity collected on the filter paper was determined using a well-type sodium iodide (thallium-activated) scintillation crystal coupled to a multi-channel gamma-spectrometer.

Iodine-131 was first detected in air on May 28, 1965, and zirconium-95–niobium-95 appeared 3 days later. Barium-140–lanthanum-140 were detected on June 1, 1965. The fact that radio-iodine was detected in surface air

prior to barium-140–lanthanum-140 may be due to the difference in transport mechanisms of gaseous fission products as compared with particulate debris. Many countries use barium-140 rather than iodine-131 as the first indicator of new fission products[2], since traces of radio-iodine may be present in the environment as a result of normal operation of nuclear installations. Table 1 shows the radio-iodine activity concentrations detected in the air.

Table 1. IODINE-131 ACTIVITY IN SURFACE AIR

Collection date	^{131}I Activity (pc./m^3)	Activity corrected to date of detonation (pc /m^3)
May 28, 1965	0 015	0·05
May 30, 1965	0·02	0·08
June 1, 1965	0·04	0·18
June 2, 1965	0 02	0·10
June 3, 1965	0·03	0·16

After June 3, the presence of barium-140–lanthanum-140 in the gamma spectrum made iodine-131 determinations difficult without resorting to chemical separations, and iodine measurements in the air samples were therefore discontinued. Following the detection in air of the short-lived isotopes, tests were started on samples of milk, as well as animal thyroids.

Milk samples. Samples of cows' milk were collected regularly from more than ten different locations scattered throughout the country and they were routinely analysed for strontium-90 and caesium-137 (ref. 3). These samples were also tested for iodine-131. Sodium hydroxide and sodium iodide were added, after which the milk was ashed at about 400° C. The elementary iodine was separated and extracted with carbon tetrachloride[4]. Table 2 shows the results of our milk tests for 3 months following the detonation.

Table 2. ^{131}I ACTIVITY IN MILK

Date of milking	No. of samples	^{131}I on date of milking (pc./l. per sample)
June 1–10	1	0
June 11–20	2	0, 0
June 21–30	4	0, 8, 10, 32
July 1–10	2	0, 20
July 11–20	3	8*, 9, 10
July 21–31	2	11*, 17*
August 1–10	5	0, 6*, 10, 11, 15
August 11–20	1	4
August 21–31	—	—

* Sheep's milk.

Thyroid glands. Thyroid glands of sheep and goats slaughtered in two municipal abattoirs were tested for the presence of iodine-131. Composite samples were prepared from the thyroids of ten to twenty animals. The sample was ashed at about 400° C after the addition of sodium hydroxide and sodium iodide in order to ensure trapping of the free or organic iodine. Iodine-131 was identified by its peak at 0·36 MeV and 0·64 MeV using a multichannel gamma spectrometer. Because of the relatively high activity in the samples it was possible to check the half-life of the activities at both peaks, which proved conclusively that the activity at 0·64 MeV was due to iodine-131 and not to 0·66 MeV peak of caesium-137. Results of iodine-131 activity tests in sheep and goat thyroids are summarized in Table 3.

Table 3. IODINE-131 ACTIVITY IN SHEEP AND GOAT THYROIDS

Date of sampling	Weight of thyroids (g)	Average ^{131}I activity on date of sampling (pc./g)
June 1–10	—	—
June 11–20	90	5·0
June 21–30	—	—
July 1–10	95	7·3
July 11–20	40	2·7
July 21–31	55	1·7
August 1–10	60	1·5
August 11–20	20	0·3
August 21–31	110	0·3
September 1–15	55	0·3
September 16–30	—	—
October 1–15	—	—
October 16–31	60	0
November 1–15	40	0

In the case of the first Chinese nuclear detonation (October 1964) no fresh fall-out was detected in Israel. A rise in gross beta activity in air was detected during the month of October 1964, but this was attributed to a seasonal increase in activity after the first winter rains, as encountered during past seasons. Short-lived nuclides associated with fresh fall-out were not detected[5]. These findings were consistent with the data presented by Krey and Rosa that the southernmost edge of the debris passed over northern Europe and did not enter the Mediterranean area[6]. A map of the path of the debris from the second Chinese test, which appeared in the same report, though not complete, indicates a more southerly route.

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CHEMISTRY

Calculations of the Racah Parameter B for Nickel (II) and Cobalt (II) Compounds

RECENTLY, numerous papers have been published on the spectra of nickel (II)[1–3] and cobalt (II)[4,5] complexes in which the Racah parameter B has been deduced. For those octahedral nickel (II) complexes where only the $\nu_2(^3A_{2g} \rightarrow {}^3T_{1g}(F))$ and the $\nu_3(^3A_{2g} \rightarrow {}^3T_{1g}(P))$ bands were observed, Dq and B were found by solving the secular equation[6] by trial and error[2]. We have found that in these circumstances it is possible to simplify the problem to that of solving a quadratic equation in Dq, ν_2 and ν_3 only. The equations for finding Dq and B from ν_2 and ν_3 for octahedral nickel (II) complexes (d^8) are:

$$340Dq^2 - 18(\nu_2 + \nu_3)Dq + \nu_2\nu_3 = 0 \qquad (1)$$

$$B = \frac{\nu_3 + \nu_2 - 30Dq}{15} \qquad (2)$$

The solution of equation (1) gives two values for Dq and thus two values of B are obtained. It is always obvious which values of Dq and B are the required solutions. The same equations will, of course, apply to ions with a d^3 configuration in an octahedral field and to ions with d^2 and d^7 configurations in tetrahedral fields.

Table 1. CALCULATED VALUES OF Dq AND B (cm^{-1})

Compound	Ref.	Observed bands ν_2	Observed bands ν_3	Calculated from equations (1)–(4) Dq	Calculated from equations (1)–(4) B	Reported values Dq	Reported values B
Ni(3Etpy)$_4$Br$_2$ (d^8, octa.)	1	12,970	23,000	781 1,123	835 152	789	830
NiMpCl$_2$ (d^8, octa.)	2	13,150	23,000	795 1,118	819 173	794	825
NiMp$_2$Cl$_2$ (d^8, tetra.)	2	9,200	15,620	497 −350	808 −884	500	810
CoO (d^7, octa.)	7	16,680	18,450	887 −98	780 −1,190	888*	780†
Cs$_3$[CoCl$_4$]Cl (d^7, tetra.)	5	5,580	14,800	320 759	719 −159	320	719

3Etpy is 3-ethyl pyridine, Mp is methylpyrazine.
* Calculated from ν_2 band.
† Calculated from diagonal sum rule.

The corresponding equations for ions with a d^2 or d^7 configuration in an octahedral field, or with a d^3 or d^8 configuration in a tetrahedral field, are given below.

For example, d^7 (octahedral field) where ν_2 is ${}^4T_{1g}(F) - {}^4A_{2g}$, and ν_3 is ${}^4T_{1g}(F) - {}^4T_{1g}(P)$,

$$340Dq^2 + 18(\nu_3 - 2\nu_2)Dq + \nu_2^2 - \nu_2\nu_3 = 0 \qquad (3)$$

$$B = \frac{\nu_3 - 2\nu_2 + 30Dq}{15} \qquad (4)$$

Table 1 records examples of Dq and B calculated using equations (1)–(4) together with those reported previously, for some octahedral and tetrahedral compounds of cobalt (II) and nickel (II).

One of us (D. E. B.) thanks Loughborough College of Technology for a research scholarship.

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Ultrasonic Absorption in Aqueous Solutions of Certain Inorganic Sulphates

It is well known[1] that for a salt solution the relationship between adiabatic compressibility, β, and molarity, c, is given by the expression:

$$\beta = A + B\,c + C\,c^{3/2}$$

Recently[2], the variation of compressibility with concentration, for aqueous solutions of various inorganic sulphates (lithium sulphate, magnesium sulphate, sodium sulphate, potassium sulphate, etc.), was reported; differences in the behaviour of each solution were related to differences in the cations—as the sulphate ion was common to all, the differences were considered to be due to differences in the ionic radius of the cations.

When the ionic radius increases, the compressibility decreases; if it is assumed that the hydration of the sulphate ion is the same in each solution for a given concentration, it would seem that a larger ionic radius would lead to greater solvation which would result in a lower compressibility.

Table 1

Magnesium sulphate in water		Mg^{++} (r = 0·65 Å)	
c molarity	ϱ (20° C) g/cm³	η centipoise 20° C	$\frac{\alpha}{\nu^2}\times 10^{17}$
0·10	1·0105	1·0670	7·1650
0·25	1·0291	1·1724	8·4508
0·50	1·0501	1·3785	9·41864
1·00	1·1014	1·944	10·4159
2·00	1·1990	3·908	15·9238
$Li_2SO_4 \rightleftarrows 2Li^+ + SO_4^{--}$			
Lithium sulphate in water		Li^+ (r = 0·60 Å)	
0·10	1·0080	1·0565	7·2383
0·25	1·0215	1·1416	7·3513
0·50	1·0440	1·296	7·7399
1·00	1·0874	1·670	8·5892
Sodium sulphate in water		Na^+ (r = 0·95 Å)	
0·10	1·0111	1·0390	6·9970
0·25	1·0279	1·1050	6·9801
0·50	1·0606	1·2260	7·2065
0·75	1·0891	1·2730	7·4120
1·00	1·1179	1·5700	7·7200
Potassium sulphate in water		K^+ (r = 1·33 Å)	
0·10	1·0325	1·0545	6·7050
0·25	1·0125	1·022	6·860
0·5	1·0734	1·109	6·4651

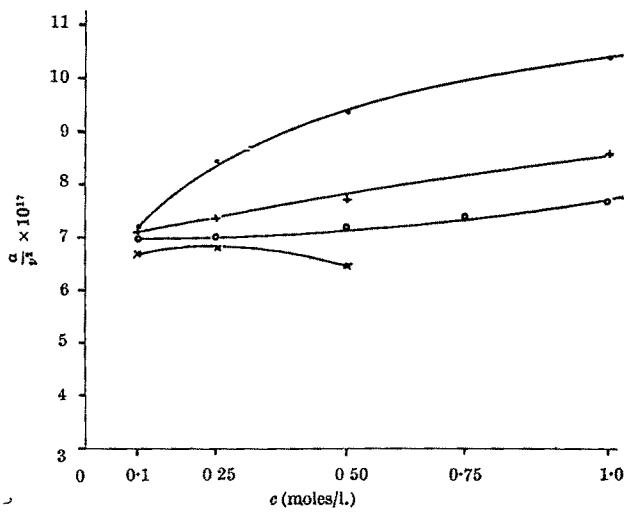


Fig. 1. Plot of ultrasonic absorption against molarity for the sulphates of magnesium (●), lithium (+), sodium (○) and potassium (×)

It is necessary to take into account the fact that the radius of Li^+ ions (r = 0·60 Å)[3] has a greater effect on compressibility in lithium sulphate than does the radius of the Mg^{++} ion (r = 0·65 Å) in magnesium sulphate.

It is possible that a larger number of water molecules is bound by the two Li^+ ions than by the single doubly charged Mg^{++} ion. We can obtain values for ultrasonic absorption $\left(\frac{\alpha}{\nu^2}\times 10^{17}\right)$ (ref. 4) from the expression:

$$\frac{\alpha}{\nu^2} = \frac{8}{3}\frac{\Pi^2}{\rho}\frac{\eta}{V^3}$$

where V is the ultrasonic velocity, ρ is the density (20° C), and η is the viscosity (Ostwald) 20° C.

In liquids, as we know, the contribution of the thermic conducibility to the ultrasonic absorption is negligible; thus, because $\alpha_{class} = \alpha_\eta + \alpha_{cond}$, $\alpha_{class} = \alpha_\eta$.

Table 1 gives $\frac{\alpha}{\nu^2}\times 10^{17}$ values, molarity, density and viscosity coefficient, and Fig. 1 a plot of ultrasonic absorption against molarity, for the sulphates of magnesium, lithium, sodium and potassium.

For a given value of concentration, we can see that $\frac{\alpha}{\nu^2}\times 10^{17}$ is largest for a solution of magnesium sulphate, and that it decreases in the order lithium sulphate, sodium sulphate, potassium sulphate.

Because dissociation of magnesium sulphate only results in one Mg^{++} ion (r = 0·65 Å), while dissociation of lithium sulphate, sodium sulphate and potassium sulphate results in two Li^+, Na^+ and K^+ ions, respectively, (the radii of which are 0·60, 0·90 and 1·33 Å, respectively), when ionic radium increases, the ultrasonic absorption decreases. It is thus necessary to take into account the fact that there is a lower effect for doubly charged Mg^{++} ion than for Li^+ and other ions. This could well confirm the hypothesis that the number of water molecules bound to ions in solution is responsible for the different behaviours of η, β, V in various solutions, that is, the larger the number of water molecules bound by ions of large ionic radius, the lower the value of ultrasonic absorption.

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Metal Clusters in a New Mixed Halide of Molybdenum (IV) and (V)

SINCE 1963 there have been several reports of the existence of trimeric clusters of rhenium atoms in anionic and uncharged halogen compounds of rhenium (III)[1–5]. Recently, Dr. R. Colton and Prof. R. L. Martin have adduced magnetic and spectral evidence for the existence of trinuclear clusters in rhenium (IV) chloride[6] and also in molybdenum (III) chloride[7]. In an investigation of the reactions of molybdenum hexafluoride as outlined in the following paragraph, a new mixed halide of molybdenum has been isolated and characterized. Magnetic, spectral and conductance measurements indicate that this compound is ionic and contains the $(Mo^{IV}_3Cl_9)^{3+}$ cation, an entity which fits very well into the scheme proposed by Colton and Martin for stable clusters in molybdenum–chlorine compounds.

When molybdenum hexafluoride reacts at room temperature with chlorides such as phosphorus trichloride, carbon tetrachloride and silicon tetrachloride, the corresponding fluorides (PF_3, CF_4 and SiF_4) are identified among the products of reaction. However, in each of these cases and in many similar reactions[8] the product containing molybdenum was found to have the empirical formula $Mo_2Cl_3F_6$. This same orange compound, together with chlorine, is obtained as a result of mixing molybdenum hexafluoride and molybdenum pentachloride. When hydrogen fluoride was distilled on to molybdenum pentachloride, the same mixed halide was the only involatile product while hydrogen chloride and chlorine could be distilled from the reaction system.

Obviously this mixed halide of odd oxidation state is a compound of considerable stability. It appeared to sublime unchanged at 80° C, but on further heating it decomposed to molybdenum trichloride and molybdenum hexafluoride. It was found to react with water and with nitrobenzene, and to be virtually insoluble in carbon tetrachloride, in perfluoroheptane and in trifluoroacetic acid. However, it was readily soluble in acetonitrile.

The empirical formula suggested two possible structures. The first of these is a bridged structure with two molybdenum atoms, each surrounded by an octahedron of three chlorine atoms and three fluorine atoms. The two octahedra could share a face containing three chlorine atoms.

This type of structure has been reported for the $W_2Cl_9^{3-}$ anion[9]. An alternative formulation would be an ionic one, for example $(Mo^{IV}Cl_6)^{+}$ $(Mo^{V}F_6)^{-}$. The hexafluoromolybdate (V) anion is well known, but the $MoCl_3^{+}$ cation has not been previously reported.

Conductance measurements in acetonitrile favoured an ionic structure. A value of 80 ohm^{-1} cm^2 was obtained for the molar conductance at a concentration of $4{\cdot}35 \times 10^{-3}$ M based on the empirical formula $Mo_2Cl_3F_6$. Electrolysis of the brown solution in acetonitrile in a three-compartment cell resulted in a brown catholyte solution which gave a positive test for chloride and a negative test for fluoride, and a colourless anolyte which gave a positive fluoride test and a very weak chloride test. This observation was consistent with electrolysis of a compound containing the MoF_6^- anion and a cation containing molybdenum and chlorine.

Determination of the magnetic susceptibility over the temperature range 93°–290° K showed it to follow a Curie-Weiss plot with a value for θ of 155°, which is of the same magnitude as the values found for other compounds containing the MoF_6^- anion[10]. Using this correction, values for the magnetic moment of 1·67–1·73 Bohr magnetons (B.M.) per molybdenum atom were obtained over the temperature range studied. Solution measurements in acetonitrile also gave a value of 1·67 B.M. However, the formulation $(Mo^{IV}Cl_3)^{+}$ $(MoF_6)^{-}$ is at variance with these magnetic data. If 'spin-only' values are assumed for the d^1 (Mo^{V}) and d^2 (Mo^{IV}) configurations a value of 2·45 B.M. is obtained. Experimental figures can be reconciled with calculated values, however, if the suggestion is made that the cation is trimeric. The compound could then be formulated $(Mo^{IV}_3Cl_9)^{3+}$ $(Mo^{V}F_6)_3^{-}$.

We assume here that the core of the $(Mo_3Cl_9)^{3+}$ cation is similar to that found in the $Re_3Cl_{12}^{3-}$ and Re_3Cl_9 structures[1–4]. In this case the molecular orbital treatment[6] leads to a ground-state electronic configuration $(a_1')^2$ $(a_2'')^2$ $(e')^2$ with two unpaired electrons per trimer. This structure would be expected to give a moment of 1·64 B.M. per molybdenum atom, a value corresponding with two-thirds of an electron per molybdenum atom.

However, the magnetic moment of the anion cannot be neglected. It is assumed here that it contributes one unpaired electron per MoF_6^- anion to the measured moment. The magnetic moment calculated for the overall formulation $(Mo_3Cl_9)^{3+}$ $(MoF_6^-)_3$ then becomes 1·68 B.M. per molybdenum atom, a value in excellent agreement with the experimental one.

Examination of the spectrum of the compound in acetonitrile shows the presence of a peak in the infra-red region at 630 cm^{-1}. In spectra of mulls and disks absorption at 623 cm^{-1} has been reported[11] as being characteristic of the hexafluoromolybdate (V) anion in the well characterized compound $KMoF_6$.

An acetonitrile solution of the compound shows absorption maxima at 21,500 cm^{-1} and 14,100 cm^{-1}. The colourless MoF_6^- anion does not absorb appreciably in the visible region and the observed absorption bands are therefore attributed to the trimeric cation $Mo_3Cl_9^{3+}$. The spectra of some related trimeric transition metal chloride clusters are summarized in Table 1 and it is a striking feature that they all show two similar bands.

Table 1. SPECTRA OF SOME CHLORIDE CLUSTERS

Compound	Electron configuration	Positions of absorption maxima cm^{-1} (ϵ_{max})	Solvent	Reference
Re_3Cl_9	d^4	13,300 (410); 19,400 (1,610)	Acetone	4
Mo_3Cl_9	d^2	13,150; 19,100	Reflectance	7
$Mo_3Cl_9^{3+}$	d^2	14,100 (160); 21,500 (1,550)	Acetonitrile	This report
Mo_3Cl_{12}	d^2	13,330; 22,200	Acetonitrile	12
$W_3Cl_{12}^{3+}$	d^1	13,100; 22,200	Acetonitrile	12

Conductance measurements and electrolysis in acetonitrile suggest that the compound reported here is ionic and its insolubility in non-polar solvents further supports this. Spectra and magnetic measurements suggest a structure based on a trimeric cation and the MoF_6^- anion. It should be noted that if the molar conductance is calculated on the basis of the formula $(Mo_3Cl_9^{3+})$ $(MoF_6^-)_3$ a value of 240 ohm^{-1} cm^2 is obtained for the molar conductance at a concentration of $1{\cdot}45 \times 10^{-3}$ M. Measurement of the molar conductance as the solution is diluted indicates a limiting value of about 330 ohm^{-1} cm^2.

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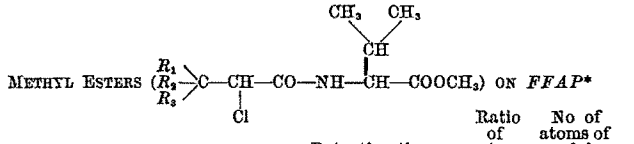
Investigation of Steric Effects about the Amide Bond by Gas–Liquid Chromatography

THE steric requirements of molecules dominate the rates and equilibria of many reactions in organic and inorganic chemistry. It is still difficult to make precise calculations of steric effects, but in many cases the qualitative principles controlling steric requirements have become apparent. For example, in reactions that involve addition to an unsaturated function containing a double bond, the empirical 'rule of six' correctly states "the greater the number of atoms in the six position, the greater will be the steric effect"[1]. The rule of six has also been used to interpret conformations of polypeptides and proteins and to explain hydrolysis rates of di- and poly-peptides[2].

We have found that the separation of diastereoisomeric pairs using gas–liquid chromatography can be used to assess steric conditions about the amide bond and that here again the rule of six correctly predicts the steric effect. In particular, we chose α-chloro alkanoyl valine methyl esters, because they are readily prepared from the respective peptides; and we had already shown that α-chloro alkanoyl chlorides are excellent reagents for the quantitative chromatographic resolution of diastereoisomers[3]. Since the presence of neighbouring polar groups may greatly modify steric factors, we have restricted our work to those compounds, derived from amino-acids, which have no hetero-atom in the side-chain. Correlation of the six-number with the ratio of retention times of the diastereoisomeric pairs is summarized in Table 1. Examination of the data suggests that the number of atoms in the 5 and 7 position is unimportant for the assessment of steric effects, but that the most hindered α-chloro acids are those with the highest six-number. This explanation is consistent with the observation that 2-chloro-3-dimethylbutanoic acid is the best reagent for the gas–liquid chromatographic analysis of D,L-amino-acid esters.

Our results suggest that differences between the vapour pressures of the diastereoisomers are enhanced by increased crowding about the amide bond, as is shown by separations on a non-polar silicone gum rubber column (*SE*-30) (Table 2). In addition, a more polar liquid phase (*FFAP*) improves the resolution (Table 1). We interpret the latter result as being due to secondary bonding between the amide carbonyl and the polyester column substrate. Since the magnitude of this effect will also be dependent on configuration it will be different for each diastereoisomer and further enhance the separation.

Table 1. CORRELATION OF THE RULE OF SIX WITH RATIO OF RETENTION TIMES OF DIASTEREOISOMERS OF A SERIES OF *N*-CHLORALKANOYL VALINE



METHYL ESTERS (R_1, R_2, R_3 = C—CH(Cl)—CO—NH—CH(CH(CH$_3$)$_2$)—COOCH$_3$) ON *FFAP**

Chloro acid	Retention times of diastereoisomers L,D	L,L	Ratio of retention times L,L/L,D	No of atoms of resolving agent in position† 5	6	7
2-Chloropropanoic acid $R_1=R_2=R_3=H$	5·75	6 5	1 13	3	0	0
2-Chlorobutanoic acid $R_1=R_2=H$; $R_3=CH_3$	6·3	7·95	1·17	3	3	0
2-Chloro-4-methylpentanoic acid $R_1=R_2=H$; $R_3=CH(CH_3)_2$	10·15	12·0	1·18	3	3	6
2-Chlorohexanoic acid $R_1=R_2=H$; $R_3=CH_2CH_2CH_3$	12·4	14 7	1·18	3	3	3
2-Chloro-3-methylbutanoic acid $R_1=H$; $R_2=R_3=CH_3$	8 1	9·9	1·22	3	6	0
2-Chloro-3-methylpentanoic acid $R_1=H$; $R_2=CH_3$; $R_3=CH_2CH_3$	10 35	12·9	1·25	3	6	3
Allo-2-chloro-3-methylpentanoic acid $R_1=H$; $R_2=CH_2CH_3$; $R_3=CH_3$	10·5	12 85	1·22	3	6	3
2-Chloro-3-dimethylbutanoic acid $R_1=R_2=R_3=CH_3$	9·6	12·5	1 30	3	9	0

* Chromatographic analyses were carried out on a Wilkens '600*C* Aerograph' equipped with a flame ionization detector. The 5 ft. × 1/8 in. column was packed with 5 per cent *FFAP* on 'Chromosorb *W*'. The separation temperature was 161° C and during analyses the nitrogen flow was 28 ml /min

† The six number for the peptide bond is obtained by adding the contribution of the valine (three atoms) to the appropriate values for the α-chloro acid. The number system used for a peptide bond is illustrated as follows.

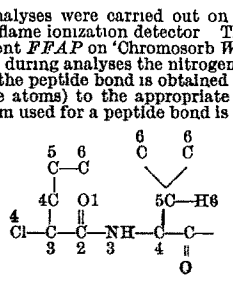


Table 2. GAS CHROMATOGRAPHIC SEPARATION OF DIASTEREOISOMERS OF *N*-CHLORALKANOYL VALINE METHYL ESTERS ON *SE*-30*

Chloro acid	Retention times of diastereoisomers L,D	L,L	Ratio of retention times L,L/L,D	Six No.
2-Chloropropanoic acid	5·9	6·25	1·06	0
2-Chloro-4-methylpentanoic acid	17·05	18 8	1·10	3
2-Chloro-3-methylbutanoic acid	11 85	13·5	1·14	6
2-Chloro-3-methylpentanoic acid	17·9	20·6	1·15	6
Allo-2-chloro-3-methylpentanoic acid	18 9	21·7	1·15	6

* The 5 ft. column was packed with 5 per cent *SE*-30 on 'Chromosorb *W*' The separation temperature was 140° C and during analyses the nitrogen flow was 28 ml /min.

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Comparison of Diffusion of Carbon Dioxide into Hydrocarbon Systems and Polymeric Solutions

METZNER recently reported diffusion coefficients of ethylene and carbon dioxide into polymeric and colloidal solutions[1]. We have measured absorption rates of carbon dioxide in mixtures of hydrocarbons of molecular weight 160–360. In this work, for a twenty-four-fold increase in viscosity the diffusion coefficients varied from $0{\cdot}73 \times 10^{-5}$ to $2{\cdot}50 \times 10^{-5}$ cm²/sec. These data can be interpreted by existing theories on diffusion[2]. The values quoted by Metzner for diffusion of carbon dioxide into dilute solutions of carbopol and carboxy methyl cellulose are all within this range ($1{\cdot}8$–$2{\cdot}4 \times 10^{-5}$ cm²/sec) although the apparent viscosities of these solutions will be very much greater than that of the highest molecular weight hydrocarbon investigated (25 centipoise).

As Astarita has shown[3], the values of the diffusion coefficients of carbon dioxide into polymeric solutions are very much higher than existing theories would predict. The fact that the diffusion coefficients are all of the same order as those for carbon dioxide into water and hydrocarbons suggests that, at the concentrations considered, the presence of the polymeric molecules has little effect on the molecular transport of the solute, although such molecules have a profound effect on the flow properties of the solution. At zero shear stress, the polymer molecules would not effect the diffusion of solute if they were spaced well apart in relation to the volume of the solute molecules. The increase of diffusion coefficients as the concentration of polymer molecules increases (apparent viscosity increasing) could then only be accomplished by ordering of the solvent around the polymer molecules, provided the intermolecular spacing between the polymer molecules was again very large.

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BIOCHEMISTRY

Insensitivity of Phytochrome Decay *in vivo* to Respiratory Uncoupling by 2,4-Dinitrophenol

PHYTOCHROME, the red, far-red photo-reversible chromoprotein that controls much of higher plant growth and development, undergoes two changes in the dark following an initial red light exposure. One change, reversion, is the transformation of the far-red absorbing form, P_{FR}, into the red absorbing form P_R. A second change, which we will call decay, is the loss of total photo-reversible phytochrome as a result of the apparent destruction of P_{FR} (ref. 1). Since there is considerable indirect evidence that P_{FR} is the active form of phytochrome, the interactions between P_{FR} decay and metabolism may be of significance in elucidating the mechanism of phytochrome action.

Butler and Lane[2] have shown that decay in etiolated *Zea* (corn) seedlings is directly proportional to oxygen concentrations between 0 per cent and 10 per cent. They further demonstrated that cyanide, carbon monoxide and azide partially inhibit both respiration and P_{FR} decay, thus suggesting that respiration and decay are closely linked. Furuya *et al.*[3], however, have shown that in coleoptiles of etiolated *Avena* (oat) seedlings ethylenediamine tetraacetic acid (EDTA) stops decay at concentrations having no effect on oxygen uptake. This suggests that respiration and phytochrome decay are not necessarily related. We have now used 2,4-dinitrophenol (DNP), an uncoupler of the respiratory chain, as a further test of the possible relationship between these processes.

Since it is well established that the uncoupling action of DNP is accompanied by a surge of oxygen uptake[4], we first determined what concentrations of DNP cause a significant increase in respiration.

The material was taken from four-day-old seedlings of *Avena sativa* cultivar 'Clintland' grown at 26° C in darkness. Leafless coleoptile segments, 5 mm in length, were cut, randomized and then placed in groups of thirty in each of the Warburg-type flasks used in the experiment. The main chamber of the flasks contained, beside the tissue, a 0·02 M sodium–potassium phosphate buffer at *p*H 6·5 with 1·5 per cent sucrose[5]. The centre well contained 10 per cent potassium hydroxide, and the side arms held either DNP or water, as the case might be. Oxygen uptake was followed at 26° C in air with a '*GME*' differential respirometer (Gilson Medical Electronics, Middleton, Wisconsin). Once the initial respiratory rate of each flask was determined over 100 min, the contents of the side arms were tipped into the main vessel. The final rate was established over 120 min after a 30-min incubation period. All data were first normalized to the initial rates for each flask. These values were then averaged for the various DNP concentrations and for the controls. With the control values taken as 100 per cent, the means obtained in this way were used to compute the net DNP effect at various concentrations[3]. Several such experiments gave values of 114, 137, 143, 154 and 135 per cent of the control for 1, 2, 3, 5 and 10×10^{-5} M DNP, respectively. These values are close to those obtained for *Zea* (corn) coleoptiles under similar conditions by Beevers[6].

We then proceeded to test the effects of uncoupling concentrations of DNP on phytochrome decay. The same type of material prepared for the respirometer experiments was used here, except that all manipulations were carried out in the dark or under a dim green safelight[7]. The segments were then incubated for 30 min in the sucrose–phosphate buffer, either with or without added DNP. After the incubation period a 5-min red-light treatment[7] was given to the appropriate segments. At 0, 1 and 2 h after the red light, twenty-five segments were quickly packed into cuvettes, 3 mm in diameter, and placed on ice. The samples were then assayed for phytochrome by methods described elsewhere[7], using a two-filter difference spectrophotometer to measure total $\Delta(\Delta OD)$ values, which are taken as estimates of total phytochrome. Results of a typical experiment are presented in Table 1 and indicate that uncoupling concentrations of DNP have no effect on decay.

Table 1. LACK OF EFFECT OF 3×10^{-5} M DNP ON PHYTOCHROME DECAY IN *Avena* COLEOPTILE SEGMENTS

Each value is the mean of three samples

Treatment	Hours after red light	Phytochrome total $10^3\ \Delta(\Delta OD)$	Percentage remaining
Controls (buffer and sucrose only)	0	53·2	100
	1	32·8	62
	2	17·2	32
Plus DNP	0	51·8	100
	1	29·0	56
	2	17·0	34

Further experiments have shown that a much higher concentration of DNP, 10^{-3} M, prevents the loss of total phytochrome in both red-light-treated and dark samples. At the same time, 10^{-3} M DNP reduces the respiratory rate to about one-tenth of the control rate. These facts indicate that decay is dependent at least on an intact metabolism and also that DNP itself does not affect phytochrome absorbance.

In conclusion, our results demonstrate that respiratory chain energy conservation is not intimately related to phytochrome decay. This finding supports the view[3] that decay requires oxygen and metal activity, not as a function of the respiratory chain, but rather as a function of other, as yet unknown, reactions. The question of the physiological importance of phytochrome decay remains open.

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Activity of Amino-laevulinic Acid Synthetase in Normal and Porphyric Human Livers

ACUTE intermittent porphyria (AIP) is well known as an inborn error of porphyrin metabolism, clinically characterized by attacks of abdominal pains and frequently associated with the neurological and mental symptoms and signs. The excretion of the two kinds of porphyrin precursors, porphobilinogen (PBG) and delta-amino-laevulinic acid (ALA) in urine, suggesting the presence of a disturbance in the porphyrin metabolism, has been thought essential for the diagnosis of this disease.

Although a block in the conversion of PBG and ALA to porphyrins has been regarded as the explanation for the accumulation and urinary excretion of these compounds, Granick and Urata[1] reported a marked elevation of ALA synthetase activity in liver mitochondria in the experimental porphyria in guinea-pig induced by feeding of 3,5-dicarbethoxy-1,4-dihydrocollidine (DDC). Recently we have had an opportunity to perform a metabolic investigation on human porphyric liver.

The patient was a man 32 years old, with a history of attacks of abdominal pain and neurological symptoms such as weakness of arms and legs lasting for a month

Table 1. ENZYMATIC ACTIVITIES IN HUMAN PORPHYRIC LIVER

	ALA synthetase in washed mitochondria		ALA dehydrase in 10,000*g*-supernatant	PBGase in 10,000*g*-supernatant	Iron–protoporphyrin chelatase in mitochondria
	mμmoles ALA formed from α-KG and glycine per mpv* per 120 min incubation at 38° C	mμmoles ALA formed from succinyl-CoA and glycine per mpv* per 10 min incubation at 38° C	mμmoles PBG formed from ALA per mg protein per 60 min incubation at 38° C	mμmoles PBG utilized per mg protein per 120-min incubation at 38° C	mμmoles protoporphyrin utilized per mpv* per 120 min incubation at 38° C
Porphyric A.H. 32 yr ♂	317	16	45·9	4·55	150
Control H.N. 34 yr. ♂	trace or 0	0	25·0	4·75	180
K.W. 32 yr. ♀	trace or 0	0	18·7	4·35	165
N.N. 30 yr ♂	trace or 0	0	13·2	4·40	187

* mpv, ml. packed volume.

3 years ago. He was hospitalized for re-examination, with the disease in remission. During his stay in hospital he continued to excrete about 200 μmoles of PBG and 80 μmoles of ALA in his urine every day without signs and symptoms of AIP. A biopsy of the liver was carried out surgically to remove 2 g of liver.

For the preparation of liver mitochondria and supernatant, 2·0 g of the fresh liver was homogenized for 1 min with 20 ml. of 0·25 M sucrose including 0·005 M sodium bicarbonate and 0·005 M potassium-phosphate-buffer (*p*H 8·0) in a Potter–Elvehjem glass homogenizer with a loose pestle, followed by centrifugation at 800*g* for 10 min. The supernatant was centrifuged at 10,000*g* for 10 min. The mitochondria portion was washed twice with sucrose solution. ALA synthetase activity was assayed by the method of Granick and Urata[1], ALA dehydrase and PBGase activity by the method of Granick and Mauzerall[2], and iron–protoporphyrin–chelatase activity by the method of Labbe[3].

As shown in Table 1, ALA synthetase activity is markedly greater in porphyric liver mitochondria than in the controls, and the ALA dehydrase activity is about twice that in the controls, while the PBGase and chelatase activity is nearly the same. The ability of porphyric liver to convert PBG to porphyrins, measured by an increase of optical density at 407 mμ in a Beckmann spectrophotometer, was about twice that of the control, although the activity of each enzyme is still under investigation.

This study indicates that overproduction of the porphyrin precursors, resulting from increased ALA synthetase activity in the liver mitochondria, is the principal feature of the disturbance of porphyrin metabolism in human acute intermittent porphyria.

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Carbon-14 in Carboxyl Carbon of Fatty Acids formed by Platelets from Normal and Diabetic Subjects

WHEN platelets from diabetic patients are incubated with acetate carbon-14, a significantly smaller than normal percentage of fatty acid carbon-14 is found in myristic and palmitic acids. Formation of longer-chain fatty acids, particularly saturated and unsaturated 20-carbon fatty acids, is much less affected, resulting in an increased percentage of fatty acid carbon-14 in these fatty acids[1–3]. Platelets from comatose diabetics show the greatest abnormality[1], while those from non-ketotic insulin-dependent diabetics[2] and insulin-independent diabetics[3] show less marked but similar changes.

Harlan and Wakil[4] have shown that myristic (14 : 0), palmitic (16 : 0), and stearic acids (18 : 0) can be made in the supernatant fraction of rat liver by *de novo* synthesis via the malonyl CoA pathway. However, the mitochondrial fraction of rat liver makes both palmitic and stearic acids by chain lengthening of relatively non-radioactive shorter chain fatty acid precursors.

We have postulated that the abnormalities in fatty acid formation which we have demonstrated in platelets from diabetic patients result from decreased formation of fatty acids *de novo* with little or no impairment of fatty acid formation by chain lengthening.

We have, therefore, decarboxylated the fatty acids formed by normal platelets to determine if both *de novo* synthesis and chain lengthening occur. Since a decrease in carbon-14 myristic and palmitic acids could be due to formation of these fatty acids by chain lengthening in the diabetic, we have decarboxylated myristic and palmitic acids formed by platelets from diabetic subjects.

Platelet-rich plasma (5 ml.) was prepared by centrifugation of heparinized whole blood at 450*g*. All glassware was siliconized. After the initial centrifugation, the platelet-rich plasma was checked for leucocyte contamination. If the platelet suspensions contained more than 200 leucocytes per mm³, they were re-centrifuged for 2–3 min. In this way, platelet suspensions containing 0–200 leucocytes per mm³ could be obtained. In a previous investigation, we have calculated that this degree of leucocyte contamination could contribute no more than 3·5–4 per cent of the total fatty acid radioactivity[5]. Following centrifugation, the platelet suspensions in plasma were incubated with 5 μc. of 1-^{14}C acetate for 4 h at 37° C under 95 per cent oxygen and 5 per cent carbon dioxide. Lipids were extracted from the platelets and plasma[5]. After saponification and extraction[6] of the crude lipid extract, the fatty acid methyl esters[7] were then separated on a 20 per cent diethylene glycol succinate column.

Each peak and the areas between the peaks were trapped separately on siliconized cellulose acetate filters by a method developed in this laboratory[8]. Fatty acids were tentatively identified by their retention time in relation to known standard fatty acids. The radioactivity in each fatty acid was determined in a liquid scintillation spectrometer. The fatty acid methyl esters were then recovered from the scintillator by passing them through a silicic acid column in 2·5 per cent diethyl ether in hexane. They were decarboxylated by the Schmidt azide degradation technique as modified by Hajra and Radin[9].

All diabetics who required insulin to prevent ketosis were classified as insulin-dependent. All diabetics who had never developed ketosis or ketonuria and had never received insulin were classified as insulin-independent.

Myristic and palmitic acids appear to be formed *de novo* from acetate 1-^{14}C by the normal platelet, since one-seventh and one-eighth of the total radioactivity was found

in the carboxyl carbon. In contrast, stearic acid (18 : 0), oleic acid (18 : 1), arachidic acid (20 : 0), eicosenoic acid (20 : 1), and the mixture of fatty acids with retention time corresponding to 22 : 0 or more appear to be formed by addition of acetyl Co*A* to a relatively non-radioactive shorter chain fatty acid, since 51–100 per cent of the total fatty acid radioactivity is found in the carboxyl carbon. Lauric acid (12 : 0) may be formed by either mechanism since 12·9–34 per cent of the total carbon-14 was found in the carboxyl carbon (Table 1).

The percentage of carbon-14 found in the carboxyl carbon of myristic and palmitic acids formed by platelets from non-ketotic insulin-dependent or insulin-independent diabetics was similar to that of normal individuals (Table 1).

In contrast, in three of four experiments, the carboxyl carbon of palmitic acid formed by platelets from acidotic diabetics contained a much greater percentage of carbon-14 than normal. This was also true of myristic acid in two of four experiments (Table 1). Therefore, both palmitic and myristic acids may be formed abnormally in diabetic acidosis, apparently by chain lengthening. Decarboxylation of 18 and 20 carbon fatty acids formed by platelets from diabetics in acidosis showed that these fatty acids are made by chain lengthening (Table 1).

Coniglio and Cate[10] have shown that starvation may result in a shift in formation of palmitic acid from *de novo* synthesis to chain lengthening in both liver and carcass. Hollenberg and Douglas[11] have demonstrated similar findings with the rat epididymal fat pad. It appears that the platelet may respond to severe diabetic acidosis in a similar way. Fatty acid formation in the platelet may be regulated in much the same way as that in better-known tissues. Another aspect of these findings is of interest. Fatty acids containing 18 carbons are presumably formed by addition of acetyl Co*A* to palmityl Co*A*. If newly formed palmitic acid served as a precursor for stearic or oleic acid, only one-ninth of the carbon-14 in 18 : 0 and 18 : 1 should have been in the carboxyl carbon of these acids. However, a much greater percentage of the radioactivity was actually found. It seems apparent, therefore, that there are two pools of palmitate within the platelet which do not equilibrate during the time of investigation. We have shown in a previous report that newly formed myristic and palmitic acids are found primarily in non-esterified fatty acids of platelets, while newly formed longer chain saturated and unsaturated fatty acids are found primarily in phospholipids and glycerides[12]. It is therefore unlikely that non-esterified fatty acids serve as direct precursors for chain lengthening. It may be that the precursors for formation of 18-, 20- and 22-carbon fatty acids are present in an esterified form at the site where chain lengthening occurs.

The existence of pathways for *de novo* synthesis and chain lengthening within the same cell magnifies the difficulties involved in attempting to measure relative rates of synthesis of various fatty acids made by the cell. If, for example, 7 per cent of the total fatty acid radioactivity is in stearic acid, but all is present in the carboxyl carbon, while 24 per cent of total fatty acid radioactivity is present in palmitic acid, but distributed equally in each alternate carbon, there will be seven moles of stearic acid formed for each three moles of palmitic acid. Similarly, in diabetic acidosis, when 30 per cent of the carbon-14 in palmitate is in the carboxyl carbon, approximately two and a half times as much palmitate will be formed per unit amount of acetate 1-^{14}C incorporated. In this situation, a decrease in incorporation of acetate carbon-14 of more than 50 per cent could still maintain normal 'synthesis'. Perhaps, however, the site of formation and the function of palmitate would be quite different from normal.

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Table 1

	FBS	Carbon dioxide (m.equiv./l.)	12 : 0 1*	12 : 0 2†	12 : 0 3‡	14 : 0 1	14 : 0 2	14 : 0 3	16 : 0 1	16 : 0 2	16 : 0 3	18 : 0 1	18 : 0 2	18 : 0 3	18 : 1 1	18 : 1 2	18 : 1 3	20 : 0 1	20 : 0 2	20 : 0 3	20 : 1 1	20 : 1 2	20 : 1 3	20 : 4 1	20 : 4 2	20 : 4 3	22 : 0+ > § 1	22 : 0+ > § 2	22 : 0+ > § 3
Controls																												
MA	70					151	21	13·5	148	21	14	86	45	53	243	183	75	216	231	100								
JH	87		64	22	34	473	66	14·6	678	85	12·6	190	124	65	160	135	85	290	165	57	205	198	97	89	61	68	162	82	57
RF	79		124	37	30	341	46	13·4	483	62	12·9	105	76	72				234	136	58	196	126	65				119	61	51
AP	76		146	19	12·9	802	101	12·5	1,166	114	9·8	258	245	95	303	297	98	506	478	94	97	66	68				173	110	64
AF	85		124	37	30	341	46	13·4	488	62	12·8	105	76	72														
Insulin-independent diabetics																												
CS	70		118	6·3	5·4	736	78	10·5	918	115	12·5																	
TR	83		253	14	5·5	1,237	131	10·6	1,704	236	13·8																	
LB	129		334	59	17·7	2,728	396	14·5	4,039	564	14·5																	
BB	111					102	12	11·7	152	18	11·8																	
EP	179					44	6	13·7	74	8	11																	
Insulin-dependent diabetics (no ketosis)																												
JA	48		287	67	23	1,053	160	15	1,110	167	15	118	133	100	31	32	100	294	311	100	165	133	80					
JK	84					785	127	16	1,080	174	16	189	182	96	138	107	78											
MS	380					160	25	15·6	240	37	15																	
WL	80					498	92	18·4	1,164	141	12·1																	
Diabetics in acidosis																												
MT	456	5·8	47	17	37	87	31	36	85	36	42																	
IN	540	7·2	161	27	16·7	418	58	13·9	296	45	15·4																	
VH	1,000	5·8				46	8	17	453	108	37																	
BA	690	6·9				146	142	97	358	273	76																	

* Total DPM recovered in fatty acid.
† DPM in $^{14}CO_2$.
‡ Percentage of carbon-14 in carboxyl carbon.
§ 22 : + > Fatty acids with retention time corresponding to 22 : 0 (behenic acid) or greater.

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Occurrence of Phytanic Acid in Rumen Bacteria

THE branched-chain fatty acid 3,7,11,15-tetramethylhexadecanoic acid (phytanic acid) has been isolated and identified in butterfat[1–4], sheep fat[5], ox fat[6], ox serum[7,8], crude petroleum[9] and also in the tissues, serum and urine of humans afflicted with the rare disease referred to as Refsum's syndrome[10–12] (heredopathia atactica polyneuritiformis). This communication reports the occurrence of trace quantities of this acid in the rumen bacteria from a fistulated cow fed clover-grass hay.

Phytanic acid has not hitherto been reported as a constituent of rumen lipids, although Keeney, Katz and Allison[13], in their quantitative studies on rumen digesta, detected C_{13}, C_{14}, C_{15}, C_{16} and C_{17} branched-chain fatty acids in rumen bacteria. In the investigation now reported, a concentrate of phytanic acid was prepared from the rumen bacterial lipids by low-temperature crystallization from acetone and mercuric acetate adduct formation followed by chromatographic separation on a 'Florisil' column[14]. The presence of the C_{20} multi-branched component was determined by gas–liquid chromatography, recognition of the chart peak being based on retention data previously established in this laboratory for the same acid isolated from butterfat[4] and from Refsum's syndrome tissues[12] and conclusively identified by techniques which included mass and infra-red spectrometry. The methyl ester of authentic phytanic acid isolated from ox fat[6] when added to the methyl esters of the phytanic acid concentrate extracted from rumen bacteria, co-chromatographed on columns impregnated with polydiethylene glycol adipate and 'Apiezon L', respectively.

The presence of branched-chain fatty acids in bacterial lipids is well established[15,16] but, whereas in some species of bacteria certain of these constituents—namely the C_{15} and C_{17} iso and ante-iso acids[17–20]—represent major proportions of the total lipids, in ruminant body and milk fats these acids occur in trace amounts only. Akashi and Saito[17] postulated that branched-chain acids of animal fats[21,22] were derived from bacterial lipids in the gut of the host, and this hypothesis is supported by the investigation of Keeney *et al.*[13] on the rumen contents of a lactating cow, by the studies of Kuzdzal-Savoie[23] and by this report.

The chemical structure of phytanic acid, as distinct from that of the iso and ante-iso acids, suggests a close relationship with the isoprenoid alcohol phytol which constitutes a substantial proportion of the chlorophyll molecule of green plant life. An examination of the lipids extracted from the clover-grass hay fed to the experimental cow did not, however, reveal phytanic acid. It seems probable, therefore, that rumen bacteria convert phytol to phytanic acid within their own tissues and that the presence of this C_{20} tetramethyl fatty acid in ruminant body and milk fats is due to assimilation of the lipids of these micro-organisms[4].

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Effect of Boiling and Storing on Cycasin Content of *Cycas circinalis* L.

RECENTLY we described a quantitative chromatographic method[1], using the extraction procedure by Riggs and Korsch[2], for the specific estimation of cycasin in the kernel of *Cycas circinalis*. Matsumoto and Strong[3] have reported a chromotropic acid method which can be used for indirectly assessing cycasin from the quantity of formaldehyde liberated. Hydrolysis of cycasin by acid[4] or by its emulsin[5] is known to yield methanol, formaldehyde and nitrogen.

Our own method has been considerably simplified. First, the soaking and washing for 8 days through three changes of water (carried out initially to emulate the cooking procedure of the Guamanians)[6] has been dispensed with since this seems to remove the cycasin from the cycad nut[1,7]. Secondly, the Riggs and Korsch[2] procedure of extraction, which was followed initially and which involves boiling for 20 min, can also be dispensed with. The method now followed is reduced to: (1) cutting the cycad nuts into small slices; (2) drying the slices overnight in an oven at 40° C; (3) powdering of the dried slices; (4) extracting by stirring in 80 per cent ethanol for 15 min; (5) centrifuging at 3,000 r.p.m.; (6) directly spotting the clear supernate on Whatman No. 1 paper, using amounts equivalent to 25–50 mg of the original kernel; (7) unidirectional paper chromatography; (8) staining; and (9) colorimetric quantitation, as earlier. Recovery of known amounts of authentic cycasin is quite adequate with this method also.

Results obtained using this simplified procedure are given in Table 1, and compared with the cycasin content of the same specimen assessed by the chromotropic acid method. It is seen that on the whole the values obtained are comparable, especially in materials '*B*' and '*D*'. There appears to be a difference, however, in the values

Table 1. CYCASIN IN CYCAD NUT POWDERS DETERMINED BY THE SIMPLIFIED CHROMATOGRAPHIC METHOD AND THE CHROMOTROPIC ACID METHOD

	Material	No. of estimations	Chromatographic method (month of estimation)	No. of estimations	Chromotropic acid method (month of estimation)
			July '64 and July '65		July '65
A	Powder of dried chips of cycad (received from Guam)	5	Nil	2	0·080
B	Powder of dried slices of nuts:		July, 1965		
(1)	II cycad sample	2	0·71	2	0·73
(2)	III cycad sample (with green coat)	2	1·10	2	0·95
(3)	III cycad sample (with black coat)	2	1·06	2	1·16
C	Unwashed cycad nut powder Received from the U.S. National Institutes of Health (*Ci*-6312-12*A*-17)	5	January–July '65 1·36	3	2·21
D	Boiled and powdered nut: II and III cycad pooled sample	3	August '64 and July '65 1·46	2	1·32

Table 2. CYCASIN IN EXTRACTS OF WHOLE CYCAD KERNELS AND POWDERS: EFFECT OF STORING AND PROLONGED BOILING, AND COMPARISON OF THE TWO METHODS

	Material	Boiling time (min)	No. of estimations	Chromatographic method Cycasin (g%)	No. of estimations	Chromotropic method Cycasin (g%)
A	Washed and dried chips of cycad received from Guam			February '64		
		20	3	Nil	—	—
		80	2	Nil		
B	Washed nuts; extract*			August '64		
(1)	I cycad sample (received in Oct. '62)	20	2	0·70	—	—
		80	2	0·68		
(2)	II cycad sample received in April, '64)	20	3	Nil		
		80	2	Nil	—	—
(3)	III cycad sample (received in May '64)	20	3	Nil		
		80	2	Nil	—	—
C	'Unwashed' nuts; extract*			March to April '64 July '65 Extract over		June to July '65 Extract over
(1)	I cycad sample	20	4	0·97	2	0·98
		80	3	0·79 0·67	2	0·98
(2)	II cycad sample	20	4	Nil Nil	2	0·24
		80	3	1·04 0·94	2	0·88
(3)	III cycad sample (with green coat)	20	4	Nil Nil	2	0·28
		80	2	1·05 1·04	2	1·14
(4)	III cycad sample (with black coat)	20	2	Nil Nil	2	0·32
		80	2	1·54 1·31	2	1·40

* Riggs and Korsch extraction procedure (ref. 2).

obtained by these two methods in the case of unwashed cycad nut powder (material '*C*') received through the National Institutes of Health, and also for the 20-min extracts of unwashed nuts prepared here (Table 2, material '*C*' 2, 3, 4). In these samples formaldehyde liberation indicated a higher cycasin-level or showed some cycasin where none was detected by the chromatographic method. Since the latter is sensitive to as little as 5 μg of cycasin and specific for it, it appears possible that the formaldehyde reaction might be given by substances in the cycad nut other than cycasin itself, thereby perhaps producing higher values than those actually present. Even the pure enzyme emulsin, β-glucosidase (kindly provided by Dr. Marjorie Whiting and Dr. H. Matsumoto), which is present in the kernel of cycads[5] (a specific enzyme generally accompanying a plant glycoside[8]) is found to give a colour reaction identical to that of formaldehyde, on treatment with chromotropic acid.

Prolonged storage may have a significant effect on the cycad nut. As will be seen from item '*B*' 1 of Table 2, sample I of the cycad nut received in October 1962 was exceptional in showing cycasin in August 1964, even when the nut had been soaked and washed in water, while samples II and III revealed no cycasin under these conditions. It is possible that this was due to some internal change in the nut stored for a long period, because samples II and III had at that time only just been received. As expected, the extract of the 'unwashed' nut from this batch also showed a moderate quantity of cycasin (Table 2, '*C*' 1). An explanation for this was made possible by our subsequent observation on the effect of prolonged boiling on cycasin content of the nuts.

In view of the foregoing, and because the nuts of *C. revoluta* and *C. circinalis* contain the enzyme β-glucosidase which is known to destroy cycasin[5,9], it was decided to inactivate β-glucosidase and see if this resulted in a higher cycasin concentration being obtained. For the purpose of doing this the kernels were boiled for a prolonged period, since boiling for 20 min in the course of the preparation of the Riggs–Korsch extract was apparently inadequate to reveal cycasin (items '*C*' 2, 3 and 4 of Table 2). On the other hand, the effect of boiling for 80 min was striking and revealed 0·5–1·5 per cent cycasin, where none was evident before (Table 2 and Fig. 1). The absence of any difference between the 20 min and the 80 min extracts of cycads of batch I ('*B*' 1 and '*C*' 1 in Table 2) is due to the operation of the aforementioned factor of prolonged storage.

It was thought that the emulsin would be destroyed by boiling for 20 min as there is probably no enzyme which resists such treatment; however, the enzyme is located within the kernel in such a position that 20 min of boiling did not affect it. It is conceivable that emulsin and its substrate, cycasin, reside in different cells of the kernel, since such a state is known to subsist in glycosides[8]. The prolonged boiling probably breaks up the cell wall, exposing the enzyme which is then destroyed by the heat. Prolonged storing of the whole nut under inadequate refrigeration probably achieves the same result and under both these conditions a higher value of cycasin is obtained.

On the other hand, repeatedly washing and soaking the nuts for 8 days, as in our earlier experiments[6], probably liberates and activates the enzyme which destroys the cycasin which cannot afterwards be detected (Table 2, '*B*' 2 and 3). A similar procedure is carried out by the Guamanians, and cycad chips received from Guam also

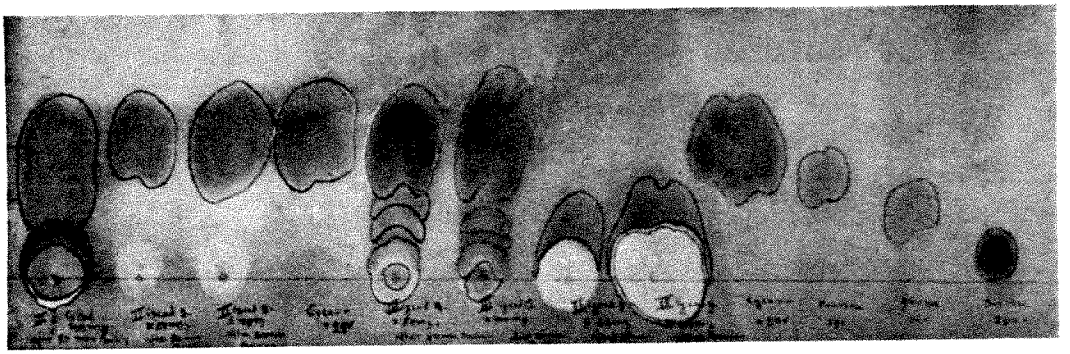


Fig. 1. Twelve spots of different preparations of various types of cycad nuts and of known sugars, as indicated below each spot. Note the effect of boiling on II cycad, spots 2 and 3 as compared with spots 7 and 8

failed to show cycasin (Table 2 *A*). Prolonged boiling did not reveal any cycasin as it had already been destroyed.

Further indirect confirmation of the foregoing observations, on the varying content of cycasin and the effect on it of boiling and storing, emerged from our long-term experiments on the feeding of various preparations of cycad nuts to different groups of monkeys. Two of three animals fed on the nut, after it had been intensely boiled, died of jaundice and hepato-toxicity while only one out of five monkeys fed on unwashed cycad suffered this fate (unpublished observations).

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Red Fluorescence in Costo-vertebral Spot of Hamsters

DURING the course of experimental production of melanomata in hamsters[1,2] and the study of red fluorescence in tumours[3,4], we discovered, by chance, that the costo-vertebral spot of some of our normal hamsters showed a well-marked red fluorescence under ultra-violet radiation. Red fluorescence has been observed in the Harderian gland of hamsters and some other rodents[5], but its significance is obscure. Red fluorescence is also seen in many tumours[3,4]. In all these cases the red fluorescence is due to the presence of porphyrins.

Fifty males and fifty females from our colony of hamsters, in which the exact date of birth was known, were shaved in the region of costo-vertebral spots and examined under ultra-violet radiation. This was done in the autumn (23.10.64) when the animals were between 5 and 18 months old. Only one male showed red fluorescence in one of its costo-vertebral spots. Twenty-one males and twelve females from the same group of animals were shaved and re-examined in the following summer (19.5.65). (The remaining animals were not available because they had been used for other experiments.) The age of the animals used in this experiment ranged from 11 to 15 months. Of the females, only one animal showed a weak reddish fluorescence in one of the costo-vertebral spots, but sixteen males showed fluorescence in both spots, two in one spot, and three showed no red fluorescence. Even in males showing red fluorescence in both spots, one spot was usually more strongly fluorescent than the other.

Histological examination of cryostat prepared sections under ultra-violet radiation showed that the red fluorescence is mainly located in the necks of the hair follicles above the large sebaceous-type gland that occurs in this region. At times a faint patchy red fluorescence was also seen in the adjacent sebaceous glands themselves.

Red fluorescence in biological material is due to the presence of porphyrins. It would therefore appear that the male hamster in the summer season produces a secretion containing porphyrins from the glands in the costo-vertebral spot. It has been suggested that the secretion from the spot of the male hamster serves to mark territory[6], but that from the female has no such function. It is intriguing to speculate whether this difference is due to the presence of porphyrins. As yet, little is known about the fluorescence of the secretion of other territory-marking animals.

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HAEMATOLOGY

Effect of Ferritin on Globin Synthesis

IN certain hypersideraemic anaemias the erythroid precursor cells in the bone marrow contain iron deposits. The iron overloaded mitochondria of these sideroblasts are not capable of synthesizing haem at a normal rate[1]. Iron-containing deposits are demonstrable not only in mitochondria, but also in other parts of the cytoplasm of these cells. It is, therefore, possible that iron overload also affects globin synthesis directly. In some of these hypersideraemic anaemias pyridoxine has been found to have a therapeutic effect which is likely to be due to the mobilization of the mitochondrial iron deposits[1]. In view of the amelioration of the anaemia of thalassaemia with pyridoxine[2], a direct influence of this vitamin on globin synthesis might also be assumed.

The effect of ferritin on globin synthesis was investigated in a cell-free system meant to imitate the conditions in sideroblasts. Reticulocytes, obtained from phenylhydrazine-treated rabbits, were lysed by short exposure to hypotonic *tris* chloride buffer containing magnesium chloride (ref. 3). The stroma, mitochondria, and intact leucocytes were removed by centrifugation at 15,000*g* for 15 min. Ribosomes and the enzyme fraction were then obtained and processed according to the method of Allen and Schweet[4] with the following exceptions: *s*RNA was not separated from the enzyme fraction; valine-1-^{14}C was used and the incubation period was 40 min. To some of the incubation mixtures (Table 1) commercial, 2x crystallized, cadmium-free ferritin was added which had been dialysed against 0·05 M *tris* chloride buffer, *p*H 7·5. Experiments were also done with the addition of buffered pyridoxal, pyridoxine, pyridoxamine, or deoxypyridoxine in concentrations from 10 mμg to 1 mg per tube.

After incubation the ribosomes and, when present, most of the ferritin were removed by centrifugation at 100,000*g* for 2 h. The supernate was freed of unincorporated valine-1-^{14}C by dialysis against running tap water for 36 h. Aliquots of the dialysed samples were then dissolved in hydroxide of hyamine and added to naphthalene-dioxane scintillation fluid[5]. Radioactivity was deter-

Table 1. THE EFFECT OF FERRITIN ON GLOBIN SYNTHESIS WITH VARIATIONS IN THE COMPOSITION OF THE INCUBATION MIXTURE

Tube content	Without ferritin (c.p.m./tube)	With 10 mg ferritin (c.p.m./tube)
Standard mixture*	1,703	473
Standard mixture, double enzyme	3,268	795
Standard mixture, double ^{14}C-valine	1,797	997
Standard mixture, double energy†	1,315	584
Standard mixture, double GSH	1,726	1,676

* 3 mg ribosomes; 5 mg enzyme; 1 μmole ATP; 120 μg pyruvate kinase; 10 μmoles phosphoenolpyruvate; 20 μmoles GSH; 50 μmoles potassium chloride; 5 μmoles magnesium chloride; 0·25 μmoles GTP; 0·05 ml. of amino-acid mixture (3); 10 μg D,L-valine-1-^{14}C; 50 μmoles *tris* chloride buffer, *p*H 7·5; total volume 1·45 ml.
† 2 μmoles ATP; 240 μg pyruvate kinase; 20 μmoles phosphoenolpyruvate.

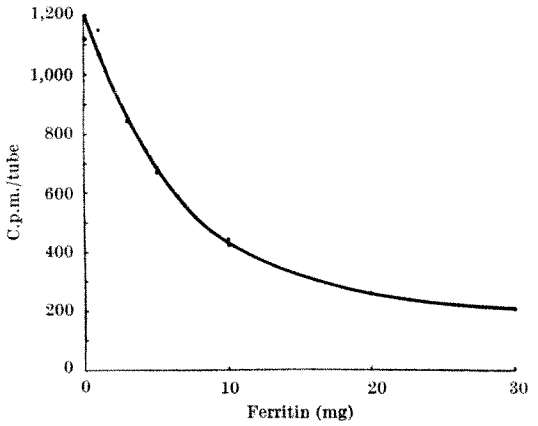


Fig. 1. The effect of increasing amounts of ferritin in the incubation mixture on the incorporation of valine-1-^{14}C

mined and adjusted for self-absorption by use of an internal standard. Incorporation was expressed in c.p.m. per incubation tube. Counts in duplicate tubes varied less than 5 per cent.

The amount of incorporated valine-1-^{14}C into the non-dialysable fraction was considered to be a measure of globin synthesis. The incorporation decreased in a hyperbolic manner with increasing concentrations of ferritin in the incubation mixture (Fig. 1). Ferritin had no effect when added to the mixture after incubation. The enzyme-*s*RNA fraction and the ribosome fraction were both rate-limiting at the concentrations employed. The inhibition by ferritin was completely abolished by doubling the concentration of glutathione (Table 1). This effect is possibly due to binding of ferritin iron by SH-groups. In the absence of ferritin a slight decrease in incorporated valine occurred following doubling of the concentration of the energy system. The mechanism of this effect is obscure as is the reason for the diminution of the inhibiting action of ferritin in the presence of doubled concentration of valine-1-^{14}C.

Pyridoxine, pyridoxal, or pyridoxamine in amounts up to 1 mg per tube neither enhanced globin synthesis in this system nor prevented the inhibition by ferritin. Deoxypyridoxine, a pyridoxine antimetabolite, was also ineffective.

The results of the described experiments suggest that ferritin inhibits globin synthesis. The fact that this inhibition is counteracted by excess glutathione in the incubation medium suggests that ferritin inactivates SH-enzymes required for the assembly of the polypeptide chains. It is possible that in thalassaemia the high ferritin content of the cytoplasm inhibits globin synthesis beyond the genetically determined limitation. Since in the incubation tube pyridoxine neither stimulated globin synthesis nor counteracted the effect of ferritin, it is likely that the slightly ameliorating effect of pyridoxine in this disease[2] is indirect through the action of pyridoxine on haem synthesis[1].

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BIOLOGY

*p*H of Grasses in Relation to Genera

IN 1964 Birch *et al.*[1] reported that the low *p*H values found in aqueous extracts of the grass *Setaria sphacelata* did not occur in similarly prepared extracts of another grass *Brachiaria ruziziensis* or in those of legumes. We have now made a *p*H survey of many grasses to find out if *S. sphacelata* is a unique species in this respect.

The majority of the grasses were sampled from the Kitale nursery (6,200 ft.) and the remainder from Molo (9,200 ft.) and Mtwapa on the coast. Five sub-families and their associated tribes were represented as follows: Bambusiformis (Bambuseae); Phragmitiformis (Stipeae and Oryzeae); Festuciformis (Festuceae, Hordeae and Phalarideae); Eragrostiformis (Eragrosteae, Chlorideae and Sporoboleae); and Paniciformis (Paniceae, Arundinelleae, Andropogoneae and Maydeae). As might be expected in a tropical country the Paniceae contained the largest number of species and the Andropogoneae the second largest. In all, 112 samples were examined for *p*H: the determination was made electrometrically after shaking 0·4 g of the dried and ground sample with 50 ml. water for 30 min.

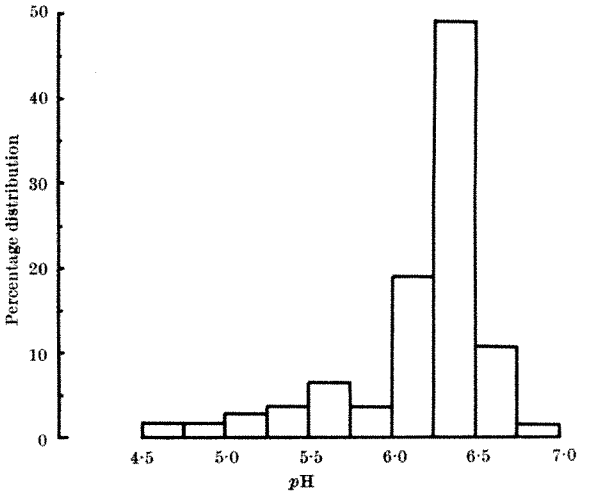


Fig. 1. Percentage distribution of *p*H in 112 samples of grass

As shown in Fig. 1, more than 80 per cent of the samples gave a *p*H value of more than 6; their mean value was 6·38. The remaining samples gave a mean *p*H value of 5·44 and they were composed as shown in Table 1.

Table 1. GRASSES WITH *p*H VALUES OF LESS THAN SIX

Genus	Species	No. of samples	*p*H range
Cenchrus	*C. ciliaris*	8	5·35–5·90
	C. setigerus	1	5·90
Pennisetum	*P. polystachyon*	1	4·75
	P. purpureum	1	5·70
	P. stramineum	1	5·15
Setaria	*S. sphacelata*	6	5·00–5·70
	S. splendida	2	4·75–5·00
	S. trinervia	1	5·45

It is apparent that *S. sphacelata* is not exceptional in its ability to produce low *p*H values in aqueous extracts, but it is remarkable that, of all the species examined, those which share this characteristic are confined to three closely related genera of the Paniceae.

Work is now in progress to see if the relationship between *p*H and ammonium accumulation as reported for *S. sphacelata* applies to these species.

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Regeneration of Yeast Protoplasts prepared by Snail Enzyme

It has previously been shown[1,2] that yeast protoplasts prepared by autolysis of the cell wall may regenerate. The rate of regenerating protoplasts grown on the surface of solid media was very low (1–2 per cent). The cultivation of protoplasts embedded into high-percentage gelatine led to almost quantitative regeneration[3].

The regeneration ability of yeast protoplasts liberated by digestion of the cell wall with enzymes of the snail *Helix pomatia* has either not been demonstrated at all[4], or has been observed occasionally only[5,6]. We have been successful in achieving a high rate of regeneration of yeast protoplasts prepared by snail enzyme by embedding the protoplasts in nutrient media containing gelatine.

Cells of *Saccharomyces cerevisiae* Hansen, strain No. 7 (VULKP, Prague), were used. The protoplasts were prepared by the modified procedure of Eddy and Williamson[7]. Log phase cells were washed with distilled water and then incubated in the concentration of 10^7–10^8 with the following medium: citrate phosphate buffer (*p*H 5·4), 0·8 M mannitol and dried gut juice of *Helix pomatia* 10 mg/ml. After about 1 h of incubation at 28° C, 95–98 per cent of cells were converted into protoplasts. The enzyme was removed by washing the protoplasts with mineral nutrient medium. The protoplasts were then partly grown on the surface of agar films in moist microscopic chambers and partly embedded in mineral nutrient medium containing 30 per cent gelatine. The procedure of embedding has been described previously[3].

The character of growth of protoplasts prepared by snail enzyme on the surface of solid media was the same as that of those prepared by autolysis of the cell wall. Plasmatic formations that have already been described in both types of protoplasts arise[1,4]. The majority of these plasmatic formations died within 48 h and only a small proportion (about 1 per cent) regenerate into new yeast cells after 5 days of incubation. Both morphology and time lapse of regeneration is the same with protoplasts prepared by autolysis of the cell wall.

The protoplasts embedded into high percentage gelatine grow (their diameter increases 2–3 times), maintaining the spherical shape. After 10–14 h of incubation (at room temperature) the formations begin to bud on one or several sites simultaneously (Fig. 1). After 20–24 h, microcolonies of regenerated yeast cells can be found in the preparations. The rate of regenerating protoplasts may reach 80 per cent under optimal conditions. The morphology of the regeneration process of protoplasts prepared by snail enzyme is entirely the same as in that prepared by autolysis. The time lapse, however, or the appearance of morphological changes, respectively, is substantially shorter (by 10–15 h). This is based probably on the physiological state of protoplasts, as the preparation of protoplasts by autolysis uses the cells of stationary cultures.

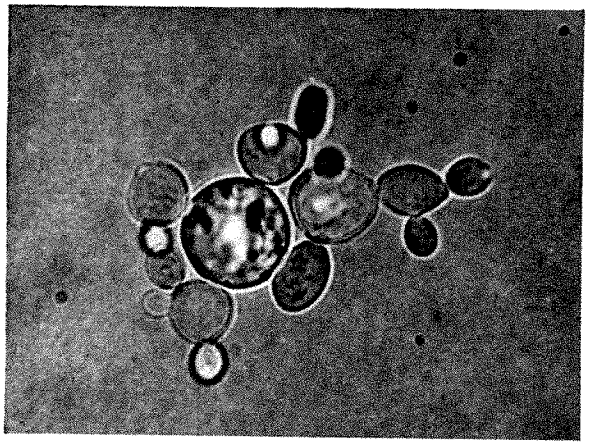


Fig. 1. Regenerating protoplast of *S. cerevisiae* after 24 h incubation in 30 per cent gelatine (× *c.* 1,133)

Formation of the cell wall also takes place in the same way in both the types of protoplasts. The fibrillar network representing the ground work of the new cell wall[8] is formed first and then the cement substances of the matrix. Just before the regeneration, the formation of a structurally and functionally complete cell wall is accomplished. The whole process of formation of the cell wall in protoplasts prepared by snail enzyme accordingly takes place faster.

In the course of further experiments, the protoplasts prepared by autolysis of the cell wall were treated with snail enzyme under the same conditions as the protoplasts prepared by snail enzyme. When embedding in gelatine medium, the regeneration rate as well as the morphology and time lapse of regeneration of these protoplasts was the same as those of controls.

The identity of protoplasts prepared by autolysis of the cell wall or by snail enzyme from the point of view of the regeneration ability has thus been confirmed. More detailed cytological aspects of the possible differences between these protoplasts prepared in different ways are under investigation at present.

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An Arthropod Intermediate Host of a Pentastomid

The life cycles of pentastomids, or tongue worms, are little known although the biology of these creatures has been the subject of investigations since the pioneer studies of Rudolf Leuckart[1] in 1860. In the adult stage, pentastomids are almost exclusively parasites of reptiles, exceptions being species of *Linguatula* from carnivores and *Reighardia sternae* from gulls and terns. The immature stages of these parasites have hitherto been found only in vertebrate animals, ranging from fish to mammals. In every case in which life cycles have been either partially or totally elucidated the intermediate host has always proved to be a vertebrate. As Baer[2] says, "It is particularly interesting to record that no larval pentastomid has ever been found in an invertebrate and that, on the other hand, many species of warm-blooded animals can act as intermediate hosts of reptilian parasites". Our discovery recently of larval pentastomids in cockroaches in Singapore is therefore of unusual significance since it appears to be the first record of the finding of tongue worms in invertebrates.

During routine dissections of the American cockroach, *Periplaneta americana*, taken from a house in a well-to-do area of Singapore, a few cockroaches were discovered harbouring hundreds of pentastomid larvae. Subsequently, systematic examinations of *P. americana* from the same and from neighbouring localities revealed a high percentage of parasitized insects. 108 cockroaches were dissected and, of these, eleven contained larvae. Most of the infestations were light, numbering not more than twelve larvae per insect, but in the case of two cockroaches, infection was so heavy that the haemocoelic cavity gave the impression of being a writhing mass. In addition to

those captured in prosperous quarters of the city, cockroaches from poorer districts, including village (or kampong) areas, were also dissected and found to have pentastomid larvae.

Since the definitive hosts of by far the great majority of pentastomids are to be found among reptiles, attention was focused on these animals in the search for the definitive host of the blattid pentastomid. Geckonid lizards occur commonly in houses in Singapore and we have found cockroach remains in their stomach contents.

100 geckoes taken in various parts of the city were examined and, of these, thirty-one were found infested with pentastomids. All the tongue worms we have collected so far from Singapore geckoes belong to a species of *Raillietiella*, tentatively identified as *R. hemidactyli* Hett 1934. *R. hemidactyli*, originally described from Burma, is widely distributed throughout South-east and Eastern Asia, where it occurs in various lizards[3–5]. It has also been recorded recently from Madagascar[6] and Puerto Rico[5].

We have found all stages of *R. hemidactyli* in the lungs of several species of geckoes, the lizards chiefly represented in our collections being *Hemidactylus frenatus* and *Platyurus platyurus*. The pentastomid material obtained ranges from advanced larvae (a little less than 1 mm in size) to adults (relatively large creatures measuring up to 100 mm and more), which may completely fill the lung cavity (see Fig. 1). Larvae are uncommon, most specimens being nymphs or adults.

Larval pentastomids, taken from the lungs of the geckonid *H. frenatus*, have been compared with those removed from the haemocoele of the cockroach, *P. americana*, and they are the same species (Fig. 2, *A* and *B*).

The life cycle of *R. hemidactyli* as we have deduced it from dissection and ecological data available is as follows: The adult female tongue worm in the lungs of the lizard liberates embryonated eggs into the trachea of the host. The eggs pass to the pharynx where they are swallowed and find their way to the exterior with the faeces of the gecko. Nymphal or adult cockroaches ingest the lizard faeces containing the pentastomid eggs. These hatch in the mid-gut of the insect. The hatched larvae penetrate the wall of the mid-gut and make their way into the haemocoele where they undergo further development. At least one moult to the second larval stage (?) takes place here.

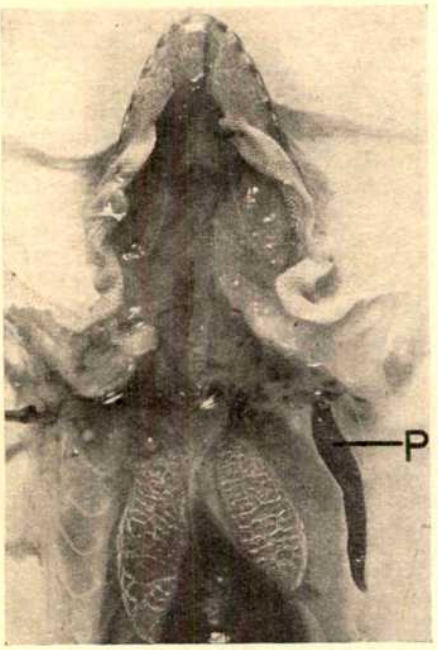
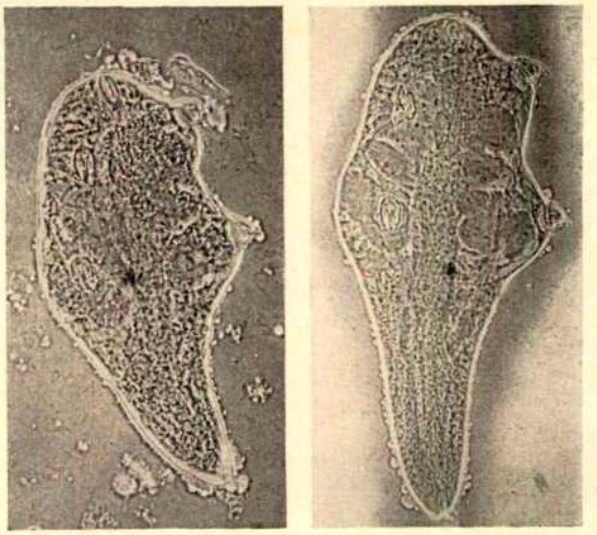


Fig. 1. Young adult female gecko (*Platyurus platyurus*) dissected so as to show the thoracic cavity. A tongue worm, *Raillietiella hemidactyli*, removed from the left lung of a lizard of about the same age is shown lying close to the left lung. The parasite (*P*), fixed in the extended state, measured 10·5 mm in length



A *B*

Fig. 2. Photomicrographs of unstained larvae of the tongue worm, *Raillietiella hemidactyli*. *A*, A larva, measuring 0·65 mm, removed from the haemocoele of the American cockroach, *Periplaneta americana*; and *B*, a larva, measuring 0·95 mm, obtained from the lung of a geckonid lizard, *Hemidactylus frenatus*

Infection of the definitive host takes place when the cockroach is eaten by a lizard. The larvae probably attach themselves to the mouth and the pharynx and migrate from there directly to the lungs. We believe that this is the route by which infection of the respiratory system takes place, since in numerous careful dissections we have never found pentastomids in the post-pharyngeal portion of the alimentary canal or neighbouring structures.

Our claim that the American cockroach, *Periplaneta americana*, is an intermediate host of *Raillietiella hemidactyli* of Singapore house geckoes is supported by experiments carried out in our laboratory. Mr. C. Rajamanickam has successfully infected cockroaches by allowing them to feed on lizard faeces containing pentastomid eggs. The repeated failure by previous workers to discover an intermediate host for certain pentastomids of insect-eating reptiles has led recent investigators to postulate that at least some species of tongue worms (including *Raillietiella* spp.) must have direct cycles[7]. The discovery of an invertebrate host for at least one species of *Raillietiella* now calls in question this postulate.

We thank Dr. Ralph Audy, director of the George Williams Hooper Foundation, for his encouragement and interest, and Mr. Yip Hoi Kee of the Department of Zoology for the photographs in Figs. 1 and 2. This work was supported by the U.S. Public Health Service.

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Cytological Investigations on some Indian Diplopoda (Myriapoda)

THE class Myriapoda has received scant attention from cytologists, the order Diplopoda even less. Among the earlier contributions to this field of study, mention may be made of Oettinger[1], Sokoloff[2] and Bessiere[3]. Barring a recent contribution to the cytology of three South Indian species of Diplopoda by Natarajan[4], no attention has been devoted to chromosome studies in this group.

This note presents the chromosomal cytology of eight South Indian species of Diplopoda investigated for the first time; these were collected from different parts of the state of Mysore. The scheme of classification adopted here is based on the investigation of Attems[5].

Three species of the family Harpagophoridae (*Harpurostreptus* sp., *Ktenostreptus* sp. and *Thyropygus* sp.) reveal different diploid chromosome numbers of twelve, twenty and twenty-four respectively. The autosomal heteropycnosis in *Thyropygus* sp., regular 'Bouquet' formation in *Ktenostreptus* sp. in early meiotic prophase and tetraploidy in *Harpurostreptus* sp. are some of the significant observations made in the course of this investigation. *Chondromorpha mammifera* Attems is a member of the family Strongylosomidae. The diploid chromosome number is fourteen. Very extensive endopolyploidy has been observed in this form. *Strongylosoma* sp., a member of the same family, exhibits the diploid chromosome number of twenty-four.

The other three forms investigated are *Arthrosphaera zebraica* and two unidentified species of the genus *Arthrosphaera* belonging to the family Sphaerotheridae. They are commonly called pill-millipedes. They present the highest diploid chromosome numbers for the group: twenty-six, thirty and thirty respectively.

In addition to the variation in chromosome numbers, the cytology of diplopods offers many points of interest. In all the species studied all the chromosomes are acrocentrics. The sex-chromosome mechanism is of the *XY* type. The prereductional meiotic division and succession are a few of the characteristics of the sex-chromosomes. The occurrence of single chromatid bridges in the meiotic anaphase of one of these (*Arthrosphaera zebraica*) is a significant observation.

It is too early to evaluate the importance of cytological data based on the numerical relationships of the chromosomes of a few species, although it indicates the probable existence of a cytological basis for taxonomic purposes. It is hoped that the completion of the cytological analysis of large numbers of species will afford a clearer perspective of the evolutionary history and phylogeny of this ancient group.

I thank Dr. B. R. Seshachar of the University of Delhi for guidance, and Dr. D. MacFarlane of the Commonwealth Institute of Entomology and the British Museum (Natural History), London, and the Zoological Survey of India, Calcutta, for their kind attention in identifying the specimens used in the present investigation.

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Ribonucleic Acid-storage Inclusions of Freshwater Sponge Archaeocytes

THE presence of RNA in the lenticular, metachromatic inclusions of freshwater sponge archaeocytes has been recently commented on by Ruthmann[1]. Typically, the RNA occurs in conjunction with a basic protein to form a combined structure of characteristic appearance (*RS* in Fig. 1).

In attempts to determine the ultrastructural organization of the RNA–protein complex, by means of RNase digestion techniques, Ruthmann was unable to determine conclusively whether the RNA was directly bound to fibrillar protein or was carried on ribosomes presumed present in the complex.

We have been examining the structure and functions of the archaeocyte inclusions of the Australian freshwater sponge, *Ephydatia multidentata*, with techniques very similar to Ruthmann's and, in general, have come to similar conclusions. However, we have also used an additional technique the results of which strongly suggest that a large proportion of RNA, if not all of it, is carried on ribosomes which are attached to the fibrillar protein of the inclusions. In this technique electron-microscope sections were prepared from archaeocyte inclusions fixed in veronal acetate buffered with 1 per cent osmium tetroxide (*p*H 7·3) for 2 h, followed by 1 per cent potassium permanganate solution for 30 min and treated with 2 per cent uranyl nitrate. Definite leaching out occurred in the ribonucleoprotein 'shell' material of the inclusions which assumed the appearance shown in Fig. 2, where the darker nucleoprotein region can be seen to be perforated with many small 'holes' the diameter of which is in the order of 40 mμ. Their arrangement is orderly and in some regions there is evidence of a lamination reminiscent of membranes or fibrils. Since potassium permanganate solutions are known to remove ribosomes from the endoplasmic reticulum, we consider that a similar event has occurred here, and the section may be interpreted to mean that the spaces and laminations have been caused to appear by the removal of ribosomes from the fibrillar protein matrix. Neither we, nor Ruthmann, have obtained so definite a picture with RNase-treated material, although a faint suggestion of it has sometimes been seen.

Ruthmann has also speculated on the source of deoxyribonucleotides for chromosomal replication during the initial phase of histogenesis. Many workers[3–6] have noted

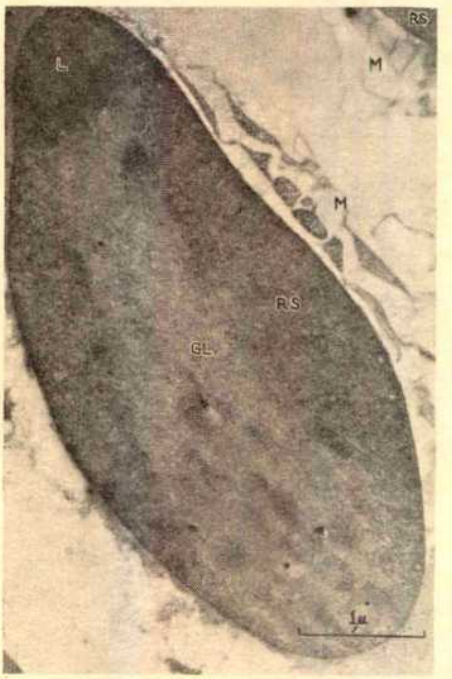


Fig. 1. Electron micrograph of an archaeocyte lenticular granule within the gemmule. Veronal acetate buffered 1 per cent osmium tetroxide, 2 h. 0·1 per cent uranyl acetate in absolute methanol, 1 h; *in toto*. *RS*, Ribonucleoprotein 'shell' material; *GL*, inner region of the granule composed mainly of acidophilic proteins and glycoprotein; *M*, phospholipid membrane surrounding granule; *L*, lipid droplet

the binucleate condition of the archaeocytes both within the gemmule and just prior to its formation. We have also noticed this in our material and agree that some early differentiation within the gemmule may be explained by a division of these binucleate cells without recourse to deoxyribonucleotide stores. Ruthmann considers, however, that it is very unlikely that morphogenesis begins with only twice the number of cells present in the gemmule since he observed mitosis within the gemmule. He therefore postulated that the deoxynucleotide precursors must have been present within the completely closed gemmule system. He considered the precursors could exist in the form of soluble mono- and di-nucleotides which had escaped detection by the histochemical tests used, or perhaps as cytoplasmic DNA, although he could provide no evidence for this.

We have repeatedly been impressed by peculiarities observed in Feulgen-treated archaeocytes taken from gemmulating *Ephydatia multidentata*. In many of these cells the DNA was all within the nucleus, showing up as numerous large granules. In many other archaeocytes, however, additional Feulgen-positive granules of a similar size were found outside the nucleus. In some cases there were no extra-nuclear granules, but the cytoplasm had localized areas which were tinged red and which could be explained by assuming the presence of small particles of DNA, the particle size being below the resolving power of the microscope. It is possible that these observations are of stages in which DNA becomes available in the cytoplasm and is broken down into sub-microscopic units and then into the soluble mono- and di-nucleotides as suggested by Ruthmann.

We should also like to draw attention to another possible source of nuclear material which can be seen in some archaeocyte inclusions. Fig. 2 was chosen because it includes, not only the evidence for the presence of ribosomes already discussed, but also a comparatively large electron-dense body the function of which is problematical. It has a double membrane and resembles a minute nucleus, but it is not Feulgen-positive nor is its appearance changed when digested with DNase or RNase. It is possible, however, that the interior substance may be deoxyribonucleotides.

Another suggestion introduced by Ruthmann is that these particular organisms possess enzymes capable of converting RNA to DNA. Although this hypothesis was initially suggested by Brachet[7,8], biochemical evidence

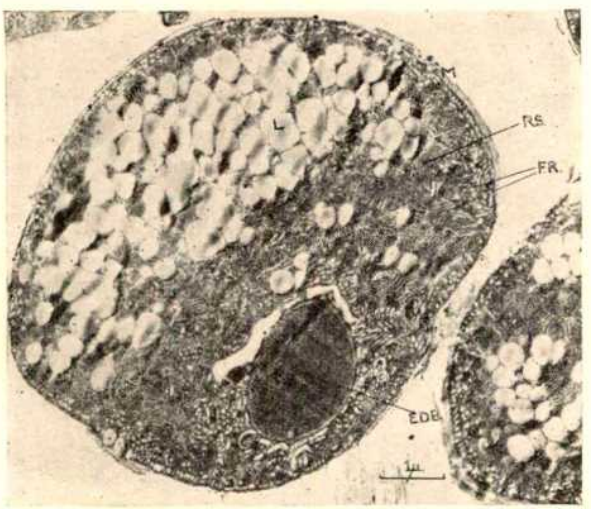


Fig. 2. Electron micrograph of an archaeocyte lenticular granule within the gemmule. Veronal acetate buffered 1 per cent osmium tetroxide (*p*H 7·3), 2 h. 1 per cent potassium permanganate solution 30 min, 2 per cent uranyl nitrate. *R.S.*, Ribonucleoprotein 'shell' material; *L*, lipid droplets; *F.R.*, ribosomes arranged in a linear fashion, leached out by potassium permanganate treatment; *E.D.B.*, electron dense body; *M*, phospholipid membrane surrounding the granule

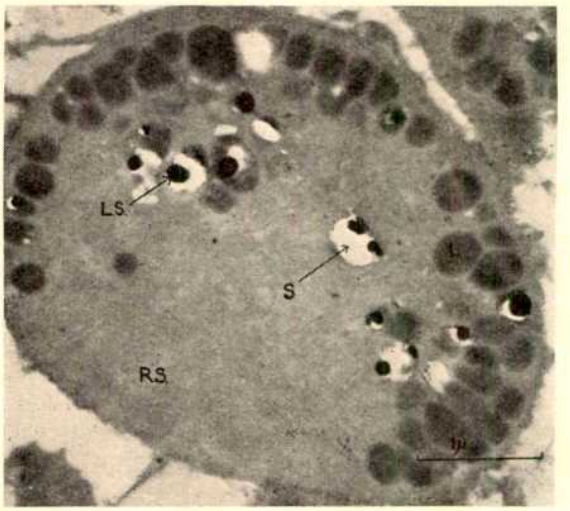


Fig. 3. Electron micrograph of an archaeocyte lenticular granule within the gemmule. Revised Gomori acid phosphatase method. Veronal acetate buffered 1 per cent osmium tetroxide (*p*H 7·3), 2h; *in toto*. *L.S.*, Lead sulphide deposit indicating the site of acid phosphatase activity; *R.S.*, ribonucleoprotein 'shell' material; *S.*, space or lacuna

has not been forthcoming to support it. However, we have found that in our material acid phosphatase, at least, is present in these inclusions. This was established by performing a revised Gomori acid phosphatase test coupled with fixation in veronal acetate buffered 1 per cent osmium tetroxide for electron microscope analysis. The acid phosphatase was observed (Fig. 3) to be deposited as black lead sulphide, precipitated in such a way as to have shrunken away from the rest of the tissue. These deposits therefore seem to be surrounded by clear spaces or lacunae and in some cases have been removed by sectioning.

The acid phosphatase is localized within the lipid inclusions of the granule and it would seem, therefore, that the enzyme hydrolyses the phospholipid which is present, but this does not rule out the possibility that it may be involved with degradation of nucleic acids of the granule, perhaps in a way similar to that described by Schmidt and Laskowski[9] for acid phosphomonoesterase. Although no definite evidence for these more specific acid phosphatases was obtained, we have definitely established that these granules do incorporate at least one enzyme species.

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Analysis of Reptilian Cortical Structure

THE evolution and organization of the reptilian cortical structures have long been a major problem to comparative neuroanatomists. Three major cortical layers are recognized[1]. In reptiles, these three regions (Fig. 1*A*) occupy the roof of the telencephalon and form distinct cellular masses which are named as follows in dorsomedial to dorsolateral succession: hippocampus (archipallium),

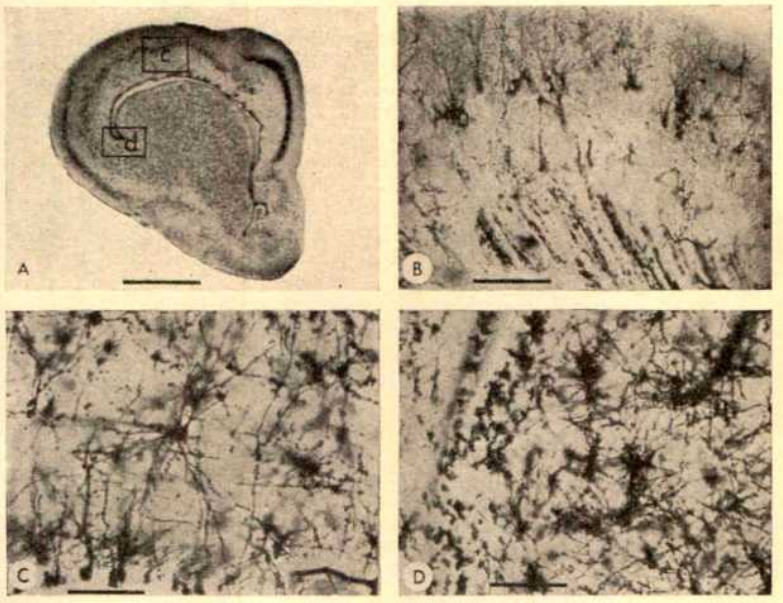


Fig. 1. Forebrain sections of *Iguana iguana*. *A*, Transverse section through the hemisphere showing nuclei and position of Figs. *C* and *D*. *B*, Golgi-Cox sagittal section through hemisphere showing dorsomedial cortex and alveus. *C*, Golgi-Cox transverse section through hemisphere showing projection neurones of dorsomedial cortex. *D*, Golgi-Cox transverse section through hemisphere showing association neurones of dorsolateral cortex. Bar scales indicate the following lengths: *A*, 17 mm; *B*, 250μ; *C*, 125μ; *D*, 166μ

general cortex (neopallium), and pyriform cortex (palaeopallium). In addition, a sub-pallial mass, the 'primordial general cortex'[2], is of pallial origin[3].

Recent electrophysiological work by Kruger and Berkowitz[4] confirms the basic organization of the pyriform and hippocampal cortices as proposed by earlier workers and suggests that these areas are true homologues of similarly named regions in the mammalian telencephalon. However, the data obtained by these workers suggest that the organization of the reptilian general cortex may not be along the lines of the mammalian neocortex.

With these facts in mind, I analysed the cortical areas of two *Alligator mississippiensis* and six *Iguana iguana* by use of the Golgi–Cox method. All animals were juveniles. The results of the analysis support Kruger and Berkowitz's statement with some modifications.

Crosby[2] in her analysis of the cortex of the alligator suggests that the axons of the large pyramidal cells of the general cortex pass ventrally into the alveus where they synapse with projection neurones of the primordial general cortex and neostriatum (dorso-lateral area of Crosby). Such a series of connexions would confirm homology with the extrapyramidal motor system in mammals and would suggest a basic organization similar to the mammalian condition. If such is the case, then the cells of this region should be of the pyramidal projection type with long axons visible extending into the underlying palaeostriatum.

The primordial general cortex in the alligator is not well developed and consists only of a slight infolding of the cortex in the rostral region of the telencephalon. The cell types of the general cortex are definitely projection cells and their axons do pass into the alveus. However, the neurones of the primordial general cortex appear to be intermediate between projection and associative neurones. No clear evidence can be obtained as to the projection of axons into the primordial general cortex. Resolution of this problem will require the use of experimental degenerative methods.

The cortex of the green iguana is more highly differentiated and offers a clearer solution to the problem. The general cortex is not continuous with the hippocampus or pyriform cortex as in the alligator. The primordial general cortex is highly developed and is located along the rostral one-third of the telencephalon. The general cortex (dorsomedial cortex) consists of large double pyramid neurones which receive their input along a highly branched dendritic tree oriented towards the superficial surface of the cortex. The axons of these cells pass into a deep alveus Fig. 1*C*).

Then neurones of the primordial general cortex (dorsolateral cortex) are not of the double pyramid type but are typical short-axon associative cells (Fig. 1*D*). Furthermore, the alveus does not extend ventrally into this region. The alvear axons pass toward the medial wall of the hemisphere, where they form the fornix complex and then fuse with the medial forebrain bundle (Fig. 1*B*).

It appears that the organization of the 'general cortex' in reptiles is fundamentally different from that of mammals. There are no direct thalamo-cortical afferent pathways to the 'general cortex'. The only observed connexions of the dorsal thalamus with the telencephalon appear to be thalamo-palaeostriatal fibres of the lateral forebrain bundle[4,5]. If the dorsolateral cortex (primordial general cortex) of the green iguana does not contain efferent cortical fibres, then it may be hypothesized on the basis of cell type that this region serves as an association area between the dorsomedial cortex and the striatal regions (Fig. 2). This is further substantiated since this is the only region of the lateral surface of the hemisphere where such connexions could be made with the dorsomedial cortex. Afferent connexions could occur via the medial forebrain bundle and alveus, but it appears that this system is primarily efferent in the region of the dorsomedial cortex.

The efferent pathway of the dorsomedial cortex does not pass into the striatal mass as in mammals but passes via the alveus to the medial forebrain bundle (Fig. 2). Analysis of these characters leaves little basis for homologizing the dorsomedial cortex of reptiles with the mammalian neocortex. Instead, this cell layer should be considered a part of the hippocampus or more likely

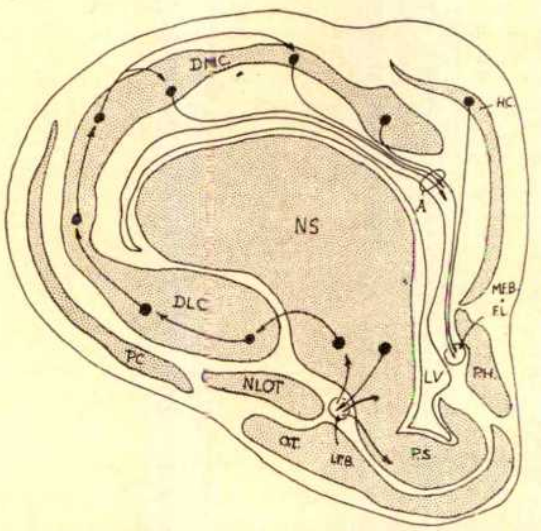


Fig. 2. Diagram of a transverse section through the hemisphere of *Iguana iguana*, at the level of the dorsolateral cortical infolding, showing major connexions. *A*, Alveus; *DLC*, dorsolateral cortex; *DMC*, dorsomedial cortex; *HC*, hippocampal cortex; *LFB*, lateral forebrain bundle; *LV*, lateral ventricle; *MFB+FL*, medial forebrain bundle and fornix longus; *NLOT*, nucleus of lateral olfactory tract; *NS*, neostriatum; *OT*, olfactory tubercle; *PC*, pyriform cortex; *PH*, primordial hippocampus; *PS*, palaeostriatum

transitional cortex (mesopallium) homologous with the subiculum or other similar mammalian cortical structures. Such a proposal was considered by Rose[6]. While the present analysis does not confirm the detailed description of Rose's work, the basic concept appears sound. This arrangement could account for the transition of evoked potentials on the dorsomedial surface of the hemisphere[4] and still satisfy the observed anatomical evidence for an efferent pathway via the alveus and medial forebrain bundle.

It seems that the dorsal cortex or pallium in tetrapods has evolved along two separate lines. Early in its evolutionary history, probably at the amphibian or very early reptilian level, selective forces of very different types acted on the primitive dorsal pallium to produce the mammalian neocortex and also quite independently the reptilian structures. The dorsomedial and dorsolateral cortices of the reptile may be considered homologous to the dorsal pallium in amphibians in the same manner that the mesopallium and neopallium of mammals are homologous to the amphibian dorsal pallium. The organization of the amphibian telencephalon would allow such an interpretation. The dorsal pallium is in contact with association fibres from both the hippocampus and striatum[7]. Based on anatomical organization, it should be possible to divide even this primitive dorsal pallium into presumptive evolutionary regions. A lateral component in theropsids would be free to expand into neocortex under striatal control with the increase in somatic input as would occur with increased complexity of locomotion. An increase in complexity is an acceptable corollary of the modifications occurring in the other body systems during the amphibian–reptilian transition of the theropsid line of evolution.

The sauropsid evolutionary line which gave rise to modern reptiles and birds did not evolve a neocortex. The amphibian lateral pallial component in these forms appears to have migrated towards the striatum. The dorsolateral cortical component in modern reptiles appears to be shifting ventrally and may mimic a stage in avian evolution in which the same homologous region fused to form, in part, the massive avian striatum.

A medial component already associated in amphibians with the hippocampus might continue its evolution in this direction in reptiles and mammals. A continued evolution would give rise to transitional cortical structures (mesopallium) important in functions involving both olfactory and somatic sensory integration. It now seems that modern reptiles and mammals share this trend.

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Evaluation of Nitrogen Fixation in Legumes by the Regression of Total Plant Nitrogen with Nodule Weight

THE relation of nitrogen fixation to nodulation is not yet well understood. Nodule position, nodule size, haemogoblin content and longevity of nodule tissues have been taken into consideration[1,2]. Chen and Thornton[3] showed, from considerations of the structure of effective and ineffective nodules of clover, pea and soybean, that differences in efficiency of nitrogen fixation were due to the volume of active bacterial tissue. Bowen[4] found a highly significant regression of dry weight of plants with nodule weight in *Centrosema pubescens*, inoculated with seventeen different *Rhizobium* strains. He suggested that *Rhizobium* strains have the same nitrogen fixing ability weight for weight of nodule tissue, and that differences in effectiveness resulted from the different amounts of nodule tissue formed.

In a number of greenhouse and field experiments, we found linear regressions of the logarithm of total plant nitrogen with nodule weight. The regressions have been a valuable tool in the investigation of nitrogen fixation under most conditions. Fig. 1 presents a typical example of such a regression.

In this experiment, in all treatments, total plant nitrogen was dependent on the amount of nodule tissue formed, its logarithm increasing linearly with the nodule weight. Following the equation for the linear regression $Y = a + b\,X$ we can calculate the amount of nitrogen fixed and that absorbed from seeds and soil. Y represents the logarithm of total plant nitrogen and X the corresponding nodule weight on each point of the regression line. a represents the point on the Y-axis where no nodules were formed, that is the logarithm of nitrogen absorbed from seeds and soil. Thus, logarithm of nitrogen fixed, $NF = Y - a$ and $Y = NF + a$. Substituting in the regression equation:

$$Y = a + b\,X$$
$$NF + a = a + b\,X$$
$$NF = b\,X$$

Thus, under the conditions of such an experiment, where nitrogen is the limiting factor of plant growth, the amount of nitrogen fixed can be calculated. It is dependent on the nodule weight and on the slope b of the regression of total plant nitrogen with nodule weight. Environmental effects which act on nodule number or size will increase the nodule weight but will not change the slope. It becomes possible to distinguish effects on nitrogen fixing efficiency, that is on the amount of nitrogen fixed per unit nodule tissue, because these effects will change the slope. Addition of nitrogen to the soil will increase the Y – intercept a and the amount of nitrogen actually absorbed from seeds and soil can be calculated as well.

These observations have been confirmed in a number of experiments. In a greenhouse experiment with soy-

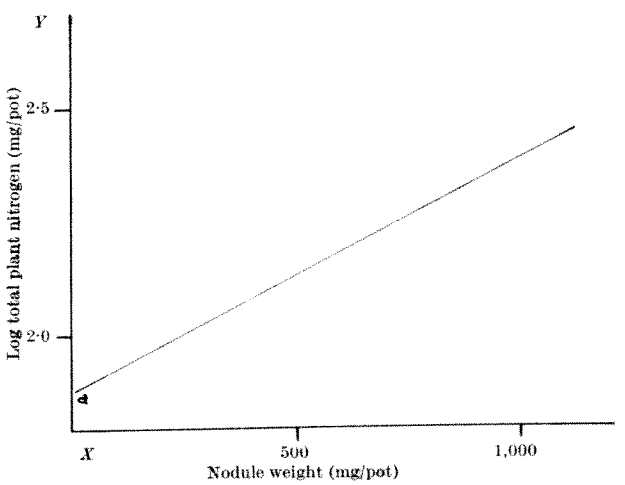


Fig. 1. Nitrogen fixation of soybeans. The greenhouse experiment included two *Rhizobium* strains, two soybean varieties and three lime treatments. It was conducted in a very poor, acid soil mixed with 1 per cent of rice straw and fertilized with *PK* and minor elements. a, log nitrogen absorbed from seeds and soil; b, regression coefficient, significant at $P = 0{\cdot}01$; NF, log nitrogen fixed. $a = 1{\cdot}88$; $b = 0{\cdot}000503$; $NF = 0{\cdot}000503X$

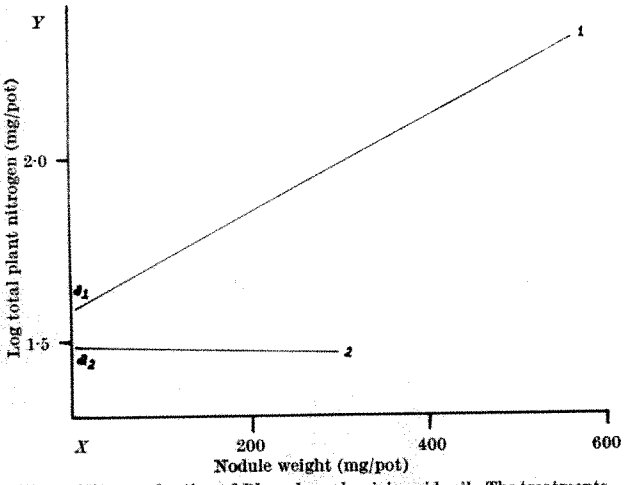


Fig. 2. Nitrogen fixation of *Phaseolus vulgaris* in acid soil. The treatments of the greenhouse experiment included six *Rhizobium* strains and three lime treatments. Line 1, five more or less effective *Rhizobium* strains; line 2, one ineffective *Rhizobium* strain; *a*, log nitrogen absorbed from soil and seeds; *b*, regression coefficient, significant at $P = 0{\cdot}01$; *NF*, log nitrogen fixed. $a_1 = 1{\cdot}58$; $b_1 = 0{\cdot}001331$; $NF_1 = 0{\cdot}001331X$; $a_2 = 1{\cdot}48$; $b_2 = 0{\cdot}000085$; $NF_2 = 0{\cdot}000085X$

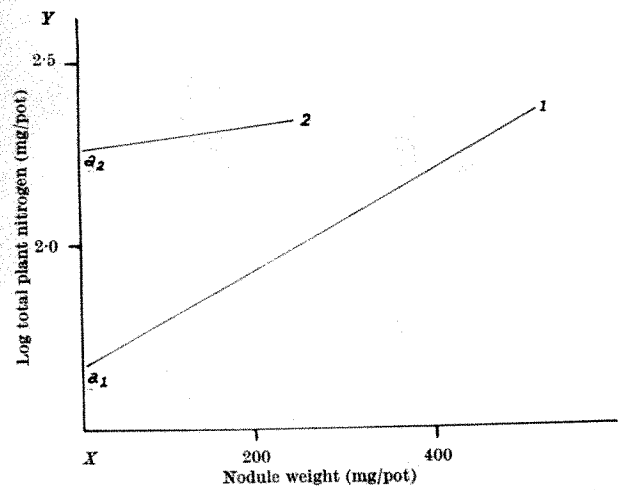


Fig. 3. Nitrogen fixation of *Phaseolus vulgaris*. The treatments of the greenhouse experiment included three fertilizers (*O*, *PK* or *NPK*) and four *p*H levels obtained by diluting an acid soil with sand. All pots were uniformly inoculated. Line 1, no mineral nitrogen added; line 2, 100 p.p.m. of nitrogen added; *a*, log nitrogen absorbed from seeds and soil; *b*, regression coefficient, significant at $P = 0{\cdot}01$; *NF*, log nitrogen fixed. $a_1 = 1{\cdot}66$; $b_1 = 0{\cdot}001386$; $NF_1 = 0{\cdot}001386X$; $a_2 = 2{\cdot}27$; $b_2 = 0{\cdot}000377$; $NF_2 = 0{\cdot}000377X$

beans, similar to that shown in Fig. 1, practically the same regression coefficient was obtained ($b = 0{\cdot}000501$ significant at $P = 0{\cdot}01$). Two field experiments with thirty soybean varieties showed similar regressions ($b = 0{\cdot}000678$, significant at $P = 0{\cdot}05$; and $b = 0{\cdot}000684$, significant at $P = 0{\cdot}01$ respectively).

In three greenhouse experiments with beans (*Phaseolus vulgaris*) it was shown that bean nodules, under similar conditions, were 2–3 times more efficient than soybean nodules, the regression coefficients being 0·001331, significant at $P = 0{\cdot}01$, 0·001386, significant at 0·01, and 0·001383, significant at $P = 0{\cdot}01$ respectively. The regressions of two of these experiments are shown in Figs. 2 and 3.

The results of the experiment presented in Fig. 2 were divided into two groups. One included five more or less effective *Rhizobium* strains at three lime treatments and the other included one ineffective strain at three lime treatments. The first group is presented in regression line 1, the second in line 2. As expected, the amount of nitrogen absorbed from seeds and soil (*a*) was similar in both groups. In group 1 the nitrogen fixation increased accordingly to the increase in nodule weight caused by the different *Rhizobium* strains or by the lime treatments. The increase in nodule weight of the plants inoculated with the completely ineffective strain did not bring about any nitrogen fixation.

The results of the experiment presented in Fig. 3 also form two distinct regressions. Here the data from the pots with mineral nitrogen form a different line. As expected, the amount of nitrogen absorbed from seeds and soil (*a*) is higher. It is almost as high as the amount needed for maximal plant growth under the given conditions, and the regression coefficient is not significant. The addition of 100 p.p.m. of nitrogen as ammonium nitrate almost paralysed nitrogen fixation, even in the presence of nodules. The regression of the data from the pots without mineral nitrogen was practically identical with that obtained in the previous experiment (Fig. 2).

Fig. 4 shows the results of a greenhouse experiment with *Centrosema pubescens*. Nodules of this plant were, under the given conditions, still more efficient than bean nodules. In this experiment the added nitrogen (50 p.p.m. nitrogen) was not enough for maximal plant growth and there was some nitrogen fixation even in these pots. The nitrogen-fixing efficiency of these nodules, however, was lower than that in the pots without mineral nitrogen and the slopes of the two regressions were significantly different.

Details of the experiments mentioned in this paper have been published elsewhere[5–7]. The treatments included the use of different *Rhizobium* strains, and different plant varieties, lime-pelleted seeds, lime and fertilizer treatments, *p*H levels and two soil temperatures. Most of these treatments increased nitrogen fixation, but few of them affected the regression coefficient (*b*) of total plant nitrogen with nodule weight. *b* is, therefore, an index of nitrogen-fixing efficiency which seems to be surprisingly constant for each plant species and appears to be independent of most environmental effects, *Rhizobium* strains and plant varieties. This confirms Bowen's[4] suggestion that differences in the nitrogen fixation of *Rhizobium* strains are generally due to differences in the numbers or sizes of the nodules produced. The fact that none of the different calcium treatments affected efficiency in nitrogen fixation per unit nodule tissue supports Andrew and Norris's[8] suggestion that calcium is essential for the meristematic activity of nodules.

The calculation of nitrogen fixation and of the amount of nitrogen absorbed from seeds and soil as well as the

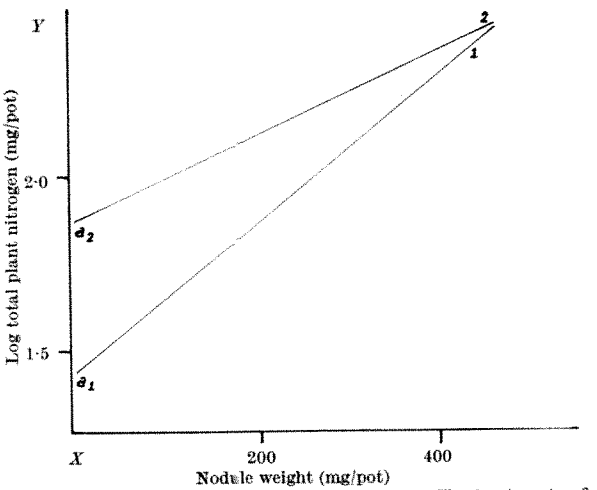


Fig. 4. Nitrogen fixation of *Centrosema pubescens*. The treatments of the greenhouse experiment included two soil temperatures, three lime treatments and two nitrogen levels (0 and 50 p.p.m.). There was one check treatment without inoculation for each nitrogen level, all other pots were uniformly inoculated. Line 1, no mineral nitrogen added; line 2, 50 p.p.m. of nitrogen added; *a*, log nitrogen absorbed from seeds and soil; *b*, regression coefficient, b_1 and b_2 significant at $P = 0{\cdot}01$, difference between b_1 and b_2 significant at $P = 0{\cdot}05$; *NF*, log nitrogen fixed. $a_1 = 1{\cdot}45$; $b_1 = 0{\cdot}00202$; $NF_1 = 0{\cdot}00202X$; $a_2 = 1{\cdot}87$; $b_2 = 0{\cdot}00110$; $NF_2 = 0{\cdot}00110X$

possibility of distinguishing efficiency of nitrogen fixation per unit nodule tissue might become of considerable help for nutrition studies in legumes, under field and greenhouse conditions. Complete results will be published elsewhere.

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Anisopus Larvae (Diptera) in Cases of Intestinal and Urino-genital Myiasis

ZUMPT[1] in his comprehensive work on myiasis in the Old World includes *Anisopus* (Anisopodidae) under intestinal myiasis. James[2], writing on myiasis from the New World, also includes *Anisopus* (as *Sylvicola*) in the same category. Smart[3] states that there are records of *Anisopus* larvae in cases of intestinal and urino-genital myiasis. Many of the early text-books of medical entomology do not include the genus.

There appears to be only one published record of intestinal myiasis involving larvae of *Anisopus fenestralis* (Scop.): this is the one reported by Shrewsbury[4]. In this case a 3-year-old girl, after an attack of vomiting, developed a mild enteritis which lasted for some 2 weeks. On two separate occasions a single larva was found in her motions and in the third week of her illness two more larvae were found in her chamber-pot immediately after urination. There was no urethral or vaginal irritation in this case, as would be expected if larvae had been present in the urino-genital system, and it is therefore assumed that the larvae were passed *per anum*. There appear to be no published records of urino-genital myiasis involving *Anisopus*, and Dr. Smart informs us that his statement was based on an unpublished case which he dealt with when on the staff of the British Museum (Natural History).

We can now record a case of urino-genital myiasis involving larvae of *Anisopus fenestralis* brought to us by Dr. H. H. Johnston, of the Public Health Laboratory, Oxford, in 1962.

The patient was a pregnant woman, of reasonable intelligence. Larvae had been passed in the urine for up to a month when she directed her private doctor's attention to them. He then sent a urine sample to the Public Health Laboratory for investigation. The sample, which was brown in colour, contained about half a dozen larvae one of which was sent to the Hope Department of Entomology, Oxford, for identification. This larva was determined by us as *Anisopus fenestralis* in the second instar (10 mm long).

Of the seventy or so species of Anisopodidae (=Rhyphidae) known, only *Anisopus fenestralis* has been accused of causing myiasis. Keilin and Tate[5] give a detailed description and figures of the third instar larva, which is 12–14 mm long. It may be that records of *Anisopus* have been overlooked, and for convenience the larva of *A. fenestralis* is illustrated here. The most important diagnostic character of *Anisopus* is a shield-like thickening, lined by very large hypodermal cells, which surrounds the anus, the shape of this structure varying with the species.

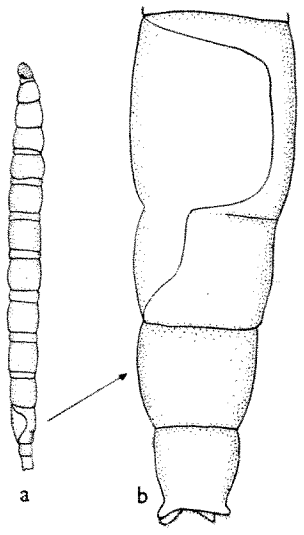


Fig. 1. *a*, Third instar larva of *Anisopus fenestralis* (L.); *b*, same showing enlarged view of anal 'shield'

Shrewsbury[4] suggested that with the intestinal myiasis case, the patient had swallowed eggs which hatched in the intestine. Zumpt[1], after considering the possibility that the larvae were already in an advanced stage when swallowed or that eggs were deposited near the anus, favours Shrewsbury's view because of the small number of larvae recovered. Smart[3] suggests that in these cases eggs may be laid directly in the pubic region.

The larvae of *Anisopus* have been recorded from various habitats such as slime flux exuding from tree wounds, decaying leaves in tree rot-holes, decaying roots of angelica and burdock, honeycombs, rotting potatoes, mouldy decaying cardboard and sewage filter beds. Edwards[6] records a remarkable case of *Anisopus* breeding in liver that had been in formalin for 7 years. The eggs are enclosed in a large gelatinous mass when laid and a gravid female will probably oviposit on any suitable moist surface. It is therefore probable that infection of the urino-genital tract by larvae of *Anisopus* is purely fortuitous.

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Lichens with Bitunicate Asci

THE division of the class Ascomycetes into the two sub-classes Ascomycetes and Loculoascomycetes in accordance with the ideas of Nannfeldt[1] is now generally accepted. Characteristic of the sub-class Ascomycetes are the unitunicate ascus and true paraphyses, of the sub-class Loculoascomycetes the bitunicate ascus and paraphysoid filaments. Two British species belonging to genera the ascocarps of which were of unknown type are now found to belong to the Loculoascomycetes. They are *Pyrenula nitida* (Weig.) Ach. and *Anthracothecium pyrenuloides* (Mont.) Müll. Arg. These two species, typical of

their genera, are notable for their brown thick-walled spores and are highly characteristic members of the Pyrenulaceae, a family which apart from these and closely related genera has been somewhat variously constituted by Zahlbruckner[2], Watson[3], and Hale[4].

Observation of the branched and occasionally anastomotic structure of the paraphysoid filaments of *P. nitida* and *A. pyrenuloides* suggested that their asci might be bitunicate, but examination of dried herbarium specimens by the method of Richardson and Morgan-Jones[5] produced negative or, in one case, equivocal results. The reason for this is that in some genera, notably those with more or less cylindrical rather than ovate or saccate asci, proliferation of the asci can be induced only in fresh material. This is worth emphasizing because no difficulty is experienced with dried specimens in most genera, especially *Arthopyrenia* and those morphologically close to it such as *Dermatina*, *Mycoporellum* and *Microthelia*.

A visit to Ireland in August 1965 was therefore made the occasion to collect young fresh specimens of *P. nitida* and *A. pyrenuloides*. The former is abundant everywhere and was obtained from West Cork (*V.C. H3*), Glengarriff, altitude *c.* 100 ft., on smooth bark of *Sorbus aucuparia* (rowan). Material of the latter was collected from North Kerry (*V.C. H2*), Torc Mountain, altitude *c.* 500 ft., on smooth bark of *Corylus avellana* (hazel). This region in the neighbourhood of Killarney is the only locality for *A. pyrenuloides* in Europe. The specimens were kept for 8 days in the humid, but not wet, environment of a plastic bag open at one end. Before they were examined, a few drops of water were added to the bag and its mouth was closed for 48 h. The result was to provide specimens with a few undoubtedly proliferated asci (see Fig. 1). In both species caps consisting of the upper part of the outer tunic of the ascus were formed. Hodgetts[6], Cain[7], and Butler[8] considered that these may be an abnormality produced in water mounts. The results obtained by Richardson and Morgan-Jones[5], who refer to this opinion, appear to confirm my own conclusion drawn from the study of asci in a variety of genera that, though the cap may be an abnormality, it is especially characteristic of plants with more or less cylindrical asci. The genus *Microglaena* as reconstituted by Morgan-Jones and Swinscow[9] is also notable for its cylindrical asci and the formation of caps.

The finding that *Pyrenula nitida* and *Anthracothecium pyrenuloides* have pseudothecia rather than true perithecia for ascocarps means that they must be placed in the Loculoascomycetes. Moreover, the family Pyrenulaceae, of which these genera are characteristic members, needs to be revised to exclude from it genera with unitunicate asci.

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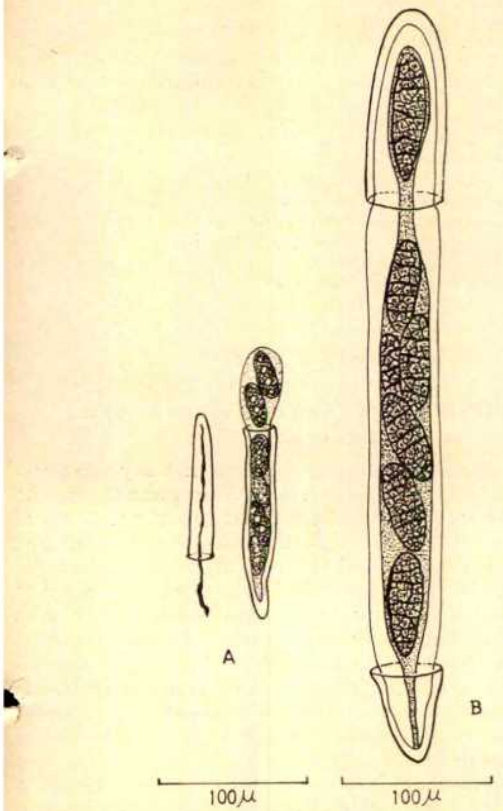


Fig. 1. *A*, *Pyrenula nitida*, proliferated ascus on right; detached cap of outer tunic from another ascus on left. *B*, *Anthracothecium pyrenuloides*, proliferated ascus, with cap of outer tunic covering inner tunic

Electron Microscope Observations of Pancreatic Islet Cells of the Early Chick Embryo

VILLAMIL[1] reported that the islet cells in chick embryo pancreas are formed from the primitive pancreatic cells at the eighth day of incubation. He observed these cells through a light microscope.

I have used an electron microscope to observe the fine structure of islet cells in the chick embryo. The pancreas was removed from the embryo at the eighth day of incubation, cut into small pieces, fixed in phosphate-buffered 1 per cent osmic acid solution for 1 h, embedded in 'epoxy' resin[2] and sectioned with a Porter–Blum ultramicrotome. Ultra-thin sections were stained with lead citrate[3] and observed using an *HU* 11*A* electron microscope.

The pancreas from a chick embryo at the eighth day of incubation is composed of a mass of parenchymal cells, some of which form an incomplete acinus and a few acinar lumens dilate into an irregular shape[4]. Among these parenchymal cells, well-differentiated α- (Fig. 1) and β-cells (Fig. 2) can be discerned. In the cytoplasm of islet cell, the rough-surfaced endoplasmic reticulum and Golgi apparatus are not so well developed, but the Golgi apparatus obviously plays a part in synthesizing

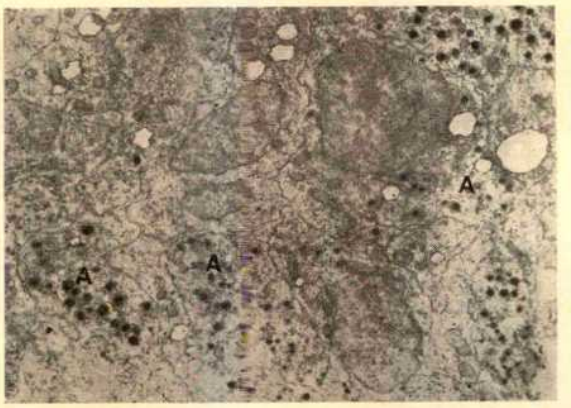


Fig. 1. Electron micrograph of chick embryo pancreas at the eighth day of incubation. Well-differentiated α-cells (*A*) can be seen among the mass of parenchymal cells. They are distinguished by a mass of spherical dense secretory granules in the cytoplasm. (× 3,100)

the secretory granules (Fig. 3). Abundant ribosomes and a few thin mitochondria are preserved in the cytoplasmic matrix. α-Cells contain many spherical dense granules enveloped in a limiting membrane, and these granules are 50–500 mμ in diameter; however, they vary considerably in density. Between the granule and the limiting membrane there is often a clear space resembling a hood. β-Cells have dense crystals enveloped in a membrane. These crystals are mostly needle-shaped or rectangular and are frequently gathered into a sheaf-like form. Therefore, depending on the direction of sectioning, the granules of β-cells have varied outlines. β-Granules are less dense than α-granules. The α- and β-granules are thought to be identical with those of chicken previously reported[5–7]. At the eighth day of incubation only a narrow intercellular space is observed between the islet cells and the undifferentiated parenchymal cells or primitive exocrine cells. Between the islet cells and the endothelial cells the basement membrane, exhibiting a low density, and connective tissue are sometimes observed.

The active secretion of hormone in the islet cells of the chick embryo is supposed to begin after the eleventh day of incubation[8–11]. Some granules appear in islet cells, however, close to the cytoplasmic membrane at the eighth day of incubation. Fig. 4 reveals two α-granules secreted into the intercellular space. This suggests that secretory granules can be passed out through any part of cytoplasmic membrane to the extracellular space even at the eighth day of incubation: the process responsible for this action is termed emiocytosis and is similar to that exhibited by merocrine glands[5,7,12–17].

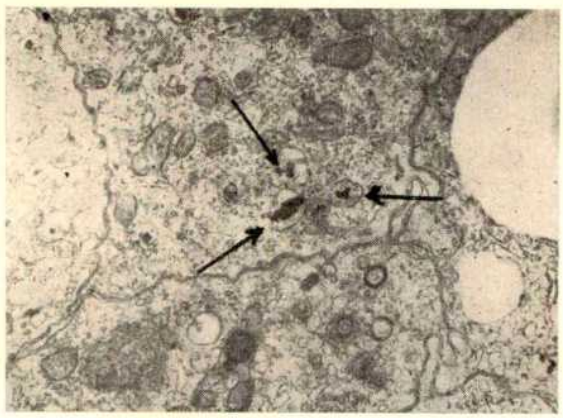


Fig. 2. Electron micrograph of chick embryo pancreas at the eighth day of incubation. In the cytoplasm of β-cell, crystal-like secretory granules can be seen with a limiting membrane (arrows). (× 9,300)

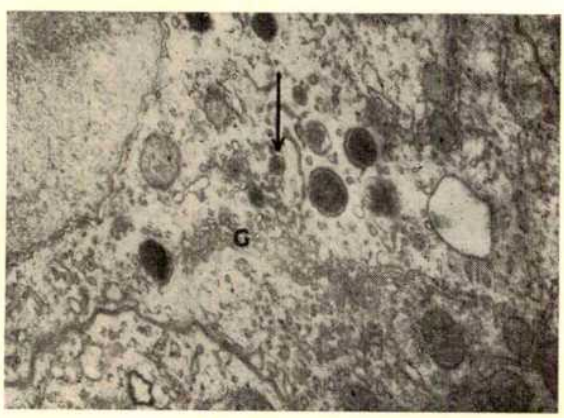


Fig. 3. Electron micrograph of chick embryo pancreas at the eighth day of incubation. The Golgi apparatus (*G*), which is composed of vesicles and small tubules, is forming α-granules (arrow). (× 15,000)

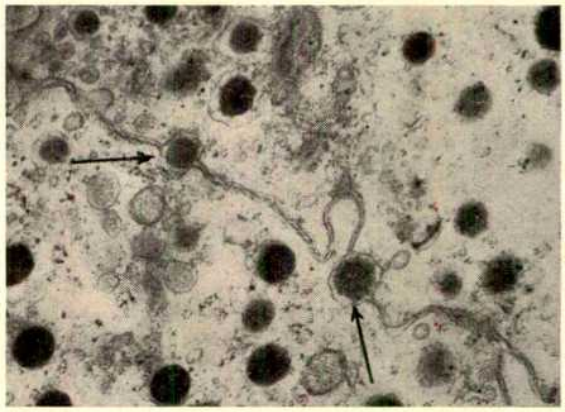


Fig. 4. Electron micrograph of chick embryo pancreas at the eighth day of incubation. Two α-granules in the intercellular space (arrows) may have been secreted from the α-cells by emiocytosis or merocrine type of secretion. (× 37,000)

Glycogen deposits have been observed in the liver of chick embryo between the seventh and the ninth days of incubation[18]. The finding at the eighth day of incubation that secretion occurs from the islet cells, and the presence of glycogen deposits in the liver, suggest that the two phenomena are related[1].

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PHYSIOLOGY

Measurement of 'Core' Temperature in the Rat

IN investigations involving changes in body temperature in the rat[1–3] we have noted discrepancies, in the control levels and changes in response to given doses of drugs such as morphine, chlorpromazine and reserpine, between our own data and those quoted for comparable situations in the literature. Although a wide variety of techniques for measuring body temperature have been used, the most commonly used method in the conscious animal is the intermittent or chronic insertion of a thermosensitive device into the rectum. Few authors state the distance to which the thermometer is inserted but, where the measurement is given, it is generally, in the rat, of the order of 3·0–5·0 cm.

Using a thermistor probe, 3·5 mm in diameter, and a tele-thermometer with which the temperature can be read to 0·1° C, the temperature was recorded at various distances from the anal sphincter of six rats weighing

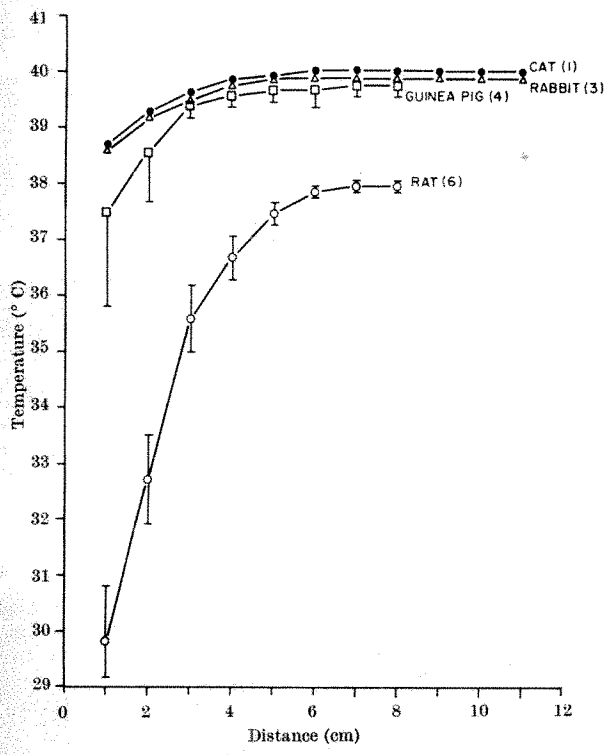


Fig. 1. Mean temperatures recorded at various distances from the anal sphincter. Numbers refer to number of animals. Vertical bars represent standard errors of the means

between 200 and 300 g (Fig. 1). The record assumes a plateau between 6 and 8 cm to give a mean of 38·0 ± 0·1° C, and it can be seen that the readings fall off rapidly at distances of less than 5 cm. If the readings were to be taken by repeated introduction of the probe to a distance of around 3 cm, a slight variation in position could produce a considerable error in the readings, and this could easily be of greater magnitude than the changes being investigated.

A more serious source of error is apparent from Fig. 2, where the fall in body temperature at the time of maximum effect of a systemic dose of 35 mg/kg of morphine sulphate

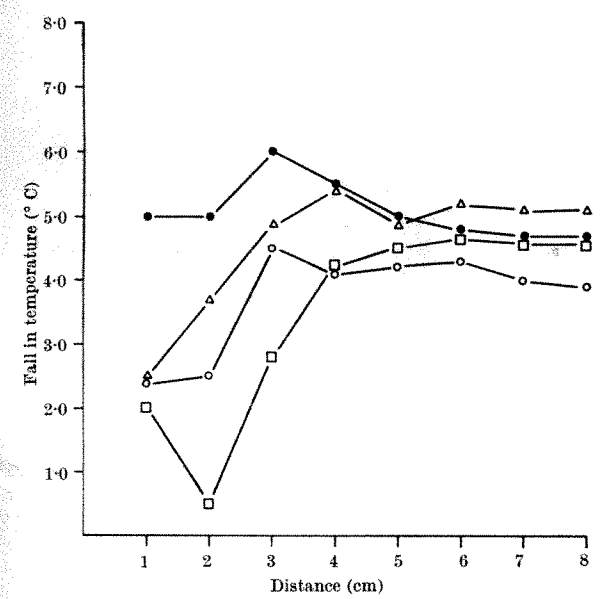


Fig. 2. Fall in temperature recorded at different distances from the anal sphincter in four rats at time of maximum hypothermia following intraperitoneal injection of 35 mg/kg of morphine sulphate

is recorded at different distances in four animals. Measurements at less than 5 cm would indicate a considerable variation in response and possibly obscure significant effects.

With a rat weighing from 200 to 300 g the maximum distance to which the probe can be inserted is approximately 8·0 cm, and post-mortem examination shows that, at this distance, the tip of the thermistor is lying behind the liver and close to the diaphragm.

Temperature measurements at various sites throughout the body of the calf and the rabbit, by insertion of thermocouples directly into the organs, reveal that the liver is at the highest temperature[4]. The temperatures of the liver and the mesentery around the portal vein are essentially the same in the rat and are the highest temperatures in the abdominal cavity[5]. Although higher temperatures may be recorded in specialized tissues such as the anterior hypothalamus[1] or brown adipose tissue during cold-induced thermogenesis[6] in the rat, it would seem that the temperature of the liver represents the best approximation to the body core temperature. Measurement of temperature at more distal points in the gastro-intestinal tract will reflect changes in core temperature but will be also variably affected by changes in local blood flow.

A similar increase in recorded temperature is seen in the guinea-pig (Fig. 1); there is appreciable variation from animal to animal at less than 3 cm (using animals of 600 g). Records for the rabbit and the cat are almost identical with the temperature reaching a steady level from 6 cm onwards.

It is concluded from these investigations that the most simple, accurate and reliable method of recording core temperature in the conscious rat is by means of a probe inserted at least 6 cm into the rectum and left in position throughout the experiment.

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Sensitivity to Acetylcholine in Rat Slow Muscle

IN normal muscle fibres from the rat diaphragm, the depolarizing action of acetylcholine (ACh) is restricted to the region of the neuromuscular junction and its immediate surrounding[1,2]. The diaphragm is a 'fast' contracting muscle and it was interesting to determine if a similar pattern of ACh-sensitivity exists in a 'slow' muscle.

As an example of a slow muscle, we used the soleus of adult rats. The muscle was isolated and placed in oxygenated solution at room temperature. ACh was applied ionophoretically to different regions of the muscle fibres and the potentials were recorded with intracellular electrodes. The local ACh-sensitivity was taken as the ratio of depolarization (mV) to the quantity of charge ($C \times 10^{-9}$) which flows through the pipette[3]. The smallest detectable sensitivity was about 10^{-4} mV/nC.

The end-plate region of the muscle fibres is located about midway along the fibre; and its position can be identified by the presence of spontaneous miniature end-plate potentials[4]. The mean frequency of these potentials

in thirty soleus fibres was 1·3/sec (range 0·3–3·0/sec) which is within the values found in rat diaphragm muscle fibres.

The entire length of practically all the fibres in the soleus muscle is responsive to ACh, although the sensitivity is not uniformly distributed. There is a peak of sensitivity which coincides with the end-plate region. The ACh-sensitivity falls rapidly to either side of the end-plate and remains at a low level (10^{-1}–10^{-3} mV/nC) throughout the rest of the fibre. At the muscle-tendon junction there is again an increase in sensitivity; but the maximal sensitivity at myotendinous junctions is still a few hundred times less than at the myoneural junction. The profile of ACh-sensitivity along soleus fibres resembles that found by Katz and Miledi[5] in some fibres in the frog sartorius.

Since the diaphragm may not be a typical fast muscle, we decided to examine the distribution of ACh-sensitivity in a fast leg muscle to make the observations more comparable with those in the soleus. In preliminary experiments, we have found that the distribution of ACh-reactive sites in the fast extensor digitorum longus is similar to that in the diaphragm: the only region of detectable sensitivity is the end-plate and its immediate surroundings.

It would seem, therefore, that in rat fast muscle fibres the ACh-sensitivity is restricted to a small part of the cell surface; while in slow soleus fibres the entire surface is usually sensitive to ACh. An interesting possibility is that the ACh-sensitivity of a muscle is determined by the type of innervation it receives, a possibility which we are testing by cross-innervating fast and slow muscle.

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Anaerobic Glycolysis in Parts of the Giant Axon of Squid

THE giant axon of the squid, *Loligo pealii*, after careful dissection and removal of auxiliary fibres, can be separated into mitochondrion-poor axoplasm and the relatively mitochondrion-rich envelope[1] (axonal membrane plus Schwann cell plus associated cell-wall material) by the simple mechanical operation of extruding the axoplasm by rolling[2,3].

Recently, the oxidative system as measured by the metabolism of ^{14}C-glucose to $^{14}CO_2$ by this preparation has been found to be limited to the envelope, whereas the axoplasm has been found to be totally devoid of such metabolic activity[3]. The use of specifically labelled glucose has also permitted a qualitative affirmation of a functioning pentose phosphate pathway in squid axon as had previously been shown in lobster axons and in the electric organ of *Electrophorus electricus*[4,5], although it might still be expected that Embden–Meyerhof glycolysis would represent the major route[6,7]. Because of the marked compartmentation of oxidative activity, it appeared of interest to examine the extent and possible localization of glycolysis in squid axons.

Anaerobic glycolysis was measured manometrically using conditions similar to those previously used in this laboratory[4,8]. Three procedures were used for these determinations: (1) cleanly dissected axons were weighed and homogenized in the desired amount of bicarbonate glycolysis buffer[8], and aliquots of the homogenate were added to the main compartments of prepared iced Warburg vessels; (2) cleanly dissected axons were freed of axoplasm by thorough rolling and flattening on a glass plate under sea-water with a small tygon-coated roller[2,3] and were later treated as the whole axons in (1); (3) axoplasm was extruded from crudely dissected axons by light rolling, weighed and added directly to Warburg vessels, where it dispersed in the aqueous medium without the necessity of homogenization. A typical Warburg vessel used in these experiments contained in the main compartment (where all components were placed), 1·08 ml. of glycolysis medium[8]; 0·12 ml. of co-factor solution such that final concentrations of ADP and ATP were 0·00033 M each, and DPN was 0·0002 M; 0·12 ml. of substrate such that the final concentrations of glucose, glucose-1-phosphate, glucose-6-phosphate and fructose-1,6-diphosphate were 0·002 M each; 0·48 ml. of 0·02 M potassium bicarbonate containing 48 mg of axonal material. Substrates and co-factors were either purchased as the potassium salts or converted to that form; gas phase was 95 per cent nitrogen–5 per cent carbon dioxide; temperature, 30° C. Ninety minutes after commencement of readings there was an 80-mm increase in manometer fluid height. A control vessel (0·12-ml. buffer in place of substrate) gave a 7-mm increase. All vessels, including the thermobarometer, were run in duplicate; vessel constants were about 0·7.

Fig. 1 shows that whole axon and axoplasm have high rates of anaerobic glycolysis compared with that for the isolated axonal envelope. In terms of μl. carbon dioxide per gram fresh tissue and hour, the glycolytic activities of whole axon, axoplasm and envelope are in the ratio 780 : 950 : 130. This is a relatively high glycolytic rate, about one-fifth of that for rat brain under similar conditions[9], a most actively glycolysing system. Other published rates[10] under other conditions may not be a suitable basis for comparison, although such comparison would make the relative squid axonal rate appear even greater. Because the envelope represents about one-tenth the weight of the whole cleanly dissected axon, the anaerobic glycolysis rate of envelope is a small fraction of the glycolysis rate for whole axon, of the order of 2 per cent. Even the small but significant production of carbon dioxide in the absence of added substrate is probably also glycolysis as indicated by the total inhibition of carbon dioxide evolution in the presence of 0·005 M iodoacetate, also shown in Fig. 1.

The question may be asked what function the high level of glycolytic activity would perform. The inward passage of nutrients, the outward passage of products, and the synthesis and subsequent hydrolysis of ATP, metabolic events probably coupled to the maintenance of ionic gradients in cellular systems[11,12], almost certainly are dependent on glycolysis as well as on oxidative metabolism. It may be speculated that, since far less ATP is formed per mole of substrate glycolysed than is formed in the subsequent corresponding oxidative cycle, the high level of glycolytic activity would represent the greater means of ATP formation. As for the matter of supplying intermediates for oxidative metabolism, it need only to be noted that previous[3] and present results indicate that, at the maximum, squid envelope can oxidize 0·15 μmoles glucose to carbon dioxide per gram and hour. The extra glycolytic activity, especially of the axoplasm, is probably more a reflexion of enzyme concentrations than of actual metabolic functioning, since, due to the Pasteur effect, the glycolysis rate under aerobic conditions may be expected to be markedly lower than that reported here. The high glycolytic enzyme-levels may supply intermediates for the continuing synthesis of cellular components. The production of such intermediates by the axoplasm, and the production of TPNH by way of the pentose phosphate pathway metabolism by the axonal envelope, may represent a means of metabolic function and control in nerve tissue.

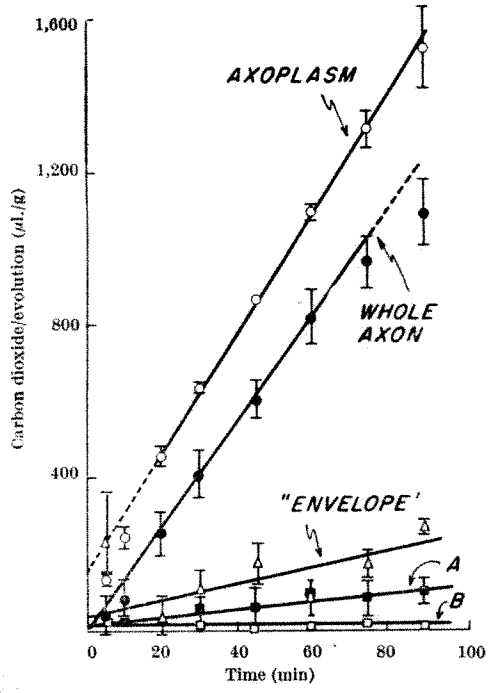


Fig. 1. Anaerobic glycolysis by whole squid axon, by axoplasm, and by envelope, that is, membrane plus Schwann cell plus associated cell wall material. *A*, Average endogenous reaction (control) for all preparations in the absence of substrate. *B*, Whole axon in the presence of added substrate and 0·005 M iodoacetate. Vertical bars, standard deviation

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Absorption by the Mouse and Rat Urinary Bladder of Glycine, Tryptophan and Glucose

THE passage of drugs, dyes and inorganic ions through the mammalian urinary bladder has been the subject of many investigations[1,2]. However, few attempts have been made to ascertain if the mammalian bladder absorbs normally occurring, physiological components of urine, for example, amino-acids and sugars. It was observed[3] during the course of investigations of the bladder carcinogenicity[4] of certain urinary metabolites of the essential amino-acid, L-tryptophan, that nearly 90 per cent of the carbon-14 present in an aqueous solution of carbon-14-labelled 3-hydroxy-L-kynurenine or 3-hydroxyanthranilic acid, instilled directly into the mouse urinary bladder, was recovered after 24 h in the respiratory carbon dioxide, carcass, and faeces. These data suggested that other normally occurring urinary substances might also be absorbed by the bladder.

To investigate this hypothesis, an aqueous solution (*p*H 5·6) of glycine-1-^{14}C (0·1 μM, 0·7 μc., 0·05 ml.), L-tryptophan-3-^{14}C (0·04 μM, 1·0 μc., 0·05 ml.), or D-glucose-1-^{14}C (0·1 μM, 0·4 μc., 0·05 ml.) was instilled, through a needle placed into the urethra under direct vision, into the urinary bladders of four female Swiss mice. To prevent possible reflux into the kidney and to avoid dilution of the label by urine, the ureters were ligated midway between the bladder and the kidneys. After ligation of the urethra[3], the mice were placed individually into a closed metabolism chamber. Respiratory carbon dioxide was collected as described[3] during the period 0–1 h, 1–6 h, 6–18 h and 18–24 h. After 24 h, the mice were killed and the urinary bladder, bladder contents, carcass and faeces were prepared[3]. The carbon dioxide samples, precipitated as barium carbonate, and aliquots of the homogenized tissue and faecal samples were placed on tared planchets and the radioactivity was measured in a Nuclear-Chicago thin-window, automatic gas-flow counter, with a background of 1·5 c.p.m. To obtain a comparative evaluation of the absorptive capacity of the urinary bladder, carbon-14-labelled glycine, L-tryptophan, or D-glucose were injected intraperitoneally into four mice, using the same conditions and dose-levels as were utilized for the instillation into the urinary bladder. Respiratory carbon dioxide was collected for the same time intervals, and all samples of carbon dioxide, tissues, faeces and urine were collected, prepared, and analysed for radioactivity[3]. As the total carbon-14 present in the animal and its faeces, urine, bladder contents, and respiratory carbon dioxide was measured, the percentage of carbon-14 present in each sample was calculated from the c.p.m. of carbon-14 recovered.

Following direct bladder instillation of glycine, $^{14}CO_2$ was detected in the breath after 1 h (Fig. 1*A*). At the end of 24 h, 51 ± 7 (*S.D.*) per cent of recovered carbon-14 was present in carbon dioxide, 47 ± 7 per cent was in the carcass and faeces, 1·1 ± 0·7 per cent was in the bladder, and only 0·2 ± 0·1 per cent was recovered from the bladder contents. Compared with the distribution of carbon-14 following intraperitoneal injection of glycine (Fig. 1*D*), the only statistically significant differences noted were in the percentage of $^{14}CO_2$ found at the end of 1 h (25 ± 3 intraperitoneal; 7 ± 3 intravesical; $P < 0·01$), and in the percentage of carbon-14 in the bladder (0·05 ± 0·03 intraperitoneal; 1·1 ± 0·7 intravesical; $0·02 > P > 0·01$). After the intravesical injection of L-tryptophan, $^{14}CO_2$ was present in the breath after 1 h (Fig. 1*B*). At the conclusion of the experimental period, 29 ± 4 per cent of the recovered carbon-14 was present in the carbon dioxide, 68 ± 4 per cent was in the carcass and faeces, 1·8 ± 0·9 per cent was in the bladder, and only 1·6 ± 1·4 per cent was recovered from the bladder contents. The only statistically significant difference in the distribution of carbon-14, in comparison with that observed following intraperitoneal injection of L-tryptophan (Fig. 1*E*), was the greater quantity of carbon-14 present in the bladder following intravesical instillation ($P < 0·01$), and the greater quantity of carbon-14 present in the urine (compared with the bladder contents) following intraperitoneal injection ($P < 0·01$). After direct bladder instillation of D-glucose, 6 ± 4 per cent was present as $^{14}CO_2$ after 1 h (Fig. 1*C*), and 77 ± 3 per cent was found in the carbon dioxide after 24 h. The carcass and faeces contained 23 ± 3 per cent, while the bladder and bladder contents contained lesser quantities of carbon-14. Compared with the distribution of carbon-14 following the intraperitoneal instillation of D-glucose (Fig. 1*F*), significantly greater

Table 1. COMPARISON OF THE DISTRIBUTION OF CARBON-14 IN SPRAGUE-DAWLEY RATS INJECTED INTRAVESICALLY OR INTRAPERITONEALLY WITH AN AQUEOUS SOLUTION OF L-TRYPTOPHAN-3-^{14}C

Sample	Intravesical injection (per cent)	Intraperitoneal injection (per cent)	*P*-value
$^{14}CO_2$			
0–1 h	0·5 ± 0·4	5 ± 4	N.S.
0–6 h	7·0 ± 3·0	22 ± 1	< 0·01
0–18 h	12 ± 6·0	28 ± 1	0·05 > *P* > 0·02
0–24 h	15 ± 8·0	32 ± 1	N.S.
Carcass and faeces	77 ± 4·0	65 ± 2	0·05 > *P* > 0·02
Bladder	8·0 ± 4·0	0·01	N.S.
Bladder contents or urine	0	4 ± 1	< 0·01

The solution used was at *p*H 5·6; and 0·1 ml., containing 0·08 μM and 2·0 μc. of L-tryptophan, were injected. The instillation into the bladder through a needle placed into the urethra, the collection and processing of the samples and tissues, and the measurement of the radioactivity were as described for the mice. The percentage of carbon-14 was calculated from the carbon-14 recovered. Three rats, weighing 150–200 g, were used for the intravesical study, and two rats of the same size were used for the intraperitoneal investigation. The values represent the mean ± 1 standard deviation.

($P < 0·01$) quantities of carbon-14 were present in the carbon dioxide at all time periods and in the urine (compared with the bladder contents) after intraperitoneal injection, while a significantly greater ($P < 0·01$) amount of carbon-14 was present in the carcass and faeces after intravesical instillation.

At the end of the experimental period, the urinary bladders were intact and of normal colour. In one experiment, the vascular supply at the base of the bladder was ligated intentionally, and at the end of 1 h, the bladder was soft and dark in colour. Unlabelled glycine, L-tryptophan and D-glucose in the same concentration and volume as used in the experiments with carbon-14 were instilled individually into the bladders of mice and the urethras were ligated[3]. The mice were killed after 1, 6, 18 and 24 h, the bladders were fixed with Bouin's solution, and histological sections were prepared[4]. The integrity of the bladder throughout the experimental period could be ascertained, for the mucosa, submucosa and musculature of the bladder were intact. When compared with a normal mouse bladder, no histological differences could be detected.

The absorption and distribution of carbon-14-labelled L-tryptophan after intravesical instillation into the bladder of the rat were compared with that observed following intraperitoneal injection (Table 1). After 24 h, following intravesical administration, all the carbon-14 was present outside of the lumen of the bladder. The rat bladder permitted the passage of this compound as readily as did the mouse bladder.

Though the passage of drugs, dyes and inorganic ions through the mammalian bladder has been demonstrated[1,2], the bladder has been thought of primarily as a storage receptacle for urine, and an organ of evacuation. These data suggest that the mammalian urinary bladder may play an active part in the conservation of substances essential for the nutrition of an animal. Because the functional capacity of the transitional cell epithelium lining the ureters, bladder and urethra is not known, it is possible that (1) the bladder mucosa, by absorbing amino-acids and sugars from the urine, may receive many essential nutrients in this way; (2) the bladder may function as an important regulator of metabolism through the absorption of physiological substances from the urine; or (3) the bladder may retain a vestigial absorbing function that permitted those species capable of conserving essential nutrients in this manner a better chance of evolutionary survival. The mechanism of absorption of these compounds is not known.

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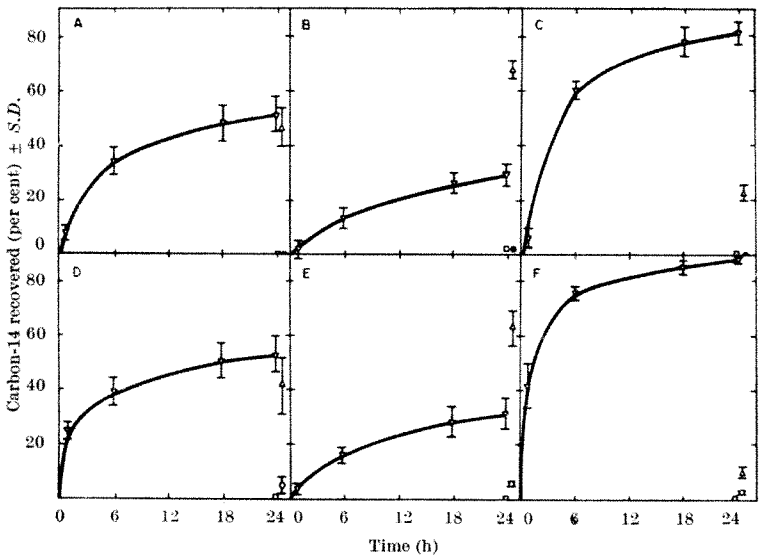


Fig. 1. Comparison of the distribution of carbon-14 in mice injected intravesically or intraperitoneally with an aqueous solution of carbon-14-labelled glycine, L-tryptophan, or D-glucose. The intravesical instillation of (*A*) glycine, (*B*) L-tryptophan, and (*C*) D-glucose is compared with the intraperitoneal administration of (*D*) glycine, (*E*) L-tryptophan, and (*F*) D-glucose. The inverted open triangles represent cumulative carbon-14 over the periods 0–1 h, 0–6 h, 0–18 h, and 0–24 h; upright open triangles the carbon-14 present in the carcass and faeces; open squares the carbon-14 present in the washed bladder; closed circles the carbon-14 remaining in the bladder lumen; and open circles the carbon-14 present in the urine

Acetylcholine and Choline Acetylase in Squid Giant Axon, Ganglia and Retina

THE giant axon of the squid stellar nerve and giant synapse of the stellate ganglia have frequently been used as test objects during investigations of the electrophysiological properties of nerve conduction and junctional transmission[1–3]. Other investigations in which these preparations have proved useful have concerned the chemical and molecular basis of nerve activity and the role of acetylcholine (ACh) in conduction[4–12].

A prerequisite for assuming an essential role of ACh in the conducting mechanism of any preparation is the presence of the ACh system: choline acetylase (ChAc), ACh itself, choline esterase (ChE), and ACh-receptor[4,5]. ChE was found in both preparations a long time ago[4], and recently by means of highly refined techniques it has been shown to be present in the envelope and axoplasm of carefully cleaned axons and in squid stellate ganglia[11,12]. Recent investigations have indicated that ACh receptor is present in the axon[9,10] as well as in the ganglia[11].

It appeared desirable to obtain more complete information by testing for the presence of ACh and ChAc. For purposes of comparison, similar determinations were made with the squid fin nerve with a bundle of axons, which lies adjacent to the stellar nerve, and with the axons from the walking leg of lobster. The last-mentioned preparation is of special interest, as ACh and related compounds directly affect conduction[13,14], and thus indicate that the permeability barrier is smaller than in the squid axon.

The squid retina was included in the present investigations as it has been reported

that this tissue has no ChAc activity[15]. This was surprising since the retinae of dog, rabbit and guinea-pig, for example, have high activities[16].

Samples of tissue were rapidly removed from the squid and lobster. The giant axons from the squid (*Loligo pealii*) were carefully cleaned of all adhering small nerve fibres and as much connective tissue as possible. Either the entire stellate ganglia was assayed or it was carefully divided into an outer portion, which contained mainly the ganglion cells of the first stellar nerve together with some of the proximal synapses, and an inner portion, which contained mainly the giant or distal synapses[17,18]. These will be referred to as the proximal and distal synaptic regions. The entire nerve bundles from the first two pairs of lobster walking legs were used.

Tissues intended for ChAc assay were placed in a refrigerator until ready for use, while those used for the direct measurement of ACh were immediately homogenized in 5 ml. of frog Ringer's solution[19] containing about 1×10^{-4} M physostigmine sulphate—except for the control samples, which were homogenized in Ringer's solution free of physostigmine.

Choline acetylase was determined as previously described[19], except that the phosphotransacetylase was purchased from Calbiochem; after incubation, the 1-ml. sample was diluted with frog Ringer's solution to at least 200 ml. This dilution avoids any interference in the bioassay from the ingredients of the incubation mixture. Control incubation tubes, both with and without boiled tissue, were always run as controls.

The ACh in the tissue, or that formed during a 1-h period of incubation in the ChAc assay, was determined by bioassay on the frog rectus abdominis muscle[19]. The following amounts of tissue were used: 50–90 mg giant axon (ten to fourteen axons); 100–140 mg fin nerve; 50–90 mg stellate ganglia (four whole ganglia); 15–30 mg proximal or distal synaptic regions (from three ganglia); 100–300 mg retina; 40–100 mg lobster axons. ($T = 18°–22°$ C.) The following tests were performed in order to ascertain that the material causing contraction of the frog rectus muscle was ACh or a choline ester: (1) tissue homogenate without physostigmine was added to the muscle; (2) the effect of acid and alkaline heating of extracts was sometimes tested. Except for the squid giant axon (see later), all contractions appeared to be exclusively due to ACh or to an ACh-like compound.

The results obtained are summarized in Table 1. In a few experiments the axoplasm of the giant axons was extruded[10], and attempts were made to determine ACh separately in the axoplasm and envelope. In order to obtain reliable quantitative results, however, it would have been necessary, based on the ACh content of the whole axon, to have at least 50 mg of axoplasm and envelope. Although as many as twenty axons were extruded for a single determination, we were unable to obtain sufficient tissue for quantitative data. The results obtained, however, seem to indicate that ACh is present in both axoplasm and envelope; the two values combined probably amount to about the same concentration found in whole axon. Investigations with a more sensitive bioassay technique may give a more exact quantitative relationship.

In the presence of physostigmine, the contraction of the rectus muscle produced by the homogenized suspension of the giant axon corresponded to an ACh concentration of 4·2 μg/g; however, in the absence of physostigmine, there was a mean contraction corresponding to 1·3 μg/g. This value was subtracted from the 4·2 to give the value of 2·9 shown in Table 1. The small contraction in the absence of physostigmine was probably due to the potassium contained in the axoplasm of the axons, as 60 mg of axoplasm contains sufficient potassium approximately to double the normal concentration of potassium (2·5 mM) in 5 ml. of frog Ringer's solution. In the absence of physostigmine, homogenates of the other tissues gave no contraction.

Table 1. ACETYLCHOLINE (ACh) AND CHOLINE ACETYLASE (ChAc) ACTIVITY OF SQUID GIANT AXON, RETINA, FIN NERVE, STELLATE GANGLIA AND PROXIMAL AND DISTAL SYNAPTIC REGIONS OF GANGLIA

Tissue	No. expts.	ACh* (μg/g)	No. expts.	ChAc* (μg/g/h)
Giant axon	7	2·9 ± 0·5†	4	263 ± 33†
Fin nerve	4	1·1 ± 0·2	4	481 ± 32
Stellate ganglia	4	11·3 ± 3·0	4	5,257 ± 235
Proximal synaptic region	3	2·7 ± 0·2	3	1,448 ± 521
Distal synaptic region	3	26·8 ± 5·5	6	8,753 ± 578
Retina	8	6·3 ± 1·0	6	887 ± 174
Lobster axons‡	13	11·3 ± 0·9	5	1,056 ± 43

* The ACh content of tissues is expressed as μg ACh/g fresh weight, and the ChAc activity as μg ACh formed/g fresh weight/h.
† Results are presented as means ± standard deviation of the mean (standard error).
‡ The lobster axons were taken from the walking legs.

The fin nerve exhibits higher ChAc activity than the giant axon but it has less free ACh. The amount of ACh in the fin nerve is about equal to that found in rabbit sciatic[20]. The giant or distal synaptic region has much higher ACh and ChAc than the proximal region, and has also been found to have more ChE[11]. The ChAc activity in the giant synaptic region is almost as great as that in the squid head ganglia, which has the highest concentration and is one of the best sources of ChAc[4]. Our values for ChAc in the walking leg of lobster are about twice as great as those recently reported[21], and indicate that the amount of ACh formed is sufficient for 800,000 impulses/h.

The relatively high ChAc activity which we found in the squid retina is in marked contrast to an earlier report by Feldberg *et al.*[15]. At the time of their investigations, however, the procedures used for determining ChAc activity were inadequate as the activity of this enzyme depended on an adequate supply of acetyl-CoA provided by another enzyme. By the addition of phosphotransacetylase to the incubation media, acetyl-CoA is produced and this source of error is eliminated.

The experiments were carried out at the Marine Biological Laboratory, Woods Hole, Massachusetts, and we are grateful for the facilities made available to us. We thank Prof. David Nachmansohn for his advice and Miss R. Spencer, Miss J. Rubinstein and Miss L. S. Ardwin for technical assistance.

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Gluconeogenesis following Hypothalamic Stimulation

In previous experiments[1,2], a rise in serum glucose, and increased endogenous gluconeogenesis in liver slices, was found 5 min after the injection of 50–100 mg of 2-deoxy-glucose (2 DG), an inhibitor of glucose utilization, into fasting rats. 2 h after administration of 2 DG, an accumulation of liver glycogen was observed. There was a constant rise in urea excretion during the first 24 h. Adrenalectomy inhibited the response to 2 DG, and no effect was observed when 2 DG was injected together with glucose or insulin.

The changes due to 2 DG were not observed in animals anaesthetized with ether or pentobarbital. This suggested that stimulation of gluconeogenesis at least in these experiments originated in the central nervous sytem, possibly in the hypothalamus. The actual stimulus in the hypothalamus may be cellular glucopenia due to inhibition of glucose utilization caused by 2 DG. Previous observations in both experimental animals[1] and man[3] support the concept that 2 DG acts on the hypothalamus. If this hypothesis is correct an electrical stimulation of the hypothalamus may induce gluconeogenesis, and the present communication reports the results of such experiments. The stimulation was carried out in the following manner.

A bipolar stainless steel electrode—insulated except for its tip—was implanted, with the aid of a 'Krieg' stereotaxic instrument, into the posterior hypothalamus or caudate nucleus of male albino rats weighing between 200 and 300 g. One week after the surgical procedure the rats were deprived of food overnight and then stimulated with a 'Grass' stimulator with pulses of 10 msec duration and at a frequency of 20/sec. The current and duration of stimulation are shown in Table 1. Other animals which were similarly treated on the same day and kept exactly under the same conditions served as controls. Within 30 min of the end of the stimulation the animals were anaesthetized and killed by bleeding. Hepatic gluconeogenesis and glycogen content were determined. The brains were removed, fixed in formalin, cut at 80μ and examined for confirmation of the position of the stimulating electrodes (Fig. 1).

It was found that electrical stimulation of the hypothalamus increased the serum glucose level and the rate of hepatic gluconeogenesis. After 3 h, a 10–20-fold rise in liver glycogen was found. The stimulation was effective with a current as low as 0·06 m.amp.

Electrical stimulation at a different area in the central nervous system, namely, the caudate nucleus, had no effect on the measured parameters.

As in the case of 2 DG, adrenalectomized rats did not show an increased rate of gluconeogenesis after electrical stimulation.

These results and our earlier observation that anaesthesia inhibits the onset of gluconeogenesis indicate the important role of the central nervous system in the regulation of gluconeogenesis and consequently of the blood glucose level. It seems that both electrical stimulation and 2 DG cause a discharge of adrenocorticotrophic hormone releasing factor, thus activating the adrenal cortex.

Table 1. Effect of Electrical Stimulation of the Hypothalamus on Gluconeogenesis in Rats

Implantation	Current (m. amp)	Duration of stimulus (h)	Serum glucose (mg%) E	Serum glucose (mg%) C	Gluconeogenesis in liver slices (μmole glucose/100 mg wet tissue/2 h) E	Gluconeogenesis in liver slices (μmole glucose/100 mg wet tissue/2 h) C	Glycogen in liver (γ/100 mg wet tissue) E	Glycogen in liver (γ/100 mg wet tissue) C
Posterior hypothalamus	1·80	3	175 ± 14·7 (7)*	101 ± 13·0 (5)	2·96 ± 0·01	1·32 ± 0·03	300 ± 36	24 ± 2
	1·80	1/2	150 ± 13·6 (3)	95 ± 12·8 (2)	3·40 ± 0·08	1·90 ± 0·09	34 ± 4	30 ± 4
	0·20	3	150 ± 9·1 (4)	103 ± 9·5 (4)	2·50 ± 0·06	1·10 ± 0·08	600 ± 53	35 ± 2
	0·20	1/2	180 ± 15·8 (3)	105 ± 9·3 (2)	2·40 ± 0·03	1·70 ± 0·05	20 ± 3	20 ± 3
	0·06	3	135 ± 23·0 (3)	105 ± 8·1 (2)	2·00 ± 0·09	1·30 ± 0·04	55 ± 6	50 ± 3
	0·06	1/2	125 ± 14·1 (3)	100 ± 9·8 (2)	2·10 ± 0·02	1·40 ± 0·06	35 ± 5	46 ± 8
Caudate nucleus	1·80	3	98 ± 5·1 (6)	97 ± 4·7 (4)	1·00 ± 0·01	1·10 ± 0·02	100 ± 12	81 ± 11

* No. of animals indicated in brackets.

The livers were removed, chilled in cold saline, sliced with a 'Stadie Riggs' slicer and weighed. 100-mg liver slices were suspended in 1 ml. Krebs Ringer bicarbonate medium (ref. 4), aerated with 95 per cent oxygen and 5 per cent carbon dioxide. Incubation was carried out in a metabolic incubator at 37° C for 2 h. The glycogen content of the liver was determined by the anthrone method (ref. 5) and glucose after deproteinization (ref. 6) by the glucose oxidase method (ref. 7).

Endogenous gluconeogenesis was estimated by subtracting the amount of glycogen that disappeared from the liver slices during incubation from the amount of glucose found in the medium at the end of the incubation.

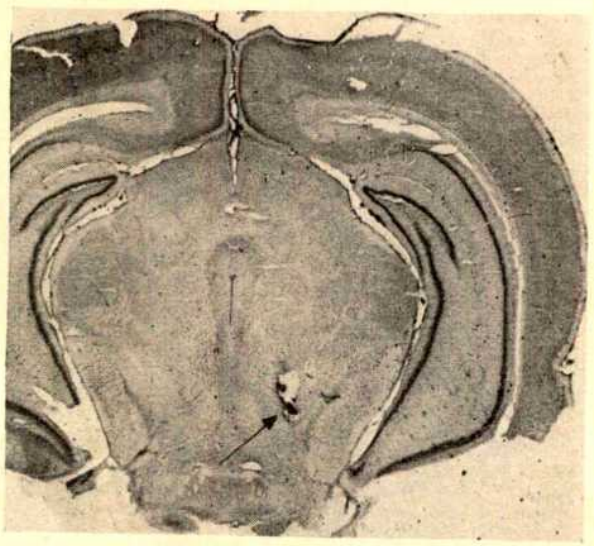


Fig. 1. Histological section of the posterior hypothalamus showing position of stimulating electrode

The fact that the hormones of the adrenal cortex are essential for the normal gluconeogenetic response is well known[8]. But it is probable that their effect in this process of gluconeogenesis is permissive. This problem is discussed elsewhere[1].

To sum up, electrical stimulation of the posterior hypothalamus in rats fasted overnight resulted in increased endogenous hepatic gluconeogenesis, hyperglycaemia and accumulation of liver glycogen. These changes did not occur in animals stimulated in the caudate nucleus. In adrenalectomized rats the effect could not be observed.

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pH Decline in the Two Layers of the Panniculus Adiposus

THE post-mortem *p*H decline in a tissue has been accepted as an indication of the underlying, concomitant physical and chemical changes[1,2]. The relationship of this acidity development to subsequent tissue processing and organoleptic properties has also been established[3,4]. Although such investigations with muscle tissue have received considerable attention[5–9], similar investigations have not been undertaken with adipose tissue. Consequently, this investigation was conducted to determine the rate and extent of *p*H decline, if any, in adipose tissue.

Ten experimental animals (*Sus domesticus*), averaging 120 kg and similar in genetic and nutritional backgrounds, were used in this investigation. Samples of the panniculus adiposus were excised from the lumbar region of the right side of the carcass immediately after exsanguination and at 30 min, 1, 2, 3, 4 and 24 h *post mortem*. The *p*H values of the outer and inner layers of this subcutaneous adipose tissue were measured with a Beckman 'Zeromatic' *p*H meter equipped with a combination probe-electrode. The *T*-test of significance and the simple correlation coefficients were calculated by the methods outlined in Steel and Torrie[10].

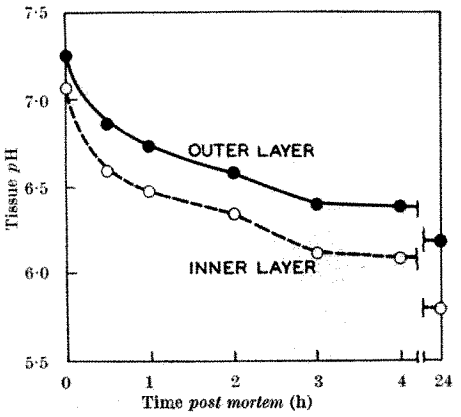


Fig. 1. Post-mortem changes in the *p*H of the outer and inner tissue layers of the panniculus adiposus

The results in Fig. 1 show the features of the post-mortem *p*H decline in the outer and inner layers of the panniculus adiposus. These data demonstrate that a gradual acidification does occur in adipose tissue following death. Further (Table 1), the two layers of this tissue are noted to differ significantly in *p*H values.

The outer layer of the panniculus adiposus always exhibited a higher *p*H regardless of the time *post mortem* at which it was measured. It can also be observed (Table 1) that the rate of *p*H decline (in 24 h) and its extent (ultimate *p*H) were more marked in the inner than in the outer layer.

Table 1. POST-MORTEM *p*H AND HYDROGEN ION CONCENTRATION (MOLES $\times 10^{-7}$) CHANGES IN THE OUTER AND INNER TISSUE LAYERS OF THE PANNICULUS ADIPOSUS

Panniculus adiposus layer	Initial (0-h)		Ultimate (24-h)		Difference	
	*p*H	H^+	*p*H	H^+	*p*H	H^+
Outer layer	7·25	0·62	6·18	8·99	1·07	8·37
Inner layer	7·07	0·92	5·79	17·53	1·28	16·60
Difference	0·18*	0·30*	0·39†	8·54*	0·15	8·23*

* $P < 0·05$.
† $P < 0·01$.

Table 2. CORRELATION COEFFICIENTS BETWEEN THE POST-MORTEM *p*H MEASUREMENTS IN THE OUTER AND INNER TISSUE LAYERS OF THE PANNICULUS ADIPOSUS

Inner tissue layer / Tissue measurement	Outer tissue layer: 0-h *p*H	0·5-h *p*H	1-h *p*H	2-h *p*H	3-h *p*H	4-h *p*H	24-h *p*H
0-h *p*H	0·95†	0·84†	0·63*	0·64*	0·54	0·49	0·54
0·5-h *p*H	0·93†	0·97†	0·93†	0·83†	0·84†	0·80†	0·64*
1-h *p*H	0·79†	0·84†	0·97†	0·71*	0·83†	0·80†	0·61
2-h *p*H	0·53	0·51	0·62	0·84†	0·85†	0·84†	0·53
3-h *p*H	0·37	0·47	0·64*	0·57	0·86†	0·88†	0·39
4-h *p*H	0·24	0·39	0·57	0·53	0·84†	0·86†	0·34
24-h *p*H	0·35	0·18	0·09	0·18	0·20	0·20	0·48

* $P < 0·05$.
† $P < 0·01$.

Although these data demonstrate the *p*H differences between the two layers of the panniculus adiposus, the simple correlation coefficients (Table 2) suggest a metabolic relationship in the development of post-mortem acidity in this adipose tissue.

The problem remains to relate these observations to the actual physical and chemical events occurring in the tissue.

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Studies on Spermatozoa and Fluid collected directly from the Testis of the Conscious Ram

RAM spermatozoa require 12 to 15 days to traverse the epididymis[1] and during this time undergo changes which have been called maturation. In order to investigate these a technique has been developed for the continuous collection of spermatozoa before they enter the epididymis so that their *in vitro* metabolism may be compared with that of ejaculated spermatozoa from the same ram. The technique has also enabled a continuous collection of the fluid which is secreted by the testis and in which testicular spermatozoa are carried to the head of the epididymis. To our knowledge this investigation represents the first evaluation of testicular fluid and spermatozoa collected from conscious animals.

Nine adult merino rams kept in a climate room at 21° C were used. Under general anaesthesia and aseptic precautions, the head of the epididymis was dissected off the tunica albuginea and raised until the efferent ducts were located and severed. A ring of silicone rubber, with a plug fitting into the top, was sewn to the tunica albuginea so that it included the cut efferent ducts in the space so formed. The fluid from the efferent ducts flowed into this space, then down one of two tubes sealed into the wall of the ring, and was collected in a receptacle attached to the scrotum; samples for chemical analysis were collected into a receptacle surrounded by solid carbon dioxide. The other tube was occluded and used for daily flushing (Fig. 1).

The testicular fluid obtained was turbid and flowed at a rate of $1·0 \pm 0·11$ ml./h/100 g of testis (range 0·76–1·45; five rams) or at a rate of about 50 ml./day from a testis of average size. Spermatozoa were present at a concentration of $67·2 \pm 17·8 \times 10^6$/ml. (range $14–123 \times 10^6$; five rams). The total daily production was $1·6 \pm 0·43 \times 10^9$ spermatozoa/100 g of testis or $3·3 \times 10^9$ spermatozoa from an average-size testis. These figures agree with the calculated sperm production of Ile-de-France[2] and Suffolk rams[3].

Freshly collected testicular spermatozoa showed prominent kinoplasmic droplets and exhibited only feeble oscillating movements. The spermatozoa became more active in fluid with a higher alkalinity, as observed previously[4], or after storage at +3° C overnight in the fluid in which they were secreted. The catheters remained patent for up to 7 days.

The initial *p*H of testicular fluid was 7·1 but rose to 8·6 several hours after collection. Cell-free testicular fluid contained very little protein (0·1–0·5 g/100 ml.), about 3 per cent of plasma values. This is in marked contrast to the high protein concentration previously described for testicular lymph collected from rams[5,6]. The fluid was either isosmotic or hypotonic with a mean osmolarity of 283 ± 8·3 mOsm/kg (six rams) compared with the plasma osmolarity of 299 ± 1·7 mOsm/kg. The mean concentration of sodium in the fluid was 134 ± 1·7 m.equiv./l., and that of potassium was 11·4 ± 0·8 m.equiv./l. These were lower than those reported for the fluid collected from rams at slaughter[7,8], but similar to those recently obtained *post mortem* from bulls[9]. Only traces of glucose and fructose were found in testicular fluid, but lactic acid was present at a concentration of 58 ± 2·6 μg/ml., which is about 10 per cent of the values obtained for slaughterhouse material[8].

The fluid collected from seven rams contained variable amounts of testosterone ranging from 0·6 to 8·8 (mean 3·4) μg/100 ml., whereas in two other rams there was no testosterone detectable (< 0·5 μg/100 ml.). When present, therefore, testosterone appeared in testicular fluid at a concentration similar to that found in testicular lymph[5] and in the lower range of that found in plasma from the internal spermatic veins[5,10], but it was not correlated with protein concentrations eliminating possible contamination with lymph. Thus the testicular spermatozoa and developing cells inside the seminiferous tubules are exposed to testosterone which has been suggested as playing an important part in the maintenance of spermatogenesis[11] and sperm survival in the epididymis[12].

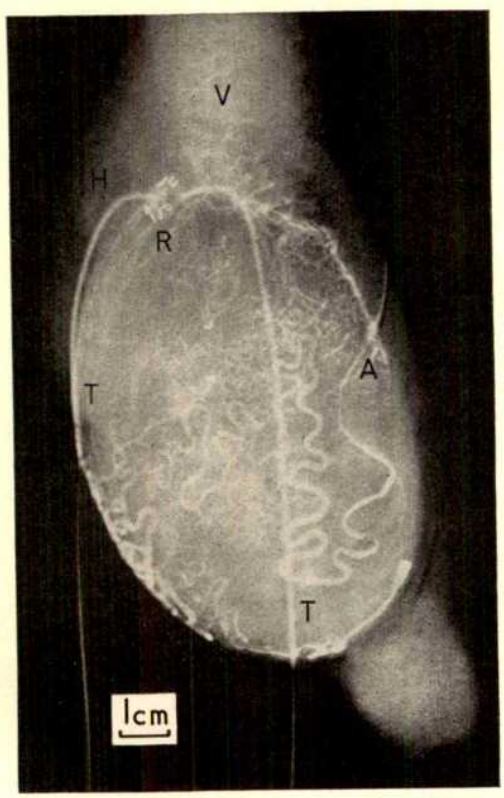


Fig. 1. Radiograph showing the ring (*R*) and tubes (*T*) of the testicular catheter in relation to the head of the epididymis (*H*) and vascular cone (*V*). *R*, 5 mm internal diameter, 8 mm outer diameter, 5 mm high; plug 2 mm thick; *T*, 1 mm internal diameter, 1·8 mm outer diameter. Sutures are attached to the stainless steel loops on the ring. Testicular catheter here filled with radio-opaque material ('Urografin', Schering *A.G.*), *A*, Catheter in testicular artery for injection of 'Urografin' to show the vessels and the vascular cone

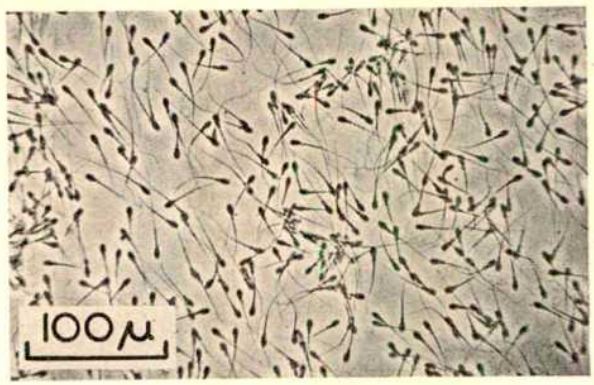


Fig. 2. Photomicrograph of washed testicular spermatozoa collected by the testicular catheter (phase contrast)

For metabolic studies the testicular spermatozoa were centrifuged (about 400 *g* for 5 min) from the testicular fluid, and were compared with spermatozoa obtained by electro-ejaculation. The spermatozoa were washed twice and resuspended in a phosphate buffer diluent with and without substrates[13]. Fig. 2 is a sample of the washed testicular spermatozoa used. The sperm suspensions were incubated in Warburg flasks under aerobic and anaerobic conditions at 37° C, usually for 3 h. Oxygen uptake, the utilization of glucose, fructose, an equimolar mixture of both and the production of lactic acid were measured[14–16].

A direct comparison of the metabolism of ejaculated and testicular spermatozoa from the same two rams is given in Table 1. The endogenous respiration of ejaculated and testicular spermatozoa was similar. The addition of a substrate stimulated the respiration of both types of cells, but to a greater extent in testicular spermatozoa. Fig. 3 shows the pattern of the oxygen uptake of testicular and ejaculated spermatozoa in the presence of a substrate. The initial rate of respiration was similar but thereafter decreased progressively in ejaculated spermatozoa. The oxygen uptake of testicular spermatozoa remained linear throughout.

In the presence of oxygen, the substrate utilization of ejaculated spermatozoa tended to be higher and also the lactic acid production was higher than that of testicular spermatozoa, but the values were not affected by substituting fructose for glucose. By contrast, under anaerobic conditions the rate of glucolysis by testicular spermatozoa was higher than by ejaculated spermatozoa. Thus although both types of spermatozoa showed a Pasteur effect—an increase in substrate utilization and lactic acid production under anaerobic conditions—it was greater in testicular spermatozoa. Both types of cells used glucose in preference to fructose when both sugars

Table 1. METABOLISM OF EJACULATED AND TESTICULAR SPERMATOZOA OF THE RAM
(Change/10^8 cells in 3 h)

Added substrate		Oxygen uptake (μl.) Ejac. sp.	Oxygen uptake (μl.) Test. sp.	Substrate utilization (μg) Ejac. sp.	Substrate utilization (μg) Test. sp.	Lactic acid production (μg) Ejac. sp.	Lactic acid production (μg) Test. sp.
—	Aerobic	20	22	—	—	—	—
Glucose	Aerobic	40	59*	262	191	191	54‡
	Anaerobic	—	—	387	473*	368	404
Fructose	Aerobic	37	55*	208	158	177	69‡
	Anaerobic	—	—	265	412†	258	329*
Glucose + Fructose	Aerobic	39	59†	G235 F65	G202* F64	180	56‡
	Anaerobic	—	—	G360 F119	G430* F154	400	397

The means of six observations on two rams are given. Similar values were obtained in six other rams, but because complete data were not obtained they have not been included.
Significance of differences between ejaculated and testicular spermatozoa:
* = $P < 0.05$ † = $P < 0.01$ ‡ = $P < 0.001$.
G and F: glucose and fructose utilization respectively from an equimolar mixture.

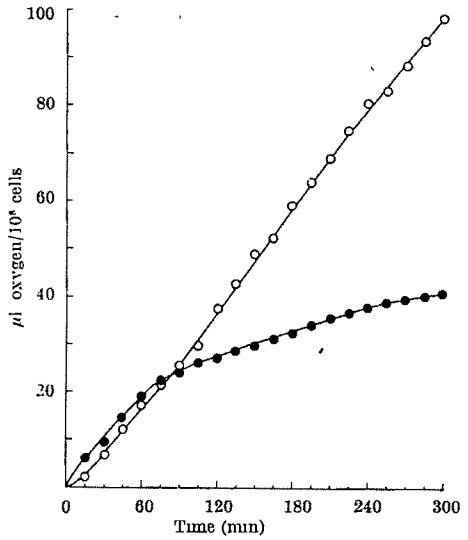


Fig. 3. A characteristic pattern of the oxygen uptake of washed testicular (○) and ejaculated (●) ram spermatozoa in a phosphate buffer diluent with glucose (0 005 M) as a substrate

were present and under both aerobic and anaerobic conditions, thus demonstrating the fructose-sparing effect previously described for ejaculated spermatozoa[17]. The lactate production by testicular spermatozoa under anaerobic conditions was not sufficient to account for the glucose utilized. Other metabolites may have accumulated in the medium and we have also shown that glucose is incorporated into lipid by testicular spermatozoa (unpublished observations).

The most striking differences found in the metabolic pattern between ejaculated and testicular spermatozoa were the greater oxygen uptake and lower lactic acid production of the latter. Differences have been observed in the metabolic pattern and enzymatic activities between ejaculated and epididymal spermatozoa from the ram and the bull, but these were only minor[18–20]. The present investigations have demonstrated that there are metabolic differences between testicular and ejaculated spermatozoa that may be the result of the process of 'maturation'.

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VETERINARY SCIENCE

Blood Adenine Nucleotide Levels in the Normal and the Acetonaemic Dairy Cow

In man, various diseases cause the concentration of the adenine nucleotides in the blood to deviate from the values found in normal circumstances[1]. Determination of the levels of the adenine nucleotides in the blood thus provides information on the state of health and the metabolic condition of the patient.

It might be expected that, in the cow, variations of the levels of these nucleotides in the blood would also give some indication of the metabolic condition of the animal. However, although the concentrations of the adenine nucleotides in human blood appear to be well documented (for example, Laudahn[1]), there are few values available for bovine blood. Consequently, in connexion with a study of metabolic changes occasioned by the disease of bovine ketosis, the levels of the adenine nucleotides in the blood of the normal dairy cow were determined, and compared with those found in ketosis. Spontaneous primary bovine ketosis, also termed acetonaemia, is a disease which can affect dairy cows in the early stages of lactation, causing a decline in milk yield, and which can therefore be of considerable economic importance.

The concentrations of ATP, ADP and AMP in blood samples taken from dairy cows were estimated using the 'Test Kits' supplied by C. F. Boehringer und Soehne *G.m.b.H.*, Mannheim, West Germany. Blood was withdrawn from the jugular vein and collected directly in ice-cold 0·6 M perchloric acid solution and the mixture shaken vigorously. Afterwards, the blood proteins were removed by centrifugation and the levels of the nucleotides were estimated in the clear supernatant either directly (ATP) or after neutralization (ADP and AMP). ATP estimations were always carried out on the day the animal was bled. ADP and AMP estimations were performed on either the same or the following day. In the latter case, the neutralized samples were stored frozen. All manipulations before assay were performed at 0°–2° C.

Table 1. Adenine Nucleotide Concentrations in the Blood of Normal Cows and of a Cow Suffering from Spontaneous Primary Ketosis

	Day	Acetoacetate mg%)	ATP (mg%)	ADP (mg%)	AMP (mg%)
Mean of 12 normal cows	—	—	6 65	1 51	0·460
± *S.E.*			±0 46	±0 15	±0 024
Acetonaemic cow	1	5 96	6·42	2 16	0·478
	2	5·17	7·59	1 28	0 622
	3	6 11	5 55	1 17	0 368
	4	4 80	6 73	1·05	0 542
	14	0 71	6 75	Not determined	
	22	0·83	7·69	Not determined	
Mean	—	—	6·79	1 42	0 503
± *S.E.*			±0·32	±0 25	±0 053

The days on which blood samples were taken from the acetonaemic cow are numbered from the day on which the disease was first apparent. Samples were taken during the height of the disease and during the recovery period as indicated by the blood acetoacetate levels measured concurrently.

Table 1 shows the mean values for the concentration of the three nucleotides in the blood of twelve apparently normal animals. The standard error of the mean for each nucleotide is also given. It is clear from these figures that in the dairy cow the blood concentrations of the nucleotides are uniformly at the lower end of the range of values for human blood[1]. In Table 2, the values for individual animals are recorded. The cows were either of the Ayrshire or Friesian breeds, and were being used in a nutritional experiment involving different ratios of carbohydrate to protein in the diet. Of the twelve animals, all but one were in milk. Inspection of Table 2 indicates that these differences did not appear to cause a significant unidirectional alteration in the concentration of any of the nucleotides. Apart from this, a rather wide variation in the levels of all three nucleotides was observed (3·98—9·40 mg per cent

Table 2. ADENINE NUCLEOTIDE CONCENTRATIONS IN THE BLOOD OF NORMAL COWS

Name	Breed	Milk yield on day (gallons)	Diet	ATP (mg%)	ADP (mg%)	AMP (mg%)
Bilberry	*PA*	3·3	*NP*	4·60	1·14	0·467
Curtsy	*PA*	3·3	*NP*	6·48	1·58	0·592
Debutante	*PA*	1·7	*NP*	7·11	0·74	0·349
Doting	*PA*	4·5	*NP*	7·50	1·87	0·569
Eva	*A*	2·8	*HP*	5·77	2·24	0·440
Fancy	*PA*	3·1	*NP*	3 98	1·29	0·467
Fashion	*PA*	2·8	*HP*	5·00	1·46	0·350
Gem	*PA*	Dry	*HP*	6·38	2·11	0·560
Goldilocks	*A/Fx*	3·3	*LP*	7·84	2 04	0·530
Grand	*PF*	1·8	*NP*	7·79	0·77	0·302
Helga	*PF*	2 6	*LP*	8·00	1·93	0·530
Junket	*PA*	1·7	*NP*	9·40	0·98	0·348

Abbreviations. *Breed: A*, Ayrshire; *PA*, pedigree Ayrshire; *A/Fx*, Ayrshire-Friesian cross; *PF*, pedigree Friesian. *Diet: NP*, Normal protein content, carbohydrate · protein = 6 . 1; *LP*, low protein content, carbohydrate . protein = 8 : 1; *HP*, high protein content, carbohydrate . protein = 4 · 1.

for ATP, 0·74–2·24 mg per cent for ADP, and 0·302–0·592 mg per cent for AMP).

Considerable evidence exists that, in acetonaemia, tricarboxylic acid cycle activity is depressed[2,3]. It might be expected that this would lead to lower ATP concentrations, and higher ADP concentrations, in the tissues and in the blood of the animal suffering from the disease, unless other energy-generating reactions (for example, fatty acid oxidation) were stimulated to compensate for decreased cycle activity. The occurrence of a case of spontaneous bovine ketosis at this institute provided an opportunity to determine whether the blood levels of ATP, ADP and AMP were altered during acetonaemia. Table 1 shows the levels of these nucleotides found in the blood of the animal while acetonaemic. Furthermore, the levels of ATP are also shown during the recovery of the animal, which is indicated by the decline of acetoacetate concentration in the blood.

It is evident from Table 1 that the mean values for the concentrations of the three nucleotides in the blood of the acetonaemic animal were similar to the mean values for these concentrations in the normal animal. Moreover, no significant change in the concentration of ATP occurred during recovery from acetonaemia, as measured by the decline in acetoacetate. Although some acetoacetate was still present at the 14th and 22nd days, this does not mean that the animal was still acetonaemic. Using the enzyme D(−)-β-hydroxybutyrate dehydrogenase for estimation of acetoacetate[4], up to 1·0 mg per cent of this substance has been found in the blood of apparently normal cows at Compton. Acetoacetate thus appears to be a normal constituent of cow's blood in these small quantities.

In summary, the levels of the three adenine nucleotides have been determined in the blood of apparently normal cows. Values within the normal range were found in a cow suffering from spontaneous primary ketosis. If tricarboxylic acid cycle activity is depressed in the acetonaemic cow, then the level of ATP may be maintained by other oxidative reactions. Alternatively, it is possible that in the cow the levels of the adenine nucleotides in the blood may not be representative of alterations in the tissues. It is hoped, therefore, to supplement this investigation with a study of adenine nucleotide levels in the tissues of the acetonaemic dairy cow.

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PHARMACOLOGY

Effect of Amphetamine and Catecholamines on Startle Response and General Motor Activity of Albino Rats

THE reactions of the mammalian organisms to external stimuli are affected, according to Hess[1], by a subcortical system co-ordinating visceral, somatic and psychic functions. This system consists of two separate and antagonistic divisions: ergotrophic and trophotrophic. The former produces a generalized increase in sympathetic activity with increased skeletal muscle tone, arousal and increased response to external stimuli. This is contrasted with the trophotrophic system which serves a recuperative and protective role and is characterized by an increase in parasympathetic activity, decreased muscle tone, sleep and decreased response to external stimuli. Brodie[2] has cited evidence that norepinephrine and serotonin may qualify as neurohormones for the two divisions respectively and claims that further evidence for norepinephrine as the ergotrophic hormone is that dopa (amino-acid precursor of norepinephrine) induces typical ergotrophic effects. It is felt by Brodie and associates that many of the lipid soluble congeners of norepinephrine easily penetrate the blood-brain barrier to stimulate the central adrenergic receptors of Hess.

In support of Brodie's position, the awakening effects of the phenylamines (specifically *m*-tyrosine and dopa) following administration of reserpine have been said to be due to their eventual transformation to catecholamines[3,4] (recalling that the sequence of steps in epinephrine formation is: tyrosine, dopa, dopamine, norepinephrine, epinephrine). However, recent work by Lee, Cho and Dobkin raised doubts as to the validity of the idea that lipid soluble precursors of epinephrine easily penetrate the blood-brain barrier and lead to the same kind of stimulation in the central adrenergic receptors of Hess's ergotrophic division. Using fairly large dose levels of catecholamines, these workers found that the norepinephrine–epinephrine precursors did not produce similar activity in their animals. Furthermore, tyrosine, tyramine and dopamine "seemed to produce a peculiar sedative state characterized by . . . the general appearance of sleep". In addition, they also noted a lack of dose-response relationships following administration of these drugs. It was felt by these investigators that if these phenylethylamines exert an effect on the ergotrophic system similar to that of norepinephrine and epinephrine (a result expected because of their structural similarity, comparable lipid solubilities and ability to undergo a stepwise transformation toward catecholamines), then they should produce a more obvious and essentially similar activity[5]. They found, furthermore, that the effect of the catecholamine precursors combined with the short-acting hypnotic, methohexital, definitely prolonged maintenance time of anaesthesia.

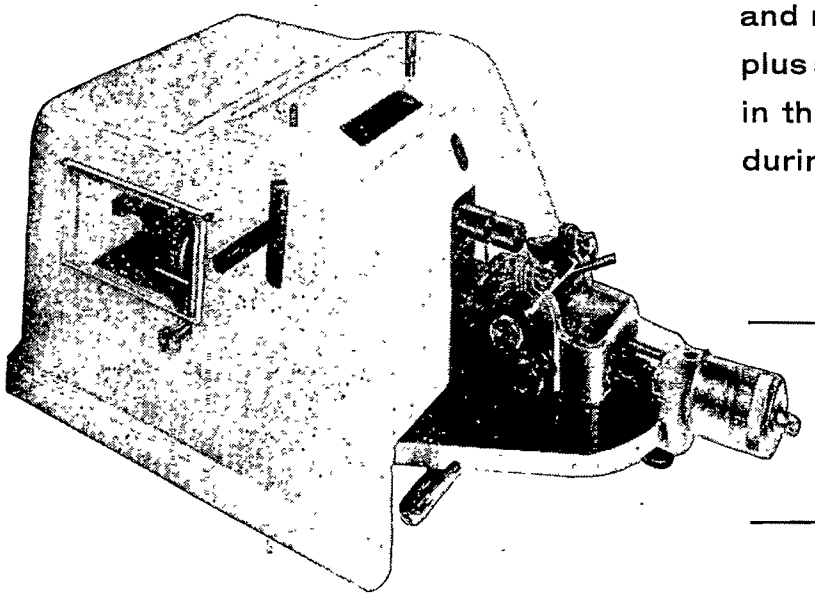
It was the purpose of this project to investigate the effect of small dose levels of the norepinephrine precursors with respect to (*a*) spontaneous motor activity, and (*b*) response to auditory stimulation. The latter parameter was selected to provide an additional means of evaluation of these drugs to determine if drug effect as measured by one means (enhancement or depression of observed activity) may be different when compared with effect on response to startle stimulation.

Because of the close structural similarity between amphetamine and the catecholamines, it was included in this experiment. It is believed to act centrally by releasing dopamine, and it appears to prevent the uptake of circulating norepinephrine by the tissues[6,7].

Subjects were eighty-four Charles River albino male rats ranging in weight from 75 to 130 g. Physical activity of the rat was measured as mm deflexion of a pen recorder through conversion of mechanical input (that is, the

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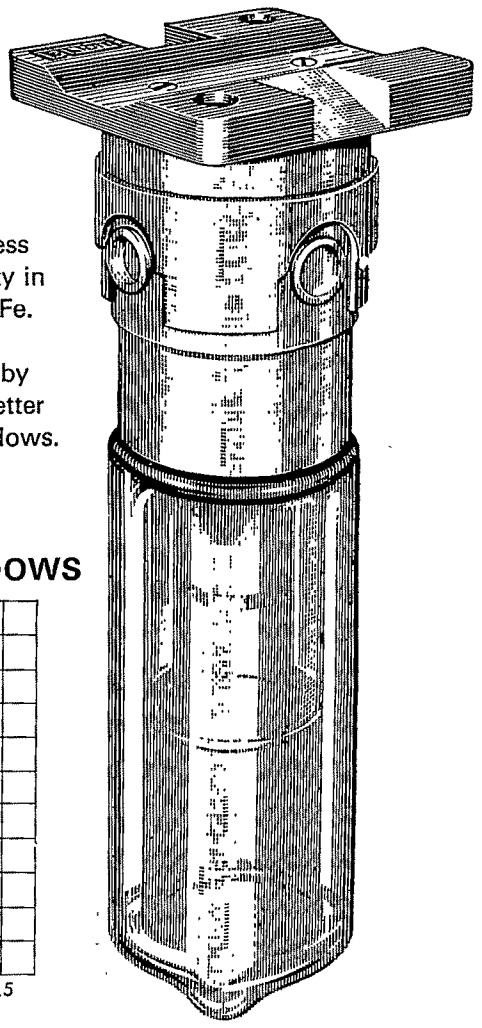
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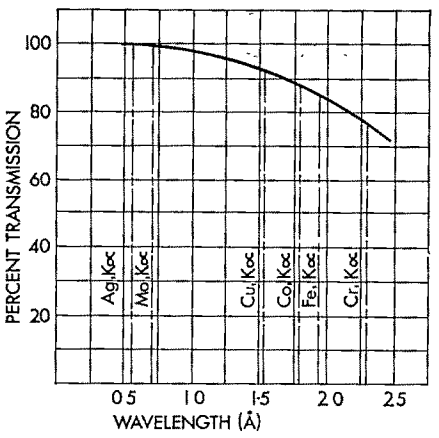
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activity of the rat) by a force displacement transducer. Each subject was placed individually in an enclosed wire cage (8 in. × 8 in. × 7 in.) which was positioned on the floating platform of a mechanical scale. Attached to this platform was an 18-gauge needle which was led through the wire loop of an independently suspended force displacement transducer. Movement of the platform was thus measured as alteration in voltage which in turn was recorded as pen deflexion on the polygraph.

The noise stimulus was produced by a Grason–Stadler white noise generator, amplified by a white amplifier to 52 dB (absolute reference scale). A Gerbrands tape timer programmed noise generation for a duration of 1 sec every 4 min.

Drugs employed were DL-tyrosine, DL-3-4-dihydroxyphenylalanine (dopa), 3-hydroxytyramine-HCl (dopamine), and amphetamine sulphate. Tyrosine and dopa were dissolved in 0·1 N sodium hydroxide saline solution and *p*H adjusted to 10; dopamine and amphetamine were dissolved in 0·9 per cent saline. Dosages used were 0·5, 3 and 10 mg/kg for each drug.

Eighty-four subjects were divided into 14 groups, with six subjects per group. Each of the twelve groups was injected with one of the three dose levels of a particular drug. The concentration of each solution was adjusted to enable a constant volume (0·1 ml.) of drug to be injected into each rat. One group of six subjects was injected with 0·1 ml. of saline as control for dopamine and amphetamine and a second group of six subjects with 0·1 ml. sodium hydroxide saline solution (*p*H 10) as control for tyrosine and dopa.

Each of six rats per dose per drug was subjected to three white noise stimuli of 52 dB, duration of 1 sec, at 4-min intervals. Ten minutes were allowed for absorption of the intraperitoneally administered drug before the first noise stimulus was applied. Pen sensitivity was adjusted such that maximum deflexion would record 45 mm above base line. The response to each stimulus was recorded and the average response for each group of six subjects was determined.

Though the response to noise stimulation was quantitated and subjected to statistical analysis, evaluation of the animal's bodily motor activity was subjectively made by direct observation. Also, the tracings revealed any subject movement made, and these permitted further evaluation of this parameter. General motor activity of any one group was graded as 0, 1 and 2, plus or minus units above or below, respectively, the activity of the control for that group of subjects. Types of behaviour on which grading was made included judgments of the subject's level of excitement or depression by his walking, sniffing, climbing with forepaws up side of cage, etc. Observations were recorded over a 12-min period, beginning 10 min after injection of the drug. The startle response data were evaluated by a simple analysis of variance and subsequent comparisons were carried out by the Newman–Keuls procedure between groups. In no case was the response to any one noise stimulus significantly different ($P > 0·10$) from the response to the other two within any particular group. Thus, for each group, responses to the three stimuli were summed and the average used in comparisons between groups. Results for each group are shown in Table 1.

As may be seen from Table 1, 0·5 mg/kg dopa exerted a quieting effect on general motor activity as compared with control, while at 10 mg/kg no appreciable difference was noted. Lee, Cho and Dobkin observed an excitation with a dose level of 600 mg/kg (ref. 5). Response to noise stimulation of the 3 mg/kg group was significantly less ($P < 0·05$) than the response of both the lower and higher dosage groups.

The sedative effect of tyrosine as observed by Lee *et al.* at a dose level of 100 mg/kg was also noted in this experiment at dose levels of 3 and 10 mg/kg. However, the 0·5 mg/kg group showed mild excitement. The results of noise stimulation for the tyrosine group were not significantly different between groups ($P > 0·05$) although qualitative differences were noted. The startle responses of the 3 and 10 mg/kg groups were slightly greater than control, while the response of the lowest dosage group was comparable to that of control. The effects of the two highest dose levels of tyrosine were different from the two parameters of measurement used; that is, in one case (startle) a greater response was elicited as compared with control, where with general motor activity a decrease from control obtained.

Table 1. RESPONSE OF NAIVE RATS TO WHITE NOISE

Drug	Dose* (mg/kg)	S.E.	Startle response† (mm pen deflexion)	Spontaneous activity
Dopa	0·5	4·45	33	−1
	3·0	4·35	24	−1
	10·0	3·24	35	Same as control
Control	—	3·06	31	—
Tyrosine	0·5	3·95	30	1
	3·0	2·39	38	−2
	10·0	2·77	38	−2
Control	—	3·06	31	—
Dopamine	0·5	4·23	19	Same as control
	3·0	4·33	29	Same as control
	10·0	3·17	31	Same as control
Control	—	4·04	24	—
Amphetamine	0·5	2·36	13	Same as control
	3·0	4·00	25	Same as control
	10·0	2·26	38	2
Control	—	4·04	24	—

* Six animals per dose per drug.
† Each indicated pen deflexion represents the mean value of 18 deflexions per group (6 rats × 3 stimuli per animal).

Dopamine at the three dose levels used had no influence on general motor activity, a result comparable with the findings of Lee *et al.* However, response to noise of the 3 and 10 mg/kg groups was greater than that of the lowest dosage group and control though results were not statistically significant ($P > 0·05$).

In the case of amphetamine, the degree of startle response varied directly with the dose. At 0·5 mg/kg this response was observed to be less than control. Both the 3 and 10 mg/kg groups had responses greater than control, with the latter significantly greater ($P < 0·01$) than the others. An increase in spontaneous activity was observed only in the highest dosage group.

In summary, the testing of catecholamines at these low dosage levels revealed variations in effect which were dependent on (*a*) dose, and (*b*) the particular parameter selected for observation. In one case, the measurement of response by one method was just the opposite of that as measured by the second method. Furthermore, changes in dose level caused a redistribution of observed effects, which were not necessarily proportional to dose alteration. The response to some injections was not what might have been expected of truly ergotrophic drugs and epinephrine precursors. Though many observations seem to support the view that catecholamine levels in the brain are involved in the control of psychomotor activity, results obtained in this experiment with low dose levels support evidence that psychomotor effects of the catecholamine precursors cannot be considered in the same category as epinephrine.

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IMMUNOLOGY

A γ-Globulin Genetic Factor related to Gm(a) but localized to a Different Portion of the same Heavy Chains

PREVIOUS investigations have indicated that Gm(a)* and Gm(f)* are genetic characters localized in various areas of the major heavy chain class[1-3] of human γG globulins. These determinants, detected as antigens, segregate in most populations, as if controlled by allelic genes, and occur independently in myeloma proteins from Caucasians. A previous report indicated that specific reagents for the human genetic factors can be produced by immunizing rabbits with myeloma proteins[4]. In order to analyse in a more satisfactory way the genetic control of different portions of the heavy chains, it seemed desirable deliberately to immunize with critical protein fragments in order to disclose additional genetic sites believed to be present but not detected by available antisera.

The Fab portion of Gm(a+) myeloma proteins lacks any detectable genetic factor[5,6] and this was used as the antigen for rabbits. The antisera obtained were absorbed with normal human serum of various phenotypes and were then tested for differential agglutination against a battery of incomplete anti-D coats on human red blood cells. Antisera which appeared specific were investigated further by inhibition with selected proteins[5,6]. In this way a new factor, tentatively called Gm(z)*, was detected.

The Gm(z) genetic factor was found in the Fab portion of papain split FII γ-globulin, while Gm(a) was detected in the Fc portion as previously described[5,6]. All Gm(a+) myeloma proteins from Caucasians and Negroes were also Gm(z+) (Table 1). Papain splitting of these doubly positive myeloma proteins showed the Gm(z) determinant in the Fab peak separated by zone electrophoresis, while the Gm(a) factor was present in the faster migrating Fc peak. Pepsin treatment destroyed the Gm(a) determinant and left intact the Gm(z). Inhibition at a concentration as low as 0·008 mg/ml. was obtained for the active fragments. Isolated light chains failed to inhibit even at 2 mg/ml. All preparations of heavy chains from FII and myeloma proteins were inhibitory, but to a lesser degree than the intact protein and Fab fragments. Parallel investigations with Gm(f) indicated that Gm(z) and Gm(f), although on different myeloma proteins, behaved very similarly in the aforementioned experiments and were localized to the Fd portion of the heavy chains. Gm(z) was found only in myeloma proteins of the We(γ_{2b}) sub-group—as was the case for Gm(f).

The phenotype frequencies of various populations are shown in Table 1. In the Caucasian, Negro and Japanese groups, Gm(a) and (z) are concordant, but there are many discordant Gm(a+z−) sera in the Chinese and Easter Islanders. The latter groups affirm the non-identity of genetic factors Gm(a) and (z). Further evidence for this difference was obtained from the study of a Chinese myeloma protein which was previously shown to be Gm(a+f+) in striking contrast to all Caucasian and Negro myeloma proteins. This protein was Gm(z−).

The data from these investigations are consistent with the possibility that Gm(z) and (f) segregate as co-dominant alleles. They are the only genetic factors found so far on the Fd portion of the heavy chain[2,3]. None of the more than 300 sera tested were negative for both (z) and (f). A significant finding is that in all the groups investigated (Table 1) the frequencies of both factors and the proportion of heterozygotes are compatible with such allelism—assuming different gene frequencies for Gm(f) and (z). In the Chinese, where the frequency of (f) is high, as would be predicted, (z) is low, whereas in the Negro and Japanese populations the reverse is true. Family investigations of 42 Caucasian and 26 Easter Island progeny support the behaviour of Gm(f) and (z) as alleles.

Table 1. GM PHENOTYPES OF DIFFERENT RACES

Sera: Gm type a	z	f	Cauc.	Negr.	Races Jap	Chin.	East.†
+	+	+	41	2	3	58	23
+	+	−	24	19	36	14	13
−	−	+	83	0	0	0	0
+	−	+	0	0	0	53	12
Myeloma proteins*							
+	+	−	5	2			
−	−	+	11				
+	−	+				1	

* Only myeloma proteins of the We(γ_{2b}) class are shown.
† Easter Islanders.

The accumulated evidence indicates that in Caucasians the genes controlling the We-type heavy chain would be Gm^{az} and $Gm^{a\text{-}f}$. In Negroes the predominant gene would be Gm^{az}. On the other hand, in Chinese and the Easter Islanders, the gene Gm^{af} as well as Gm^{az} must be postulated to account for the large number of discordancies between Gm(a) and (z), their phenotype distributions, and the Chinese myeloma protein. The previous suggestion that the Gm^{af} gene arose by an intragenic cross-over[7] is compatible with the present observations, as the association of Gm(z) with Gm(a) disappears when Gm(f) becomes associated with Gm(a).

Two genetic factors are therefore now available which seem to be present on the same heavy chains, but in different areas readily separated by papain splitting. There has been considerable speculation on the possibility that the heavy chains of γ-globulin actually represent two chains under independent genetic control[8]. Some evidence regarding this point has been obtained from the present investigations. Gm(z) located on the Fd fragment seems to be controlled by the same gene as Gm(a) on the Fc fragment. The exact position of Gm(z) on the Fd fragment remains to be determined and the possibility is not ruled out that a separate unit could still exist in another portion of the fragment.

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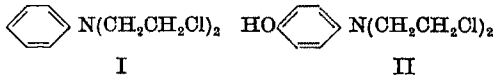
* The suggested notation of the W.H.O. for Gm(a) is (1); for Gm(f), (4); for Gm(z), (17).

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PATHOLOGY

Cure of Mice bearing Advanced Plasma Cell Tumours with Aniline Mustard: the Relationship between Glucuronidase Activity and Tumour Sensitivity

THE unique action of aniline mustard (I) and closely related analogues in curing mice bearing advanced plasma cell tumours (up to 8 g in weight) was recently described[1]. From an investigation of a series of derivatives related to aniline mustard it was postulated that this compound was hydroxylated in the liver to form the highly toxic *p*-hydroxyaniline mustard (II), and then detoxified by conversion to the glucuronide or sulphate. A high glucuronidase or sulphatase activity of the tumour would result in the selective release in the tumour of the toxic *p*-hydroxy derivative:



I II

The levels of the enzymes β-glucuronidase and aryl sulphatase have been determined in two plasma cell tumours (*ADJ/PC5* and *ADJ/PC6A*), both very sensitive to treatment with aniline mustard, and compared with the levels of these two enzymes in two other solid transplanted tumours (sarcoma 180 and *NK* lymphoma) which, although growing in the same strain of mouse (*BALB/c-*), are quite refractory to treatment with aniline mustard when well established. β-Glucuronidase was assayed in whole tumour homogenates by the method of Talalay *et al.*[2], except that incubations were carried out at *p*H 7 and 10 per cent sulphosalicylic acid (SSA) was used to precipitate proteins at the end of the incubation period. While giving a clear supernatant SSA had the disadvantage that it also caused precipitation of some of the released phenolphthalein, the amount of phenolphthalein lost being directly related to the protein concentration of the homogenate. It was found that tissue homogenized in ten times its weight of isotonic potassium chloride provided the best conditions for the assay. At this protein concentration there was only a small loss of phenolphthalein on precipitation with SSA and sufficient enzyme was present to release a suitable amount of phenolphthalein during the 1 h incubation period. Errors due to loss of phenolphthalein were allowed for by setting up internal phenolphthalein standards with each experiment, and ensuring the protein concentration of each homogenate was similar. An aliquot of the homogenate was used for estimation of protein content and results were expressed as μg phenolphthalein released per mg protein per hour. Each value recorded in Table 1 is the mean of at least six independent determinations. Sulphatase was assayed as described by Dodgson and Spencer[3]. Internal *p*-nitrophenol standards were used and the results were expressed as μg *p*-nitrophenol released per mg protein per hour. Tumours were taken when about 1–2 g in weight and non-necrotic areas were selected.

Table 1

Tissue	Aryl sulphatase (μg *p*-nitrophenol/mg protein/h)	β-Glucuronidase (μg phenolphthalein/mg protein/h)	Established tumour regressions† (%)
Liver	7·2±0·9	11·4±2·2	—
Spleen	—	14·6±3·0	—
Plasma cell tumour (*PC5*)	1·6±0·5	59·8±8·7	100%
Plasma cell tumour (*PC6*)	3·6±0·7	57·6±5·2	100%
Sarcoma 180	8·5±1·1	10·0±1·7	0%
NK lymphoma	4·9±1·0	13·1±2·0	0%
Spontaneous mammary carcinoma*	—	9·7±3·4	0%

* In the *BALB/c*+ mouse.

† Percentage of complete regressions of established tumours (1–2 g in weight) after treatment with the maximum tolerated dose of aniline mustard.

Table 1 shows the sulphatase and glucuronidase activities of established tumours (1–2 g in weight) and their response to treatment with maximum tolerated doses of aniline mustard. A direct relationship can be seen between β-glucuronidase activity and sensitivity to the nitrogen mustard. The two plasma cell tumours with levels five times higher than liver regress completely on treatment, while the two tumours with enzyme-levels of the same order as liver are insensitive to treatment. No such correlation applies between sulphatase-level and sensitivity. Liver and spleen are two tissues rich in β-glucuronidase[2] and all the tumours studied had levels at least as high, which is in agreement with the observation that tumours have a high level of β-glucuronidase. The two plasma cell tumours are outstanding in that their glucuronidase-level is five times higher than those tissues normally considered to be rich in β-glucuronidase. In both liver and *ADJ/PC5* plasma cell tumour the *p*H optimum of the β-glucuronidase was about 5·0 (Fig. 1). Since tumours are claimed to have a lower intracellular *p*H than other tissues[4,5] then, *in vivo*, the difference in β-glucuronidase activity between liver and tumour may be even greater

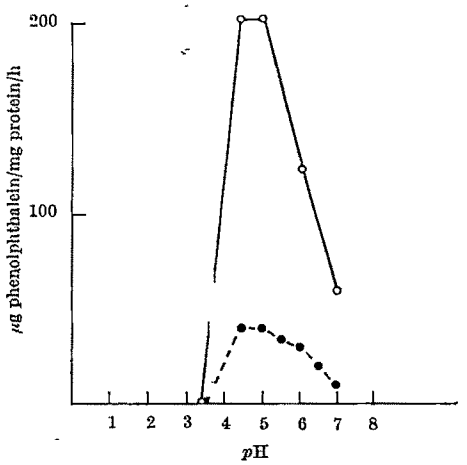


Fig. 1. β-Glucuronidase activities of homogenates of liver and *PC5* plasma cell tumour at different *p*H. ○, *ADJ/PC5* plasma cell tumour; ●, mouse liver

than indicated by the values in Table 1. In both tissues the glucuronidase was equally inhibited by 1 : 4 saccharolactone. Whether the intracellular location of β-glucuronidase differs between liver and the *PC5* tumour is not certain, although in both tissues addition of 'Triton *X*', which destroys lysosomes, before incubation gives a 50 per cent increase in enzyme activity. The enzyme uridine diphosphoglucuronyl transferase, which can be taken as a measure of the ability of the tissue to carry out glucuronide formation, was present in liver homogenate but not in *PC5* tumour homogenate when measured by the method of Storey[6].

These findings add support to the hypothesis that the extreme sensitivity of the two plasma cell tumours to aniline mustard is a consequence of glucuronide formation in the liver followed by breakdown of the glucuronide in the tumour leading to the formation of a toxic derivative. If these findings are confirmed, for example, by synthesis of the glucuronide of *p*-hydroxyaniline mustard and demonstration of its selective action against plasma cell tumours, then they may be exploited in a number of ways: (*a*) Assays of biopsy specimens of human tumours may reveal tumours which have levels of β-glucuronidase much higher than liver and which may be expected to be particularly sensitive to aniline mustard. (*b*) In tumours with high β-glucuronidase levels, this may be exploited by using not only aniline mustard glucuronide but also the glucuronides of other cytotoxic compounds which should be released selectively in the tumour. (*c*) Analogously, if tumours are found with exceptionally high sulphatase- or phosphatase-levels, the sulphate or phosphate of *p*-hydroxyaniline mustard may be used with advantage.

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VIROLOGY

Rapid Method for demonstrating Intracellular Pleuropneumonia-like Organisms in a Strain of Hamster Kidney Cells (*BHK* 21 *C*13)

THERE is as yet no ideal method for demonstrating pleuropneumonia-like organisms (PPLO) in tissue culture. Isolation of an infecting strain, particularly if this is intracellular, is frequently technically difficult and is always time-consuming. Cytological examination of monolayer cultures for evidence of PPLO infection is rapid, but staining artefacts may give rise to difficulty in interpretation. Indirect evidence of infection by transmission to susceptible 'indicator cultures'[1] is only valid if these can be maintained free from accidental contamination by PPLO.

We became acutely aware of these problems when PPLO were isolated from a strain of hamster kidney cells (*BHK* 21 *C*13)[2] in use in this laboratory. Two factors led us to believe that infection was intracellular. PPLO could rarely be isolated from the supernatant medium from cell cultures grown in the absence of antibiotics, but could readily be isolated when the cells were mechanically disrupted in a Griffiths tube and the cell debris plated on a suitable medium (Difco PPLO agar with 20 per cent inactivated horse serum and 5 per cent fresh yeast extract) which was then incubated anaerobically at 37° C for 5 days. In addition, PPLO could still be isolated from disrupted cells even after continuous cell culture for 3 weeks in medium containing kanamycin sulphate ('Kannasyn') in a concentration of 500 μg/ml., although the organism, when tested *in vitro*, was sensitive to a dose of 25 μg/ml.

Since PPLO are known to contain deoxyribonucleic acid (DNA), a modification of the fluorescent Feulgen staining technique[3] was developed which demonstrated the presence of abnormal foci of extranuclear DNA in infected cells. Subsequent investigations showed that these contained PPLO antigens.

Monolayer cultures of *BHK* 21 *C*13 cells infected with PPLO were grown in coverslips until a semi-confluent monolayer was obtained. Cultures of *BHK* 21 *C*13 cells from which PPLO had never been isolated served as a control. These preparations were washed twice in phosphate buffered saline (*p*H 7·2) and fixed for 10 min in formaldehyde saline (4 per cent) at room temperature. Preparations were then processed as follows. (Unless specifically stated otherwise, all procedures were carried out at room temperature.)

(1) Washed twice in distilled water (5 min each). (2) Immersed in 1·0 N hydrochloric acid at 60° C for 10 min to hydrolyse cell ribonucleic acid (RNA). (3) Hydrolysis stopped by plunging preparation into ice-cold water. (4) Washed twice in distilled water (5 min each). (5) Stained for 20 min in acriflavine–Schiff solution (acriflavine hydrochloride, 250 mg; potassium metabisulphite, 500 mg; 0·1 N hydrochloric acid, 50 ml.). (6) Washed twice in distilled water (5 min each). (7) Brought to 70 per cent ethyl alcohol by successive immersion in 20, 40 and 70 per cent ethanol. (8) Rinsed twice for 10 min in acid alcohol (0·1 N hydrochloric acid, 30 ml.; absolute ethanol, 70 ml.). (9) Rehydrated by immersion successively in 40 per cent and 20 per cent ethanol; mounted in buffered glycerol (glycerol, 90 ml.; phosphate buffered saline, *p*H 7·2, 10 ml.) and sealed with paraffin wax.

The preparations were then examined microscopically in short wave-length light (approximately 400 mμ: light source '*HBO* 200' mercury vapour lamp: Schott exciter filters *BG* 38/2·5, *BG* 12/4, Schott barrier filters 53/44).

In infected and in control preparations, cell nuclei fluoresced orange against a background of very pale yellow cytoplasmic fluorescence. Orange fluorescent foci were observed in the perinuclear region of 15–80 per cent of cells in infected preparations. The number of foci varied from one to one hundred per cell. The majority of cells had more than ten foci. In control preparations of uninfected cells, fewer than 3 per cent showed fluorescent foci. No cell had more than one focus. In both infected and control cells foci were still seen after treatment with ribonuclease (recrystallized five times; 100 μg for 8 h) but were not seen in cells which had been treated with deoxyribonuclease (recrystallized once; 100 μg/ml. for 8 h).

A comparison was made between cultural methods and fluorescent Feulgen staining as a means of detecting PPLO in cultures of *BHK* 21 *C*13 cells. Culture and cytological examination were carried out independently. The results are shown in Table 1.

Table 1. COMPARISON BETWEEN CULTURE AND FLUORESCENT FEULGEN STAINING AS A MEANS OF DETECTING PLEUROPNEUMONIA-LIKE ORGANISMS (PPLO) IN STRAINS OF HAMSTER KIDNEY CELLS (*BHK* 21 *C*13)

No. of specimens from which PPLO were isolated	Percentage of cells showing fluorescent foci	No. of specimens from which PPLO were not isolated	Percentage of cells showing fluorescent foci
22	15–84 (average, 30)	10	0·5–1·9 (average 1·0)

The agreement between the results of culture and of fluorescent Feulgen staining is good, and it is suggested that fluorescent Feulgen staining is a reliable, quick method for detecting intracellular PPLO.

The likelihood that the majority of fluorescent Feulgen-positive foci in infected cells might represent sites of PPLO multiplication was increased when it was found that they fluoresced specifically when infected cell preparations were exposed to rabbit prepared antiserum against *Mycoplasma hominis* followed by sheep anti-rabbit serum conjugated with fluorescein isothiocyanate. Control uninfected preparations when examined by this technique showed no specific fluorescence. It was concluded that the fluorescent Feulgen-positive foci observed in a small preparation of these cells are probably micronuclei.

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AGRICULTURE

Absence of Bitterness in Navel Oranges from Rooted Cuttings

MOST Washington Navel oranges yield a juice which eventually becomes unacceptably bitter. The bitterness, which develops slowly on standing or rapidly on heating, is due to limonoid compounds, principally limonin, present in the fragments of albedo and segment walls incorporated into the juice during extraction. As the fruit ripens, the bitterness becomes less evident, and the rate at which bitterness decreases during ripening is affected markedly by the rootstock on which the tree is grown. Thus, Marsh[1] in California and Kefford and Chandler[2] in Australia showed that some rootstocks (for example, trifoliate orange) promoted early disappearance of bitterness, while others (for example, rough lemon) encouraged high levels of bitterness at and beyond normal harvesting maturity. In the latter work, organoleptic tests were supported by the yields of crude limonin extracted from the peel of the oranges used in preparation of the juice.

When an unidentified Navel cutting was used as a rootstock for a Washington Navel scion the fruit showed

no bitterness late in the season[1]. We now report negligible bitterness and low limonin contents in juices from Washington Navel fruits taken from trees grown as cuttings on their own roots. Qualitative observations on the absence of bitterness made on glasshouse fruit in 1962 were extended to field fruit in the 1963 and 1964 seasons and confirmed by assays of the limonin content of the juice[2].

The cuttings were taken from Washington Navel trees growing on rough lemon rootstock, and were propagated by the method of Gates, Bouma and Groenewegen[4]. After two months the rooted cuttings were either planted into the field not far from the mother trees or grown in a glasshouse in clean river sand treated with nutrients and trace elements. The plants grew vigorously in the field and in the glasshouse and have remained healthy and productive throughout the 2–6 years since they were propagated.

Fruit was taken for analyses from the first crop produced by the glasshouse cuttings, from the fourth and fifth crops from the field cuttings, and also from mature trees growing on rough lemon, sweet orange and trifoliate orange rootstocks, including trees in the same field experiment as the mother trees. The juices were extracted and processed by standard procedures[2], and were stored for 2 weeks at 0° C before examination for soluble solids, citric acid, limonin and bitterness. The results are recorded in Table 1.

Table 1. JUICE CHARACTERISTICS OF WASHINGTON NAVEL ORANGES FROM TREES ON OWN ROOTS AND ON ROOTSTOCKS

Root system	Age of tree (months)	Date of pick	Soluble solids (per cent)	Citric acid (per cent)	Limonin (p.p.m.)	Bitterness
Own roots	18*	30.6.63	9·2	0 82	3·5	None
Own roots	18*	30.6 63	10·1	0·88	3·0	None
Own roots	50	30.6.63	8 7	1·15	7 5	None
Own roots	50	18.7.63	11·2	1·18	4 5	None
Sweet orange	276	9 7.63	11·0	1·31	16·0	Strong
Rough lemon†	276	9.7.63	8·8	1 29	24·0	Extreme
Own roots	19*	27 7.64	11·2	1·13	3·0	None
Own roots	63	27.7.64	9·7	1·18	7·5	Doubtful
Trifoliate orange	60	21.7.64	12·0	1·15	8 0	Slight
Sweet orange	60	21 7.64	10·1	1·13	11·0	Moderate
Rough lemon	60	21.7.64	9·6	1·07	17·0	Strong
Own roots	64	4.9 64	12·2	0·99	6·5	None
Rough lemon†	290	4.9.64	11·6	1 02	11 5	Moderate

* Grown in glasshouse; other trees grown in field.
† Similar scion-rootstock relationship to mother trees.

The relationship between bitterness and limonin content agrees with our observations[3] that orange juices containing less than 6 p.p.m. of limonin are not bitter, those containing more than 9 p.p.m. are bitter, and those containing 24–30 p.p.m. are extremely bitter. The low limonin contents of the juices given by fruits from rooted cuttings point to a direct influence of the root system of Washington Navel trees on the amount of the bitter principle present in the fruit. At the same time, oranges from rooted cuttings were normal in composition and general quality, and gave juices of satisfactory flavour, both before and after processing. The practical application of these observations will depend on the health and productivity of rooted cuttings as commercial orchard trees.

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Effect of Calcium and Strontium on the Preferential Formation of an Adenosine Triphosphatase in Wheat Roots

THERE are a number of factors which may induce the formation of a specific enzyme in living tissue homogenates[1]. In keeping within the definition established in ref. 1, the prefix 'induced' restricts the type of enzyme formation to one in which a specific inducer molecule is shown to be necessary in the formation of a particular enzyme. The present communication concerns the effects of the inorganic cations calcium and strontium on the selective formation of an adenosine triphosphatase (ATPase) of wheat roots. To distinguish this type of rather indirect effect, the term 'preferential' enzyme formation will be used.

Wheat roots were germinated in the dark and allowed to grow into solutions containing only the individual calcium or strontium salts. After 7 days the roots were excised and ground in the cold in a 0·05 M *tris*-(hydroxymethyl)-aminomethane buffer at *p*H 7·5 containing 0·005 M sodium ethylenediamine tetra-acetate (EDTA). A solution volume : root weight ratio of 2 was used in grinding. After removal of the cell debris, the mitochondria were separated by centrifugation at 20,000*g* for 15 min. The pellet was resuspended in the original volume of buffer and dialysed for 24 h in 0·001 M *tris* at *p*H 7·5. Separation in this manner undoubtedly resulted in the mitochondria being broken[2] and incapable of oxidative phosphorylation.

Table 1. EFFECTS OF CALCIUM AND STRONTIUM CONCENTRATION ON ROOT GROWTH AND ATPASE ACTIVITY

Growth treatment	Root weights (g/50 plants)	Cation added to assay*	ATPase activity†
H_2O	4 70	—	5 6
		Ca	15 2
		Sr	2·4
10^{-4} M calcium chloride	8·20	—	23·2
		Ca	47·7
		Sr	5 8
10^{-2} M calcium chloride	7·60	—	20 8
		Ca	42·6
		Sr	5·0
10^{-4} M strontium chloride	6·40	—	13·0
		Ca	26 3
		Sr	4·2
10^{-2} M strontium chloride	2·70	—	6·6
		Ca	15·3
		Sr	2·3

* 5 μmoles per tube, added as chloride salts.
† Expressed as μmoles P_i released per mg protein in 30 min.
Other conditions as presented in the text.

The ATPase activity was measured by determination of inorganic phosphate liberated from adenosine triphosphate (ATP) with an assay procedure similar to that used with spinach chloroplasts[3]. The basic reaction mixture contained 0·05 M *tris*-maleate buffer at *p*H 5·5, 5 μmoles ATP and an appropriate portion of the particulate enzyme preparation in a total volume of 2 ml. The incubation was conducted at 30° C for 30 min and reaction stopped by the addition of 4 ml. of cold 10 per cent trichloroacetic acid. Protein content was estimated from an aliquot of the enzyme preparation by the Folin-Ciocalteau method[4].

In an investigation undertaken to determine the possible involvement of an ATPase from wheat roots in ion uptake[5], it was noted that the specific activity of the ATPase varied with the calcium level of the root growth medium. The addition of calcium to the enzyme reaction mixture considerably enhanced the ATPase activity, whereas the chemically similar ion strontium inhibited the enzyme activity[5] (see also Table 1). The effects of varying concentrations of these two cations on root growth and synthesis of the ATPase are presented in Table 1. Root growth was increased in the two higher-level calcium treatments and in the 10^{-4} M strontium solution, but was reduced with 10^{-2} M strontium. The specific activity of the ATPase associated with the par-

ticulate fraction was sharply increased by all the calcium and the 10^{-4} M strontium growth treatments (Table 1, column 4). The ATPase activity of the 10^{-2} M strontium treatment was similar to that of the control, even though root growth definitely was impeded. It is re-emphasized that the results are expressed per unit of protein, thus indicating preferential formation of the enzyme. When the divalent cations calcium and strontium were added to the enzyme reaction mixture, however, two opposite effects were always noted as indicated previously. This observation is quite vital as it provides evidence that the properties of the enzyme are not altered by calcium and strontium levels of the root growth medium. Thus the actual quantity of the enzyme must be changed by their presence in the growth medium.

The results of an experiment in which the strontium concentration of the growth medium was varied over a wider range are presented in Fig. 1. The values for specific activity of the ATPase are those with calcium added to the reaction mixture and the weights are for fresh roots of ten representative plants from each treatment. At 10^{-5} M strontium the specific ATPase activity was greatly enhanced, thus indicating preferential enzyme formation. In contrast to the previous experiment, no increase in specific activity of the ATPase was obtained at 10^{-4} M strontium. Our inability to define precisely the effective strontium concentration was probably due to failure in maintaining constant strontium levels in the dilute solutions throughout the growth period.

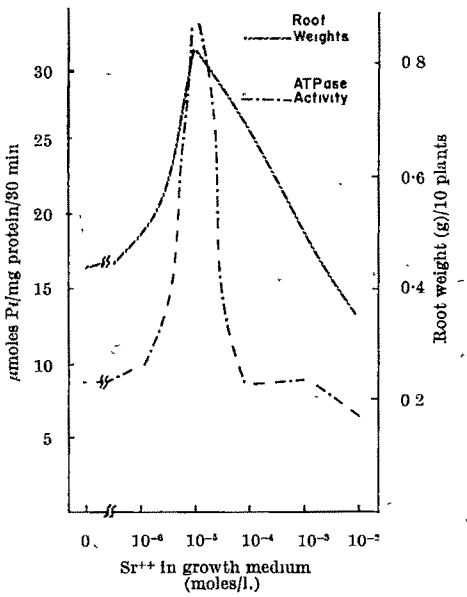


Fig. 1. Effects of altering strontium concentration of growth medium on wheat root growth and ATPase activity (particulate fraction). Strontium added as the chloride salt. ATPase assay conditions presented in the text

Considering the increased root growth associated with the higher calcium levels and limited strontium concentrations, the increase in ATPase level per plant is even more striking than that expressed on a unit protein basis. Since root growth was promoted by some strontium concentrations which did not result in increased ATPase activity, it would seem that the effect of strontium on overall protein synthesis was not identical with its effects on ATPase formation. A calcium- or strontium-activated enzyme system from guinea-pig liver which incorporates a variety of amines into proteins has been reported[6], but no attempt was made to assess the enzymatic properties of the synthesized protein.

The present results provide evidence for a rather striking and unique relationship between two possible roles of calcium and strontium in wheat roots. In one enzyme system (ATPase formation) calcium and strontium, the latter within a limited concentration range, behave in a like manner; whereas their effects on the action (ATP hydrolysis) of the very enzyme the formation of which they promote are directly opposite.

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PSYCHOLOGY

Biology and Schizophrenia

Huxley, Mayr, Osmond and Hoffer[1] have recently discussed transmission of what they consider the schizophrenic genotype. They have advanced the explanation of a simple, single-locus, Mendelian basis for what they regard as 'schizophrenia'. Moran[2], more recently, has combined such a hypothesis with the observations of socio-economic differences in incidence of schizophrenic diagnosis and concluded a socio-economic class-related genetic polymorphism. None of these authors, however, discussed the inconsistent observations regarding distributions of psychiatric diagnoses by different diagnosticians. The studies of Mehlman[3], as well as those of other writers[4–8], have supported doubts regarding the objectivity of recent systems of psychiatric classification and their reliability for the construction of simple, general biological concepts. Psychiatric taxonomy is essentially a symptom-oriented classification scheme, which groups patients according to symptoms rather than according to the disorders underlying the symptoms[9,10]. A simple hypothesis regarding the aetiology of 'schizophrenia', which is based on particular groups of diagnostic data, may partially reflect the concepts of the particular diagnosticians. The inconsistencies in diagnostic distributions and the biological irrelevance of the diagnostic systems may indicate that 'schizophrenia' represents a heterogeneity of disorder constellations rather than a single, universally- and reliably-diagnosed entity. A simple biological theory designed to explain the aetiology of such a category, or class differences in incidence, may be somewhat premature in this context.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, May 23

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2)—Colloquium on "A New Look at Energy Storage".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (joint meeting with the I.E.R.E. Medical and Biological Group, at Savoy Place, London, W.C.2), at 10 a.m. and 2.30 p.m.—Discussion Meeting on "Electrical Aids to the Handicapped".

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Prof. Raoul H. Bott (Harvard University): "The Riemann–Roch Question in Various Guises" (Further lectures on May 24 and 26).*

ROYAL INSTITUTION, LIBRARY CIRCLE (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Mr. W. B. Proudfoot: "Copying Methods: Past and Present".

UNIVERSITY OF LONDON (at the Wright–Fleming Institute of Microbiology, St. Mary's Hospital Medical School, London, W.2), at 5.30 p.m.—Dr. C. E. G. Smith: "The Effects of Civilization on the Viral Zoonoses" (Last of a series of four Almroth Wright Lectures).*

SOCIETY OF COSMETIC CHEMISTS OF GREAT BRITAIN (at the Washington Hotel, Curzon Street, London, W.1), at 7 p.m.—Annual General Meeting.

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.15 p.m.—Mr. G. de G. Sieveking: "In the Tracks of Early Man in Thailand".

Tuesday, May 24

INSTITUTION OF CHEMICAL ENGINEERS (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 2.30 p.m.—Meeting on "The Chemical Engineer and Mastery of Information". Mr. R. Snel: "Information Retrieval and Decision Taking"; Dr. W. E. Batten: "Information System as an Essential Tool for the Engineer"

OPERATIONAL RESEARCH SOCIETY (at the Royal Aeronautical Society, 4 Hamilton Place, London, W.1), at 4.30 p.m.—Annual General Meeting.

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Anniversary Meeting.

INSTITUTE OF INFORMATION SCIENTISTS (joint meeting with Aslib, at 3 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. Harry K. Bourne: "The Work of the United Kingdom Scientific Mission".

UNIVERSITY OF LONDON (at the Institute of Archaeology, 31–34 Gordon Square, London, W.C.1), at 5.45 p.m.—Mr. C. A. Burney: "New Evidence on Urartu from the Excavations at Kayalidere, 1965".*

INSTITUTE OF SCIENCE TECHNOLOGY, LONDON BRANCH (at Guy's Hospital Medical School, St. Thomas' Street, London Bridge, S.E.1), at 6.30 p.m.—Mr. I. Roberts: "Immunology".

Wednesday, May 25

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Dr. W. H. Tonking: "Project Mohole—Exploring the Earth's Crust".

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Annual General Meeting, followed by Miss Mamie Olliver: "Basic Research in the Food Industry".

Wednesday, May 25—Thursday, May 26

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N.W.1), at 10.30 a.m. daily—Symposium on "Echinoderm Biology".

Thursday, May 26

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. L. P. Kadanoff and Prof. A. B. Pippard, F.R.S.: "Ultrasonic Attenuation in Superconductors"; Mr. S. G. Lipson: "The Righi–Leduc Effect in Copper at Low Temperatures".

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Informal Discussion on "Computer Developments as a Tool for Civil Engineering Design and Control" introduced by Mr. J. R. H. Otter,

UNIVERSITY OF LONDON (at the Royal Free Hospital School of Medicine, 8 Hunter Street, Brunswick Square, London, W.C.1), at 5.30 p.m.—Dr. Frederick S. Philips (Sloan–Kettering Institute for Cancer Research, New York): "Cancer Chemotherapy: Inhibition of DNA Synthesis and Cell Death In Vivo".*

Friday, May 27

UNIVERSITY OF LONDON (at Queen Mary College, Mile End Road, London, E.1), at 5.30 p.m.—Prof. J. G. Valatin: "The Scientist's Endeavour to Understand and to Learn" (Inaugural Lecture).*

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

STAFF TUTOR (preferably candidate with a good honours degree in physics) IN THE PHYSICAL SCIENCES IN THE DEPARTMENT OF EXTRA-MURAL STUDIES—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (May 25).

LECTURER or ASSISTANT LECTURER (preferably with an interest in social psychology) IN GENERAL PSYCHOLOGY IN THE DEPARTMENT OF GENERAL STUDIES—The Staff Officer, The University of Aston in Birmingham, Gosta Green, Birmingham, 4, quoting Ref. 468/2 (May 26).

TEMPORARY LECTURER IN GEOGRAPHY (physical geography and/or surveying will be of special relevance to the post)—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (May 27).

ASSISTANT EXPERIMENTAL OFFICERS or EXPERIMENTAL OFFICERS (2) (with a degree or equivalent in science or horticulture), one for work on herbicides in the Weeds Section (male only), and the other to assist in plant breeding programmes and to do cytological checks on breeding material—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (May 28).

CHAIRS (2) OF PHILOSOPHY—The Assistant Registrar (Establishment), The University of Sussex, Stanmer House, Stanmer, Brighton, Sussex (May 28).

LECTURER and an ASSISTANT LECTURER IN THE DEPARTMENT OF BIOLOGICAL CHEMISTRY—The Secretary, The University, Aberdeen (May 28).

LECTURER (preferably with an interest in transition metal or co-ordination chemistry) IN THE DEPARTMENT OF INORGANIC CHEMISTRY—The Registrar, The University, Newcastle upon Tyne, 2 (May 28).

LECTURER (with qualifications in physiology or applied physiology) IN PHYSIOLOGY—The Registrar, Welsh College of Advanced Technology, Cathays Park, Cardiff, quoting Ref. No. CN756 (May 28).

SENIOR LECTURER or LECTURER IN THE DEPARTMENT OF PHYSICS—The Registrar, University of Keele, Keele, Staffordshire (May 28).

ASSISTANT LECTURER (Probationary) or LECTURER (man or woman, graduate, with special interests in stratigraphy, including palaeontology and/or sedimentary geology) IN GEOLOGY—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (May 30).

LECTURER or ASSISTANT LECTURER IN THE DEPARTMENT OF PATHOLOGY,—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (May 30).

ASSISTANT LECTURER IN PURE OR APPLIED MATHEMATICS—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (May 31).

ASSISTANT LECTURER (with interests in formal logic) IN THE DEPARTMENT OF PHILOSOPHY—The Registrar, The University, Leicester (May 31).

CHEMISTS (preferably with research experience) for fundamental work on brewing materials and fermentation—The Director, Brewing Industry Research Foundation, Nutfield, Redhill, Surrey (May 31).

DEMONSTRATOR/SENIOR DEMONSTRATOR IN GENETICS—The Registrar, The University, Newcastle upon Tyne, 2 (May 31).

LECTURER and an ASSISTANT LECTURER IN THE DEPARTMENT OF COMPUTER SCIENCE—The Registrar, University of Strathclyde, George Street, Glasgow, C.1 (May 31).

PRINCIPAL OF THE NORTH WEST KENT COLLEGE OF TECHNOLOGY—The Clerk to the Governors, Divisional Education Office, Essex Road, Dartford (May 31).

ASSISTANT LECTURER IN ZOOLOGY—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (June 1).

ASSISTANT DEPARTMENTAL LIBRARIAN (preferably with a degree) IN THE DEPARTMENT OF GEOGRAPHY—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (June 2).

ASSISTANT LECTURER (preferably with experience in the application of Mossbauer or E.S.R. techniques to the study of solids) IN THE DEPARTMENT OF PHYSICS—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (June 4).

ASSISTANT LECTURER (with a particular interest in personality, abnormal or social psychology) IN PSYCHOLOGY—The Registrar, University College of Swansea, Singleton Park, Swansea (June 4).

LECTURER IN MATERIALS SCIENCE IN THE FACULTY OF APPLIED SCIENCE, Queen's College—The Secretary, University of St. Andrews, Queen's College, Dundee (June 4).

ASSISTANT LECTURER or LECTURER (graduate in chemistry or pharmacy) IN PHARMACOLOGICAL or PHARMACEUTICAL CHEMISTRY IN THE DEPARTMENT OF PHARMACOLOGY—The Registrar, The University, Leeds, 2 (June 6).

TUTORIAL STUDENT IN THE DEPARTMENT OF MECHANICAL ENGINEERING—The Registrar, King's College (University of London), Strand, London, W.C.2 (June 6).

RESEARCH ASSISTANT (preferably graduate in psychology or zoology) IN THE LABORATORY OF EXPERIMENTAL PSYCHOLOGY for duties which will include carrying out behavioural experiments on various species of animals—The Assistant Registrar (Establishment), The University of Sussex, Stanmer House, Stanmer, Brighton, Sussex (June 7).

JUNIOR FELLOW (with a university degree) IN THE DEPARTMENT OF VETERINARY MEDICINE—The Registrar, University of Bristol, Senate House, Tyndall Avenue, Bristol, 8 (June 10).

JUNIOR LECTURER (graduate with medical qualifications) IN ANATOMY—The Registrar, King's College (University of London), Strand, London, W.C.2 (June 10).

LECTURER (with special interests in the theoretical aspects of the subject) IN COMPUTATIONAL SCIENCE; and a LECTURER or ASSISTANT LECTURER (preferably with interests in numerical analysis of fluid dynamics) IN APPLIED MATHEMATICS in St. Salvator's College—The Secretary, University of St. Andrews, College Gate, St. Andrews, Fife, Scotland (June 10).

ASSISTANT or ASSISTANT LECTURER (with a higher degree with a minimum of three years' post-graduate experience) IN EXPERIMENTAL PHYSICS to take part in the teaching work of the department and to undertake research—The Secretary and Bursar, University College, Earlsfort Terrace, Dublin 2, Republic of Ireland (June 11).

CHAIR OF STRUCTURAL ENGINEERING WITHIN THE DEPARTMENT OF CIVIL ENGINEERING—The Registrar, University of Strathclyde, George Street, Glasgow, C.1 (June 11).

LECTURER IN BIOCHEMISTRY—The Registrar, King's College (University of London), Strand, London, W.C.2 (June 13).

LECTURER IN THE PHILOSOPHY OF SCIENCE—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (June 13).

RESEARCH ASSISTANTS (with a good honours degree in an appropriate subject and some research experience) for studies in the biophysical fluid mechanics field under the supervision of Dr. R. L. Whitmore—The Registrar, The University, Nottingham (June 15).

LECTURER (with special interests in physical chemistry) IN THE DEPARTMENT OF CHEMISTRY, University of Tasmania—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, June 17).

TECHNICIAN (with L.R.I.C. or similar qualifications, experience in instrumental analysis and maintenance, especially in the field of gas chromatography, and preferably some knowledge of instruments, electronics and glass-blowing) to work in the chemical laboratory which is engaged on research into plant nutrition and soil problems, including a study of disease resistance in plants—The Director, Cawthron Institute, P.O. Box 175, Nelson, New Zealand (June 18).

SENIOR LECTURER or LECTURER (medically qualified with adequate experience) IN THE DEPARTMENT OF CHEMICAL PATHOLOGY, University of Ibadan, Nigeria—The Secretary, Senate Committee on Higher Education Overseas, University of London, Senate House, London, W.C.1 (June 20).

LECTURER (with a superior academic record in mechanical, civil or electrical engineering) IN ENGINEERING at the University of New South Wales,

Broken Hill Division—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, June 30).

RESEARCH ENTOMOLOGIST (with a degree in entomology or equivalent with specialization in entomology) at the Tussock Grasslands and Mountain Lands Institute, to study the effect of insects on the vegetation of eroded areas in the Southern Alps—The Registrar, Lincoln College, Canterbury, New Zealand (June 30).

SENIOR LECTURER IN BACTERIOLOGY—The Secretary, The Medical College of St. Bartholomew's Hospital, West Smithfield, London, E.C.1 (June 30).

ASSISTANT DIRECTOR (with a degree in agricultural science or in sciences basic to it, preferably experience in soil fertility work, a reading knowledge of languages and editorial experience, and the ability to write good concise English) of the Commonwealth Bureau of Soils, Rothamsted Experimental Station, Harpenden, Herts—The Secretary, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Bucks (July 15).

SENIOR LECTURER (with special interests in physical oceanography, including dynamic oceanography and/or marine geology) IN OCEANOGRAPHY—The Registrar, University College of Swansea, Singleton Park, Swansea (July 30).

ASSISTANT EXPERIMENTAL OFFICERS or EXPERIMENTAL OFFICERS (at least 21 with a pass degree or an appropriate Higher National Certificate) to assist in developing and producing radioisotopes in variety to meet the worldwide growth in sales of radio-chemicals—The Personnel Officer (Ref S.8934), The Radiochemical Centre, Amersham, Bucks.

CHAIR OF CHEMISTRY; and CHAIR OF ENGINEERING (Chemical and Materials) at the University of Auckland, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1.

LECTURERS (preferably physicists who are interested in continuing research in the fields of X-ray crystallography, infra-red spectroscopy, N.M.R., or microwave physics) IN THE DEPARTMENT OF PHYSICS—The Registrar, Portsmouth College of Technology, Hampshire Terrace, Portsmouth, Hampshire.

PROFESSOR OF CHEMICAL ENGINEERING—The Registrar, Bradford Institute of Technology, Bradford, 7.

PROFESSOR OF PHYSICS—The Staff Officer, The University of Aston in Birmingham, Gosta Green, Birmingham, 4, quoting Ref. 470/2.

SCIENCE GRADUATE (interested in publishing) to join the editorial staff of *Metallurgical Abstracts*—The Secretary, The Institute of Metals, 17 Belgrave Square, London, S.W.1.

SENIOR LECTURER (with an honours degree in biochemistry and preferably some research and/or industrial experience) IN BIOCHEMISTRY; and a LECTURER (honours graduate preferably with some research and/or industrial experience) IN MICROBIOLOGY—The Registrar, College of Technology, Park Square, Luton.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Machine Tool Technology: Decisive Factor in Industrial Development. By Prof. Janez Peklenik. (An Inaugural Lecture delivered in the University of Birmingham on 2nd December 1965.) Pp. 12. (Birmingham: The University, 1966.) 2*s*. 6*d*. [253

Pig Trypanosomiasis in Tropical Africa. By Dr. L. E. Stephen. (Review Series No. 8 of the Commonwealth Bureau of Animal Health, Weybridge.) Pp. ix+65. (Farnham Royal: Commonwealth Agricultural Bureaux, 1966.) 15*s*.: 2.25 dollars. [253

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Science Museum. Chemistry 1: Chemical Laboratories and Apparatus to 1850. By Frank Greenaway. (A Science Museum Illustrated Booklet.) Pp. viii+20. (London: H.M. Stationery Office, 1966.) 5*s*. net. [253

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Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Bulletin 125: Stratigraphy of the Rocky Mountain Group in the Southeastern Cordillera of Canada. By D. K. Norris. Pp. 82+3 plates. 2.75 dollars. Memoir No. 335: Vancouver North, Coquitlam, and Pitt Lake Map-Areas, British Columbia, with special Emphasis on the Evolution of the Plutonic Rocks. By J. A. Roddick. Pp. xiv+276 (51 plates). 5.25 dollars. Paper 64–23: Groundwater Resources of Winnipeg Area, Manitoba. Pp. v+30. 75 cents. Paper 66–1: Report of Activities, May to October 1965. Edited by S. E. Jenness. Pp. xiii+210. 75 cents. (Ottawa: Queen's Printer, 1965 and 1966.) [83

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A GOOD START

THERE are two contradictory readings of the first report of the Council on Scientific Policy, and only time will tell which is correct*. Thus it may be that the newly appointed Council has been so overwhelmed with a sense of responsibility that it has been hamstrung by respectability, and easy prey to those appeals for sober statesmanship with which H.M. Treasury is accustomed to silence the most gullible of its pensioners. Alternatively —and this is the cheerful view—the Council has calculated that the real battle to ensure the needs of science and technology in Britain will come not now but next year or the year after. In that spirit, so the argument will go, the immediate need is not a spate of polemics but the establishment of a sound philosophical base for struggles ahead. (Yet a third interpretation of the first report is that the Council has been so attracted by the intellectual delights of what is sometimes called the 'science of science' that it conceives of itself as a kind of academic institution—a rival, perhaps, to one of the university units the formation of which is described on p. 885 of this issue.)

The passive interpretation is prompted by the Council's own statement of what science policy is for, and by some ominously ambiguous pronouncements in the report. By emphasizing that the object of the science policy should be "to maintain the environment necessary for scientific discovery", the Council comfortably echoes the Haldane doctrine, but also echoes the more recent statements of Mr. Quintin Hogg when he was Minister for Science about the need that those who administer science should regard themselves as midwives and not as procreators.

Mr. Hogg, of course, was right. The Government should not set itself up as competent to decide what science is good and what science is bad. But there is no obvious reason why the Council for Scientific Policy should suffer the same restraints. On the contrary, the Council should be ready to become full-bloodedly partisan when circumstances require that it should do so. From time to time, it will find it necessary to defend the interests of the scientific community with nothing but conviction for support.

In this spirit, a lack of objective proof that a rapid expansion of postgraduate education in science would be a social and economic boon should not inhibit those concerned with the health of science (and the British economy) from proclaiming this. The Council is strangely silent on this vital point. Yet if it chooses to fight only battles which can be won by pure reason, it cannot hope to "ensure that there is balance between fields and that others are not avoidably neglected". If funds are going to be scarce, the Council will have to become skilled at inspired guesswork.

The view that the Council's first statement implies passivity is only one side of the coin, and the least attractive. The other is that the Council is seeking methodically to prepare itself for a vigorous future by creating the instruments of overwhelming authority. To be fair, this is the interpretation which the Council itself makes. And the first report is plainly an interim document—more a prospectus than a report. The consequences of the Council's forward planning are likely to be admirable. It will be splendid if the Council succeeds in developing criteria on which the making of decisions about the management of science can be predictably and soundly based. Nobody will deny the potentially great value of the proposed studies of the interactions between the different components of the British educational system and the stock of skilled manpower in the United Kingdom. Then the Council's style of working has a great deal to commend it. Evidently there will be a steady output of detailed and therefore convincing studies of particular problems—some of them commissioned from outside. Then the Council's devotion to principle has not prevented it from tackling problems such as the need to provide adequate computer facilities at British universities and, now, the paradox that Britain has a flourishing tradition of research in molecular biology, but much less university teaching in this field than circumstances require. (It will be fascinating to see how much of this trouble is traced to the fact that the Medical Research Council's Laboratory for Molecular Biology at Cambridge is not a part of the University.)

It would, of course, be easy to compile a list of similar problems that would keep the Council's members busy for a decade to come. There are a great many educational questions, including the desirability (or otherwise) of a four-year undergraduate course, and the problem of how best to marry university courses the ends of which are vocational (such as engineering, medicine and architecture) with the need for a liberal scientific education. There are problems concerned with research fellowships and studentships; rightly or wrongly, the Science Research Council's rules do not always seem sensible to those most closely affected. Post-doctoral Fellows sometimes find themselves unable to continue working in the most sensible places. Then there is the problem of what to do with the scientific literature and what attitude to take towards the proposals for the management of the literature by computers. And what about some way of making easier the transfer of people between industry and university research and teaching, or the need somehow to predict what problems and opportunities would be thrown up in Britain if the British Government should join the Common Market?

The truth is that the practical problems are so many that they will always multiply so as to outstrip the Council's resources, intellectual and material. In the circumstances, it is wise to emphasize its long-term objective of creating a framework within which decisions can be made more accurately and economically. Within such a framework, pure reason cannot be a sufficient guide. In fixing the balance between academic and more practically directed research, for example, it is improbable that everything can be determined by calculations of an economic kind. It will also be necessary to take a view about the long-term importance of science in British society.

In ways like these, the Council will have to carry the scientific community with it. The most obvious gap in its thinking so far is that it does not regard itself as the focus for public debate within the profession. But, just as a democratic government cannot move without public support, so this Council cannot hope to find criteria for making decisions without convincing working scientists

* See also pp. 877 and 885 of this issue.

that they are sound. The simplest and the best way of doing this would be to stimulate public debate among all professionally interested parties. The recent growth of interest in science policy at the universities shows that the time is ripe. Yet the Council, which is evidently anxious to reconcile the general public (including the Treasury) to the cost of science, is surprisingly cavalier in its apparent assumption that scientists will follow where it leads. Indeed, by assuming that it will usually be for research councils to make detailed decisions on the spending of money, the Council is assuming that the expert advisory committees which decide these things are fully in command of professional opinion. But is this necessarily the case, especially when the proceedings of the committees are in private and not in public? Indeed, unless the Council can set its own methodical and high-principled stamp on the legion of committees which actually spend money, there is a danger that good management at the top may be accompanied by what seems like arbitrariness lower down.

LONG MEMORIES

MR. HAAKON CHEVALIER has undermined a strong case with his book *Oppenheimer—The Story of a Friendship* (Deutsch, London, 25*s*.). The objective is an account of the relationship between Mr. Chevalier and Dr. Robert Oppenheimer when Mr. Chevalier was a professor of French at Berkeley and when Dr. Oppenheimer had not yet become the director at Los Alamos. The period was significant for both parties because it provided the raw material on which Oppenheimer later compromised Chevalier with the security authorities in the United States. Mr. Chevalier's subtitle is, of course, ironical.

By now the story of this shabby incident is a part of folk-lore. It was first brought into the open in 1954 when the Personnel Security Board of the U.S. Atomic Energy Commission examined in public the reasons why it should or should not grant a security clearance to Dr. Oppenheimer, the head of the Institute of Advanced Studies at Princeton and then a principal consultant to the Atomic Energy Commission. In the course of the evidence it emerged that Chevalier had informed Oppenheimer of a suspicious attempt to obtain secret information in the early years of the Second World War. Oppenheimer retailed this tale to the security people incorrectly. As he put it himself, he told a "cock and bull story". One consequence was to compromise Chevalier and his career. What surprised the hearings in Washington in 1954, and what rankles with Chevalier, is that Oppenheimer did not take steps to make good the damage done.

All this is now part of the public record, and a small part of the poignant history of Oppenheimer's security clearance. Chevalier's book has nothing new to say. Bitter accusations were predictable, but the result is frequently a form of words too unreflective to be communicative. Chevalier tells his tale without much feeling for the emotions concerned—even his own. When he might have thrown light on the character of his relationship with Oppenheimer, or on the tenor of their political discussions, he tends to write about the salad they had for dinner. The result is that his attempt to analyse Oppenheimer's personality reads like a briefing for a puppet show. Chevalier may feel better for having written his book, but few of his readers will be edified.

MALAISE OF CULTURE

Science and Culture

A Study of Cohesive and Disjunctive Forces. Edited by Prof. Gerald Holton. (The Daedalus Library, Vol. 4.) Pp. xxxiii+348. (Boston, Mass.: Houghton Mifflin and Company, 1965.) 6 dollars.

EVERYONE knows that the world is out of joint, but not everyone takes this to heart so much as the physicists, whose brainchild, the bomb, has brought them a profound sense of guilt. What has gone wrong? Is it nothing more than an estrangement between C. P. Snow's 'Two Cultures'? Or has science run amok and technology gone berserk? Is culture suffering from a degenerative disease called 'applied science'? Or, on the contrary, is it precisely this that we so desperately need, namely, a universal acceleration of the pace of scientific advance, which alone can triumph over the hydrogen bomb, overpopulation, and the gap between affluence and poverty? Such soul-searchings led Gerald Holton, professor of physics at Harvard, with fourteen co-authors, among them Harvey Brooks, R. Dubos, H. Marcuse, D. K. Price and E. Weil, to reflect on the mutual relationships of science and culture in the world of to-day.

Their contributions fall into three sections. The first is concerned with definitions and history. The second, more provocatively, deals with elements making for cultural coherence, and the third comes to grips with elements making for disjunction and alienation.

The second section opens with a study of the diffusion of basic concepts from one science to another, and from science to the arts and humanities. Thus the images and vocabulary of chemical equilibrium theory constantly recur in descriptions of social and economic phenomena; the idea of relativity has spread from physics to anthropology, psychology and history; and the notions of feedback and information are common to many disciplines in which 'environment' is equated with 'input' and decision with 'output'. Furthermore, there is a general acceptance that the observer interacts with the system under observation, whether the system be physical, historical or political. And there is a parallel between the abstract world picture provided by physics and the trend to abstraction in modern art and poetry, a parallel which contemporary representatives of optical and kinetic art, such as J. R. Soto, would no doubt warmly endorse. These reflexions lead Prof. Brooks to ask whether a psychologist, by studying present-day tastes in poetry, could predict the kind of theories likely to be acceptable in elementary particle physics, or vice versa.

In the same section Prof. Holton develops his challenging thesis that when one looks closely at the manner in which scientific hypotheses are initially formed, tested, and accepted or rejected, the alleged duality of culture seems to vanish. A three-dimensional propositional space, he suggests, may be used to chart the components of a scientific theory: the x-axis for empirical data, the y-axis for the analytical-heuristic dimension, and the z-axis for the thematic component—that which is rooted outside science.

Gloomy thoughts begin to emerge in the third section: modern science is an ally of a monstrous technology that threatens man's existence; and it is the growth medium of an equally monstrous ideology that engulfs the life of the mind. In the United States, we are told, the means of society are unrelated to its professed ends. American culture, because of the "permanent mobilization of the people for the eventuality of nuclear war", is swallowed by civilization. As a result, education for intellectual and emotional independence is "an all but subversive programme which involves violation of some of the strongest democratic taboos".

A number of contributors are profoundly critical of the establishment. They urge us to specify the criteria

whereby we choose between different value systems. So long as we treat science as value-free we shall continue to have a bad methodological conscience when considering questions of meaning in human life. Unaided science cannot provide a political strategy for the guidance of a blind technology. There is evidently a growing scepticism about the power of science, by itself, to improve the human lot. If there is a dualism, it lies in our two-fold image of man: on the one hand, "the lowly puppet-like figure described by science" and, on the other, "the bold, pioneering, self-possessed and self-determining fellow who invented science and appears to control the scientific establishment".

Science and Culture concludes with a report on the integrity of science prepared by a committee of the American Association for the Advancement of Science. This important document, which should be studied by statesmen and administrators as well as by scientists, puts a finger on the social hazards of experimental interference with nature, and it draws attention to the need for assembling facts relevant to value judgments. How, for example, can the benefits of nuclear testing be balanced against increased medical hazards from fall-out? The need for foresight is highlighted by glaring failures, exemplified by the adverse effects of large-scale spraying of pesticides. Above all, the report emphatically insists on free scientific debate which, for example, did not precede the *Starfish* experiment in which a 1·4 megaton bomb was detonated with melancholy repercussions now widely familiar.

Students of this book may feel it necessary to read between the lines. For it is hard to escape the impression that some contributors, skating on thin ice, would have liked to refer openly to the war in Vietnam or to the absence of China from the United Nations. If they refrained from doing so, it is because they are scholars, not politicians. Nevertheless, they see the writing on the wall and they have made it possible for others to share their vision.

J. COHEN

WOMEN IN SCIENCE

Women and the Scientific Professions

Edited by Jacquelyn A. Mattfeld and Carol G. Van Aken. (The M.I.T. Symposium on American Women in Science and Engineering.) Pp. xvii+250. (Cambridge, Mass., and London: The M.I.T. Press, 1965.) 6.95 dollars; 53*s*.

IN October 1964, the Association of Women Students of the Massachusetts Institute of Technology sponsored a symposium on the problems faced by American women in the scientific and engineering professions. *Women and the Scientific Professions* gives an account of the proceedings. The contributors included psychologists and sociologists as well as scientists. The welcome extended by the President of the Institute, where the enrolment of women is increasing, is encouraging.

The account is divided into four parts. The first, a discussion on the commitment required of a woman entering a scientific profession, was begun by Bruno Bettelheim, professor of education in the University of Chicago. The moderator was Mrs. Bunting, who as president of Radcliffe has promoted a scheme to help women with families to pursue advanced projects on a part-time basis. Prof. Bettelheim gives his reasons for accepting the invitation, adding disarmingly, "I knew it might put me out on a limb". He is deeply concerned with the adjustment of all human beings to living with modern technology. It is probably natural that I find myself in agreement with the comments of the physicist, Chien-Shiung Wu, and the anatomist, Mrs. Nachmias, on his contribution to the specific problems of a woman's commitment to her work. I am glad to see the point made that, although the majority of girls may not want to be scientists, those who do should not be made to feel odd. There is a realistic article by Mina Rees, who urged that women "must bring to the task the competence and objectivity that the task requires". Rita McCabe, of I.B.M., discussed the position of women in the computer industry, a field new to everyone and in which women have not been hampered by a long tradition that it is for men only. Dorothy Simon, vice-president of Avco Defense and Industrial Products Group, compared the position of American women in science and engineering with that of women in other countries, as deduced from the representation of, for example, the United Kingdom, India and Japan at the first International Conference of Women Engineers and Scientists held in New York in June 1964. This volume will be of interest to all those who hope to attend the second of these conferences, which is to be held in Cambridge in 1967.

The second part, on "Who wants Women in the Scientific Professions?", begins with an extremely interesting study by Alice Rossi of "Barriers to the Career Choice of Engineering, Medicine or Science among American Women". This occupies more than a quarter of the book and should be useful for reference. It is followed by a valuable article by the chairman of the Corporation of the Massachusetts Institute of Technology, who gave evidence from several sources that the proportion of women in professional occupations is dropping and that few women reach the executive and more highly paid ranks of their profession. Also, out of the 707 members of the National Academy of Sciences, only five are women. This is lower than the corresponding ratio among Fellows of the Royal Society. Both he and Prof. Jessie Bernard made it clear that it is idle for women to talk of discrimination, when there are not enough women with high qualifications. Richard Bolt gave a factual account of the present situation in industry and government.

Part 3 is a discussion on the case for and against the employment of women and includes contributions from two men experienced in recruitment in industry and the civil service. It emerges that it is only in quite recent years that the United States Civil Service has required that all appointments be made regardless of sex, with very rare exceptions.

The closing part consists of addresses by Mrs. Gilbreth, an example to us all of a woman who lives the lives of several ordinary women, and Erik H. Erikson, professor of human development at Harvard. He asked the delegates whether they had not listened too much and said too little and it would be interesting to know what was said from the floor. The whole volume is characterized by an objective approach to the problems involved, neither minimizing the difficulties nor presenting them as insuperable.

With a reasonably careful search I have not found out whether the credit for the mathematical gibberish on the dust jacket goes to a man or a woman, but the recurrence of "$m = c^2$" rather offends the eye of a physicist.

BERTHA JEFFREYS

CANCER BIOCHEMISTRY

Biochemical Approaches to Cancer

By Dr. Eric Reid. (The Commonwealth and International Library of Science, Technology, Engineering and Liberal Studies: Biochemistry Division.) Pp. xi+198+3 plates. (London and New York: Pergamon Press, Ltd., 1965.) 21*s*. net.

TWELVE years have elapsed since the publication of the second edition of the late Dr. J. P. Greenstein's classic, *Biochemistry of Cancer*. This work was authoritative, comprehensive and well documented. The advances in biochemical knowledge since 1954 make a new work on the subject most desirable. Dr. Reid's *Biochemical*

Approaches to Cancer, despite its small size, must be considered on its merits as a possible successor to the earlier work.

The first three chapters are concerned with the nature of cancer, how it is induced and the areas which are open to biochemical experimentation. Particular emphasis is laid on the necessity of comparing like with like (that is, tumours with their cell-types of origin) in biochemical investigations, even though this limits the field almost entirely to the comparison of hepatomas with normal and preneoplastic liver. The need for selecting the viable parts of a tumour is emphasized even though (p. 24) ". . . in the special case of hepatomas and perhaps of other tumours . . . necrosis may not invariably connote that the cell is dead". A short chapter follows on the effects of a tumour on its host.

The major part of the book is concerned with a comparison between the metabolism of carbohydrates, fatty acids, proteins and nucleic acids in tumours and in comparable normal tissues. The volume of fact included in this discussion is so great as to make the text too much of a catalogue to be easily readable. The lack of selectivity is symptomatic of the difficulty biochemists have in deciding changes which are and are not significant to the neoplastic process. In conclusion, those biochemical control mechanisms which are modified in cancer and the effects of cancer chemotherapeutic agents are considered.

The least satisfactory feature is the bibliography. This consists of fifty references to books and reviews followed by nearly a hundred and seventy more to 'key' papers. It is explained in the preface that a complete list of references would have been too expensive and that readers, with patience, will be able to find the references they need from the abstract literature. Since most workers are likely to want to use a book as tightly packed with facts as this as a guide to the original literature, the system adopted is unfortunate.

There is a short but adequate biochemically orientated index. It is felt that the author has attempted to write a comprehensive text on the subject in the space which would normally be allocated to a more superficial account.

D. B. CLAYSON

MEDICAL CARE

Medicine in Transition

By Dr. I. Galdston. Pp. xii + 220. (Chicago and London: The University of Chicago Press, 1965.) 5.95 dollars; 44*s*. 6*d*.

EVERYONE knows that medicine is in transition; but some doubt still clings to the questions whence it came, where it has got to, and whither it is going.

As an erudite man with a strong interest in and feeling for medical history, the author of *Medicine in Transition* is at his best in dealing with the first of these questions. In his chapter on "Patterns of Practice" (pp. 92–115), he brings out the antiquity of present-day forms of practice, finding in Greek times not only a salaried service, but also demonstrations of "free choice, fee for service, the exclusion of all parties intervening between patient and physician, and the unbreachable confidential nature of patient-physician relations"—things which have more recently been put forward as "fundamental principles, the neglect or subversion of which it is thought degrades the practice of medicine and radically reduces its effectiveness". He develops (pp. 100–105) Henry Sigerist's concept that "Social insurance is a result of the industrialization of the world". The paternalism (sketchily exercised) of feudalism was replaced by association of workers for mutual aid in sickness, and later by assumption of this responsibility by the State. We can accept the force of this analysis without necessarily following Dr. Galdston in his further implication (Chapter 9) that the growth of the affluent society will once again diminish the need for State participation in medical care. It is also something of an injustice, one hopes, to our professional forebears to write (p. 110) that "The vast majority were treated by 'doctors of the poor', most of whom were, in the extended sense of that term, also likely to be poor doctors".

In his analysis of the present state of medicine, Dr. Galdston emphasizes the increasing cost of medical care, the growing emphasis on hospital practice, the rise of specialization. These are not unfamiliar themes, but his account of them is factual and of value as a picture of present-day medicine in the United States, where the submergence of family practice by specialist practice has gone much further than in Britain. Dr. Galdston reveals himself as a strong defender of private medicine against the "threatened bureaucratization of medicine" (p. 206). Before accepting the sum of his advocacy, I would suggest three criticisms. Although not limited in time, he is parochial in space; and he generalizes what are essentially local problems, as in his long chapter on "Organized Labor and Medicine" (pp. 116–133). Although medically qualified, he does not appear to have engaged to any large extent in the actual practice of medicine. Finally, his use of the National Health Service in Britain as a foil is weakened by his dependence for information on recognized critics of the Service. As Lord Cohen says in his foreword, "the practice of medicine cannot be isolated from the social and political philosophy of a nation", and "medicine is but one limb of the body politic". It perhaps follows from this that the real determinants of the future pattern of medical practice will be social and not strictly professional.

In looking to the future, Dr. Galdston calls for a new pattern of medical education. His central theme is summarized on p. 167, where he sees the doctors of the future as "divisible into two groups, not as they are today into general practitioners and specialists, but rather into those trained and expert in dealing with serious types of episodic and emergency diseases and disorders and those trained and expert in the practice of maintenance medicine". The latter group are "as much involved in the normal as in the pathological", and on p. 174 he emphasizes the importance of nutrition and mental hygiene. This outlook he traces back to Hippocrates. Once more, I find myself a sceptic. The practicability of educating such people is not discussed; and I would even question their value. As Celsus said, "A man in health should be under no obligatory rules, and have no need, either for a medical attendant or for a rubber and annointer". Perhaps Americans have ears keenly attuned to the exhortations of the prophets of positive health; but that figure of stolid sense, the British working-man, is jealous of the fifth freedom, to do things that we are told are bad for us. For example, the evidence of association between smoking and lung cancer is enough to have made many doctors give up smoking; yet the expenditure on tobacco by the general population increases. If people could be persuaded to lead healthier lives, they would no doubt be the better for it, if not necessarily the happier; but I doubt the expediency of educating large numbers of doctors to tell them what to do. It is manifest that sanitarians have made a massive contribution to the health of us all; but they have done this by manipulating the environment, and not by exhorting individuals to lead better lives.

This speculation looks to me a piece of harmless idealism; and I would like in conclusion to return to my deprecation of Dr. Galdston's obvious dislike of a free health service. To his patent belief that the cost of a commodity increases its value, I would oppose the equally emotional and unprovable beliefs that it is bad enough to be ill without having to pay for it, and that one can accept free help from a fellow-man without respecting him less than if he charged a fee.

DOUGLAS A. K. BLACK

SCIENCE POLICY FOR BRITAIN

THE first report of the Council for Scientific Policy is concerned with the definition of objectives for the years ahead rather than with the solution of specific problems. In his introduction to the report (*Report on Science Policy*, H.M.S.O.), the chairman of the Council, Sir Harrie Massey, states that he and his colleagues have not found it easy to "ensure the development of science in an environment of relatively slow economic growth". He says that there must be a fuller understanding of the mechanism and the benefits of scientific growth, and describes his Council's first report as "our preliminary analysis".

The Council explains its concern for the principles of science policy by saying that "science needs confidence for its development", which means, in turn, "widespread public confidence in science as a necessary, desirable and well-managed investment for the future, and a main enablement for the attainment of national objectives". It goes on to say that the growth of the scale of science has made a self-conscious concern for its conduct more urgent, and announces, in a somewhat delphic manner, "the temptation may have to be resisted to throw all our limited resources into the exploitation of present knowledge, thus cutting back our capacity to advance in the future".

Strictly speaking, the Council is concerned simply to advise the Secretary of State for Education and Science, which implies responsibility for the research councils and a right to a hearing from the University Grants Committee.

At the beginning of its first report, the Council sets out to exorcize "the misconception that the advance of scientific knowledge itself can be directed from the centre. This would be to misunderstand the original and spontaneous nature of science. The advance of scientific knowledge cannot solely be achieved by the arbitrary selection of national goals and by committing resources of men and money to them".

The objective of science policy is not to direct the advance of science, but to "maintain the environment necessary for scientific discovery; to ensure the provision of a sufficient share of the total national resources; to ensure that there is a balance between fields and that others are not avoidably neglected; to provide opportunities for inter-fertilisation between fields, and between the scientific programmes of nations". It is for universities and research councils to decide what scientific objectives to aim at. The task which the Council sets itself is to look ahead nationally and internationally, to relate scientific activities to others competing for resources, and to "provide the basis for national acceptance of the scale of resources which are needed for the healthy evolution of science".

The Council refers to the recent argument about the proper level of government expenditure on research and development by the understatement that "it has been represented to us that the capacity of the national economy will not permit the growth of research council expenditure at present rates—about 13 per cent annually in real terms, or a doubling every six years—within the limits of planned national expenditures. This situation was not before our predecessors, and so far as we are aware is not yet faced by other countries in the van of scientific advance". The Council accepts that there must at some stage be a levelling off. "The question at issue is when, and at what rate, and on what criteria, the levelling-off of the growth rate should take place". With something akin to resignation, the Council goes on to say that a constructive solution should be one that maintains the morale of scientists and the vitality of science even on "relatively stable economic resources", which in turn implies greater flexibility in the management of research and development.

The Council has a powerful argument in support of planning ahead. In discussions with the research councils, it has apparently emerged that existing commitments to the recipients of grants would make necessary a substantial increase of expenditure in the financial year 1966–67. The forward commitments of the Medical Research Council are apparently such that even if there were no new grants for biological research in the universities in the current financial year, expenditure in 1966–67 would be 15 per cent greater than in the previous year. The report argues that in such a situation, comparatively small restrictions of the funds available for research can grievously hamper the starting of new projects. (This is an echo of one of the recurrent themes of the recent report of the U.S. National Academy of Sciences on the condition of physics in the United States.) In the event, the Council has settled for an increase in 1966–67 of 18·7 per cent above the £19·07 million spent by the research councils in its charge during 1965–66, although the increase in cost to public funds will amount only to 12·2 per cent in real terms, partly because of the offsetting effect of recent restrictions of capital expenditure.

For the time being, however, the Council is evidently more concerned to establish criteria for determining the strategy of science than with haggling about decimals of a per cent. Thus the Council is working with the research councils on plans to estimate in advance the consequences of any "tapering off" in research expenditure that there may eventually be.

The Council is also planning to keep a careful watch on the development of higher education in science, and it holds that "one of the important parameters for scientific growth should be the expansion of university teaching functions in science and technology, especially at the more advanced levels . . . we consider that the volume of research conducted in universities should keep pace with the growth in teaching functions".

So as to know what 'rates of growth of science are permissible in the universities and the research councils without creating serious problems for the intake of highly-qualified manpower into industry and for the long-term quality of science teaching in the schools", the Council has persuaded the Committee on Manpower Resources to establish a working party under Prof. Michael Swann.

The improvement of the "scientific environment" is given as another goal. The recommendation of the sub-committee under Prof. B. H. Flowers that expenditure on university computers should run at about £3 million a year has clearly persuaded the Council that there will be benefit in other similar exercises. Thus Sir Gordon Sutherland has been asked to lead a working party to find out whether universities could make better use of the equipment of the public research establishments, while Dr. J. C. K. Kendrew has been asked to lead a group reviewing "the problem of the development of Molecular Biology in the United Kingdom". Finally, Sir Harrie Massey will be the chairman of a group which is continually to review the conduct of international relations in science. In this connexion, the report declares there to be a distinction "between measures which facilitate the natural and essential interchange of men and ideas which is at the heart of science, and the creation of specific co-operative arrangements for the solution of particular scientific problems". The Council is in favour of arrangements of the first kind, but evidently determined to look most critically at the second.

PLASTIC FLOW AND PRESSURE MELTING IN THE DEFORMATION OF ICE I

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THERE has been little work of a unified nature dealing with the deformation of ice over a temperature range from close to the normal melting point (0° C) to say −15° C. Investigations have been made of the creep properties mainly below the pressure melting point by Glen[1], Steinemann[2] and others. They conclude that the behaviour of ice resembles that of a metal: the deformation is plastic and the creep involves dislocation climb similar to that involved in metallic creep. The usual relation for the steady-state creep rate ($\dot{\varepsilon}$) in metals is:

$$\dot{\varepsilon} = A_1 . \sigma^m . \exp\left(\frac{-Q}{RT}\right) \qquad (1)$$

where σ is the applied stress and T is the absolute temperature. Glen investigated the creep of ice in uniaxial compression for stresses up to 10·8 bars and temperatures from −0·02° to −12·8° C. He found that after an initial stage of decreasing creep rate (the range of transient creep, $\dot{\varepsilon}_{tr}$), a steady state was reached in which the creep rate was constant and given by equation (1); the following constants were deduced:

Q = 31·8 kcal/mole

m = 3·2 or 4·2 depending on the particular analysis used

$A_1 = 8{\cdot}9 \times 10^{23}$ years^{-1} . bar^{-m}.

Weertman[3] has proposed a dislocation climb model for hexagonal crystals such as ice I. Assuming the stresses are not too high, he derives a relation similar to equation (1), where Q represents the activation energy for self-diffusion.

On the other hand, some investigations of deformation close to the melting point (0 to −1° C) undertaken by Telford and Turner[4], Kamb and LaChapelle[5] and others, show that at the pressures developed between a metal wire and a block of ice, other processes may be involved. The classical experiments of a similar nature by Faraday and others[6] suggest that, in this restricted temperature range, the main process is pressure melting. For example, using the standard Clausius–Clapeyron equation, the depression in the melting point (dT) for a hydrostatic pressure (dP), when both ice and water phases are present, is given by:

$$\frac{dT}{dP} = \frac{-\Delta V}{\Delta S} = -0{\cdot}0074 \text{ °C/atmosphere} \qquad (2)$$

where ΔV and ΔS are the ice–water changes in volume and entropy. In a direct experiment, Moser[7] found that for a pressure change of about 1 atmosphere this was very close to the observed value. In the literature there are two main alternatives to the classical Clausius–Clapeyron equation. In physical terms, both of these appear to be somewhat unrealistic. In one, due to Poynting[8], it is assumed that the melted ice is instantaneously released from the applied pressure. As a result, the lowering of the melting point is eleven times greater. In the other, Riecke[9] considers the behaviour of ice under a uniaxial compression. He assumes that the external forces do no work and the only effect is due to the release of elastic strain energy in the ice when melting occurs. The depression of the melting point is given by:

$$\frac{\Delta T}{\sigma^2} = \frac{-V_S}{2E.\Delta S} = -451 \times 10^{-9} \text{ °C . bar}^{-2} \qquad (3)$$

where E is the relevant elastic modulus, and V_S the specific volume of the ice phase. This lowering is minute: even for stresses of 1,000 bars, ΔT is less than 0·5° C, whereas for a hydrostatic pressure of the same magnitude the classical Clausius–Clapeyron relation gives a value of ΔT of about −7·4° C.

This article describes a systematic study of deformation of cold ice and ice up to its normal melting point. The deformation of ice in frictional processes has already been discussed[10]. Apart from its theoretical interest, the work may have a useful bearing on glaciological investigations.

The experiments were carried out in a large refrigerator; the temperature in specified regions could be maintained constant and monitored to an accuracy of better than 0·1° C. A study of grain sizes and orientations was also made before and shortly after deformation using polarized light. The specimens were cut with a cold saw and the cut regions (which were disturbed) removed by gentle melting until the specimens were about 1 mm thick.

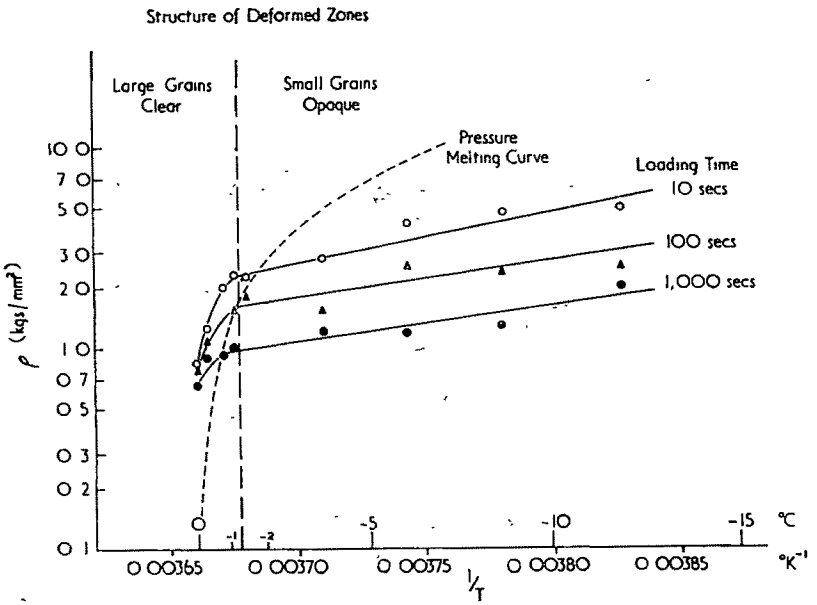


Fig. 1. Figure showing the indentation hardness (p) of ice (logarithmic scale) obtained with a spherical indenter for different loading times and temperatures (reciprocal scale). At temperatures lower than about −1·2° C (that is, to the right of the broken line), the curves are approximately straight in accordance with expected exponential variation (equation 5). At temperatures higher than −1·2° C, the hardness falls abruptly. The dotted line is a phase transition curve indicating $p - 1/T$ co-ordinates at which pressure melting is expected to occur. It is seen to cut the hardness plots at about −1·2° ± 0·3° C. The structures of the deformed zones observed in these two distinct regions are indicated

Deformation Behaviour

A few experiments were performed in which a hard indenter was dragged over the surface of the ice specimen and the width of the resulting groove determined. However, in most of the experiments, indentation hardness measurements were made under conditions of

static loading. A hard steel indenter (sphere, cone, or pyramid) was pressed with a known load for a specified time on to the surface of a smooth block of ice. After the indenter had been removed the diameter of the permanent indentation was measured and the hardness calculated as the ratio of the load to the projected area of the indentation. Sections of the ice were cut through the indentations as already described and examined through crossed 'Polaroids'.

Typical results are given in Fig. 1 for a spherical indenter of diameter 5 cm under a load of about 100 kg on ice with a crystal size of the order of 1 cm in the surface plane. Subsidiary experiments (see also ref. 11) have shown that hardness depends on crystal orientation, but in the series quoted the indenter is supported on at least four grains so that orientation effects are considerably, though not completely, "smoothed out". Indeed, very similar results are obtained on ice of very small grain size. The results show three main features:

(*a*) The hardness falls with increased loading time (t).

(*b*) The hardness increases exponentially as the temperature falls. These effects are due to creep and resemble the results obtained by Glen. They are also similar to the indentation hardness results on metals at elevated temperatures obtained by Atkins and Tabor[12]. Atkins, Silverio and Tabor[13] have derived a relation for hardness assuming the material to be in a state of transient creep. The relation between transient creep rate ($\dot{\varepsilon}_{tr}$) and steady-state creep rate ($\dot{\varepsilon}$) is assumed to be of the Mott type[14]:

$$\dot{\varepsilon}_{tr} = A_2 \dot{\varepsilon}^{1/3} t^{-2/3} \qquad (4)$$

A_2 being a constant and $\dot{\varepsilon}$ given by equation (1). The simplified relation finally obtained for hardness (p) is:

$$p = A_3 \exp \frac{+Q}{mRT} \cdot t^{-1/m} \qquad (5)$$

where Q and m are the same constants as those used in the steady-state creep equation (1). From the results of Fig. 1, m is found to have a value of $3{\cdot}85 \pm 0{\cdot}3$ which agrees well with the limiting values 3·2 and 4·2 obtained by Glen. Q/m was found to be $7{\cdot}7 \pm 1$ kcal/mole. Using our value of m, this yields $Q = 29{\cdot}7 \pm 4{\cdot}4$ kcal/mole. This also agrees well with the activation energy value of 31·8 kcal/mole quoted by Glen. The agreement is somewhat surprising in view of the fact that the stresses involved in these hardness measurements are some 10–100 times greater than those in Glen's experiments.

(*c*) The last and most striking feature is the marked change in hardness behaviour at about $-1{\cdot}2 \pm 0{\cdot}3°$ C. Between this temperature and 0° C the hardness falls very markedly.

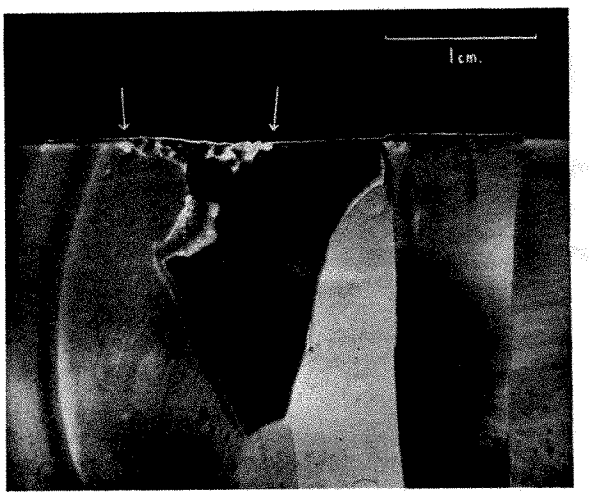
Changes in Crystal Structure

The change in hardness behaviour at $-1{\cdot}2°$ C is associated with a very marked change in the deformation produced by the indentation process. Distortion of the ice around the indentation is followed by recrystallization: this is shown by studying the sectioned specimens within a few hours of making the indentation. (If the ice is left for a few days, appreciable crystal growth can occur.) With cold ice (below $-1{\cdot}2°$ C) the recrystallized grain size is always very small (Figs. 2 and 3), being about 0·5 mm in diameter. The depth of this zone is small at $-10°$ C and larger at $-1{\cdot}9°$ C. This is essentially the consequence of "stress-enhanced recrystallization" within the plastic zone; the amount of recrystallization is naturally greater at the higher temperature. However, with ice warmer than $-1{\cdot}2°$ C, the recrystallized grains are consistently larger, lying between 2 mm and the original grain size (Fig. 4). This is strong evidence for pressure melting in this temperature range.

This view is supported by a visual examination of the sections. With cold ice the aggregate of small-grain ice around the deformation zone scatters light strongly and appears white in contrast to the relatively transparent surrounding ice (Fig. 5). The behaviour of the warmer ice is shown most clearly by using ice specimens deliberately prepared with inclusions of fine air bubbles. The undeformed specimen appears "cloudy". After indentation the contact zone becomes clear (Fig. 6). This provides very strong evidence indeed for the occurrence of local melting and the subsequent escape of the air bubbles. These results are not isolated examples. Numerous experiments show that there is always consistent behaviour below about $-1{\cdot}2°$ C and consistent behaviour of a different type above $-1{\cdot}2°$ C.

Magnitude of the Hardness

If the indentation process between 0° and $-1{\cdot}2°$ C is mainly due to pressure melting, we should be able to



Figs. 2–6. Examination of indentations in ice using both polarized and ordinary white light. The specimens are sectioned in the plane of the indentation (perpendicular to the ice surface) and are thinned to about 1 mm for crossed-'Polaroid' examination. The specimens shown were indented with loads of 70–120 kg and loading times of 1,000 sec, or more. The dark curved bands seen in the background of Figs. 2, 3 and 4 are caused by the circular aperture of the specimen support

Fig. 2. Indentation of ice at about $-10°$ C. Crossed-'Polaroid' examination shows the distorted zone of the grains under the indentation region (given by the arrows). The recrystallized grain size is about 0·5 mm in contrast with the large undisturbed grains. The depth of the zone is small at these temperatures

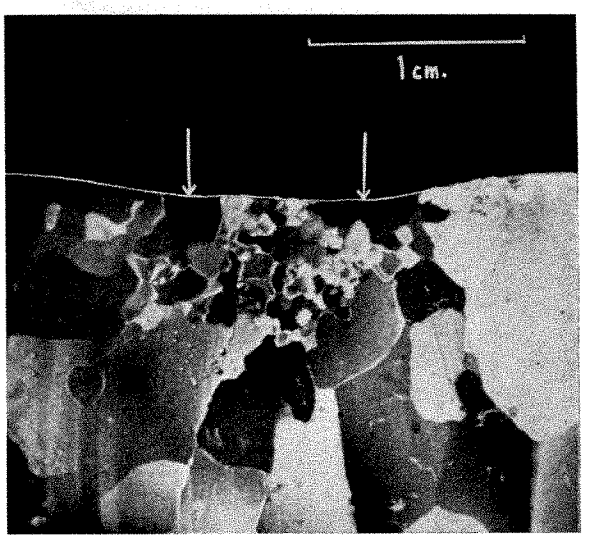


Fig. 3. Indentation of ice at $-1{\cdot}9°$ C. Crossed-'Polaroid' examination shows the distorted zone of the grains under the spherical indentation region (given by the arrows). The recrystallized grain size is about 0·5 mm. The depth of the zone is quite large

calculate from equation (2) the indentation pressure at which melting just occurs. For example, ice at $-1{\cdot}0°$ C ought to melt at a hydrostatic pressure of about 135 atmospheres if the ice and water are both subjected to the applied pressure. If we consider the stresses around a hardness indentation to be primarily hydrostatic, the hardness ought therefore to have an equilibrium value of about 135 atmospheres (or 1·4 kg/mm²) at $-1{\cdot}0°$ C. The dotted line in Fig. 1 has been calculated in this way. It is seen to cut the observed hardness curves very near the transition at about $-1{\cdot}2 \pm 0{\cdot}3°$ C.

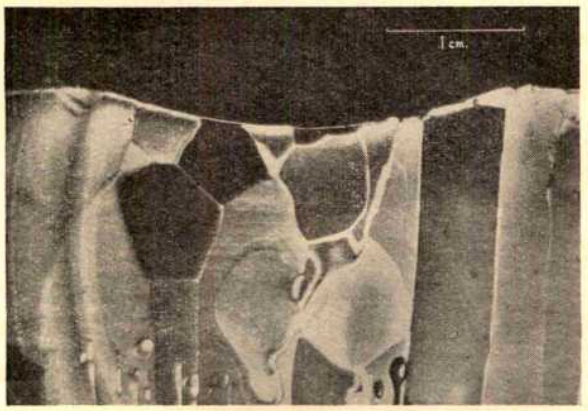


Fig. 4. Indentation of ice at 0° C. Crossed-'Polaroid' examination shows that there is no breakdown of crystal size. This is strong evidence for pressure melting. The ice is in the pressure-melting temperature range and presumably on removing the indenter the water refreezes to form large grains of size comparable with the undisturbed grains. The curved surface produced by the spherical indenter is easily seen

Although this transition agrees with the observed results it will be seen that between $-1{\cdot}2°$ and 0° C the ice is somewhat harder than one would expect on this model. The reasons for this discrepancy are two-fold. First, the pressure over the ice is not uniform in the indentation experiment, nor is it solely of a hydrostatic nature. From the theory of plasticity[15], the maximum hydrostatic stress occurs near the centre of the indentation and has a value of about 60 per cent of the overall hardness value (see, for example, ref. 16). The pressure melting curve ought therefore to be shifted a little to the left. It may be noted

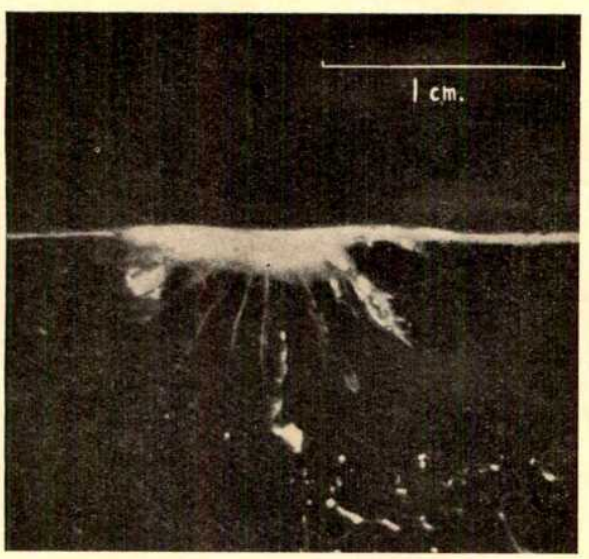


Fig. 5. Indentation of ice at $-10°$ C. Direct optical examination shows that the zone under the indented region scatters light strongly and appears white in contrast to the relatively clear undisturbed region

that indentations in this temperature range showed a well-formed hollow with sharp edges as though extruded water had frozen on escaping from the contact zone. Secondly, equilibrium can only be achieved after the liquid produced by pressure melting has been squeezed out from between the indenter and the ice. This takes a finite time so that the indentation hardness is always higher than the equilibrium value: in addition, the value is less the longer the time of application of the load. This dependence on time can, in a semi-quantitative way, be explained in terms of the viscous resistance of the liquid during extrusion.

With ice colder than $-1{\cdot}2°$ C, the pressures at which plastic deformation occurs are less than those required to produce melting. Consequently, the deformation behaviour falls into two fairly well defined zones. Presumably for shorter times it should be possible to extend the pressure melting phenomenon into a lower temperature range, and, at longer times, into a higher temperature range. This is the subject of further investigation.

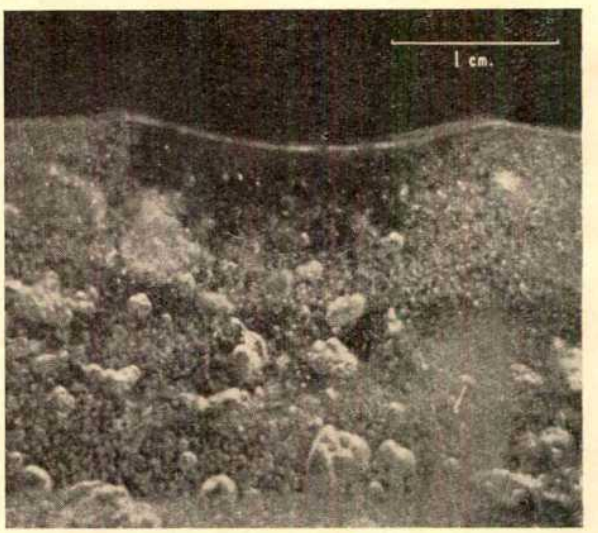


Fig. 6. Indentation of ice at $-0{\cdot}2°$ C. Optical examination of specimens specially prepared with many air bubble inclusions shows that the region under the indentation becomes relatively clear in contrast to the cloudy bubble-rich background. This again supports the view that, at these temperatures, pressure melting of ice has occurred; this makes it possible for air bubbles to escape before refreezing on removal of the indenter

It is interesting to compare these results with those of Glen obtained under conditions of uniaxial compression. Rewriting equation (1) in terms of the stress (σ),

$$\sigma = A_4 \dot{\varepsilon}^{1/m} \exp \frac{+Q}{mRT} \qquad (6)$$

It is seen that this equation is analogous to equation (5) for hardness (p) so that we can likewise draw plots of log σ against $1/T$. Varying $\dot{\varepsilon}$ will give a family of such curves just as in the hardness plots for varying loading times (t). We have recalculated Glen's data in this way and the results are plotted in Fig. 7. It is at once apparent that these results show two striking resemblances to the hardness data. First at temperatures below $-1{\cdot}2°$ C, where plastic flow and creep are occurring, the slopes are the same. Secondly at a temperature ($-0{\cdot}02°$ C) very close to the pressure melting curve, the stress for a specified creep rate is smaller than expected. Glen used Riecke's relation to show that pressure melting is impossible, but the agreement with the pressure melting curve suggests that the use of the Riecke's relation is probably unjustified. Our results suggest that crystalline creep of the ice is augmented in this temperature range by melting of ice be-

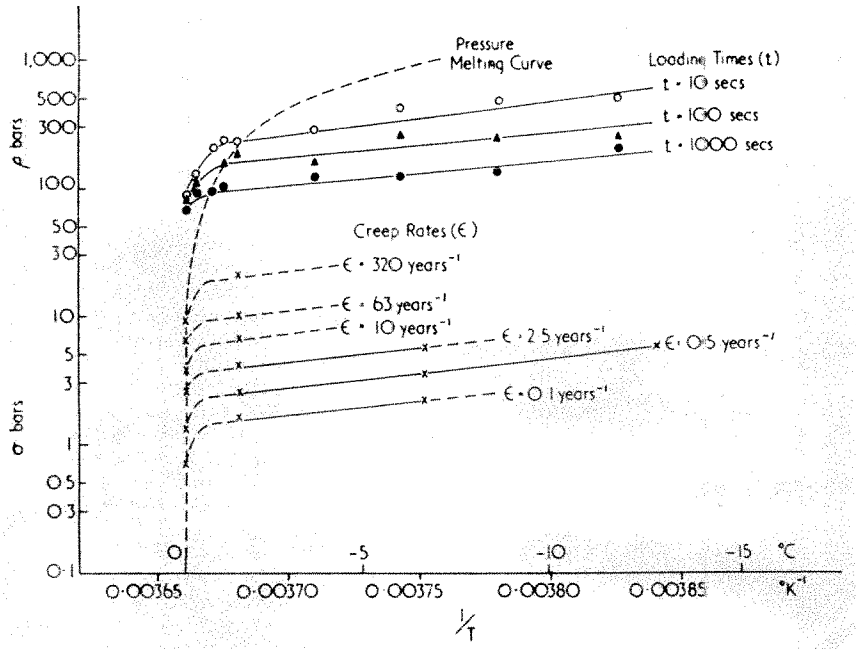


Fig. 7. Figure showing the comparison between the hardness (p) results of Fig. 1 and Glen's creep data. The latter curves (shown by crosses) represent the stress (σ) required at a temperature (T) to produce a given strain rate. The similar abrupt fall in this stress near the pressure melting curve at $-0{\cdot}02°$ C is striking. The pressure melting curve is calculated here assuming the hydrostatic pressure to be given by the compressive stress (σ)

tween the plattens and the ice specimen. In this region the magnitude of the effective hydrostatic pressure lies between the compressive stress σ and the associated hydrostatic stress (the stress invariant) equal to σ/3. This is illustrated in Fig. 8 where the pressure melting curve has been calculated for both these limiting cases. The enlarged temperature scale clearly shows that Glen's results at $-0{\cdot}02°$ C lie close to these limiting curves.

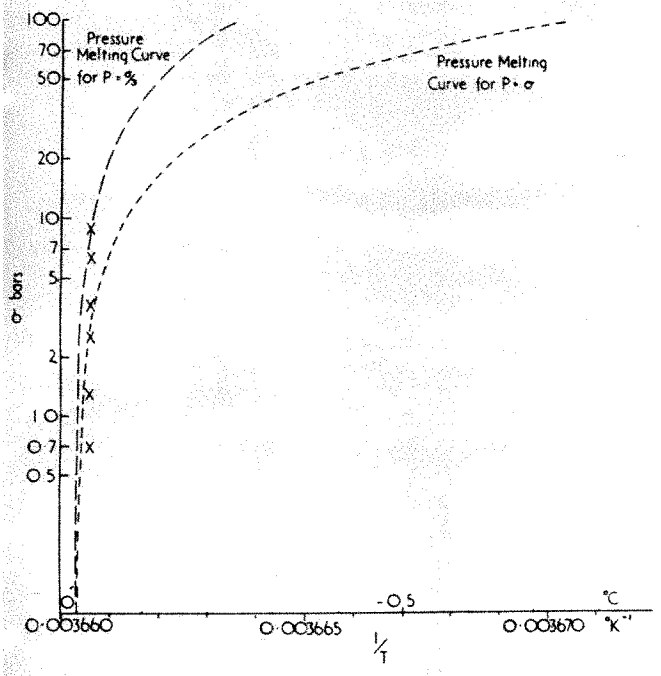


Fig. 8. Figure showing the position of Glen's data at $-0{\cdot}02°$ C on a magnified temperature scale. The pressure melting curve has been calculated in terms of the hydrostatic pressure (P) assuming that P is equal to (i) the compressive stress (σ), and (ii) the stress invariant ($\sigma/3$). It is seen that the points lie close to these limiting curves, suggesting that, in this region of Glen's experiments, pressure melting was playing an important part

Accelerated Creep and Transient Creep

Glen and Steinemann showed that after prolonged loading the creep rate accelerated because of recrystallization of the ice in an orientation favourable for easy glide along the basal plane. Our low-temperature experiments (at a much higher stress) should, according to their extrapolated data, bring us into the range of accelerated creep in about 1–1,000 sec and produce a recrystallized grain size of about 0·5 mm. This agrees with our observed grain size although with our experimental arrangement we could not detect a preferential orientation. However, even if the material near the contact zone is in a condition suitable for accelerated creep, the creep process as a whole is governed by the diffusion of the plastic zone into the virgin ice below it. This is apparently the rate-limiting part of the process and for this reason hardness results are explicable in terms of the transient creep process occurring in this region.

Conclusions

Our experiments show that in the indentation of ice for loading times greater than 10 sec and for temperatures colder than $-1{\cdot}2°$ C, the hardness behaviour may be explained in terms of transient creep. The crystal size around the deformed zone is small and provides evidence for local plastic deformation. Above this temperature there is a rapid fall in hardness and it is suggested that this is due primarily to the onset of pressure melting. This is confirmed by a study of the crystalline structure of the ice around the deformed zone; the grains are large and often continuous with the underlying ice crystals. Presumably for ice colder than $-1{\cdot}2°$ C the pressure required to produce plastic deformation, under our experimental conditions, is not sufficient to produce pressure melting.

We thank Prof. F. P. Bowden for his interest, Dr. G. de Q. Robin, Dr. J. W. Glen, and Dr. M. Imai for many helpful discussions, Dr. J. F. Nye and Dr. W. H. Ward for comments at an early stage of the work and the

Science Research Council for a research grant to one of us (P. B.) arranged by the Scott Polar Research Institute.

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IMPLICATIONS FOR GLACIOLOGY

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BECAUSE temperate glaciers consist of ice at, or within, 1° C of the pressure melting point, the results of Barnes and Tabor[1] on the flow of ice in this temperature range are likely to have critical implications in relation to the flow of glaciers.

In general, the main bulk of a glacier undergoes slow strain, and changes of strain rate and stress are likely to be relatively slow. However, processes of sliding, involving regelation against the bedrock and side walls, are likely to produce rapid changes of stress which may be of the same order as those used in the experiments described by Barnes and Tabor. The most generally accepted theory of sliding is that of Weertman[2], who has built up a theoretical model in which much of the ice is supported on a thin film of water giving low frictional resistance, while the ice is retarded by obstacles projecting through the water film into the basal ice. Stress concentration around these obstacles results in rapid rates of strain and in regelation of the ice. Kamb and LaChapelle have shown[3] that such a model is realistic, but have developed their equation of sliding on the regelation process alone.

The Sole of the Glacier

The most direct suggestion from the hardness experiments is that the sole of the glacier, a layer of relatively clear ice, sometimes containing debris and about 0·5 m thick, is the zone in which regelation–deformation processes occur, corresponding to the changed character of the hardness tests for temperatures between −1·2° C and 0° C. Kamb and LaChapelle's comments on this zone are:

(*a*) "The lowermost 0·5 m of ice above the regelation layer was relatively bubble-poor and appeared unfoliated. Above this zone, typical bubble foliation was prominent."

(*b*) "A stake anchored in the ice a distance of 10 cm above bedrock showed an average motion of 1·6 cm day^{-1} parallel to the bed. A stake 150 cm above bedrock moved 12 per cent faster. By means of a vertical profile of marker pegs spaced 10 cm apart, it was found that most of this 12 per cent differential motion occurred within the lowermost 50 cm of ice, as shear uniformly distributed except for two irregularities suggestive of 'shear zones', which, however, had only intermittent activity. In any case essentially all the motion of 1·6 cm day^{-1} took place as slip at the bedrock-ice interface."

Observations recorded by McCall[4] on the 30-cm thick sole of Skauthoe cirque glacier in Norway are broadly similar to the observations of Kamb and LaChapelle. McCall suggests that the bubble-free nature of the sole "probably originated" by meltwater percolating down the headwall of the glacier and freezing against the base of the ice. Such an explanation is unlikely to apply to the tunnel in which Kamb and LaChapelle worked.

The bubble-free nature of the ice and the relatively rapid strain rates observed in the sole of the Blue Glacier and Skauthoe both suggest strongly that regelation–deformation is occurring in the same manner as in the hardness experiments at temperatures above −1·2° C. The large crystal size in Fig. 5 of Kamb and LaChapelle's paper also suggests regelation–deformation, as the relatively rapid shear in this layer would otherwise be expected to produce smaller crystal sizes.

It must be remembered that the pressures involved in the laboratory experiments of Barnes and Tabor are of the order of 50–500 bars. Such pressures are not typical of the main ice bulk of temperate glaciers: however, Weertman's model of sliding involves stress concentrations proportional to the relative areas of obstacle and intervening glacier bed. For a mean shear stress on bedrock of 1 bar, the stress concentration factor could produce stresses up to 50 bars near the obstacles. Direct measurements of such stress concentrations have not been made. Our suggestion that the sole of the glacier is a regelation–deformation zone is based on the characteristics of the sole rather than Weertman's theory.

Catastrophic Advances of Glaciers

The proposal that temperature effects may be responsible for triggering off glacier surges in sub-polar glaciers[5] receives further support from the results of Barnes and Tabor. The slow warming of the basal ice of a glacier into the temperature range in which regelation–deformation and regelation against bedrock protuberances can occur is likely to produce an instability which could lead to rapid basal sliding and a catastrophic glacier advance. The relative roles of such processes and of the sub-glacial water film of Weertman[2] in explaining the rapid rate of flow once an instability develops need further investigation.

With regard to catastrophic advances of temperate glaciers, the results are suggestive but the answer is not clear. The time effect indicates greater apparent hardness values for shorter time intervals, which in terms of sliding would mean more resistance for higher rates of sliding, as is also indicated from the results of Glen[6]. However, in contrast to this, higher rates of sliding imply greater stress concentrations and increased flow rates due to the onset of regelation–deformation processes. It is not clear which of these two processes is the more important, but the fact that some temperate glaciers, such as the relatively flat lobes of the Vatnajökull ice cap[7], undergo catastrophic advances suggests that the onset of accelerated flow may be due to the processes shown to exist by Barnes and Tabor.

General Conclusion

The regelation–deformation process outlined by Barnes and Tabor appears likely to be of importance to short period transient processes involving relatively rapid changes of pressure, rather than to relatively slow strain rates for which much glacier flow theory has already been

developed. Its relevance to problems of sliding of ice appears to be of particular importance.

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GLACIER DEPTH-SOUNDING FROM THE AIR

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EARLIER[1] we announced the successful use of very-high-frequency radio echo apparatus to sound the depth of the Greenland ice sheet, providing a continuous profile of the bedrock surface by continuous recording from a moving vehicle. The detailed analysis of the records obtained in 1964 is now complete. The results mainly relate to a comparison with depths obtained by the seismic reflexion shooting technique, the relation between the radio echo strength and the ice temperature distribution, and the relation of ice surface features to bedrock topography on the small scale.

Following a generous invitation from the Defence Research Board of Canada (D.R.B.) the same radio echo apparatus was installed in a chartered *DHC-3 Otter* aircraft for flying operations based on Tanquary camp, Ellesmere Island, N.W.T. (81° 25′ N., 76° 55′ W.). The only new feature of the apparatus was the aerial arrangement designed and installed by H. Serson of the D.R.B. Telecommunications Establishment, Shirley Bay, and illustrated in Fig. 1. It was found to be more satisfactory to use one aerial for transmitting and the other for receiving, rather than to use a single aerial for transmitting and receiving, and the coupling between the two was about −45 dB. There was no discernible effect of drag on the performance of the aircraft.

A number of flights during April 10–20, 1966, covered some major glaciers and several crossings of ice caps on northern Ellesmere Island and one flight over the McGill ice cap and the Thomson glacier on Axel Heiberg Island. At 1,000 m above the surface, the echo from sea ice was about 80 dB over receiver noise (in confirmation of the overall system performance), from land about 70 dB, and from the top surface of a glacier of the order of 60 dB over receiver noise. Thus the glacier surface has a reflexion coefficient of the order of −20 dB. The echo from the bottom of the glacier was almost always weaker by 10 or 20 dB and, without giving an analysis of echo strength, ice temperature and depth, we can say that this is the order of magnitude of the attenuation due to dielectric absorption in the ice under the prevailing conditions.

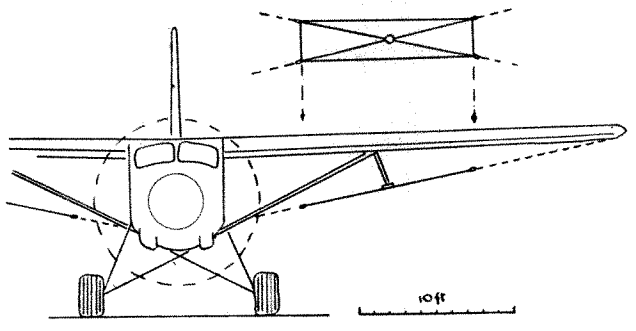


Fig. 1. Four-wire 35-Mc/s half-wave dipole aerial suspended beneath each wing of the *Otter* aircraft. Two of the conductors are driven from a coaxial cable which carries a ferrite-cored matching transformer at the centre. Conductors and supporting wires are assembled from 7/0·032 in. copperweld, 'Nico-press' sleeves, and 1-in. egg insulators (all tested to 2,000 lb.)

Thus the bottom surface of the glacier has a reflexion coefficient comparable with that of the top surface. It is possible to find exceptions to any of these statements, which are offered only as a guide.

Navigation was limited to line-of-sight paths, or sun-compass courses from identifiable landmarks on air-photo mosaics. Because the glacier surface elevations above sea-level on the maps are in error by as much as 1,000 m in some places, it was our practice to record the aircraft aneroid altimeter reading at frequent intervals. From this the true elevation of the aircraft above sea-level could be derived with an error less than ±50 m. The cruising speed was 50 m/sec (100 knots) and a height of the order of 500 m above the glacier was convenient. The top and bottom surface echoes could then be recorded on a time scale of 20 μsec total length and the error of ice depth measurement on this scale is ±8 m.

In Ellesmere Island, only the Gilman glacier depths have been surveyed previously, from gravity profiles and seismic soundings[2]. Radio echo recordings were taken along the same longitudinal profile and three distinct sections are illustrated in Fig. 2.

The first example, *a*, shows the ice cap in the vicinity of Mount Oxford. The smooth top surface is in contrast to the complex pattern of overlapping echoes from the bedrock, seen at various angles to the vertical but at right angles to the lines of sight from the aircraft. The record is typical of this ice cap, revealing mountain peaks just below the surface in addition to the visible nunataks. Example *b* shows the deepest section of the Gilman glacier, 20 km from the snout, 1,100 m above sea-level, and 660 m deep, which can be compared with a value of 700 m derived from the gravity survey at this point. Example *c* shows the snout (440 m above sea-level) which it would not have been possible to record from a surface vehicle because the finite receiver recovery time limits the minimum measurable range to about 100 m.

A comparison of the continuous echo from the smooth top surface of the Gilman glacier with that from the Otto fiord glacier in Fig. 2*d* reveals some interesting features. Between 1950 and 1959 the snout of this glacier surged forward by 3 km[3], and the chaotic ice structure is revealed by rapid fading of the top surface echo. It is probable that some of the intermediate echoes, of longer time delay than the minimum from the top surface, arise from discontinuities in structure below the surface. They cannot be differentiated from those which arise at some angle to the vertical unless there is a regular or continuous pattern beneath the surface, and we do not expect that here.

The next two examples are of thinner ice recorded on a faster time sweep. The ice rise in the Ward Hunt Ice Shelf, off Cape Discovery, is shown in *e*. We find the thickness to be 125 ±5 m and the smooth top surface (by which it is recognized visually) produces a different echo from the Ward Hunt Ice Shelf shown in example *f*. Here the thickness is 40 ± 5 m and the peaks in echo strength from the top surface occur at a rate of four or five per kilometre on the ice shelf surface, which is of the same order as the visible troughs (containing water

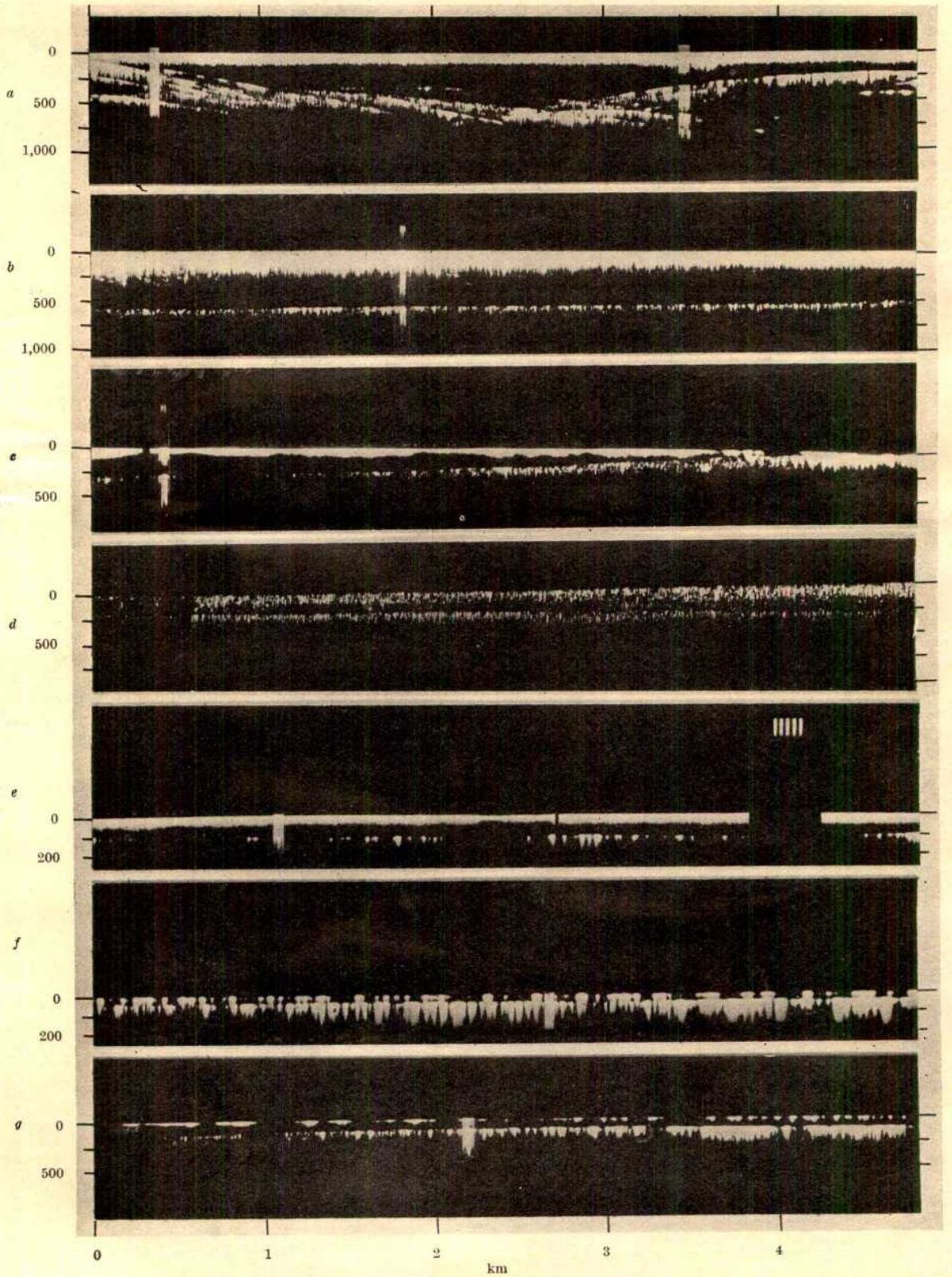


Fig. 2. *a–g*, Continuous records of radio echo soundings from the air showing various glaciological situations described in the text. In these examples, the time sweep runs vertically downwards across the film starting from the instant of the transmitter pulse, so the delay to the start of the echo from the top edge of the film represents the travel time through the air. The ragged trailing edge of the surface echo is due to the longer travel time of waves which are reflected at some distance from the perpendicular point on the top surface. Some of the power which penetrates the ice mass is reflected in the same manner from the ice/rock or ice/water interface below; the ice depth can be estimated, in metres, from the scale given at the ends of each record

in summer). Note that the bottom echo is stronger than the top echo on the floating ice shelf but much weaker on the grounded ice rise: however, both bottoms appear to be rough.

The transition from floating to grounded ice is illustrated in the final example, *g*, taken in Milne fiord. To the left, the glacier surface is smooth and the echo is stronger than the bottom echo from rock. To the right, the top surface is irregular and the echo is broken but of similar strength to the smooth section, whereas the bottom echo, from sea water, is about 15 dB stronger than that from the adjacent rock.

Echoes from intermediate layers within the ice (reported previously[1]) were not identified in this work—probably because, if they exist, they are very different in strength from the main top and bottom echoes and would easily be overlooked.

In the use of airborne techniques, it is the obvious factor of speed of coverage which will be the greatest asset for the Antarctic and Central Greenland. In mountainous country or in crevassed regions, however, the great advantage of aircraft lies in their ability to travel routes which would be dangerous or quite inaccessible on the ground: this is apparent in the present results. Our greatest debt is to the Chairman, D.R.B. (and partly the McGill Arctic Research Expedition), for the provision of the flying time, to the Department of Transport, Meteorological Branch, for hospitality at Arctic Weather Stations, and to G. Hattersley-Smith for inspired organization and direction of the field operations. Construction of the apparatus was financed by the Paul Instrument Fund, and the present development is supported by the Natural Environment Research Council.

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NEWS AND VIEWS

Pattern of Science Expenditure

THE first report of the Council for Scientific Policy says that for the first time in recent history the proportion of the gross national product in Britain devoted to research and development has not increased in the period of the three years 1963–66. The total expenditure on research and development has increased at a rate of 6 per cent a year from £634 million to £756·6 million in the 3 years. Direct government spending has continued to decline and now amounts to slightly more than a quarter of all the money spent on research and development in Britain. The principal reason for this decline has been a reduction in expenditure on defence research and development. In 1955–56, expenditure under this head was £65·7 million, or 21·9 per cent of the total expenditure on research and development; in 1964–65 the amount spent on defence research had increased absolutely to £91·5 million but had declined as a proportion of the total to 12·1 per cent. In parallel with the decline of defence expenditure there has been a rapid increase in the value of research and development in universities from £14·4 million in 1955–56 to £55·9 million in 1964–65. The statistics on manpower collected in the report show a steady increase in the output of trained scientists and engineers, and a sustained recruitment of potential professionals to the universities. In 1958, 14,050 qualified people began work, 8,275 of them after taking first degrees at universities. By 1964 the total output had increased to 20,581, including 12,303 with first degrees. It is estimated that by 1968 the total output of trained people will have further increased to 26,060—most of the increase occurring between 1967 and 1968. The growth of the numbers of technically qualified people seems to have been uniform among specialists of different kinds. Mathematicians, however, are being produced in rapidly increasing numbers, and in the decade 1958–68 it is expected that the output of trained mathematicians will have multiplied four times.

On the recruitment of young people from the schools into undergraduate courses at universities and colleges of advanced technology, the Council quotes with approval the conclusion of the Dainton Sub-Committee that fears that the numbers of recruits to science and technology were declining were based on a misinterpretation of available statistics. It says that the correct interpretation is that there has been a growth of social studies, and a "striking growth in sixth form studies in subjects which do not normally lead to university education".

University Science Policy Units

A RASH of courses and research activities in the sociological antecedents of science is evident in British universities. At Sussex, Stirling, Edinburgh, Manchester and Bath (or the Bristol College of Advanced Technology) formal academic units have been or are being set up. At other universities, existing departments are tending to include studies of what has been called "the science of science" in their curricula.

Sussex is farthest ahead in this as—the university would no doubt say—in other things. Setting up a research unit for science policy at Sussex has been talked of since 1963, when Dr. Stephen Toulmin urged such a course on the university. Edinburgh became interested in a similar concept at much the same time, and it would be valuable to know how much of the planning at Stirling stems from the fact that the Vice-Chancellor, Dr. T. L. Cotterell, is an Edinburgh man. Manchester appears to have been stimulated in its plans more by its connexions with the Council on Scientific Policy and from the interest of the Departments of Economics and of Public Administration in matters closely related to science policy. There is no obvious motive but good sense for what Bath is planning.

Sussex

THE Unit for the Study of Science Policy at Sussex was established in January 1966 under Dr. Christopher Freeman. It has three full-time members already, and four more are to be appointed by the autumn. The intention is to carry out research both in the natural and social sciences relating to the economic, historical and social aspects of science policy. At present there are two main projects under way, one under Dr. Roy McLeod, a Harvard biochemist and historian, on the changing patterns of support for science. Another, on "Invention and Innovation in Capital Goods in Relation to the World Market", is directed by Dr. Freeman and is a continuation of his work at the National Institute of Economic and Social Research. The unit plans also to study the problems of science policy in developing countries; it is extending its international contacts and has already made arrangements for joint research work with the *Studiengruppe für Systemforschung* in Heidelberg, with CENSIS in Italy, and with the University of Lund in Sweden. One undergraduate seminar entitled "Science and Public Policy" is currently being offered at the University, as a contribution to the Arts–Science Scheme. More undergraduate and graduate

courses are planned, but the emphasis of the Unit will remain on research.

Bristol

THE Bristol College of Science and Technology has established a Centre for Scientific and Technological Affairs. Since the Centre is primarily a research unit, its emphasis will at first depend on the availability of funds for designated pieces of research. Some work has already begun on a comparative study of scientific policies at different times and in various countries. Other projects are planned on the internal development of the sciences and their interrelation; on the external relations of the sciences with other disciplines and the influence of basic discoveries on technological and social change; and a statistical documentation of scientific growth in the modern period. In addition, Dr. Gerald Walters, who as head of the humanities department is initially organizing the Centre, plans a monthly seminar on the social, economic, political and philosophical aspects of science.

Manchester

THE University of Manchester has decided to concentrate on teaching, and is concerned to train what the director of the unit, Prof. F. R. Jevons, describes as "journalists, teachers, administrators, and decision makers who are well grounded in science on a broad front and yet can express themselves". There will be a small entry of students in October 1966 and possibly two dozen in 1967. Central concepts in the physical sciences will be taught in conjunction with economics, sociology, history and philosophy. According to Prof. Jevons, the department will be interested in questions such as the financing of science, the study of innovation, the possibility of applying cost–benefit analysis to scientific research, science and social policy, and the methods of teaching science. He intends to offer natural or geological sciences as alternatives to the physical sciences later on, if there is sufficient interest. He hopes "that the school will form an important link, the first of its kind, between the arts and the sciences".

Stirling

THE University of Stirling will also teach undergraduates but will have no students until October 1967. In what will be called the School of Technological Economics the intention is somehow to design a course to train industrial or public administrators, analysts of industrial operations and "the industrial researcher whose work depends on a correct appraisal of sociological and economic issues". To judge from what has been said so far, the School is aimed not so much at students interested in the social and political basis of science and science policies but rather in the application of science and scientific techniques, including games theory, linear programming and statistics, to industry.

Edinburgh

AT Edinburgh the intention is to use the Science Studies Unit (under Dr. David Edge) to teach undergraduates and to conduct research "in those areas where scientific activities overlap with more general concerns of human society". There will be a short course of introductory lectures and seminars in September this year, and in the following academic year a one-year optional course for second- or third-year science undergraduates which, it is hoped, will soon become a two-year series. The teaching will be concerned with "the problems of economic growth, science policy decisions and sociological results of scientific advance".

Dr. Van Zandt Williams

THE death occurred in London on May 13 of Dr. Van Zandt Williams, director of the American Institute of Physics, at the age of fifty. He was making a short visit to discuss plans for a co-operative programme on physics abstracting and physics information services.

Dr. Williams was born in Providence, Rhode Island, gained a doctorate at Princeton University in 1941, and then entered the research laboratories of American Cyanamid Corporation, where, with Dr. R. B. Barnes, he played a leading part in the development of spectroscopic techniques for use in industry. He was among the pioneers in the application of infra-red measurements to analysis, to problems of molecular structure, and in the differentiation of some of the newer chemical products such as synthetic rubbers. He soon became the assistant director of the physics division of the Cyanamid laboratories, but it was not surprising that shortly after the Second World War he joined the recently formed Perkin Elmer instrument company. There he guided the design and rapid manufacture of commercial infra-red spectrometers and other optical instruments, some of which have become classical and have acquired universal fame. He was director of research and sales, manager of the instrument division, and later executive vice-president of the company.

A year ago he became the director of the American Institute of Physics, and would shortly have completed his term of office as president of the American Optical Society, after leading the very successful meetings in Washington two months ago to celebrate its fiftieth anniversary. He had also recently become the secretary of the committee for physics under the National Academy of Sciences, with the responsibility of maintaining relations with the International Union of Pure and Applied Chemistry.

Dr. Williams's death is a loss to American physics, to optics and spectroscopy, and to all who have international scientific co-operation at heart. Not only his colleagues in the United States, but also others the world over, and particularly in Europe, will miss his friendship, loyalty, sound counsel, wit and humanity, and share a great personal loss with his wife and two sons.

Sociology of Engineering

AN attempt to form a rounded picture of the character of the engineering profession in Britain is being made by the Council of Engineering Institutions with the financial backing of the Ministry of Technology. More than 25,000 chartered engineers are included in the sample being sent a detailed questionnaire intended to find out how engineers were trained to do their present jobs, and how they are rewarded. The survey will cover roughly 20 per cent of the membership of the thirteen engineering institutions affiliated to the Joint Council. The chairman of the Council, Sir Robert Wynne-Edwards, says in his introduction to the questionnaire that the results of the survey will be used to "help plan for the future by providing pointers towards better usage of engineering talent". Many working engineers will, no doubt, hope that the survey will also justify objectively the suspicion that engineers are paid too little.

The survey should provide sociological information of value outside engineering as such, partly because it has set out to define the levels of responsibility at which individual engineers function. To be sure, it will be a considerable test of character for a man to assign himself to category "A" by confessing that his work is "reviewed for accuracy and adequacy and for conformity with prescribed procedures", but the results could provide an important insight into the sharing of responsibility in modern industry. It is therefore all the more surprising, but entirely characteristic of the common conviction among engineers, that theirs is a job for men, that the questionnaire does not permit a woman to declare herself as such. Yet information about the employment of women engineers could have been particularly valuable in the recruitment of more.

Cyclotron for Medical Research

THE only Cyclotron Unit in Britain to be used in conjunction with medical radiobiological study and clinical applications has been operating for more than a decade at the Hammersmith Hospital. The Unit is completely owned and run by the Medical Research Council and is used by staff within the Unit, by other workers and post-graduate students at Hammersmith, and by visitors from other medical centres. In addition to the 15-MeV cyclotron, the Unit operates an 8-MeV linear accelerator and a 2-MeV Van de Graaff machine, thereby giving a wide range of radioisotopes needed for radiobiological research.

The cyclotron, built in 1955 at a cost of £250,000, has the great advantage of producing isotopes of very short half-life—ones that will last in the patient's body long enough for measurement but no longer. Since the Unit is on hospital grounds, it is practicable to use such isotopes as carbon-11, nitrogen-13 and oxygen-15, which have half-lives of 20 min, 10 min and 2 min respectively. It has also proved possible to use bone-seeking isotopes with short half-lives. In this respect, fluorine-18 has been used successfully to find bone lesions.

Radiation studies within the Unit are largely concerned with neutron beam experiments. According to the director of the Unit, Mr. D. D. Vonberg, one of the reasons for installing the cyclotron is to study the use of neutrons as an alternative to X-rays for radiotherapy, especially on cancerous tissues. Some cancers have a poor blood supply so that many of the cells are anoxic. The efficiency of X-rays on anoxic cells is reduced by as much as two-thirds, but neutron beams lose very little of their cancer-destroying efficiency. This line of research was first studied at the University of California at Berkeley but was stopped by the Second World War. Over the past ten years at Hammersmith, much research has been done on animal tumours but none on human patients. However, the hope is that the fast neutron beam can be used to treat certain types of malignancy and will be available soon for clinical use. Dr. R. L. Morgan, a radiotherapist involved in this study, stresses that while this may prove to be a useful application of the neutron beam, it is not the all-purpose cancer cure; it can only be considered a technical advance that will be useful in certain cases.

Other experiments involving the cyclotron include a tracer experiment studying the control of ventilation by carbon dioxide concentration and comparing the experimental results—the control loop being simulated by an analogue computer.

Another interesting clinical application is the use of oxygen-15 to trace heart lesions in the septum wall. In a very simple and brief test, oxygen-15 is breathed in and traced as it passes from the lungs in the form of carbon dioxide; if blood passes through a hole into the right side of the heart, a small decrease followed by a small increase, or a 'recirculation peak', would be noted as the extra blood is carried to the lungs.

In addition to the collaborative medical work with radioisotopes, the Unit provides its own engineering, radiochemical and radiation protection sections to deal with problems of accelerator design, chemical separation and purification of isotopes, and problems of radioactive contamination, leakage, and waste disposal.

Announcements

W. O. BAKER, vice-president of research at the Bell Telephone Laboratories, has been awarded the Priestley Medal by the American Chemical Society.

J. W. GARDNER, secretary of Health, Education and Welfare, has been awarded the Public Welfare Medal of the U.S. National Academy of Sciences for "eminence in the application of science to the public welfare".

MR. C. S. RICHENBERG, a senior examiner at the British Patent Office, is embarking on a study of the policy, law and practice relating to patents in a number of countries overseas. He is particularly concerned with "problems arising out of the present state of technology", and says that he would welcome the views of interested persons on "what they consider to be serious basic shortcomings of law and practice".

THE third international Congress of Radiation Research will be held in Cortina d'Ampezzo during June 26–July 2. Further information can be obtained from the Secretary-General of the Congress, Casella Postale 2359, Rome A-D.

A SYMPOSIUM on "The Molecular Biology of Viruses" will be held in the University of Alberta during June 27–30. Further information can be obtained from Dr. J. S. Colter, Department of Biochemistry, University of Alberta, Edmonton.

A MEETING of the Physical Biochemistry Group of the British Biophysical Society will be held at the Unilever Research Laboratory, Bedford, on June 29. Further information can be obtained from Dr. P. Johnson, Colloid Science Laboratory, Free School Lane, Cambridge.

THE eleventh annual meeting of the Health Physics Society will be held in Houston during June 27–30. Further information can be obtained from O. L. Pirtle, jun., Hastings Radiochemical Works, P.O. Box 60448, A.N.S., Houston, Texas.

THE second Rochester conference on "Coherence and Quantum Optics" will be held in the University of Rochester during June 22–24. Further information can be obtained from Dr. Emil Wolf, University of Rochester, Rochester, New York.

A CONFERENCE on "Precision Electromagnetic Measurements" will be held at the Boulder Laboratories of the National Bureau of Standards during June 21–23. Further information can be obtained from Mr. J. Cronland, Bureau of Continuation Education, 328 University Memorial Center, University of Colorado, Boulder.

THE second Canadian wood chemistry symposium, sponsored jointly by the Chemical Institute of Canada and the Technical Section of the Canadian Pulp and Paper Association, will be held in Sainte Marguerite, Quebec, during June 22–24. Further information can be obtained from Dr. D. B. Mutton, International Cellulose Research, Ltd., Hawkesbury, Ontario.

A SYMPOSIUM on "Discontinuous Waves" will be held by the Department of Mathematics, University of Strathclyde, in collaboration with the Institute of Mathematics and its Applications, in the University during June 30–July 1. Further information can be obtained from the Registrar, Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex.

THE third congress of the International Federation of Automatic Control, organized by the United Kingdom Automation Council with the co-operation of the Secretariat of the Federation and of its national member organizations, will be held in London during June 20–25. Further information can be obtained from the Secretariat of the Congress, c/o the Institution of Electrical Engineers, Savoy Place, London, W.C.2.

THE seventh International Congress of Gerontology, organized by the Austrian Society for Geriatrics, will be held in Vienna during June 26–July 2. The main themes of the Congress will be: the role of environment in ageing; adaptability in ageing persons; methodology, research and education in gerontology; current trends in gerontology; age, work and social change; problems of therapy in gerontology. Further information can be obtained from the Secretariat of the Congress, Wiener Medizinische Akademie, Alser Strasse 4, Vienna IX.

A HIGH-RESOLUTION STUDY OF THE RADIO SOURCE 3C273

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SINCE the first detailed study of 3C273 by the "method of lunar occultations" and its subsequent identification with a stellar object[1,2] which led to the recognition of a new class of astronomical objects, it has proved to be one of the most interesting and puzzling objects in the entire sky. The early occultation observations which were made using the 210-ft. radio telescope at Parkes at frequencies of 136, 410 and 1,420 Mc/s showed that basically the source consists of two components (called *A* and *B*). A preliminary analysis of the records showed that the majority of the radiation from *B*, which is associated with a 13th magnitude star-like object, arises in a source of angular size less than or equal to 0·5″ while component *A* appeared to consist of a central core about 2″ wide embedded in a halo about 7″ wide and to be associated with a jet extending outwards from the stellar object. The jet which lies along a position angle of 43° starts at about 11″ from this object and terminates abruptly at a distance of 20″, while it was estimated that the two radio components were oriented along a line at position angle 44° and separated by 19·5″. A more detailed analysis of the 1,420 Mc/s observations[3] using the convolution procedure of Scheuer confirmed these general conclusions but indicated the presence of additional weak sources near *B* and a faint extension extending from *A* towards *B*. It also appeared that *B* was resolved with an angular size of about 0·6″, a conclusion confirmed by a similar analysis of the same data by Scheuer[4]. Later observations by Hughes[3,5] confirmed the conclusion that *A* is elongated along the direction of the jet, a conclusion also reached by Von Hoerner[6] from a series of occultation observations at Green Bank. A comparison of the observations at different frequencies showed a marked difference in spectral index between the two components, that of *A* being about −0·9 compared with a value for *B* of about 0·0.

Since the foregoing series of observations the extremely interesting observation has been made by Dent[7] that at 8,000 Mc/s the flux of 3C273 has increased over a period of 3 years by about 40 per cent. Because of the difference between the spectral indices of the source components this increase in flux must be due to component *B* and it sets an upper limit to its angular size of about 0·001″. However, the absence of any evidence of a cut-off in the spectrum of *B*, at least down to a frequency of 410 Mc/s, shows that if the emission from *B* is due to synchrotron radiation the angular size must be greater than about 0·2″, which is in better agreement with the occultation observations. This suggests either that the emission is not synchrotron radiation or that there is a genuine change in apparent angular size between 1,420 Mc/s and 8,000 Mc/s.

Because of the seriousness of the discrepancy in the estimates of angular size we have taken advantage of an occultation visible from the Arecibo Ionospheric Observatory in Puerto Rico to make a more accurate measurement of the diameter of component *B* and at the same time a more detailed comparison of the optical and radio brightness distributions. In addition we have re-analysed the earlier 1,420 Mc/s observations, as in this case the resolution limit set by the time constant and the bandwidth was about 0·5″, so close to the estimated size of *B* that there must be some doubt as to whether it had really been resolved.

Observations of the occultation which occurred on June 7, 1965, were made using the 1,000-ft. aperture telescope of the Arecibo Ionospheric Observatory which has been described in detail by Gordon[8]. The main primary feed of this instrument consists of a line feed 96-ft. long operating at 430 Mc/s and designed to correct the spherical aberration of the reflecting surface and to fill the full 1,000-ft. aperture. However, it was decided not to use this feed but to construct a special set of simpler feeds operating at 41 Mc/s, 195 Mc/s and 430 Mc/s, feeding 1,000 ft., 500 ft., and 350 ft. of the aperture respectively. There were several reasons for this decision. First, it was decided to attempt a resolution of 0·1″ and, as pointed out by Hazard[3], the finite size of the aperture sets a limit to the resolution and for an aperture of 1,000 ft. this limit is about 0·2″. Secondly, it was desired to investigate the source spectrum in more detail. Finally, the occultation occurred near the edge of the field of view of the telescope and there was some danger that part of the occultation curve would be missed using the line feed, while with the simpler feeds the field of view could be extended by mounting them offset from the main feed support. It was estimated that the apertures illuminated by the simple feeds would be adequate to achieve the desired resolution.

Because of low-level interference and the higher background noise at the lower frequencies, the 430-Mc/s record is the only record which permits a detailed study of the source structure down to a resolution of the order of 0·1″ and we will confine our attention to the observations made at this frequency. The spectral measurements have turned out to be very complex and will be discussed later.

At 430 Mc/s, a Dicke-type receiver was used with two separate channels, one channel having a bandwidth of 125 kc/s and the other 1 Mc/s. The observations were made by directing the telescope beam to the position of the source and recording the received power with the telescope in automatic motion tracking the source. The received power was recorded on magnetic tape using a time constant of 0s.1. With this time constant the limit to the resolution is set only by the receiver bandwidth and the signal-to-noise ratio. The effect of the bandwidth on the source distribution when restored using the method of Scheuer is to convolve the source distribution with a beam of half-power width about equal to 0·62 $(\Delta\lambda/D)^{1,2}$, where $\Delta\lambda$ is the width between half-power points and D is the distance of the Moon from Earth. At 430 Mc/s this corresponds to 0·09″ at 125 kc/s and to 0·26″ at 1 Mc/s. The 430-Mc/s record, smoothed with a gaussian beam 2 sec of time wide in order to show its main features, is shown in Fig. 1. The angle between the limb of the Moon and the line joining the two source components was only 15° so that the two components are not clearly separated, but their presence is shown by the beating in the fringe pattern. The extensive diffraction pattern shows the presence of fine-scale structure in the source of less than 1″.

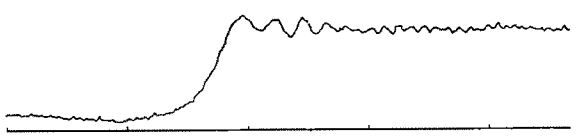


Fig. 1 Facsimile of observed occultation curve at 430 Mc/s using 1-Mc/s bandwidth and smoothed with a time constant of 1 sec. Horizontal scale: universal time, 1 div. represents 1 min

* The Arecibo Ionospheric Observatory is operated by Cornell University with the support of the Advanced Research Projects Agency under a research contract with the Air Force Office of Scientific Research.

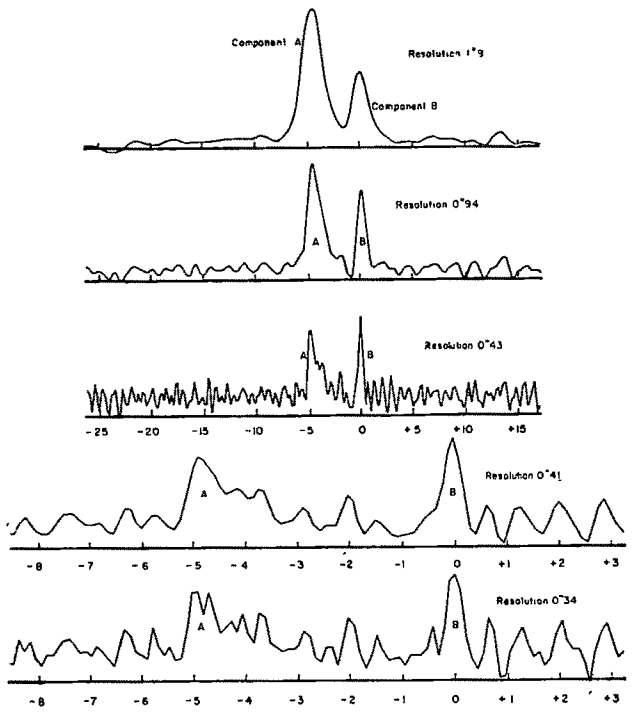


Fig. 2. Derived distributions across 3C273 using different resolutions and obtained using the 1-Mc/s bandwidth observations. The effective resolution is indicated on each curve. All records are normalized so that an unresolved source would have constant height at all resolutions. Horizontal scale: distance from component *B*, along position angle 299°, in seconds of arc

The record was analysed using a procedure based on Scheuer's convolution method for recovering the true strip brightness distribution, as seen by a beam of some assumed size, from the observed occultation curve. It differs mainly from the procedures outlined by Von Hoerner[9] and by Scheuer[4] in that account is taken of the curvature of the limb of the Moon, for, as shown elsewhere, this is an important consideration when aiming at a resolution of 0·1″ where the diffraction pattern and the restoring curve extend to a distance of 3′ from the limb of the Moon. In calculating the time scale of the diffraction pattern the source position given by Hazard, Mackey and Shimmins[1] was adopted and initially the Moon was assumed to be truly circular. However, a preliminary analysis showed that the use of the scaling factor derived in this way produced a marked asymmetry associated with a pronounced overshoot in the restored distribution of component *B*. As discussed elsewhere, this is a clear sign of an error in the restoring function (as well as evidence that the source is of small angular size) and, as the position of the source is known to the order of 1″, must be due to the relevant section of the limb of the Moon deviating from a true circle by about 2°. The scaling factor was therefore adjusted experimentally until all signs of the overshoot had disappeared and the narrowest distribution for *B* obtained, a procedure analogous to focusing. This procedure is justified as there is no mechanism which could produce a narrower distribution than the true distribution.

Having obtained the true scaling function, the observed occultation curves were restored with a series of restoring functions each using a different assumed beam width. The restored curves for a 1-Mc/s bandwidth are shown in Fig. 2. These curves represent the true brightness distribution convolved (*a*) with the assumed beam and (*b*) with a gaussian of half-power width 0·26″ due to the bandwidth. The effective beam width in each case is indicated on each curve; at the maximum resolution used it is set almost entirely by the receiver bandwidth. The curves are normalized so that an unresolved source would have a constant height at all resolutions.

Some interesting features are immediately obvious from these curves. As the height of component *B* is constant for all resolutions within the limits of experimental error and as there is no significant broadening at the highest resolution it must have an angular size $\leqslant 0{\cdot}3''$. On the other hand, the curves for component *A* show an appreciable diminution in height going from a resolution of 3·6″–1·8″, showing that it contains structure wider than 2″ of arc. As the resolution is decreased below 1″ the nature of this structure becomes clear. There is a weak extended source just visible in the noise and at least 5 sec of arc in extent on which sits a narrower source in which the majority of the emission originates; the extension from *A* to *B* noted earlier is probably part of this extended source. A comparison of the areas under the curves at different resolutions indicates that the extended region contains 40 per cent of the total flux from *A* while the central region contains the remaining 60 per cent. At the highest resolution, structure begins to appear in the central region itself which may be considered to consist of two separate components. One component has a half-width of 1·5″ and overall extent of about 2″ with a remarkably flat top and contains 40 per cent of the total flux of *A*; the second component contains 20 per cent of the total flux, is asymmetrically placed with respect to the extended central component and has a half-width less than or equal to 0·3″. The exact configuration of these two sources is somewhat uncertain as the shape of the restored curve depends on the scaling factor adopted for the restoring function and this is not necessarily the same for *A* as for *B*. However, there is no doubt that the asymmetry of *A* is real and that the small angular size component is situated on the side of the extended source more remote from *B*. Apart from a suggestion of a weak discrete source between *A* and *B* there is no evidence of any other source in the region.

In order to increase the resolution we now consider the observations made with a bandwidth of 125 kc/s which sets a limit to the resolution of about 0·1″. These restored curves are shown in Fig. 3. At a resolution of 0·35″ the restored curve is similar to that obtained for the same resolution using a bandwidth of 1 Mc/s, but with a poorer signal/noise ratio. As the resolution is increased still further component *A* disappears into the noise while *B* is still clearly visible. Because of the low signal/noise ratio of the narrow component of *A* and the possibility that the scaling factor is not the same as for *B* there is no evidence that it has been resolved. Its angular size is therefore $\leqslant 0{\cdot}3''$. The signal/noise ratio also limits the resolution for *B*. The half-width of this source at a resolution of 0·22″ appears to be slightly increased to 0·29″, but on the other hand there is no significant change to its height. The broadening is therefore not significant and we conclude that its half-width is $\leqslant 0{\cdot}15''$. A comparison of the total fluxes observed at different resolutions

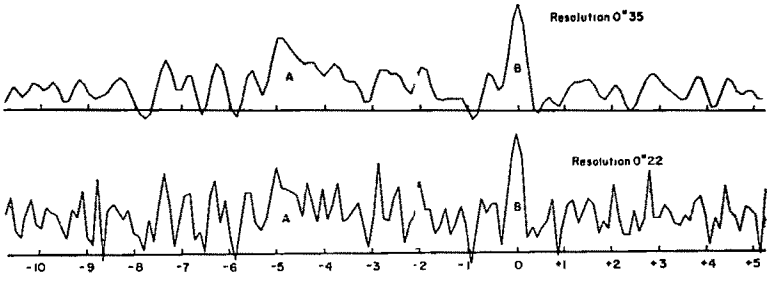


Fig. 3. Derived distributions across 3C273 using the 125 kc/s bandwidth observations and effective resolutions of 0·35″ (upper curve) and 0·22″

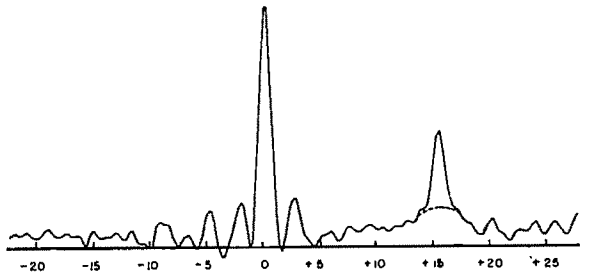


Fig. 4. 1,420 Mc/s distribution across 3C273 at position angle 103° using a resolution of 0·6″. The broken line represents the possible separation of *A* into a narrow central source situated on a more extended region of emission

shows that within the limits of experimental error all the flux from *B* arises in this small angular size component.

In order to compare the results of the present investigation with those made earlier at 1,420 Mc/s using a comparable resolution, we show in Fig. 4 the restored 1,420-Mc/s distribution using an effective beam of 0·63″. This is close to the maximum possible resolution which is limited to 0·52″ by the bandwidth and time constant. The basic structure of component *A* is the same at the two frequencies. Thus there is a narrow component superimposed on a weaker, more extended source, and running towards *B* and more clearly visible at coarser resolutions a faint flat extension. Unlike the 430-Mc/s curves the narrow source is not asymmetrically placed with regard to the extended source which appears to extend away from *B* on the far side of *A*. The width of the narrow component is difficult to estimate because of the uncertainty in the base level. If we consider the extended source and central component as a single source then its half-width is 0·9″, indicating a source size of about 0·6″, while if we draw the zero as indicated by the broken line the central region is not significantly resolved and has a width $\leqslant 0{\cdot}4''$. The structure seen around component *B* on the 1,420 Mc/s curve is not visible at 430 Mc/s and must be considered spurious and probably due to irregularities in the limb of the Moon, although the possibility remains that it is genuine but that the sources have a steeper spectral index than *B*. It is the presence of these sources which apparently led to the conclusion that *B* has a core-halo type of structure—a conclusion which is certainly not true at 430 Mc/s. However, the 1,420 Mc/s results still indicate that *B* is resolved, for the observed half-width is 0·92″, corresponding to an angular size of 0·7″. As the presence of the irregularities near *B* indicates that for this occultation the irregularities in the limb of the Moon may be a significant source of error it is possible that they are responsible for its apparent resolution although the narrow width observed for the central region of *A* is a strong suggestion that the resolution may be real. In this case, the structure at 1,420 Mc/s must be different from that at 430 Mc/s. This is, the source may be elongated or it may indeed have a core-halo structure but with the halo having a steeper spectral index than the core. Alternatively there may be two sources present with different spectral indices, only one being visible at 430 Mc/s and both visible at 1,420 Mc/s. A further possibility is that the two sources are oriented so that they passed simultaneously behind the Moon on June 7 but with an apparent separation of about 0·5″ on October 26, 1962. Whatever the explanation, it is now clear that component *B* has structure less than about 0·15″ of arc and there is no discrepancy between the occultation observations and alternative methods of estimating the source size.

It is now of interest to compare the optical and radio distributions. It seems reasonable to assume that component *B* is in fact coincident with the stellar object and that this object can be used as a reference point in making the comparison. Hughes and Van Hoerner have shown that the radio emission is essentially confined to a line joining *A* and *B*, a conclusion which was suggested by the early occultation observations. If we make this assumption then the position and extent of the various components of *A* can easily be deduced from the angles between the limb of the Moon and the axis of the source at the times of occultation.

The results of this analysis are summarized in Fig. 5. The narrow component at both frequencies lies very close to the end of the jet, 19·3″ and 20·1″ at 430 and 1,420 Mc/s compared with the optical value of 20·0″. The flat extended component at 430 Mc/s merges into the noise at a distance of about 8″ from *B* while the long flat extension at 1,420 Mc/s becomes visible at a distance of 10″, which is close to the start of the optical jet at 11″ from *B*. The sharp fall in the flux from the extended source visible at 430 Mc/s with a resolution of 0·4″ is 14″ from *B* which corresponds closely at 1,420 Mc/s to the edge of the extended source lying beneath the narrow component.

It seems clear that this extended source at 1,420 Mc/s is associated with the flat central component observed at 430 Mc/s, the main difference being that while at 1,420 Mc/s it reaches at least 6″ beyond the edge of the jet there is no evidence for any extension of the central component at 430 Mc/s. However, the 0·9″ resolution curve in Fig. 2 suggests that the extended region of *A* does reach beyond the edge of the jet which indicates that at least part of the extension at 1,420 Mc/s may be associated with the long flat extension rather than the central extended component. There is therefore a remarkable correlation between the optical and radio features; of particular interest is the constancy of the flux at 430 Mc/s over a large region of the visible jet and the strong suggestions that the narrow component arises in a shock-front at the leading edge of the jet. It may be noted that the change in the structure of the source with frequency will lead to an apparent change in position with frequency unless the resolution is adequate to reveal the detailed structure.

A comparison has been made of the ratio of the fluxes of *A* and *B* from the 430 Mc/s records of August 20, 1962, and those of June 7, 1965. The comparison is somewhat complicated by the different angles of cut across the source in both cases and by the presence of some weak structure which may not lie along the line joining *A* and *B*. However, adopting all possible interpretations, it is clear that the ratio of *A* to *B* has not changed by more than 7 per cent and more likely by less than 2 per cent over the 3-year period. This suggests that unless both components are varying in the same way the flux of *B* has changed by less than 7 per cent which may be compared with the value of 51 per cent estimated by Dent at 8,000 Mc/s over approximately the same period.

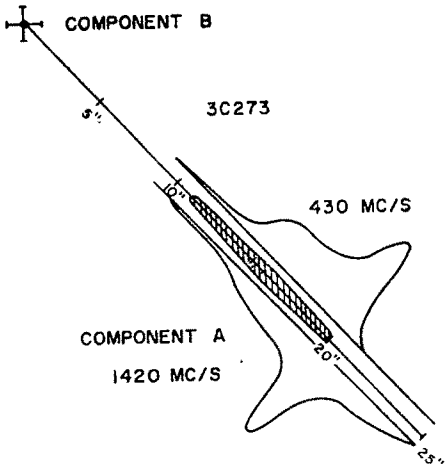


Fig. 5 Schematic representation of the optical, 430-Mc/s and 1,420-Mc/s distributions along the axis of 3C273. It has been assumed that component *B* is coincident at both frequencies with the stellar object. The shaded area represents the faint optical jet

We thank Prof. W. E. Gordon for his interest and encouragement throughout the course of the work.

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BRIGHTNESS TEMPERATURE OF SOLAR ACTIVE REGIONS DETERMINED FROM DRIFT CURVES OF THE SUN ACROSS A BROAD ANTENNA MAIN LOBE

By DR H. W. URBARZ
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TWO methods are being used in investigating solar active regions of small diameter at radio frequencies; these are: scanning the Sun by high-resolution radio telescopes with fan beams or pencil beams and discussing solar eclipse curves providing high resolution in one dimension. Another method and its results are briefly discussed here, which can be described as the superposition of wide-beam transit curves.

From October 1960 to March 1964 continuous measurements of the total solar radio flux density were made with some interruptions at Tubingen at 3,750 mc/s. The main lobe of the 10 ft. horn fed reflector was about 110 min of arc wide. A number of solar transits were recorded from time to time in order to check the antenna characteristics. The integrated brightness temperature and flux density were derived from the antenna temperature of the continuous records, which is the maximum transit value[1,2]. The measurements were made to find the daily mean values of the quiet and slowly varying components and to record burst events. The slowly varying component is due to coronal condensations, which appear as small areas of enhanced temperature on the solar disk and disappear over periods of weeks or months. The apparent brightness temperature of the small active areas may be derived by superposing drift curves, provided the location and diameter of the enhanced areas on the solar disk are known. These data were available from optical maps and high-resolution radio observations. The results show that small active areas may be determined by the superposition method with small radio telescopes as used for daily measurements of mean values. The method may in particular be interesting at other frequencies than 9·1 cm and 21 cm at which solar radio maps are produced by high-resolution instruments. The 21-cm maps were edited from 1959 to October 1962 by Fleurs Observatory and the 9·1 cm radio maps are being edited by Stanford Observatory[3,4]. The essential idea of the superposition method is to regard the solar brightness distribution as a disk of uniform temperature superposed by Gauss-type sources of defined location and maximum temperature and width. A measured drift curve may be considered a result of the superposition of individual drifts of the main lobe across the quiet Sun described by a cylindric source model and a number of small areas of much higher temperature described by Gauss-type sources. The drift curve of an antenna main lobe described by a Gauss distribution $F_n = \exp(-\zeta^2/\alpha'^2 - \gamma^2/\beta'^2)$ across a cylindric source characterized by radius $R_\odot$ and uniform temperature T_c is given[1] by the relation:

$$T_{Ac} = T_c\left(1-\beta_s\right)\frac{R_\odot^2}{\alpha'\beta'}\exp\left(-\frac{\zeta^2}{\alpha'^2}-\frac{\gamma^2}{\beta'^2}\right)\left\{1-\frac{R_\odot^2}{2\alpha'^2}\left(\frac{1}{2}-\frac{\zeta^2}{\alpha'^2}\right)-\frac{R_\odot^2}{2\beta'^2}\left(\frac{1}{2}-\frac{\gamma^2}{\beta'^2}\right)\right\} \quad (1)$$

T_{Ac} is the main lobe contribution to antenna temperature caused by the source, and α', β' are the Gauss parameter of the main lobe, β_s is the stray factor of the antenna and ζ, γ are the distances of the source centre relative to the main lobe-centre in a rectangular co-ordinate system.

The term in parentheses is a correction if the source radius $R_\odot$ is not very small relative to α', β'.

The drift curve of a Gauss-type source across the main-lobe described by maximum temperature T_g and width parameters α, β is given by:

$$T_{Ag} = \left(1-\beta_s\right)T_g\frac{\alpha\beta}{\alpha'\beta'}\exp\left(-\frac{\zeta^2}{\alpha'^2}-\frac{\gamma^2}{\beta'^2}\right) \quad (2)$$

In equation (2) it is assumed that $\alpha, \beta \ll \alpha', \beta'$, that is the widening of the transit curve due to finite source extension is neglected, since the half power width (HPW) would be $\mathrm{HPW} = \sqrt{\ln 2}\ \alpha\ \{1+1/2\ (\alpha/\alpha')^2\}$

The response of antenna temperature to a drift in ζ-direction through the centre of the solar disk and a number of small sources imposed located at (a_n, b_n) may now be written:

$$T_{A,tot} = T_{Ac}\left(\zeta, 0\right) + \sum_{n=1}^{n} T_{Ag}\left(\zeta - a_n, \gamma - b_n\right) \quad (3)$$

The summation of the stationary mean values of noise powers is correct if the contributions of the individual areas are incoherent. This is no doubt the case for any surface element of the solar disk emitting noise. Fig. 1 shows the location of the sources relative to the main lobe centre and the corresponding drift curves, where ζ and γ may be identified as hour angle and declination respectively. The resulting drift curve across the quiet Sun and one or more small hot areas is essentially the sum of exponential functions with different amplitudes K_n and different deviations a,b of the maximum with respect to the centre $\zeta = 0$ of the quiet Sun drift curve.

The maximum excentric deviation is $\zeta_{max} = R_\odot$, representing a source on the solar limb located at $\gamma = 0$. The resulting maximum of the superposed transit curve will be located between $\zeta = 0$ and $\zeta = R_\odot$. Since $\alpha', \beta' \sim 4\,R_\odot$ the amplitude at ζ_{max} will be practically the same as at $\zeta = 0$; at $\zeta = 1/2\ R_\odot$ we have $\exp(-1/69) = 0{\cdot}985$. So one may conclude that in any case the resulting maximum of $T_{A,tot}$ (ζ) will be situated close to $\zeta = 0$. The approximation $\zeta_{max} = 0$ simplifies the analysis considerably, the error being the smaller, the smaller the ratios $(R_\odot/\alpha'; R_\odot/\beta')$. If, for example, two or more hot areas are located on one half of the solar disk, the effect of unsymmetry will accumulate. But in general the sources will be distributed over the east and west half, so that the unsymmetry of $T_{A,tot}$ (ζ) will be partially cancelled. The best way, it is felt, of studying a special case of source distribution is to write equation (3) with all numerical values. The numerical values of the parameter of our telescope not related to the superposed sources are[1]:

$T_\odot = 27 \times 10^3$ °K, $\beta_s = 0{\cdot}415$, $R_\odot = 32'$, $\alpha' = 4{\cdot}14\ R_\odot$, $\beta' = 3{\cdot}85\ R_\odot$

Inserting these values in equation (3) and setting $\zeta = 0$ we get the relation:

$$T_{A,tot,\max} = 958 + \sum_{n=1}^{n} T_{g\,n} \frac{\alpha_n \beta_n}{30{\cdot}34\ R_\odot^2} \exp\left\{ - \frac{a^2 n}{17{\cdot}13\ R_\odot^2} - \frac{b^2 n}{14{\cdot}83\ R_\odot^2} \right\} \quad (4)$$

This relation may be applied to any distribution of enhanced areas on the solar disk, if the maximum antenna temperature of the transit curve $T_{A,tot,\max}$ has been recorded with our telescope.

Now this relation is to be used in connexion with the solar radio[3] may of October 31, 1960, reproduced in Fig. 2. The optical solar map of the Fraunhoferinstitute[7] shows two large sunspots near the radio spots in the north-east and south-east quadrant of the disk, coinciding with Ca-plage regions. Table 1 gives the co-ordinates a,b and the Gauss parameters of the sources gathered from the 21-cm radio map. It may be assumed that the temperature maxima of the 21-cm spots coincide with the maximum at 8 cm. With the data of Table 1 and equation (4) we get a simple relation for the T_g and α,β values at 8 cm:

$$1{,}529 = 958 + 0{\cdot}034\ T_{g1} \frac{\alpha_1 \beta_1}{R_\odot^2} + 0{\cdot}036\ T_{g2} \frac{\alpha_2 \beta_2}{R_\odot^2} \quad (5)$$

This is an equation with four unknowns, and, to get more relations, two assumptions on the spectrum are made which may include considerable error: that the ratio of maxima temperatures and that of the source parameters is the same at 8 cm as it is at 21 cm. From Table 1 we have:

$$T_{g1}/T_{g2} = 1/2{\cdot}58 \quad (6)$$

$$\frac{\alpha_{8\ cm}}{\alpha_{21\ cm}} = \frac{\beta_{8\ cm}}{\beta_{21\ cm}} \quad (7)$$

$$\alpha_1\beta_1/\alpha_2\beta_2 = 2 \quad (8)$$

The brightness temperatures of the quiet Sun were found conventionally by extrapolating the daily mean values of the flux density to the sunspot number 0 plotted in a scatter diagram. Data of the period from October 15,

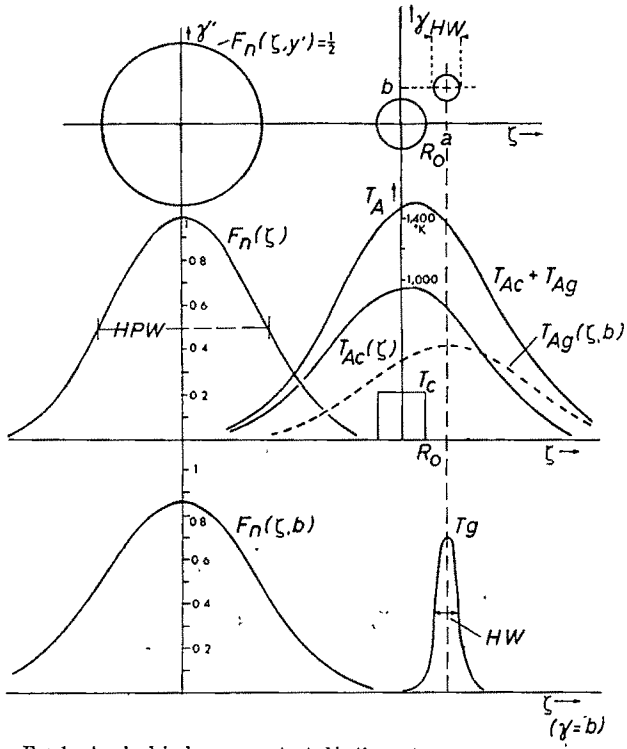


Fig 1. A cylindrical source is situated in the centre A Gauss-type source is at (a, b) of the ζ γ system A Gauss-type antenna main lobe is swept across the two sources in ζ direction. The resulting response of antenna temperature is the sum of the individual drift curves $T_{Ac}(\zeta)$ and $T_{Ag}(\zeta,b)$. The following parameters are used in the true scale graph: $a' = 4\ 14R_\odot, \beta' = 3{\cdot}85R_\odot, a = 2R_\odot, b = 1{\cdot}5R_\odot, \alpha,\beta = 0\ 6R_\odot, HW = R_\odot$

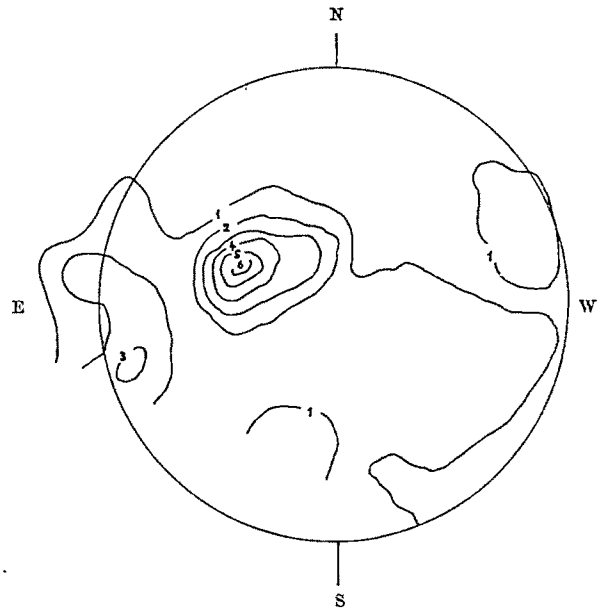


Fig. 2. October 31, 1960 ζ=21-cm spot scan, Fleurs, Sydney; $T_\odot = 10^6$ °K: isophote level

1960, to February 28, 1961, were included[2,3,5]. Labrum[6] has found that the quiet Sun component of the total solar flux density and consequently the apparent disk temperature show variations in the ratio 1 : 2 from sunspot minimum to maximum. The adopted values ($T_{\odot,21\ cm} = 1{\cdot}1 \times 10^6$ °K and $T_{\odot,8\ cm} = 2{\cdot}7 \times 10^4$ °K) represent the quiet sun level during the period in question. The daily mean values of the Heinrich-Hertz-Institut at 21 cm were plotted in the scatter diagram with the proposed correction factor of 0·7 (ref. 8).

Little is known about the morphology and the spectrum of radio condensations, but from known observations one may state qualitatively that the area of a condensation as well as the apparent brightness temperature increases with wave-lengths. In our case this means the 8 cm area is not larger than the 21-cm area and the same holds for the maxima temperatures. To find some 8-cm parameters, the following procedure was used. First, the β values of Table 1 were inserted in equation (5) and the values T_{g1}, T_{g2} were determined using equation (6). The temperatures of Table 1 were then inserted in equation (5) and the source parameters α,β were found from equations (7) and (8) and Table 1. Table 2 gives the results. The parameters of the model describing the 8-cm condensation found by this procedure are the minimum values. The true 8-cm values of T_g and α,β will be closer to the 21-cm data. Interpolating simply between the two models found by the superposition method, $T_{g21\ cm}$ $\alpha_{8\ cm}$ $\beta_{8\ cm}$ and $T_{g,8\ cm}$, $\alpha_{21\ cm}$ $\beta_{21\ cm}$, might lead to a better approximation of the true distribution (Table 3).

Examining 21-cm and 9·1-cm radio maps one finds a variety of enhanced areas regarding diameters and maximum temperature. Unfortunately these recordings only overlap a few months. Furthermore, we now know that mapping the Sun with 0·5 min of arc pencil beams is required instead of a 3 min of arc beam width. More statistical material of very high resolution at three or more frequencies is desirable in order to find the spectral features and to determine the emission process of condensations. It can be supposed that two basic types exist: purely thermal emission and thermal emission with gyromagnetic emission. These may be ascribed to weak condensations of coronal or lower temperatures or to intense condensations exceeding it, both giving different spectral features. The gyro mechanism of radiation from condensations was proposed in ref. 12. The collected observations in ref. 8 tend to indicate that higher tempera-

Table 1. 21-CM DATA OF SOLAR RADIO MAP

Date	Source No.	Fraction of $R_\odot$: Co-ordinate of maximum	Fraction of $R_\odot$: HPW of sources	Fraction of $R_\odot$: Source parameter	$T_\odot$ and flux density	$T_g+T_\odot$ (°K)[3]	T_g (°K)	$\frac{T_{g1}}{T_{g2}}$	$\frac{\alpha_1}{\alpha_2}$	$\frac{\beta_1}{\beta_2}$	Flux density 10^{-21} jan.
Oct. 30 1960	1	−0 863 −0 196	0·392 0 549	0·236 0 329	$1\cdot1\times10^6$ °K	3×10^5	$1\cdot9\times10^5$	1:2 58	1	2	1·31
	2	−0 392 0 176	0 392 0 274	0 236 0 165	4 65 10^{-21} jan.	6×10^5	$4\cdot9\times10^5$				1·7

Table 2 8-CM DATA EVALUATED

Source No	Maximum temp. of reduced drift curve	$T_\odot$ and flux density	T_g (°K)	$T_g+T_\odot$ (°K)	(α,β) 8 cm	$\frac{\alpha 8\text{ cm}}{\alpha 21\text{ cm}} = \frac{\beta 8\text{ cm}}{\beta 21\text{ cm}}$	α fraction of $R_\odot$	β fraction of $R_\odot$
1	1,529° K	$2\cdot7\times10^4$ °K	$9\cdot06\times10^4$	$1\cdot18\times10^5$	$3\cdot72\times10^{-2}\,R_\odot$	0 693	0·161	0·114
2		$7\cdot9\times10^{-21}$ jan	$2\cdot61\times10^5$	$2\cdot61\times10^5$	$1\cdot86\times10^{-2}\,R_\odot$		0·161	0 228
			assumed: $\alpha 8\text{ cm}=\alpha 21\text{ cm}$, $\beta 8\text{ cm}=\beta 21\text{ cm}$		assumed: $T_{g21\text{ cm}}=T_{g8\text{ cm}}$			

Table 3. 8-CM DATA CORRECTED BY INTERPOLATING SOURCE MODELS

Source No.	$T_{cor.}=\frac{T_{g21}+T_{g8}}{2}$	Fraction of $R_\odot$: α or $=\frac{\alpha_{21}+\alpha_8}{2}$	Fraction of $R_\odot$: $\beta_{cor}=\frac{\beta_{21}+\beta_8}{2}$	HPW_ζ	HPW_γ	$(HPW_\zeta)(HPW_\gamma)$ square min of arc	Flux density 10^{-21} jan
1	$1\cdot4\times10^5$ °K	0 198	0 278	0·327	0 461	38	2·24
2	$3\cdot26\times10^5$ °K	0·198	0 14	0 327	0 232		2·59

tures than coronal exist, and this should be confirmed by observations at very high resolutions.

Some spectral features regarding size seem to be definite. Plotting the diameter of fourteen different condensations against frequency given in refs. 8–11 shows no variation of diameter from 3 cm to about 15 cm, while from 15 to 30 cm wave-length the simple relation $A=1\cdot6\lambda+b$ can be adopted, where A is the area in sq. min of arc (product of HPW of profiles), λ is the wave-length in cm, and b is a constant varying from one condensation to another. According to Table 1, source 1 has an area of 55 sq. min at 21 cm, and $b=21$ gives 44 sq. min at 8 cm. The interpolated values of Table 3 give 38 sq. min which is in fairly good agreement.

It is not, however, possible to find a similar relation for peak temperatures from the present observations. Eclipse measurements give the extent of sources in one dimension correctly, but to deduce temperature from brightness requires both diameters in perpendicular directions. An estimate of the lacking diameter leads to an estimate of peak temperature only[9–11]. On the other hand, as the present pencil beam observations yield flattened values of peak temperatures, the estimates of Table 3 cannot be improved, and interpolating in this way may be adopted as a feasible procedure.

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TRIGGERING OF THE ALASKAN EARTHQUAKE OF MARCH 28, 1964, AND MAJOR AFTERSHOCKS BY LOW OCEAN TIDE LOADS

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DURING the investigation of the tsunami in South-central Alaska, many of the interviewed inhabitants of the damaged harbours, villages and isolated homesteads stated that it was very fortunate that the 'quake had occurred during the low tide levels. The tsunami thus did not cause nearly as much damage as if it had occurred at high tide level which is generally some 4–5 m higher. The low tide has therefore been considered as a possible trigger for the main earthquake and larger aftershocks. Stresses and strains induced in the crust either from atmospheric disturbances or from the solid earth tides have been considered possible influences on the occurrence of earthquakes. Knopoff[1], however, has shown that the solid earth tides do not have a statistically meaningful influence in California. The peak-to-peak strains induced are about $1/2\times10^{-7}$.

Those induced in the area of the Gulf of Alaska are considerably higher. The results of other investigations seem to indicate the incidence of earthquakes in regions where some anomalous crustal loading occurs. D. S. Carder[2] reports earthquakes associated with the reservoir loading of the Boulder Dam area and his calculations indi-

cate the correct relation between earthquake energy and deformation under the water load. Dr. and Mrs. Gough also reported a higher incidence of shocks as a result of waterloading in a dam area from Rhodesia[3]. McGinnis[4] investigated the earthquake incidence due to water load changes in the Mississippi Valley Region, where the earthquake frequency increases with an increase in the rate of change of river stage, and the rate of energy release increases with an increase in water mass, the addition of a water load only aiding the movement of a subsiding crust.

Very pronounced occurrence of earthquake swarms under low ocean tidal loads have been described in the Bay of Sagami area by Matuzawa[5] (after research by the Imamura group). In this case, however, the tide variations at the coast are only about 1 m (peak to peak), whereas those in the shelf area of the Gulf of Alaska are about 4–5 m (peak to peak). In Japan, as in Alaska, the earthquakes occur during the low water half of the tide cycle.

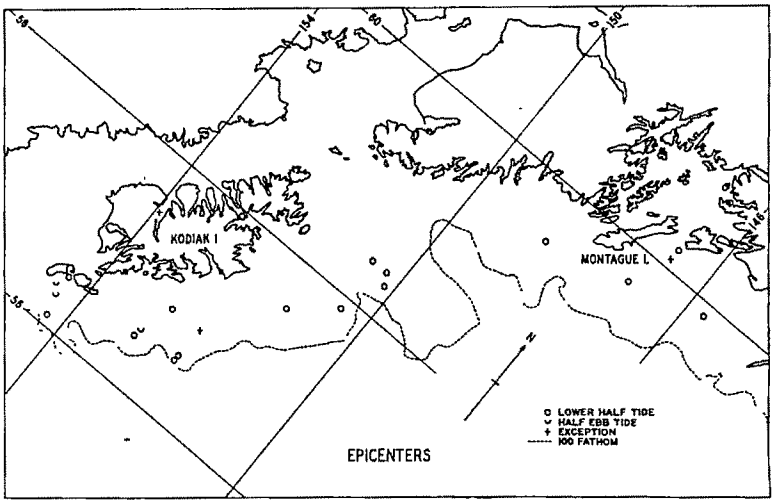


Fig. 2. Epicentre locations of shocks with $m_B \geq 5{\cdot}5$, O, lower half tide; U, half ebb tide, +, exception; - - -, 100 fathoms

The tidal diurnal ranges in the Gulf of Alaska are very high compared with elsewhere. In the preparation of Fig. 1, only those locations very close to open water have been chosen, to give an idea of the loading in the shelf area itself. This shelf area is here (more or less arbitrarily) considered to be limited by the 100-fathom line, since from there on the depth increases very rapidly towards the Aleutian Trench (or its extension) to about 2,000 fathoms within 70 km in the middle section of the aftershock area, and twice as fast south of Kodiak Island[14]. The tide ranges are highest in the north-eastern part and diminish progressively further out along the Aleutian Chain. Since Middleton Island, situated almost on the edge of the shelf, has a diurnal range of 10·3 ft., I feel that 3 m is a representative level for most of the Gulf area, compared with diurnal variations some 20 per cent higher (12·3 ft.) at Cordova in the Prince William Sound area. Also, the absolute water levels have drastically changed throughout Alaska (by about 6 ft. at Cordova, 20 ft. in most of Montague Island and 5 ft. in Kodiak) although the tidal variations stood basically the same. Therefore, the predicted tides still could be used for the present investigation.

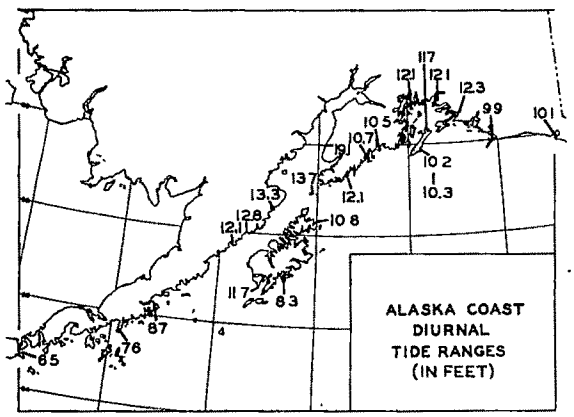


Fig. 1. Tide ranges along the Alaskan coast (in ft.)

Fig. 2 shows the location of the epicentres of all the shocks of magnitude $m_B \geq 5{\cdot}5$ to August 30, 1964, listed in the first part of Table 1, except for the main shock. The table includes all the aftershocks of $m_B \geq 5{\cdot}5$ listed by the U.S. Coast and Geodetic Survey starting 24 h after the main shock. The aftershocks for the first 24 h are not included, since their occurrence is predominantly determined by the aftershock release mechanism.

Fig. 3 shows the tide levels for Cordova and partly for Kodiak (dashed lines) and the time (A.S.T.) of occurrence of the earthquakes with respect to the tides up to April 19. It is found that most of the quakes occur in the lower

Table 1. EPICENTRES WITH $m_B \geq 5{\cdot}5$ TO AUGUST 30 FROM U.S. COAST AND GEODETIC SURVEY PRELIMINARY EPICENTRE CARDS

64	03	28	033612·7	0·6	87	61·1	N.	147·6	W.	H	20	M		8 6	8·4	8·6	PAL	PAS	BRK
64	03	29	060444·5	0·6	56	56·1	N.	154·3	W.	H	30	M5·6	0·2						CGS
64	03	29	164057·9	0·7	72	59·7	N.	147·0	W.	H	15	M5·6	0·3						CGS
64	03	30	021806·3	0·9	75	56 6	N.	152·9	W.	H	25	M5·8	0·4						CGS
64	03	30	070934·0	0·8	80	59·9	N.	145·7	W.	H	15	M5·6	0·3						CGS
64	03	30	160928·4	0·6	67	56·6	N.	152 1	W.	H	25	M5·5	0·3						CGS
64	04	03	084627·0	1·4	13	57·9	N.	150·5	W.	H	15	M5·5	0·0						CGS
64	04	04	045401·7	0·8	76	60·1	N.	146·7	W.	H	40	M5·6	0·2	Deep					CGS
64	04	04	091055·1	0·7	74	56·9	N.	152·7	W.	H	15	M5·9	0·4						CGS
64	04	04	174608·6	0·8	78	56·3	N.	154·4	W.	H	25	M5 7	0·4						CGS
64	04	04	175943·3	0·7	48	56·4	N.	154·5	W.	H	25	M5·5	0·3						CGS
64	04	05	192818·1	0·8	85	60·2	N.	146·7	W.	H	15	M5·8	0·3						CGS
64	04	07	192824·7	0·7	55	55·7	N.	151·9	W.	H	20	M5·6	0·3	Off shelf					CGS
64	04	10	010800·2	0·7	65	58·4	N.	150·6	W.	H	15	M5·5	0·4						CGS
64	04	12	012431·2	0·9	95	56 6	N.	152·2	W.	H	22	M5·6	0·4						CGS
64	04	13	140500 0	0·6	57	57·6	N.	151·2	W.	H	25	M5·5	0·2						CGS
64	04	13	212633·0	0·8	68	57·5	N.	153·9	W.	H	30	M5·5	0 3	On Kodiak					CGS
64	04	15	153047·1	0 6	74	56·5	N.	154·4	W.	H	35	M5·5	0 4						CGS
64	04	16	192657 4	0·7	87	56·4	N.	152·9	W.	H	30	M5·5	0 4						CGS
64	05	29	101734·5	0·9	24	60·2	N.	146·8	W.	H	5	M5·6	0·0	Exception					CGS
64	05	30	031808·3	0 9	21	59·5	N.	148·5	W.	H		M5·5	0·4						CGS
64	06	28	190905·4	0 9	55	58 3	N.	150·2	W.	H	23	M5 5	0·0						CGS
64	08	06	182450·5	0·4	15	56 9	N.	152·1	W.	H	39	M5·6	0·0	Deep half ebb tide					CGS
64	08	24	215654·2	1·0	46	58·4	N.	150·3	W.	H	22	M5·8	0·0						CGS
Earthquakes not mapped																			
64	04	03	223342·2	0·7	80	61·6	N.	147·6	W.	H	40	M5·7	0·4	Inland					CGS
64	04	10	214406·7	0·6	63	60·1	N.	153·7	W.	H	10	M5·6	0·4	Inland					CGS
64	04	20	115641·6	0·8	87	61·4	N.	147·3	W.	H	30	M5 7	0·4	Inland					CGS
64	06	29	072132·8	0·8	58	62·7	N.	152 0	W.	H	33	M5·6	0·0	Inland					CGS
64	08	02	030416·9	1·0	19	56·1	N.	156·1	W.	H	33	M5·6	0·0	Out of map Possible exception					CGS

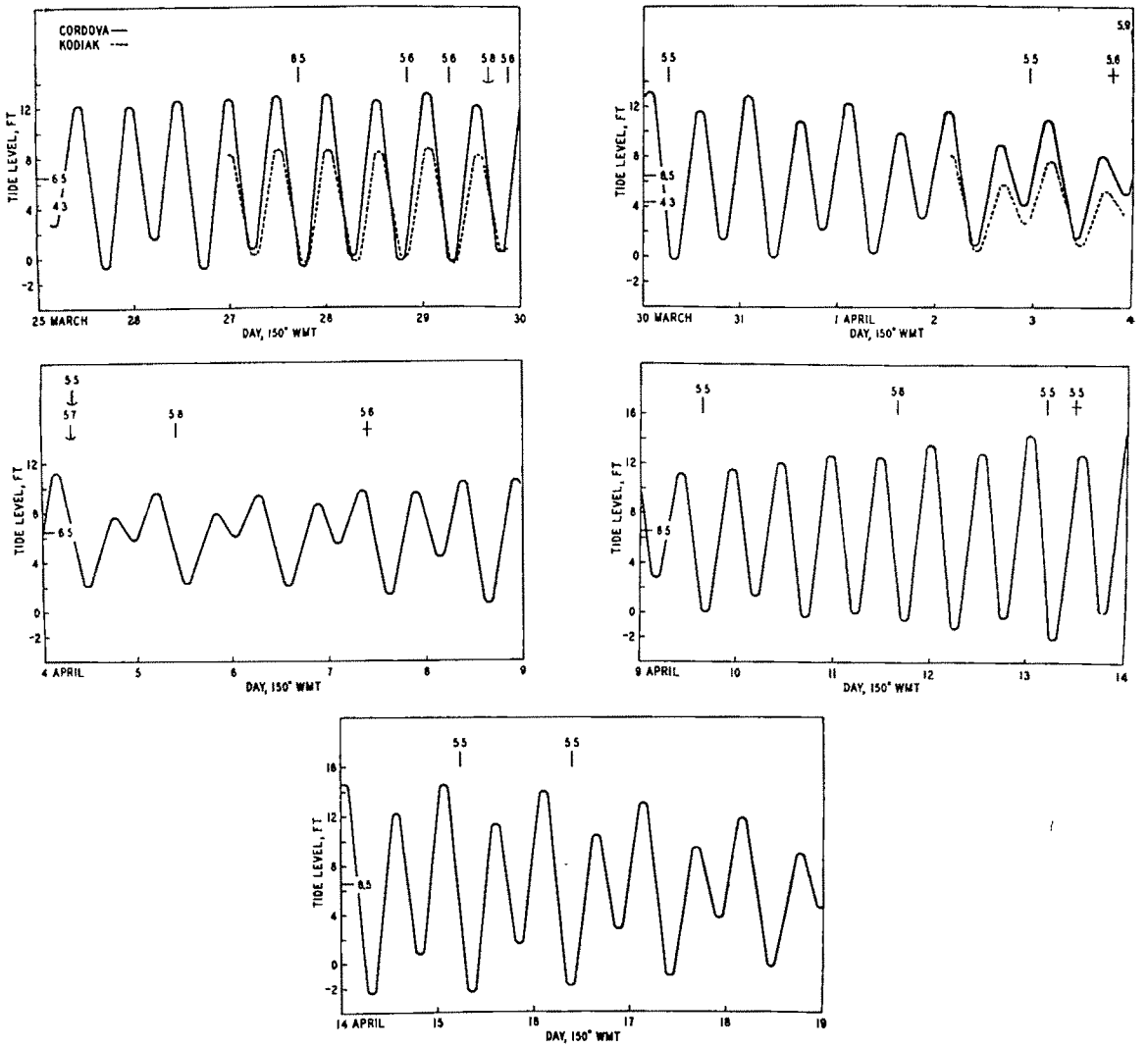


Fig 3 Earthquake occurrence and tide levels[6]

half of the tide cycle or very close to the half ebb tide. The exceptions which may be explained are: two 'quakes at a depth of 39 and 40 km, the 'quakes inland or on Kodiak Island, and one off the shelf area. Since the stresses induced by the tide load diminish with depth and with the distance from the coastline and the tide levels also diminish with the depth of water, these exceptions may be expected.

The exceptions are marked on Figs. 2 and 3 by a cross. The 'quakes marked with an open half circle are those very close to the half ebb tide.

The remaining two 'quakes which are exceptions are those of May 29 at the north-eastern end of the aftershock zone and the one of August 2 at the very south-western end (off the map). No attempt has so far been made to consider shocks of magnitude $m_B < 5{\cdot}5$.

No attempt is made to solve the problem of the fracture mechanism of rocks *in situ*, that is, at the earthquake failure zone. But if it is admitted that the 'quakes examined here have been effectively triggered by the unloading of the crust, some more data limiting the figures for rock strength in an important earthquake belt are obtained.

During a half-tide cycle of about 6 h duration the pressure varies approximately by 0·3 kg/cm² on the ocean floor of the shelf area. This pressure variation seems to act as a trigger for the main 'quake and the subsequent aftershocks (excluding the first 24 h) of $m_B \geqslant 5{\cdot}5$. Since brittle fracture seems to be excluded at some depth, and on the other hand strain and tilt observations prior to large earthquakes point towards rapidly increasing values as the failure approaches, it is assumed that a 'creep' fracture is involved. The rocks *in situ* are already stressed far beyond the yield point into the strain-hardening portion of the stress-strain curve, possibly into the strain-softening area, so that the additional shear force generated by the unloading of the crust (see Fig. 4) in the shelf area and applied during a few hours is sufficient to bring the material to the fracture point. It may be that the very shallow 'quake (5 km) of May 29, which did not occur during the unloading, is an indication that the creep or flow fracture is confined to greater depth and does not apply to very shallow depth earthquakes.

In laboratory experiments the shear strength of rocks is of the order of 100–400 kg/cm²,[7] but several workers pointed out that strengths of rocks *in situ* are reduced by

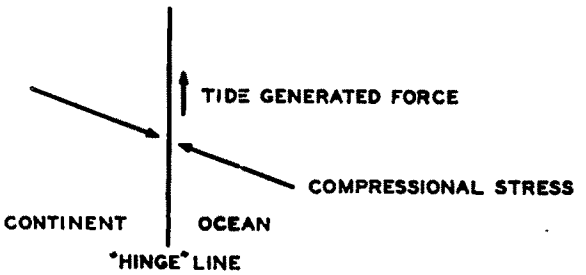


Fig 4 Stress system along the hinge line

up to 2 orders of magnitude[8]. It seems reasonable, therefore, to assume that the crustal unloading is able to initiate fracture when the pressure variation is of the order of 1 per cent or less of the total fracture stress. Also, laboratory stress experiments with other materials indicate that failure occurs much more rapidly if a very small cyclic stress is superimposed on a continuous one, where the amplitude of the cyclic stress is even less than 1 per cent of the continuous one. In a particular kind of steel under an 'equilibrium' stress of 120,000 lb./in.^{2} and strain of 4×10^{-2} large flow rates of 5×10^{-3}/sec in strain are initiated by only a 1·5 per cent overstress[9].

Assuming similar behaviour for rocks *in situ*, an upper limit for the failure stresses encountered can be obtained. Using 0·3 kg/cm^2 × 100, a value roughly of the order of 30 kg/cm^2 is found which will, however, diminish with increasing depth.

Balakina[10] has shown that for all the major earthquakes (with one exception) in the Aleutian Chain and the Alaska belt, the principal stress axis is compressional and more or less perpendicular to the tectonic trend. In addition it is directed slightly upward on the Pacific Ocean side for focal depths less than 100 km. I found[11] that this holds also for the main shock and major aftershocks (partly unpublished data) in the Alaska 1964 event.

The periodical unloading of the crust generates an additional upward directed component of force, introducing a periodic shear enhancing the existing stress system and the tectonic movement along the 'hinge' line. The 'hinge' line separates zones of land rise on its ocean side from land subsidence on its continental side[12,13] (Fig. 4).

The unloading of the crust by large ocean tides may be considered as a triggering mechanism for the Alaska earthquake of March 1964 and major aftershocks. The stress system is compatible with that deduced from fault plane solutions and land-uplift and subsidence which occurred during the main shock.

Since brittle fracture seems to be excluded at some depth, the time interval of a few hours and the pressure variation involved as a result of the ocean tide cycle seem to be significant in carrying the last part of the stress-strain curve in the 'strain-softening' region towards failure.

I thank Dr. Maurice Ewing, Dr. Jack Oliver and Mr. William Best for valuable discussions and strong encouragement to publish these data.

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DEPENDENCE OF CONTACT ANGLES ON TEMPERATURE: POLAR LIQUIDS ON POLYETHYLENE

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INVESTIGATIONS of the dependence on temperature of the advancing contact angle of liquids on 'low energy' solids, notably the thermoplastic polymers, have been reported by Phillips and Riddiford[1] and Johnson and Dettre[2]. Before these investigations, relatively few data existed for the effect of temperature on the contact angle[3]. This communication presents the results of some experiments and calculations on the effect of temperature on advancing contact angles between polar liquids and polyethylene. The experimental data on contact angles for the glycerol–polyethylene system are analysed in the light of current theoretical approaches to interfacial phenomena[4,5]. The impetus for this investigation has been the extensive wettability investigations of Zisman[6] and the theoretical work of Fowkes[4] and Good *et al.*[5]. Moreover, the implications in the area of adhesion are considerable[7].

The glycerol, which was used without further purification, was a 'Baker Analysed' reagent from the J. T. Baker Chemical Company. The surface tension at 20° C was 63·4 dynes/cm as measured with a Du Noüy tensiometer.

The polyethylene was a 'Marlex 5003' obtained from the Phillips Chemical Company, Bartlesville, Oklahoma. The polymer had a melt index of 0·3 and a density at 20° C of 0·95. A 0·010-in.-thick film was formed by moulding at about 1,000 lb./in.^{2} and 160° C.

For the measurements of contact angle, the polyethylene film was mounted on a standard microscope slide, using double-backed adhesive tape, then placed on a hot stage. A surface thermometer with an accuracy of ±1° C, manufactured by the Pacific Transducer Corporation, Los Angeles, California, was placed on the polyethylene surface external to the area used for contact angle measurement to provide a record of the interfacial temperature.

The advancing contact angles were measured directly using a telescopic device equipped with an ocular protractor which was built by the Gaertner Scientific Corporation, Chicago, Illinois. Three separate drops were placed on the polymer surface with opposite edges of the drops being measured. The drops were equilibrated for 10 min at each temperature before reading the contact angle. The reproducibility of the readings was about ±2°. The comparatively high vapour pressure of both water and formamide precluded their study under the conditions of the present experiment.

The results of the temperature dependence of the contact angle of glycerol on polyethylene are presented in the last column of Table 1. The contact angles were between 78° and 80° in the temperature range from 20° C to about 120° C, just below the melting point of the polyethylene. The readings were taken at 10° C intervals, although only the 20° C and the 90° C are reported in Table 1. We shall attempt to interpret these results in view of the recent approaches by myself[8], Fowkes[4] and Good[5].

Schonhorn[8] has proposed on the basis of the surface-tension experiments of Schonhorn and Sharpe[9,10] that at 25° C:

$$\gamma_{LV} = \gamma_c \qquad (1)$$

Table 1. TEMPERATURE DEPENDENCE OF THE WETTABILITY OF POLYETHYLENE BY WATER, GLYCEROL AND FORMAMIDE

T °C	$(\gamma_{LV})_p$ dynes/cm	(γ_{LV}) dynes/cm	k	(γ^d_{LV}) dynes/cm	$\cos\theta$	θ deg.	θ^* deg.	θ^{exp} deg.
Water–Polyethylene								
0	37·6	75·60	0·300	22·7	−0·2275	103	—	—
10	36·9	74·22		22·2	−0·2280	103	—	—
20	36·2	72·75		21·8	−0·2275	103	94	—
30	35 5	71·18		21·3	−0·2273	103	—	—
40	34·7	69·56		20 8	−0 2266	103	—	—
50	33·9	67·91		20·3	−0·2254	103	—	—
60	33 2	66·18		19·8	−0·2248	103	—	—
70	32·4	64·40		19·3	−0·2236	103	—	—
80	31·7	62·60		18·7	−0 2204	103	—	—
100	30·2	58·90		17·6	−0 2156	102	—	—
Glycerol–Polyethylene								
20	36·2	63·4	0·584	37·0	0·1546	81	79	78–80
90	31·0	58 6		34·2	0·1126	83	—	78–80
Formamide–Polyethylene								
0	37·6	59 9	0 679	40 7	0 3055	72	—	—
20	36·2	58·2		39·5	0·2989	73	77	—
50	33·9	55·7		37·8	0 2854	73	—	—
75	32·0	53 5		36·3	0 2748	74	—	—

where γ_{LV} is the surface tension of the supercooled liquid polymer and γ_c is the critical surface tension of wetting[6]. Further, we propose that equation (1) is valid at all temperatures. Then we may state that:

$$d\gamma_{LV}/dT = d\gamma_c/dT \quad (2)$$

When γ_{LV} is known as a function of temperature, we automatically know the temperature dependence of γ_c.

The Young equation:

$$\gamma_{SV} = \gamma_{SL} + \gamma_{LV}\cos\theta \quad (3)$$

in conjunction with Fowkes's expression[4]:

$$\gamma_{SL} = \gamma_{SV} + \gamma_{LV} - 2(\gamma^d_S\gamma^d_{LV})^{1/2} \quad (4)$$

and equation (1) yields:

$$\cos\theta = \frac{2[(\gamma_{LV})_p\gamma^d_{LV}]^{1/2}}{\gamma_{LV}} - 1 \quad (5)$$

where p refers to the polymer.

To estimate cos θ as a function of temperature the temperature dependence of $(\gamma_{LV})_p$, γ^d_{LV} and γ_{LV} must be known. Schonhorn and Sharpe[9] have measured the surface tension of polyethylene over an extended temperature interval. The values for surface tension listed in Table 1 were obtained by extrapolating the data of Schonhorn and Sharpe[9] to low temperatures. The γ_{LV} values for water[11], glycerol[11] and formamide[11] are also recorded in Table 1. To estimate γ^d_{LV}, we propose that the ratio $\gamma^d_{LV}/\gamma_{LV}$ is invariant with temperature. Fowkes[12] has estimated the γ^d_{LV} values at 20° C for water, glycerol and formamide to be 21·8, 37·0 and 40·7 dynes/cm, respectively. Now equation (5) becomes:

$$\cos\theta = \frac{2k^{1/2}(\gamma_{LV})_p^{1/2}}{\gamma_{LV}^{1/2}} - 1 \quad (6)$$

where $k = \gamma^d_{LV}/\gamma_{LV}$. The results of these computations for the systems under investigation are summarized in Table 1. The validity of the foregoing proposal is readily apparent from the agreement between the experimental and calculated values of cos θ or θ for the glycerol–polyethylene system.

The water contact angle remains remarkably constant, but does show signs of decreasing at elevated temperatures. The predicted value of θ = 103° is in agreement with the work of Fowkes[12] but does not agree with the experimental value of Zisman[6]. Fowkes demonstrated that a plot of cos θ vs. $\sqrt{\gamma^d_{LV}/\gamma_{LV}}$ yields a γ_c of about 35 dynes/cm for polyethylene. This agrees quite well with the γ_{LV} value of 36·2 dynes/cm obtained by Schonhorn and Sharpe[9]. If the experimental value of θ = 94° is employed, poor agreement is obtained in a Fowkes-type plot. This implies that the measured value of θ = 94° is somewhat low.

The calculated contact angles for the formamide–polyethylene system are remarkably constant over an extended temperature range. There is better agreement between the calculated and the experimental values of θ at 20° C for the glycerol–polyethylene and formamide–polyethylene systems than the water–polyethylene system.

Since the accuracy of any measurement of contact angle is about ±2°, no change in the wettability should be observed in any of the foregoing systems. Although both $(\gamma_{LV})_p$ and γ_{LV} decrease with increasing temperature, the ratio of their square roots remains essentially constant for the polyethylene–polar liquid systems. It would be fortuitous indeed if the ratio $(\gamma_{LV})_p^{1/2}/(\gamma_{LV})^{1/2}$ always remained constant. In reality, we might expect:

$$d(\gamma_{LV})/dT > d(\gamma_{LV})_p/dT$$

and, therefore, that there may be a point where $(\gamma_{LV})_p = \gamma_{LV}$. If this occurs, then cos θ = 1 and θ = 0.

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COBALT-60 GAMMA-RADIOLYSIS OF DEUTERIUM–OXYGEN MIXTURES

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THIS laboratory has been examining the radiological health hazard involved from an accidental release of tritium gas in a confined area. Pinson and Langham[1] have shown that the internal radiation hazard from tritiated water vapour is several orders of magnitude greater than that from molecular tritium at equivalent concentrations. Hence, much of our work on this problem has been directed toward measurement of the rates of the self-initiated reactions of tritium and water vapour (exchange) to form tritiated water vapour, and of tritium and oxygen to form tritium oxide.

Yang and Gevantman[2] investigated the isotopic exchange of tritium with water vapour. Using initial tritium activities of 0·05–0·7 mc./c.c., they found the rate of exchange to be second-order with respect to initial tritium concentration. The reported rate constant with respect to tritium was $1·67 \times 10^{-3}$ c.c./mc./h. The rate was also found to be constant over a four-fold variation in the density of water vapour.

The self-oxidation of tritium in oxygen to form tritium oxide has been investigated by Casaletto, Gevantman and Nash[3]. The rate of reaction was found to be second-order with respect to initial tritium concentration over the concentration range 0·02–1 mc./c.c. The rate constant reported was $1·19 \times 10^{-3}$ c.c./mc./h. Dorfman and Hemmer[4] have also investigated this reaction but at

much higher initial concentrations of tritium. The rate of reaction was directly proportional to the initial tritium concentration. A rate constant of $7{\cdot}14 \times 10^{-3}$/h was reported. The range of initial tritium concentration was between 94 and 324 mc./c.c.

In both investigations of the self-oxidation of tritium, the methods used to follow the rate of reaction precluded the detection of products other than the assumed tritium oxide. Casaletto *et al.* determined the amount of product formed by condensing the product, pumping off the unreacted reactants, and counting the condensate with a liquid scintillation spectrometer. Dorfman and Hemmer followed the reaction by direct measurement of the pressure change of the reaction system.

The present investigation of the cobalt-60 γ-radiolysis of mixtures of oxygen and deuterium (used as a tritium substitute) was undertaken to determine whether any other product, such as deuterium peroxide, would be formed, and to gain further insight into the mechanism of the oxidation reaction.

Deuterium (Bio-Rad Laboratory, Richmond, California) of a stated purity of 99·5 per cent was used without further purification other than passing it through a U-tube immersed in liquid nitrogen. Extra-dry-grade oxygen (Matheson Co.) of 99·6 per cent minimum purity was used without further purification. Research grade argon (Matheson Co.) was used for blank runs. Nitrous oxide (Edwards Dental Supply, San Francisco), before it was used for absorbed dose measurements, was purified by trap-to-trap distillation, using liquid nitrogen-isopentane and liquid nitrogen-toluene slushes. Analysis of the purified nitrous oxide showed less than 10^{-3} mole per cent nitrogen.

All gas handling was performed on a high vacuum system of conventional design. The base pressure of the system (as measured by a 'Veeco' ionization gauge) was 1×10^{-7} torr. A calibrated Toepler pump was built and incorporated into the vacuum system to permit pressure-volume measurements of product gases.

The irradiation cell, of 'Pyrex', consisted of two volumes connected by a break-seal; the larger volume had another break-seal which was used to empty the cell after a run. The larger volume, nominally 125 c.c., was cylindrical and had an internal diameter of 1·6 cm and length of 15 cm. The smaller spherical volume, nominally 25 c.c., was joined to one end of the cylindrical volume by the connecting break-seal. Each volume had a 2-mm capillary tubulation for filling and subsequent sealing. This cell design enabled the deuterium and oxygen to be introduced (deuterium in the spherical volume and oxygen in the cylindrical volume) and sealed off separately to avoid any thermal reaction between deuterium and oxygen due to the heat of the sealing operation. After both volumes had been filled and sealed off, the connecting break-seal was broken with a 'Pyrex' slug (previously put into the cylindrical volume during the fabrication of the cell) and the deuterium and oxygen allowed to mix.

The general procedure used for filling a cell was as follows. After attachment of the cell to the vacuum system, it was flamed *in vacuo* with a gas–air torch and evacuated to 10^{-7} torr. On cooling to room temperature, the gas was introduced into the cell up to the desired pressure, as measured by a mercury manometer. The deuterium was passed through a U-tube, immersed in liquid nitrogen, before entering the cell. The oxygen was kept condensed at liquid nitrogen temperature in a trap outside the cell during the filling operation, and it was allowed to warm up to the desired pressure by lowering of the liquid nitrogen dewar from the trap. The filling of cells with nitrous oxide for dosimetric measurements was performed in a manner similar to that for oxygen. Argon was expanded directly into the evacuated cell. In all instances a trap cooled by either liquid nitrogen, a liquid nitrogen-toluene slush, or a dry-ice-acetone bath was used to prevent mercury vapour from entering the cell during the filling operation. All cells were filled to a total gas pressure of 600 torr.

All irradiations were carried out at ambient temperature in a 2,000-c. cobalt-60 radiation source at a dose rate of $5{\cdot}61 \times 10^{16}$ eV/c.c./h, as determined by nitrous oxide dosimetry[5].

After irradiation, the cell was sealed to the inlet of the calibrated Toepler pump and the product deuterium oxide condensed in liquid nitrogen. The break-seal was then broken, and the unreacted deuterium and oxygen pumped out. After the cell had been evacuated to 1×10^{-7} torr, the cell was warmed to room temperature and the amount of deuterium oxide determined by pressure-volume measurements.

The search for reaction products other than deuterium oxide, presumably deuterium peroxide and ozone, was carried out using both ferrous sulphate[6] and titanium sulphate[7] reagents. The technique used was the following. After irradiation, a bulb containing the reagent was attached to the cell via a ball-and-socket joint and the break-seal was broken. The ferrous sulphate or titanium sulphate reagent was introduced into the cell, the cell was shaken for approximately 10 min, and the solution was then centrifuged to clear it of small glass particles. A Cary 'Model 11' recording spectrophotometer was then used to analyse the solution for either ferric ion or the $[TiO_2(SO_4)]^=$ complex[7] at 3020 Å and 4100 Å, respectively. Blank runs of irradiated mixtures of deuterium and argon and of oxygen alone were treated in an identical manner.

The data obtained from measurement of the amount of deuterium oxide formed as a function of absorbed dose for each initial concentration of deuterium were treated with conventional kinetic expressions. The data for the low initial deuterium concentration ($3{\cdot}7 \times 10^{-4}$ mole/l.) were found to fit first-order kinetics, giving a rate constant, k_1, of $5{\cdot}3 \times 10^{-3}$/h with respect to deuterium concentration. The high initial deuterium concentration ($3{\cdot}7 \times 10^{-3}$ mole/l.) data fitted zero-order kinetics, giving a rate-constant, k_0, of $4{\cdot}5 \times 10^{-6}$ mole/l./h with respect to deuterium concentration. The two plots are shown in Figs. 1 and 2. Data obtained for two intermediate initial deuterium concentrations fitted a zero-order kinetic plot most closely. The reaction was carried out to at least 30 per cent completion in all cases. The amounts of deuterium oxide formed in irradiated mixtures of deuterium and argon were negligible.

Nitrous oxide was used as the dosimeter: the nitrogen produced was measured and the G value, $G(N_2)$, of 11·4 (11·4 molecules of nitrogen formed per 100 eV energy absorbed) was used. The dose rate as measured in nitrous oxide was $5{\cdot}61 \times 10^{16}$ eV/c.c./h.

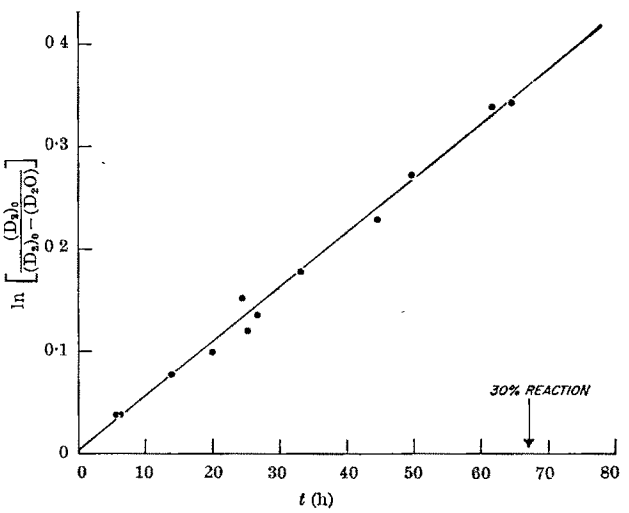


Fig. 1. First-order plot for $(D_2)_0 = 3{\cdot}7 \times 10^{-4}$ mole/l.

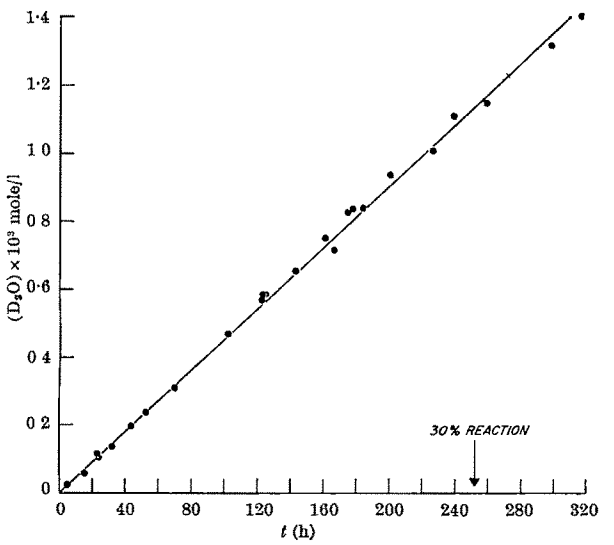


Fig 2. Zero-order plot for $(D_2)_0 = 3\,7 \times 10^{-3}$ mole/l.

The initial G values for deuterium oxide formation were calculated in the following manner. A general expression for dose absorbed, D, in eV/c.c. can be written:

$$D = \left(\frac{N \text{ molecules}}{\text{mole}}\right)\left(\frac{[P] \text{ mole}}{\text{litre}}\right)\left(\frac{100 \text{ eV}}{G(P) \text{ molecules}}\right)\left(\frac{\text{litre}}{1{,}000 \text{ c.c.}}\right) = \frac{N[P]}{G(P)10} \quad (1)$$

where N is Avogadro's number, $[P]$ the concentration of a product in mole/l., and $G(P)$ the G value for the formation of P. The expressions for the dose absorbed by nitrous oxide, D_{N_2O}, and by the deuterium–oxygen mixture D_M, are:

$$D_{N_2O} = \frac{N[N_2]}{G(N_2)10} \quad (2a)$$

$$D_M = \frac{N[D_2O]}{G(D_2O)10} \quad (2b)$$

D_{N_2O} and D_M are related by:

$$\frac{D_{N_2O}}{D_M} = \frac{Z_{N_2O}}{Z_M} \quad (3)$$

where the Z's represent electron densities of the respective systems. Combining equations (2) and (3) and solving for $G(D_2O)$:

$$G(D_2O) = \frac{Z_{N_2O}}{Z_M} \cdot \frac{[D_2O]\,G(N_2)}{[N_2]} \quad (4)$$

Substituting the equivalent kinetic expression for the concentrations of deuterium oxide and nitrogen into equation (4) gives, for the low initial concentration of deuterium:

$$G(D_2O) = \frac{Z_{N_2O}}{Z_M} \cdot \frac{G(N_2)[D_2]_0(1 - e^{-k_1 t})}{k_0' t} \quad (5)$$

where $[D_2]_0$ is the initial concentration of deuterium, k_1 the first-order rate constant for deuterium oxide formation, k_0' the zero-order rate constant for the formation of nitrogen from nitrous oxide, and t the time in hours. The limit of equation (5) as t approaches zero is:

$$G_0(D_2O) = \frac{Z_{N_2O}}{Z_M} \cdot \frac{G(N_2)[D_2]_0 k_1}{k_0'} \quad (6)$$

The analogous expression for $G_0(D_2O)$ at the high initial deuterium concentration is:

$$G_0(D_2O) = \frac{Z_{N_2O}}{Z_M} \cdot \frac{G(N_2)k_0}{k_0'} \quad (7)$$

where k_0 is the zero-order rate constant for deuterium oxide formation at the high initial deuterium concentration.

Analogous expressions (each applying to the appropriate concentration region) for the calculation $G_0(T_2O)$ derived from the data of Casaletto *et al.*, and Dorfman and Hemmer are:

$$G_0(T_2O) = \frac{k_2 NB[T_2]_0}{C\,100}; \; [T_2]_0 \text{ in mc./c.c.} \quad (8)$$

where k_2 is Casaletto's second-order rate constant, in c.c./mc./h, for the formation of tritium oxide, B is $1{\cdot}71 \times 10^{-8}$ mole/mc. and C is $7{\cdot}58 \times 10^{12}$ eV/mc./h; and:

$$G_0(T_2O) = \frac{k_1' NB}{C\,100} \quad (9)$$

where k_1' is Dorfman and Hemmer's first-order rate constant.

A comparison of pertinent quantities between the tritium–oxygen and deuterium–oxygen systems is shown in Table 1. The observed value of $1{\cdot}04 \times 10^{-5}$ mole/l./h for k_0' was used in the calculations.

The results using the ferrous sulphate reagent, which is known to react with all suspected oxidizing products, are summarized in Figs. 3 and 4. For the low initial deuterium concentration the difference in absorptivity of ferric ion between the irradiated mixture and oxygen blank is still about 0·2 at about 30 per cent reaction, while for the high initial deuterium concentration it is zero at about 15 per cent reaction. If this difference can be attributed to deuterium peroxide, its maximum concentration is of the order of 1×10^{-5} mole/l. Blank runs of irradiated argon–deuterium mixtures and argon alone gave negligible ferric ion absorbances.

Table 1. Comparison of Pertinent Quantities for the Deuterium–Oxygen and Tritium–Oxygen System

Radiation	Initial concentration of T_2 and D_2	Reaction order	Rate constant	$G_0(T_2O)$, $G_0(D_2O)$
			T_2–O_2 system	
Internal T_2 β	0 02–1 mc./c.c. ($3{\cdot}4 \times 10^{-7}$–$1\,7 \times 10^{-5}$ mole/l.)	2nd	$1{\cdot}19 \times 10^{-3}$ c.c./mc./h ($6{\cdot}96 \times 10\,1$/mole/h)	1 62 $(T_2)_0$ [$(T_2)_0$ in mc./c.c.] $9\,47 \times 10^4$ $(T_2)_0$ [$(T_2)_0$ in mole/l.]
	95–328 mc./c.c. ($1\,6 \times 10^{-3}$–$5\,6 \times 10^{-3}$ mole/l.)	1st	$7{\cdot}14 \times 10^{-3}$/h	9·7
			D_2–O_2 system	
External ^{60}Co γ	$3{\cdot}7 \times 10^{-4}$ mole/l.	1st	$5\,3 \times 10^{-3}$/h	$7{\cdot}7 \times 10^3 (D_2)_0$
	$3{\cdot}7 \times 10^{-3}$ mole/l.	0	$4{\cdot}5 \times 10^{-6}$ mole/l./h	7 3

Titanium sulphate, a reagent known to be specific for hydrogen peroxide in the presence of ozone[7], was used in an attempt to establish the presence of deuterium per-

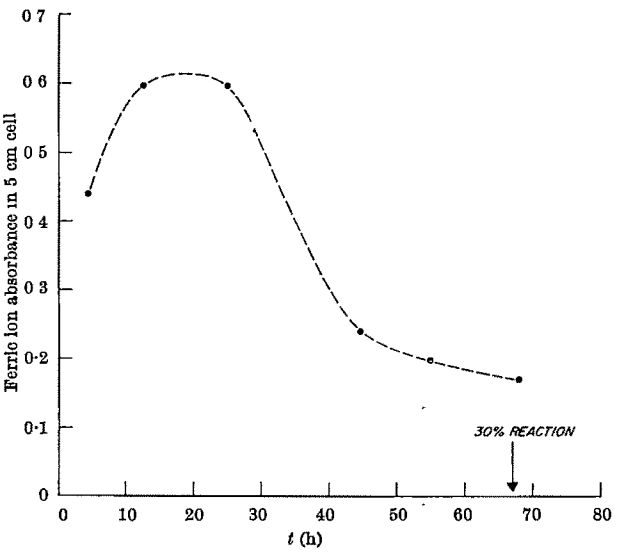


Fig 3. Difference in ferric ion absorbance between mixture and oxygen blank versus irradiation time. Initial concentration of deuterium in mixture = $3\,7 \times 10^{-4}$ mole/l.

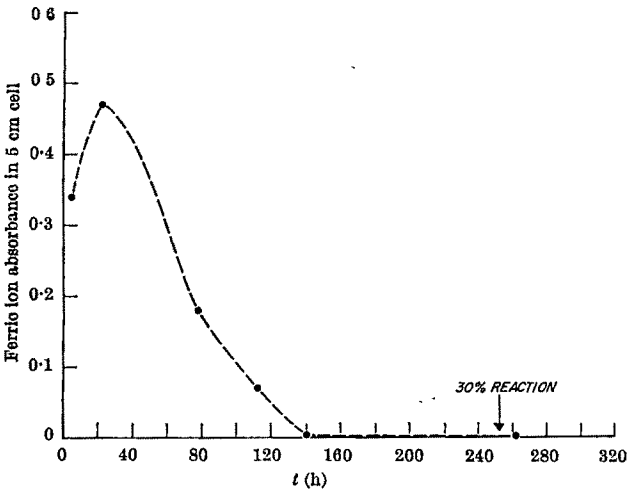


Fig. 4. Difference in ferric ion absorbance between mixture and oxygen blank versus irradiation time. Initial concentration of deuterium in mixture = $8{\cdot}7 \times 10^{-5}$ mole/l.

oxide. Calibration of this reagent with known amounts of hydrogen peroxide established that a concentration of 5×10^{-6}/l. of hydrogen peroxide will give an absorbance of 0·025 at 4100 Å in a 10-cm absorption cell. The absorbance increased linearly with added peroxide. Therefore, the amount of deuterium peroxide attributed to the difference in ferric ion absorbance between the mixture and oxygen blank, 1×10^{-5} mole/l., should have been easily measurable. However, when a mixture of deuterium and oxygen and an oxygen blank were irradiated for a time corresponding to the maximum shown in Fig. 3, no change in absorbance was observed at 4100 Å using a 10-cm absorption cell when the titanium sulphate reagent was used. Several runs were made and each gave a negative result, indicating that some species other than deuterium peroxide is oxidizing the ferrous sulphate in the irradiated deuterium–oxygen mixtures.

Several runs were made using the ferrous sulphate with cells the surface-area-to-volume ratio of which was increased eight-fold by placing lengths of small diameter 'Pyrex' tubing in the irradiation cell. The difference in ferric ion absorbance between the irradiated mixtures of deuterium and oxygen and oxygen blanks obtained for the cells with larger surface-area-to-volume ratio was essentially the same as that obtained for the cells with normal surface-area-to-volume ratio. The amounts of deuterium oxide formed were also about the same for both types of cell.

It is of interest to make a speculative comment on the orders of reaction observed for the tritium–oxygen and deuterium–oxygen systems. For the rate expression suggested by Casaletto *et al.*:

$$\frac{d(T_2O)}{dt} = k_2[T_2]_0[T_2] \qquad (10)$$

(the subscript '0' denotes initial conditions), it was suggested by them that one of the tritium terms remained as the initial concentration (since it represented the radiation intensity) and thus was not consumed in the normal sense during the reaction. If this is true and if their second-order kinetics hold at the lowest initial deuterium concentration used here, the use of deuterium and external radiation would lead to the observed first-order kinetics for the deuterium–oxygen system. Extending this line of thought to Dorfman and Hemmer's study, the observed zero-order kinetics for the deuterium–oxygen system would be expected.

In contrast to the results reported here (that is, no deuterium oxide formation in blank runs using argon in place of oxygen), Casaletto *et al.* found no decrease in the rate of tritium oxide formation using reagent grade helium in place of oxygen. It must be noted that as little as 0·005 per cent of oxygen in the inert gas would be sufficient to give their observed results. They explained the apparent result that the formation of tritium oxide was independent of oxygen concentration by suggesting an ion cluster mechanism in which O_2^-, formed by attachment of free electrons to oxygen, neutralized positive ion clusters, the resulting active species ultimately leading to the formation of stable products. Since the reaction is with O_2^-, it would be zero-order with respect to oxygen. Since the results using deuterium showed a dependence on oxygen concentration, the mechanism involved here must overshadow the one suggested by Casaletto *et al.*

The initiating step of the mechanism involved here would be the absorption of energy by the oxygen to form ozone and oxygen atoms by way of a mechanism such as that suggested by Magee and Burton[8]. An additional route subsequent to ionization of oxygen is the formation of the perhydroxy radical by an ion-molecule reaction[9], followed by neutralization:

$$O_2^+ + D_2 \rightarrow DO_2^+ + D \qquad (11a)$$

$$DO_2^+ + e \rightarrow DO_2 \qquad (11b)$$

The possible fates of the deuterium and oxygen atoms include reactions such as:

$$D + O_2 \rightarrow DO_2 \qquad (12)$$

$$O + D_2 \rightarrow OD + D \qquad (13a)$$

$$OD + O_3 \rightarrow DO_2 + O_2 \qquad (13b)$$

Although little or no deuterium peroxide is found as a product, probably it is formed as a transient species as a consequence of reactions such as:

$$2DO_2 \rightarrow D_2O_2 + O_2 \qquad (14)$$

The deuterium oxide may then be formed from the direct decomposition of deuterium peroxide and from reactions of the type:

$$O + D_2O_2 \rightarrow D_2O + O_2 \qquad (15)$$

The ion-molecule reaction:

$$O_2^+ + D_2 \rightarrow D_2O^+ + O_2 \qquad (16a)$$

$$D_2O + e \rightarrow D_2O \qquad (16b)$$

may also contribute[10].

The results using the titanium sulphate reagent show that very little, if any, deuterium peroxide is formed as a measurable reaction product. It is noteworthy to mention here that Volman[11] has observed almost complete removal of hydrogen peroxide when ozone and hydrogen peroxide are brought together in the gas phase. However, it is felt that the difference in the absorbance of ferric ion produced between the irradiated mixtures and irradiated oxygen blanks is real. It seems unreasonable to expect that the small amount of deuterium ($[D_2]\,[O_2]^{-1} = 0{\cdot}012$) present and D_2O formed would increase the production of ozone in the mixture relative to that produced in the oxygen blank to the extent represented in Figs. 3 and 4.

The product measured by ferric ion absorbance must then be due to some other species capable of oxidizing ferrous ion but not reacting with titanium sulphate. Since D_2O, D_2O_2 and O_3 are the only products that can exist in the gas phase, this species must be on the wall of the cell since it is stable for at least several minutes after removal from the radiation field. The results using cells with increased surface-to-volume ratio indicate that the species is formed in the gas phase and diffuses to the wall where it is stabilized. No attempts were made to establish the identity of this species.

Comparison of Figs. 3 and 4 shows that the difference in absorbance between the mixture and the oxygen blank approaches zero much sooner in terms of percentage reaction for the mixture with the high initial deuterium concentration. This observation suggests that at the low initial deuterium concentrations the wall 'competes' favourably with other reaction paths for the unknown

species, while at the high initial deuterium concentration almost all of it is available for reactions leading to formation of deuterium oxide. This may account, in part, for the change in reaction order and increased yield of deuterium oxide at the high initial deuterium concentration.

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FOAM FRACTIONATION OF PHENOL

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FOAM separation processes have been used for the removal from aqueous solution of proteins and enzymes including catalase, pepsin and albumin, of fatty acids and detergents, of dyes including bromothymol blue, congo red and methyl orange, of cations such as calcium, magnesium and strontium, and of anions including picrate, gallate and 1- and 2-naphthoic acid[1]. For non- or weakly-surface-active materials, foaming agents have been added. For inorganic and organic cations and anions, various mechanisms have been postulated by which the ions, together with the surface-active additive, are carried into the foam phase. These include the formation of a surfactant–ion complex which is then adsorbed at the air–aqueous solution interfaces of the foam-producing bubbles; and the electrostatic attraction between the anionic or cationic surfactant adsorbed at the interfaces of the bubbles and the non-surface-active ion of opposite charge.

Phenol is of particular interest to sanitary chemists and biologists due to its influence on the quality of water supplies. Phenol and phenolic compounds impart strong tastes and odours to water, particularly in the presence of chlorine, and they can have deleterious effects on aquatic life. The U.S. Public Health Service drinking water standard for phenol is 0·001 p.p.m. Phenol is weakly surface-active, producing a maximum depression of the surface tension of water from 72 to 41 dynes/cm. The aeration of an aqueous solution of phenol produces a very wet, unstable foam.

Eldib[2] has studied the foam separation of phenolate from spent caustic solutions produced from the sweetening of petroleum fractions. Using 0·04 per cent patent blue dye as a foaming additive, a limited separation was obtained. Other surfactants were not investigated, but the use of cationic surfactants of opposite charge to the phenolate anion appears to be a logical choice. Phenol has been chemisorbed successfully on to strongly basic anion exchange resins in the quaternary ammonium salt form[3,4]. There is some evidence of the formation of a compound of the form $—[R_4N]^+ C_6H_5O\text{-}Cl^-$ by the adsorption of phenolate ($C_6H_5O^-$) at the amine group[4].

The objective of this research was the investigation of the foam fractionation of phenol (phenolate) from aqueous solution using a cationic surface-active agent, a quaternary ammonium salt. The effects of *p*H, phenol concentration and surfactant concentration were elucidated, and the separation is discussed in terms of the residual phenol concentration, the enrichment ratio, and the relative removal of phenol and surfactant.

The foam fractionation apparatus was similar to that used in previous investigations[5,6]. All experiments were carried out in a cylindrical glass column, 9·5 cm in diameter and 52 cm in height. Nitrogen gas was saturated with water, metered with a calibrated 'Rotameter', and passed through a sintered glass diffuser, 3 cm in diameter and of 50μ porosity. The average nitrogen flow rate was 350 ml./min (at 25° C and a pressure of 1 atm.). For each experiment 2 L. of feed solution in distilled water were prepared containing from 13·5 to 672 mg/l. ($1·44 \times 10^{-4}$ to $7·15 \times 10^{-3}$ M) phenol (Analytical Reagent Grade) and from 200 to 600 mg/l. ($5·28 \times 10^{-4}$ to $1·59 \times 10^{-3}$ M) ethylhexadecyldimethylammonium bromide (EHDA-Br), a cationic surfactant. The *p*H was adjusted with 0·2 M sodium hydroxide. The feed solution was placed in the column and the nitrogen flow was begun. Foam was continuously collected at a port located 44·5 cm above the base of the column, 16·3 cm above the feed solution level, and each experiment was terminated after all foaming had ceased, which required approximately 2·5 h operation. Temperature was maintained within the range 24–26° C. At the end of each experiment the volume of the residual solution was measured, the residual concentration of phenol was determined using the modified aminoantipyrine method[7], and the residual concentration of EHDA-Br was measured by a two-phase titration technique[8]. The phenol analysis was accurate to within 2 per cent and the EHDA-Br analysis to within ± 2 mg/l. Several control experiments were conducted to establish if any phenol was removed from aqueous solution (no EHDA-Br) by volatilization into the gas stream. In all cases, negligible reductions were obtained.

Effect of *p*H. For each experiment, the following material balances can be written:

$$V = V_r + V_f \quad (1)$$

$$z_i V_i = z_r V_r + z_f V_f \quad (2)$$

$$x_i V_i = x_r V_r + x_f V_f \quad (3)$$

V_i, V_r and V_f are the volumes in litres of the feed solution, residual solution and collapsed foam, respectively; z is the concentration of phenol and x is the concentration of EHDA-Br in the appropriate solution; the subscripts i, r and f refer to the feed, residual and collapsed foam, respectively. For feed solutions containing 200 mg/l. of EHDA-Br and 23·2 and 92·6 mg/l. phenol, the effect of *p*H on the ratio of the residual phenol concentration to the feed phenol concentration is presented in Fig. 1. In each case, little reduction is obtained until the *p*H is elevated to approximately 9·0, and minima in both curves are produced at *p*H ≈ 11·5. The greater reduction for the feed solutions with z_i = 23·2 mg/l. is clearly brought about by the higher EHDA-Br to phenol ratio in the feed (2·14 g ion EHDA/g ion phenolate as compared with 0·54 g ion/g ion for z_i = 92·6 mg/l.).

As the *p*H is increased from 6·0, the ratio of phenolate ion ($C_6H_5O^-$) to phenol continually increases. At *p*H 10, the ratio is 1·0, at *p*H 11 the ratio is 10, and at *p*H 12 the ratio is 100. Since the foam fractionation process either relies on the production of a complex between the phenolate anion and EHDA cation or on the electrostatic

attraction between the ions at the air–solution interfaces associated with the bubbles, lower residuals would be expected as the *p*H is elevated and phenolate becomes the predominant form[5]. The increase in the values of z_r/z_t above *p*H 11·5 is more difficult to explain. Since quaternary ammonium bases are considered to be strong electrolytes and no dissociation constants are reported, it is unlikely that the EHDA ions react with the hydroxide ions to form ethylhexadecyldimethylammonium hydroxide. Decomposition of the EHDA ions to an amine and an alcohol should occur to a very slight degree at the atmospheric temperatures used. The most logical explanation is that above *p*H 11·5 the hydroxide ions are in sufficiently high concentration to interfere with the phenolate ions from a consideration of complex formation with or attraction to the EHDA cations. From this behaviour, it is most likely that the mechanism of the foam fractionation process involves ion attraction at the bubble interfaces. The predominance of phenolate over bromide as far as the attraction is concerned is undoubtedly brought about by the hydrophobicity and surface activity of phenol.

In these experiments, the volumes of collapsed foam produced and residual concentrations of surfactant were very insensitive to *p*H and to the feed concentration of phenol. For the twenty-four experiments shown in Fig. 1, the collapsed foam volume ranged from 0·07 to 0·21 l. in a random manner. The concentration of phenol in the foam was thus maximized at *p*H $\approx$ 11·5. For the same runs, the residual concentration of EHDA-Br ranged from 6·0 to 12·0 mg/l., also in a random manner.

Effect of feed phenol concentration and surfactant concentration. A second series of experiments was conducted to establish the influence of the concentrations of phenol and EHDA-Br in the feed solutions on the foam fractionation process. The *p*H was maintained at approximately 12·9; this value was selected to avoid inconsistencies in results which would have been realized if 11·5 had been chosen. In the *p*H range 11–12 better reductions in phenol would have resulted, but in this region the data would have been very sensitive to small *p*H variations. A total of twenty-seven experiments was made with the feed solutions containing from 13·5 to 672 mg/l. of phenol and 200, 400 and 600 mg/l. of EHDA-Br. Feed ratios thus ranged from 6·15 to 0·074 g ion EHDA to g ion phenolate. The residual concentrations of phenol (actually phenolate but referred to as phenol herein) are plotted along the abscissa in Fig. 2 versus the feed concentrations of phenol along the ordinate. The three curves are for x_t = 200, 400 and 600 mg/l. and the straight line (dotted) for x_t = 0 (no phenol separation). At x_t = 200 mg/l., z_r/z_t ranged from 0·27 to 0·93, at x_t = 400 mg/l. from 0·15 to 0·82, and at x_t = 600 mg/l. from 0·12 to 0·79. At constant x_t, z_r/z_t always increased with z_t, and clearly at constant z_t, z_r/z_t always decreased as x_t was increased. For the same data, enrichment ratios, defined as the concentration of phenol in the collapsed foam divided by that in the residual solution, ranged from 1·4 to 71·2. A value of 1·0 corresponds to no separation.

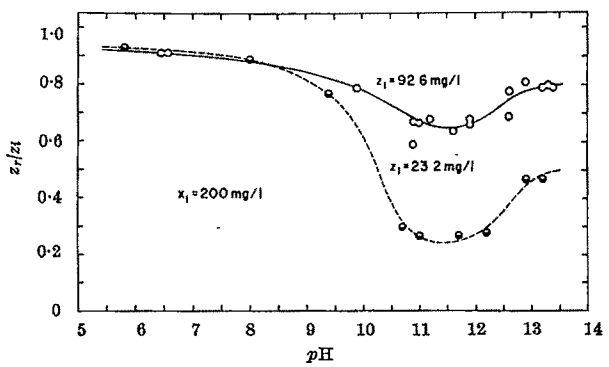


Fig. 1. Effect of *p*H on ratio of residual phenol concentration to feed phenol concentration

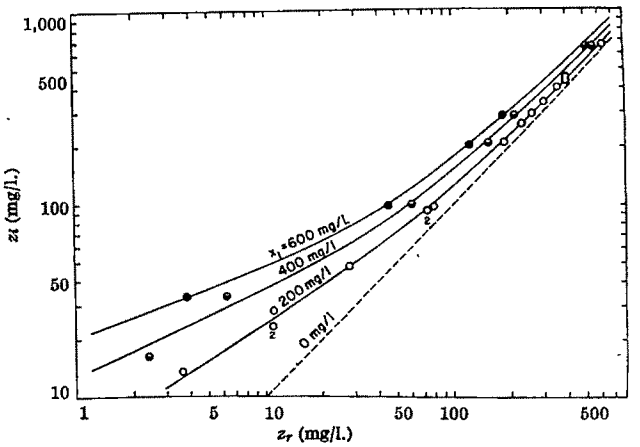


Fig. 2. Relationships between residual phenol concentration and feed phenol concentration for three feed surfactant concentrations

In these experiments, the volume of collapsed foam was independent of the feed concentration of phenol and was related only to the feed concentration of EHDA-Br. On the average for x_t = 200 mg/l. V_f = 0·21 l., for x_t = 600 mg/l. V_f = 0·42 l., and for x_t = 600 mg/l. V_f = 0·61 l. The residual concentration of EHDA-Br was independent of both z_t and x_t, ranging from 4·0 to 14·0 mg/l., in a random manner. It is interesting to observe that the presence of phenol had no effect on the collapsed foam volume or residual surfactant concentration, compared to the foam fractionation of pure EHDA-Br solutions.

A useful parameter which provides some insight into the nature of the foam fractionation process is $z_f/x_f]_{\text{Fractionation}}$[5]. In any foam separation process the surface-active agent and other solutes of interest are carried into the foam phase in two manners: one in the surface layers at the air–solution interfaces of the bubbles, the other as entrained bulk solution carried into the foam mechanically. Of course, a large percentage of the entrained bulk solution drains from the foam if a column of sufficient height is provided. In order to compute the relative concentrations of phenol and EHDA-Br in the surface layers, the quantities of both in the entrained bulk solution must be subtracted from the quantities of both in the total foam stream. For this investigation this was done in an approximate manner first by assuming that the fraction of the foam consisting of entrained bulk solution was very large compared with the fraction corresponding to the air–solution surface layers; and secondly by using the arithmetic average of the feed and residual concentrations of phenol and EHDA-Br to compute the composition of the entrained bulk solution, which decreased continuously from z_t to z_r during a run. The former assumption has been validated in an examination of the foam fractionation of pure surfactant solutions[9]. The latter assumption was validated partially by several rate experiments which indicated a linear decrease in the residual phenol concentration with time. With these assumptions:

$$z_f/x_f]_{\text{Fractionation}} = \frac{z_f - \left\{\frac{z_t + z_r}{2}\right\}}{x_f - \left\{\frac{x_t + x_r}{2}\right\}}$$

From the experimental data the following relationship between $z_f/x_f]_{\text{Fractionation}}$ and z_t and x_t was obtained:

$$z_f/x_f]_{\text{Fractionation}} = 0{\cdot}06\ (\exp)^{-0{\cdot}00443 x_t}\ z_t^{(0\ 0134 x_t^{\ 0{\cdot}608})} \qquad (4)$$

At constant x_t, $z_f/x_f]_{\text{Fractionation}}$ increases as z_t is increased; at constant z_t, the parameter decreases as x_t is increased for

small z_i but increases as x_i is increased for large z_i. For the twenty-seven experiments considered, $z_f/x_f]_{\text{Fractionation}}$ ranged from 0·035 to 0·306. If one phenolate anion is removed in the bubble surface layers with each EHDA cation, $z_f/x_f]_{\text{Fractionation}}$ will be 0·249, the ratio of the molecular weights of phenol and EHDA-Br. All but two of the experimental values were less than 0·249, indicating that more surfactant is required than the theoretical amount, and that there is some competition between bromide and hydroxide with phenolate for the EHDA cations[5].

Phenol has been foam fractionated using a cationic surface-active agent, ethylhexadecyldimethylammonium bromide. The mechanism of the process involves either the formation of a surface-active complex between EHDA cations and phenolate anions or the electrostatic attraction between the ions at the air–aqueous solution interfaces associated with the bubbles. From these investigations it appears that the latter is the true mechanism. Little separation is obtained until the *p*H is elevated to 9, and a minimum in the residual phenol concentration is obtained at *p*H $\approx$ 11 5. Considering a broad range of feed phenol and EHDA-Br concentrations, the residual concentration of phenol may be reduced to 0·05 of the feed concentration, but this requires about 50 g EHDA-Br/g phenol. A new parameter is introduced relating the quantities of phenol and EHDA-Br in the surface-layers of the foam bubbles. Values are compared with the theoretical value.

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STRUCTURAL SPECIFICITY OF α-CHYMOTRYPSIN: POLYPEPTIDE SUBSTRATES

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THE specificity of proteolytic enzymes towards model substrates has been extensively investigated during the past two decades. The relation of these investigations to the biological specificity of proteolytic enzymes for proteins is still an open question. It is the purpose of this discussion to relate the available information on the specificity of chymotrypsin towards polypeptides[1] with the considerable information on its specificity towards acylated amino-acid esters. A similar analysis of data for pepsin has recently been published[2].

The suggestion that the specificity of proteolytic enzymes is determined by the number, constitution, configuration and sequence of amino-acid residues in a peptide chain was first advanced more than 50 years ago by Emil Fischer[3]. The importance of specific residues was questioned during the early decades of this century by several groups of workers, but the data available were not sufficient for a clear picture to emerge. (Pertinent references and a brief history are given by Bergmann, M., *Adv. Enzymol.*, 2, 49; 1942).

The researches of Bergmann and his collaborators in the 'thirties gave a concrete foundation for the first time to general concepts of enzyme specificity. Bergmann synthesized a large number of small peptides of known structure and stereochemistry, containing most of the amino-acids commonly found in proteins, and he subjected these to semi-quantitative enzyme catalysed hydrolysis. He concluded[4] that the specificity observed for proteolytic enzymes, and especially for pepsin, trypsin and chymotrypsin, depended primarily on the nature of the side chain of a single, specific amino-acid and secondarily on the nature of amino-acid residues farther removed from the susceptible bond.

During the following two decades, from approximately 1940–60, considerable attention was focused on the aforementioned primary factor, the nature of the side chain of the carboxyl residue of susceptible bonds.

In particular, the specificity of α-chymotrypsin has been investigated thoroughly. This work gained impetus from the discovery of the esterase activity of chymotrypsin[5] and from the development of rapid kinetic techniques based on the use of *p*H-stats[6]. Extensive investigations by Niemann and his collaborators have resulted in a specificity theory which correlates the kinetic data obtained for the enzyme catalysed hydrolysis of more than 150 substrates of chymotrypsin[7,8].

In the past few years, the question of the specificity of chymotrypsin for larger peptides has received renewed attention from the use of this enzyme, among others, in the elucidation of amino-acid sequences of polypeptides and proteins. A number of 'abnormal' splittings have been observed[9] and the concept of secondary specificity, determined by amino-acid residues adjacent to the susceptible amino-acid on the carboxyl side of the bond which is to be split, has been reintroduced[1,10,11].

Classification of Enzyme Specificity

It is probably advantageous to discuss levels of enzyme specificity for proteolytic enzymes, in terms similar to those used for protein structure. Thus, one may speak of the primary, the secondary and the tertiary specificity of proteolytic enzymes. Primary specificity refers to the minimum structure required for enzyme specificity to be manifest. For proteolytic enzymes this is usually an amino-acid residue containing a side chain, an amido group and a carbonyl function. For chymotrypsin, trypsin and pepsin, the primary specificity is observed in the carboxyl end of the susceptible peptide bond. Specifically, the primary specificity of trypsin is towards arginyl and lysyl bonds; of chymotrypsin for aromatic, leucyl and perhaps methionyl bonds. The secondary specificity of proteolytic enzymes refers to the influence of adjacent amino-acid residues in a polypeptide chain on the observed hydrolytic reaction. A common example is the negative influence of prolyl residues, when attached to a carboxyl group, on hydrolytic rate for both chymotrypsin and trypsin catalysed hydrolyses. The tertiary specificity refers to the action of proteolytic enzymes on

native, active protein molecules in solution. Typical examples are the activation of chymotrypsinogen by trypsin—in which only a few of the many bonds which should be split by trypsin on the basis of primary specificity of this enzyme are susceptible[12]—or the unique splitting of a single peptide bond in ribonuclease by subtilisin[13].

The similarity of the proposed nomenclature to that introduced by Linderstrøm-Lang[14] and commonly used for proteins is no accident. He derived his classification scheme from a consideration of the effect of proteolytic enzymes on native and denatured proteins. The common first reference to the Linderstrøm-Lang terminology is to a lecture entitled, "The Initial Stages in the Breakdown of Proteins by Enzymes"[14]. However, the present usage differs from the conventional subdivision of protein structure into primary, secondary and tertiary. For convenience, we have defined the primary and secondary specificity of proteolytic enzymes so that both refer to aspects of the primary structure of a protein substrate. Tertiary specificity of proteolytic enzymes refers to all other aspects of the structure of protein substrates. There is too little information available at this time concerning the effects of secondary and tertiary protein structure on susceptibility towards enzymatic hydrolyses to try to subdivide there. This task remains to be undertaken in the future.

A partial understanding of the primary specificity of α-chymotrypsin was achieved only after investigation of the kinetics of a very large number of model compounds. A similar level of knowledge of secondary specificity would require a corresponding experimental effort. Some qualitative work along this line was performed by Bergmann and his collaborators. Fortunately, information concerning the secondary specificity of chymotrypsin can be obtained from results in the literature on its action towards polypeptides and denatured proteins. It is the purpose of this article to examine some of the available literature and to propose, so far as possible, a secondary specificity pattern for α-chymotrypsin catalysed reactions. A preliminary attempt along these lines has recently been reported[1].

The question of the tertiary specificity of any proteolytic enzyme cannot yet be discussed intelligently. On the one hand, too little is known about the tertiary structure of proteins in solution. On the other hand, relatively little information is available on the effect of proteolytic enzymes on native proteins. Especially in the case of proteins for which the primary structure, the amino-acid sequence, is known, quantitative studies on enzyme kinetics may prove to be of increasing value[15–18].

Specificity of Chymotrypsin

We have chosen to examine the specificity pattern shown by α-chymotrypsin for a limited number of peptides and proteins. Only compounds the complete amino-acid sequence of which has been determined have been included in the survey. Those analysed are human haemoglobin γ chain[19], human haemoglobin α chain[20], ribonuclease[21], α-corticotropin[22], glucagon[23], insulin[24], horse heart cytochrome *c*[25], baker's yeast cytochrome *c*[26], and tobacco mosaic virus[27,28].

The analysis of data in the literature which was not compiled for this purpose raises several difficulties. The chains discussed contain a total of 910 amino-acid residues. The size of the sample, as well as the diverse origin of the proteins, indicates that a representative sample is available. The distribution of amino-acids, ranging from eleven tryptophan residues to seventy-eight alanine residues, is not unusual for amino-acid compositions[29,30]. The number of different kinds of peptide linkages is also compatible with the tabulation of Sörm *et al.*[31].

A more difficult question concerns the problem of minor splittings observed in enzyme-catalysed peptide hydrolyses. These could be due to bonds which are split more slowly than others, to an impurity in the enzyme preparation, or they could even be present before enzyme is added. Such free groups could account for trace end-group analyses which are sometimes reported. We have chosen to include essentially all bonds reported broken by chymotrypsin. Other enzyme impurities in modern chymotrypsin preparations are exceedingly small. Also, in cases of minor splittings observed, there was usually no pattern which would implicate another enzyme. For example, chymotrypsin causes the splitting of a lysyl-lysine bond in both α- and β-corticotropin. Although in the latter case this split has been attributed to a trypsin impurity in the chymotrypsin preparation[32], this was the only one of several trypsin-sensitive bonds cleaved[33]. There is no doubt that the problem of minor cleavages raises some questions. In the absence of control experiments, that is, when the same specificity pattern is observed in the presence of an inhibitor for the suspected contaminating enzyme[33], either inclusion or rejection of this class of observations will introduce some error. We have chosen the former course.

If the concepts of primary, secondary and tertiary specificity have any significance, as defined, the latter two should manifest themselves as perturbations of the primary specificity. The ideal, and ultimate, criterion for any questions concerning relative specificity is kinetic information about the rate of cleavage of the bonds involved, Such information should be obtained under precisely determined conditions of concentration, temperature, *p*H, etc. Needless to say, this level of data is not at present available. One must, therefore, rely on a statistical analysis of relative frequency of occurrence of any particular peptide bond hydrolysis for a number of experiments performed under different conditions. Fortunately, the conditions used for protein analysis fall within a narrow range. The substrate-to-enzyme ratio is usually between 300 : 1 and 50 : 1; the reactions are carried out near room temperature for long times: 6–24 h; the *p*H is usually near the optimum for α-chymotrypsin between 7 and 9, but efforts have not been made to control ionic strength. Recent experiments have demonstrated that variations in *p*H[34] and ionic strength[35] can affect the relative specificity of α-chymotrypsin.

The variations in experimental conditions make any analysis of the recorded results tentative. However, the enzyme-catalysed hydrolysis of peptides was usually carried out until the rate had slowed down considerably. Therefore, it is probable that further hydrolysis would not have significantly altered the results. At best, the number of minor splittings observed might have been increased slightly. As the experiments were performed until all the more susceptible bonds were split, only those variations in reaction conditions which would vary the relative specificity of the enzyme might affect the conclusions.

Primary specificity. Table 1 lists the number of bonds split involving any of the twenty naturally occurring amino-acids as the acyl component, as well as the fraction of all bonds involving that particular acid which were reported hydrolysed by chymotrypsin. The amino-acids range from the 'classic' substrates of chymotrypsin to only four amino-acids for which no bonds were split. Table 1 also lists kinetic constants obtained for acylated amino-acid methyl esters as substrates of α-chymotrypsin.

Of the twenty amino-acid species listed, sixteen are involved in hydrolysis at least once. Although some small fraction of these hydrolytic cleavages may be due to an impurity in the enzyme preparation, it is clear that chymotrypsin has a very wide primary specificity. As indicated, the amino-acids can be divided into three groups. Group I includes those which seem to be hydrolysed a large percentage of the times they appear: the classic aromatic substrates—tryptophan, phenylalanine and tyrosine—and leucine. Somewhat arbitrarily, methionine has also been included. Of a total of 171 residues containing these amino-acids, 123 (72 per cent), are

Table 1. PRIMARY SPECIFICITY OF CHYMOTRYPSIN FOR BONDS IN POLYPEPTIDES

	Amino-acid	Total residues	Number split	Percentage	Kinetic data for *N*-acetyl-L-amino-acid methyl esters* K_0 (mM)	r_0 (sec^{-1})	k_0/K_0 (M^{-1} sec^{-1})	Ref.
Group I	Tryptophan	11	11	100	0 12	51	$4{\cdot}2 \times 10^5$	37
	Tyrosine	32	29	91	0 32	117	$3\ 6 \times 10^5$	38
	Phenylalanine	42	35	83	0·62	67	$1{\cdot}1 \times 10^5$	38
	Leucine	72	42	58	2·9	4·6	$1{\cdot}6 \times 10^3$	39
	Methionine†	14	6	43	8·0‡	18·0‡	$2{\cdot}3 \times 10^3$‡	40
Group II	Histidine	32	10	31				
	Glutamine	32	7	22	48§	20·0§	$4{\cdot}2 \times 10^2$§	37
	Asparagine	42	7	17	23‖	22 0‖	$9{\cdot}5 \times 10^2$‖	41
	Threonine	67	5	7	136	0·21	1·5	42
	Lysine	76	4	5				
	Cysteine¶	22	1	5				
	Isoleucine	27	1	4	49	0·16	3·3	43
	Valine	64	2	3	112	0·15	1·3	44
	Aspartic acid	44	1	2				
	Glycine	71	1	1	30·7	0·013	0·4	45
	Serine	67	1	1				
Group III	Alanine	78	0	0	611	1·3	2·1	46
	Glutamic acid	49	0	0				
	Proline	36	0	0				
	Arginine	32	0	0				

* Unless otherwise noted, kinetic data were obtained in aqueous solutions at 25 0° C, *p*H 7·9 and 0 10 M with respect to sodium chloride.
† Includes four methionine sulphone residues, two of which were split.
‡ *N*-benzoyl derivative in 30 per cent methanol.
§ *N*-acetyl-L-glutamic acid dimethyl ester.
‖ *N*-acetyl-L-aspartic acid dimethyl ester at *p*H 7·2.
¶ Includes fourteen cysteic acid residues, one of which was split.

hydrolysed by the action of chymotrypsin. The second group involves eleven amino-acids which undergo hydrolysis at least once. Arbitrarily, for purposes of further comparisons, methionine has also been included in this group. Only forty-six out of 558 residues (8 per cent) are hydrolysed among this group. Finally, Group III amino-acids are not hydrolysed although they account for 195 residues.

The classification of substrates into groups permits a 'statistical' comparison to be made. For Group I amino-acids, the primary specificity is probably dominant: hydrolysis is the rule with only a few exceptions. By examining the amino-acid sequence near those group I residues which are not hydrolysed, factors which hinder hydrolysis may become apparent. Conversely, for Group II amino-acids, the secondary specificity may be dominant. By examining the amino-acid sequences surrounding those Group II residues which are hydrolysed, factors which favour hydrolysis may become apparent.

A comparison of the peptide hydrolysis data with the kinetic data on *N*-acylated-L-amino-acid esters indicates that the primary specificity pattern for polypeptides is a direct reflexion of the specificity observed for the model compounds. To the extent of the kinetic data available, k_0/K_0, the catalytic rate constant divided by the observed Michaelis constant[36], for models and the susceptibility of bonds in denatured proteins to hydrolysis can be correlated. This comparison is an important step forward in the evaluation of the relevance of investigations of model compounds to the *in vivo* action of chymotrypsin.

Secondary specificity. In order to examine the secondary specificity pattern, it is necessary to tabulate the residues found adjacent to the bond hydrolysed Each peptide was classified by use of a tabulation which listed the amino-acid residues on either side of the site of cleavage. These were labelled CO^1, the carboxyl component of the peptide bond hydrolysed, NH^1, the amino component of the bond hydrolysed, CO^2, the *N*-acyl amino-acid on CO^1, etc. An example of this system of nomenclature, which is identical with that used by Tang[2], is shown in the following:

CO^3	CO^2	CO^1	NH^1	NH^2	NH^3
Ser	Val	$CySO_3H$	Ser	Leu	Tyr
Ser	Leu	Tyr	Glu	Leu	Glu
Glu	Asp	Tyr	$CySO_3H$	Asp	

↖ Peptide linkages hydrolysed

From the number of bonds hydrolysed, 163, and the total number of sequences available, it became clear that only for the CO^2 and NH^1 positions were enough data available to arrive at even tentative conclusions.

The effect of adjacent residues on the susceptibility of any particular bond to hydrolysis must be modified to account for 'vacant' positions due to other splittings or terminal groups. Thus, an NH^1 residue with a free carboxyl group may have a different secondary effect than the same NH^1 residue attached to an NH^2 residue. If it is assumed that each splitting observed is the last to occur, it is possible to tabulate the maximum possible number of 'vacant' positions for each of the groups of specific residues for which hydrolysis is observed. Table 2 lists the total number of bonds split for each group as well as the number and percentage of CO^2 and NH^2 positions which are vacant. Group −I refers to sequences where the CO^1 position is occupied by a Group I residue, tryptophan, tyrosine, phenylalanine, leucine or methionine, but no hydrolysis is observed.

Table 2. EFFECT OF "VACANT" POSITIONS ON THE SECONDARY SPECIFICITY OF CHYMOTRYPSIN

Residues at CO^1	Total bonds	"Vacant" CO^2	Percentage of total	NH^2	Percentage of total
Group I	123	14	11	17	13
Group −I*	47	7	15	12	25
Group II	46	4	8	2	4

* No hydrolysis with a Group I residue at the CO^1 position.

The significant data in Table 2 concern the Group II and Group −I residues. The Group II residues include those which are only sometimes split. It might be expected that secondary specificity would play an important part among this set. For Group −I, the lack of observed hydrolysis must be interpreted in terms of unfavourable secondary specificity requirements. It can be seen from Table 2 that vacant CO^2 and NH^2 positions are found for Group II residues in only 8 per cent and 4 per cent, respectively, of the observed hydrolyses, although they account for 15 per cent and 25 per cent, respectively, of the unsuccessful Group −I positions. In other words, of the total of forty-six splittings observed in which the carboxyl end contained a Group II residue, only six of these, or 13 per cent, have a vacancy at either the CO^2 of the NH^2 position. In contrast to this, of the forty-seven 'non-splittings' observed for Group I residues nineteen, or 40 per cent, occur when either the CO^2 or the NH^2 position is vacant.

These observations lend support to the classification of chymotrypsin as an endopeptidase. Location at the

N-terminal end or penultimate to the C-terminal end of a peptide chain appears to be a major reason for lack of hydrolysis of a normally susceptible, Group I, residue.

In the discussion of the effect of residues at the NH^1 and CO^2 positions on hydrolysis, presented below, residues with vacancies at CO^2 or NH^2 were not included where this might influence the results.

The effect of structure at the NH^1 position on hydrolytic susceptibility is presented in Table 3. Again, the significant information can be gleaned from the listings under Group II, the less suceptible but sometimes hydrolysable residues, and Group −I, the non-hydrolysis of high susceptible residues. For each type of residue we have listed the number of times its appeared as the amine component of a peptide bond which involves one of the highly susceptible amino-acids at the carboxyl end (Group I); a peptide bond which involves one of the less susceptible amino-acids at the carboxyl end (Group II); and finally the number of times it appeared as the amine component of a peptide bond which involves one of the highly susceptible amino-acids at the carboxyl end but with no hydrolysis observed (Group −I). These values ("Found") are compared with values which might be observed ("Expected") simply on the basis that any particular residue will appear in any position as often as its fractional contribution to the total number of residues. For example, of the 910 total residues, seventy-eight or 8·6 per cent are alanines. Thus, we would expect 8·6 per cent of the 123 amino components at NH^1 associated with Group I residues, or ten residues, to be alanines.

A few types of residues appear to have a favourable effect on hydrolysis when in the NH^1 position. These residues are found more than the "expected" number of times in association with Group II hydrolyses and less than the expected number of times for Group −I "non-hydrolyses". The three favourable residues appear to be alanine, glycine and lysine.

Conversely, a few residues seem to prevent hydrolyses. These residues are found less than the expected number of times in association with Group II hydrolyses and more than the expected number of times for Group −I "non-hydrolyses". Aspartic acid, proline and possibly histidine are such residues.

That proline in the NH^1 position is unfavourable for hydrolysis has been known for some time. The effect of aspartic acid can be ascribed to the free carboxylate group, although the same effect does not appear to be observed for glutamic acid. Confirmation of the secondary specificity of chymotrypsin for glycyl and alanyl residues in the NH^1 position must await quantitative experiments on suitable model compounds.

In Table 4 the possible secondary specificity towards CO^2 residues is examined. The data are presented in the same manner as in Table 3, except that the total number of residues has been reduced by the number found at

Table 3. SECONDARY SPECIFICITY OF CHYMOTRYPSIN: AMINO COMPONENT

Residue	Total No. in proteins	Group I NH^1 Found	Group I NH^1 Expected†	Group II NH^1 Found	Group II NH^1 Expected†	Group −I* NH^1 Found	Group −I* NH^1 Expected†
Alanine	78	6	10	10	4	1	3
Arginine	32	4	4	1	2	0	1
Aspartic acid	44	5	6	0	2	6	2
Asparagine	42	2	6	1	2	0	1
Cysteine	22(14)‡	2(2)‡	3	0	1	2(1)‡	1
Glutamic acid	49	8	7	1	2	2	2
Glutamine	32	2	4	2§	2	0	1
Glycine	71	14	10	6	4	1	3
Histidine	32	3	4	0	2	2	1
Isoleucine	27	7	4	2	1	1	1
Leucine	72	13	10	1	4	1	3
Lysine	76	12	10	8	4	1	3
Methionine	14(4)‖	1	2	0	1	1(1)‖	0
Phenylalanine	42	3	6	1	2	0	1
Proline	36	1	5	0	2	7	1
Serine	67	15	9	4	3	3	2
Threonine	67	14	9	0	3	1	2
Tryptophan	11	0	1	0	1	0	0
Tyrosine	32	2	4	0	2	0	1
Valine	64	9	9	9	3	3	2
Total	910	123		46		32	

* No hydrolysis with Group I residue at CO^1 position.
† Number of residues expected at this position in the absence of any specificity. Obtained by multiplying the total number of residues of any particular amino-acid in the sample by the total number of residues found at that position/total number of residues.
‡ Figures in parentheses refer to cystelc acid.
§ Both glutamine residues are carboxyl terminal.
‖ Figures in parentheses refer to methionine sulphone.

Table 4. SECONDARY SPECIFICITY OF CHYMOTRYPSIN: *N*-ACYL COMPONENT

Residue	Total No. in proteins	No. found at CO^1	Effective total No.	Group I CO^2 Found	Group I CO^2 Expected†	Group II CO^2 Found	Group II CO^2 Expected†	Group −I* CO^2 Found	Group −I* CO^2 Expected†
Alanine	78		78	15	12	3	4	0	3
Arginine	32		32	4	5	2	2	0	1
Aspartic acid	44	1	43	6	6	0	2	3	1
Asparagine	42	7	35	6	5	0	2	3	1
Cysteine	22(14)‡	1(1)‡	21(13)‡	1(1)†	3	0	1	1	1
Glutamic acid	49		49	8	7	0	3	2	2
Glutamine	32	7	25	4	4	2	1	2	1
Glycine	71	1	70	5	10	2	4	0	2
Histidine	32	10	22	4	3	1	1	5	1
Isoleucine	27	2	25	4	4	2	1	0	1
Leucine	72	42	30	5	4	3	2	2	1
Lysine	76	4	72	13	11	9	4	0	3
Methionine	14(4)§	6(2)§	8(2)§	1	1	0	0	1(1)§	0
Phenylalanine	42	35	7	1	1	0	0	0	0
Proline	36		36	3	5	5	2	1	1
Serine	67		67	8	10	2	4	4	2
Threonine	67	5	62	12	9	4	4	1	2
Tryptophan	11	11	0	0	0	0	0	0	0
Tyrosine	32	29	3	0	0	0	0	0	0
Valine	64	2	62	9	9	8	4	1	2
Total	910	163	747	109		43		26	

* No hydrolysis with Group I residue at CO^1 position.
† Number of residues expected at this position in the absence of any specificity. Obtained by multiplying the total number of residues of any particular amino-acid by the total number of residues found at that position/total effective number of residues.
‡ Figures in parentheses refer to cystelc acid.
§ Figures in parentheses refer to methionine sulphone.

CO^1 to arrive at an effective total number of residues equal to 747.

Again, comparison of the figures for Group II and Group −I occurrence suggests that some residues are particularly favourable for promoting hydrolysis while others hinder it. Lysine, valine and perhaps isoleucine when present in the CO^2 position favour hydrolysis, while aspartic acid, asparagine, histidine and serine may all hinder hydrolysis when in the CO^2 position.

Perusal of Tables 3 and 4 reveals that all conclusions concerning secondary specificity must be tentative at this time. The results are suggestive and this approach may be fruitful, but it is clear that a much larger sample is required before more definitive conclusions can be on the basis of statistical analyses. With the present accelerating rate of sequence determinations, the necessary data may soon be available for defining the secondary specificity of chymotrypsin and other proteolytic enzymes.

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STRUCTURE OF ACETYLCHOLINE AND OTHER SUBSTRATES OF CHOLINERGIC SYSTEMS

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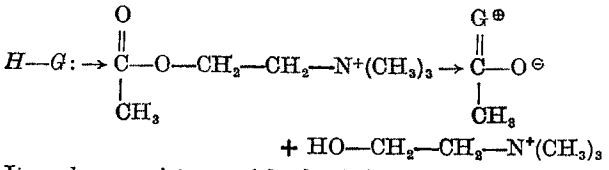
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FOR many years a great deal of effort has gone into the investigation of the properties of many substances agonistic and antagonistic to cholinergic transmission systems in order to characterize such systems and determine their mechanisms. Our understanding is still far from complete. For various reasons[1], we have undertaken a re-investigation of the crystal structure of acetylcholine bromide and have recently completed the X-ray diffraction single-crystal structure analysis. The structure is necessarily in the solid state but has certain features which can be compared with known crystal structures of other relevant molecules and with the pharmacological evidence available on the action of various molecules in cholinergic systems.

The three-dimensional single-crystal X-ray diffraction data of Sörum[2,3] have been corrected for the presence of twinned crystals and the structure solved by a careful examination by one of us (F. G. C.) of the observed Fourier synthesis phased on refined co-ordinates of the bromine atom determined by Sörum. The structure was solved in spacegroup $P2_1$, but we observed that the two independent molecules determined in this space-group are racemates related by a glide plane. The proper spacegroup is thus $P2_1/a$ or $P2_1/c$, confirming the suggestion of Dunitz[4] from certain unusual systematic absences in the diffraction data that the crystals are twinned $P2_1/a$ and not $P2_1$. The structure has been refined by least squares methods and the residual $R = 0{\cdot}10$ for the 770 observed diffraction maxima.

A drawing of the molecule in the solid state giving interatomic distances and angles is shown in Fig. 1. The interatomic distance from one substituted ammonium methyl carbon atom C_1 to the ester oxygen atom O_1 is 3·02 Å and the interatomic angles are such that this methyl group seems to form a bent hydrogen bond through one of its hydrogen atoms to the ester oxygen atom, though the hydrogen atom positions have not been determined in this analysis. This distance can be compared with the sum of the van der Waals radii of a methyl group and an oxygen atom of 2·0 + 1·4 = 3·4 Å. The nitrogen atom N to ester oxygen atom O_1 distance is also fairly short at 3·29 Å. The ester oxygen atom O_1 to carboxyl carbon

atom C_6 distance is 1·32 Å, just significantly less than the single-bond distance 1·44 Å. This implies that the ester oxygen to carboxyl carbon bond O_1—C_6 has partial double-bond character, consistent with the co-planar arrangement of the group C_5 O_1 C_6 O_2 C_7. This partial double-bond character is relevant to the first step of the mechanism of the hydrolysis of acetylcholine by acetylcholinesterase (H–G:) proposed by Wilson[5,6]:



It is also consistent with the infra-red evidence on the nature of the C=O bond of acetylcholine in various solvents investigated by Canepa and Mooney[7].

There are three planes of atoms in the molecular structure of acetylcholine: (1) the plane of the ester oxygen carboxyl group just mentioned, C_5 O_1 C_6 O_2 C_7; (2) the plane of one methyl carbon atom, the nitrogen atom and the two CH_2 group carbon atoms, C_3 N C_4 C_5; (3) the plane of the ester oxygen–CH_2 bond and of one methyl carbon–nitrogen bond, O_1 C_5 C_1 N, though the last four atoms are not quite co-planar.

Some of these features of the crystal structure of acetylcholine bromide are very similar to those of the two related solid-state structures which are known, choline chloride[8] and muscarine iodide[9]. The structure of choline chloride with interatomic distances and angles is shown in Fig. 2 and that of muscarine iodide in Fig. 3. In each of these structures there exists a close methyl carbon atom to (ester) oxygen atom distance, C_1–O_1 = 3·07 Å in choline chloride and 2·87 Å in muscarine iodide, and a fairly close nitrogen atom to (ester) oxygen atom distance, N–O_1 = 3·26 Å and 3·07 Å in the compounds, respectively. In choline chloride, one methyl group carbon atom, the nitrogen atom and the two CH_2 group carbon atoms lie in one plane, C_3 N C_4 C_5, and the oxygen–carbon bond and one methyl carbon–nitrogen bond almost lie in another plane, O_1 C_5 C_1 N, identical within the experimental error to the molecular arrangement in acetylcholine bromide. In muscarine iodide, one methyl group carbon atom, the nitrogen atom, the two CH_2 group carbon atoms and one ring carbon atom lie in one plane, C_3 N C_4 C_5 C_8, and four atoms of the ring, C_5 O_1 C_6 C_8, lie in another plane in which one methyl group carbon nitrogen bond C_1–N very nearly lies. Though the pharmacological effects of these three compounds active on cholinergic receptors are very different, they have these similar structural features in the common molecular portion. The differences in the structures of the common portions of the three molecules as analysed are not statistically significant, being less than three times the estimated standard deviations. Some similarities between the choline, acetylcholine and betaine chains involved in plant physiology have been pointed out by Canepa[11] on the basis of crystallographic and radiation decomposition evidence.

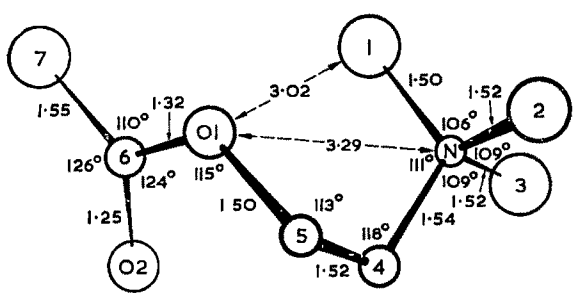


Fig. 1. The structure of the acetylcholine molecule in crystals of acetylcholine bromide, with interatomic distances and angles. Estimated standard deviation of the distances is less than 0·06 Å. In crystals both the molecule shown and its mirror image exist

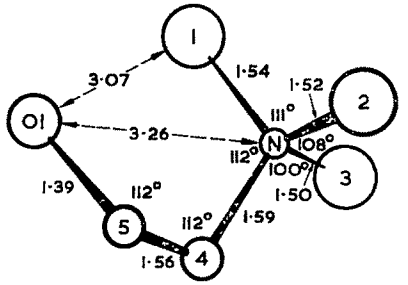


Fig. 2. The structure of the choline molecule in crystals of choline chloride[8], with interatomic distances and angles. Estimated standard deviation of distances is 0·03 Å

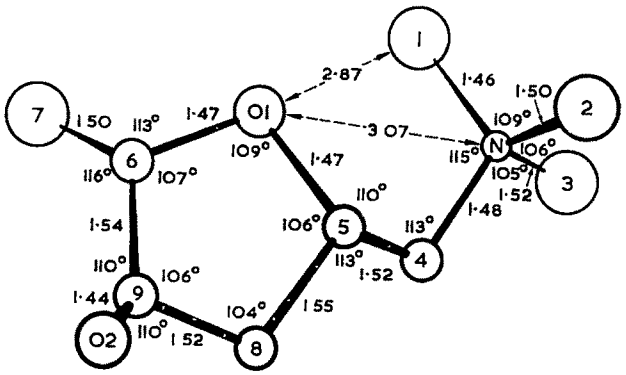


Fig. 3. The structure of the muscarine molecule in crystals of muscarine iodide[9], with interatomic distances and angles. Estimated standard deviation of distances is 0·08 Å. The configuration shown is the absolute one for that of the active L(+) form[10]

A number of investigators have endeavoured to map the active sites of cellular acetylcholine receptors and of acetylcholinesterase by determining binding strengths or rates of hydrolysis of various substances and building models of the relevant molecules. The structure of acetylcholine can be compared with these results.

Foldes, van Hees, Davis and Shannor[12] and Wilson and Quan[13] endeavoured to determine the distance between anionic and esteratic sites of acetylcholinesterase and presumably the distance between the nitrogen atom and the carboxyl group of acetylcholine by an investigation of the binding strength of various inhibitors of acetylcholinesterase as a function of $(CH_2)_n$ chain length. They concluded that the nitrogen atom to carboxyl group distance in acetylcholine should be of the order of 4·84 Å. In the solid-state the nitrogen atom to carboxyl carbon atom distance N–C_6 is 4·10 Å and the nitrogen atom to carboxyl oxygen atom distance N–O_2 is 4·42 Å.

The absolute configuration and relative pharmacological activities of the stereoisomers of acetyl-β-methylcholine have been determined and compared with that of L(+)-muscarine by Ellenbroek and van Rossum[14] and by Beckett *et al.*[15,16]. It is easy to see why the L(+) isomer is very active on muscarinic receptors and the D(−) form is not. The extra methyl carbon atom occupies the position of C_8 of muscarine in Fig. 3, just adding to the chain C_3 N C_4 C_5 of acetylcholine in Fig. 1 as it is added to by C_8 in muscarine. The extra methyl group of D(−) acetyl-β-methylcholine, however, interferes in two ways. First, it fits into the important and crowded region between the ester oxygen atom and the trimethyl ammonium group, and secondly, steric interference between this extra methyl group and the methyl group C_2 forces the molecule to change its conformation to some extent. It is interesting that the structure of muscarinic substrates determined by these solid-state investigations agrees well with the structure suggested by Beckett *et al.*[15,16] solely from pharmacological data.

Belleau and Lacasse[17] found that of a series of 1,3-dioxolane inhibitors of acetylcholinesterase, optimum affinity is shown by L(+)-*cis*-2-methyl-4-trimethyl-

ammoniummethyl-1,3-dioxolane iodide and deduced therefrom the stereospecificity of the active site. This molecule presumably has exactly the conformation shown in Fig. 3 (L(+)-muscarine) with O_2 removed and C_5 changed to an oxygen atom. Similar stereospecific effects of 1,3-dioxolanes on muscarinic receptors were shown by Belleau and Puranen[18].

Although there is no direct evidence that these solid-state investigations are relevant to the environment *in vivo*, that these three known structures have similar conformations in the common portion of the molecules encourages us to believe the analyses relevant to the biological mechanism of cholinergic synaptic transmission.

We thank Mrs. Ann Graeme-Barber for assistance, Dr. G. B. Robertson for some calculations, and Mr. John Cresswell for the drawings.

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SUCROSE PERMEASE OF *Proteus mirabilis*

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EXTRACTS of most *Proteus mirabilis* strains possess fructofuranosidase activity, but intact organisms usually do not ferment sucrose since they lack a permeability factor which controls the penetration of the disaccharide into the cell[1]. To study this permeability factor, strain 24 and a sucrose fermenting variant (24 *suc*$^+$) were used. This strain belongs to the group 1*a* previously described[1]. 'Difco' nutrient agar and a minimal medium[2] with 0·006 per cent nicotinic acid and 0·01 per cent tetrazolium chloride (medium GNM) were used. Medium GNM with 0·2 per cent sucrose substituted for glucose is called SNM. Other media used have been described[1]. Spontaneous *suc* mutants in large populations of strain 24 *suc*$^+$ were selected by a penicillin enrichment technique[2] in fluid SNM and replication was from GNM to SNM. Intrastrain transductions were done with the use of phage 34/13 with the general techniques previously described[3]. After 30 min at 37° C the adsorption mixture was centrifuged, the deposit suspended in 1 ml. saline and 0·1 ml. plated on SNM. Sucrose positive transductants appear as large red colonies after 47 h incubation. Bacterial suspensions were prepared as before[1] and the concentration estimated by determination of the deoxyribonucleic acid content[4]. Cell extracts for enzyme and sugar assays were prepared as follows: Bacterial suspensions (20 ml.) were centrifuged and the pellets resuspended in the same volume of ice-cold water. Suspensions were then sonicated in a Raytheon 50-W 9 kc/s oscillator for 30 min at 4° C. Unbroken organisms and cell debris were removed by centrifugation and the supernatant used for assays. Penetration of sucrose, lactose and fructose into cells was measured as follows: To ice-cold bacterial suspensions (5 ml.) a cold solution (0·5 ml.) of sucrose, lactose or fructose in broth was added to a final concentration of 1 per cent sugar. The experimental samples were then immersed in a water-bath at 36° C and identical controls kept at 0° C. After 5 min the mixtures were diluted with 30 ml. ice-cold broth and centrifuged (15,000*g*) for 15 min at 0° C. The pellets were washed five times to remove exogenous sugar, and cell-free extracts were used for sugar determinations[5–7]. β-Fructofuranosidase in cell-free extracts was determined as previously described[1]. The glycolytic enzymes fructokinase[8], hexokinase[9], phosphofructokinase[10] and aldolase[11] in the cell-free extracts were also determined. Protein was estimated by the biuret method[12] and paper chromatography of sugars was done according to Partridge[13].

Nineteen spontaneous *suc* mutants of strain 24 *suc*$^+$ were obtained from thirty-five different isolation experiments. Colonial growth of these mutants was identical to strain 24 on GNM, nutrient agar or 1 per cent sucrose nutrient agar, and growth curves of the mutants in a casein hydrolysate medium in the presence or absence of sucrose were identical. The reversion rates of the *suc* mutants varied from 4×10^{-7} to 1×10^{-9}/bacterium/generation. Many revertants were examined phenotypically and enzymatically. They all resembled the parent *suc*$^+$ strain and may be back mutations. Strain 24 *suc*$^+$ and mutants *suc*-3, -5, -6, -9, -10 took up sucrose under test conditions. Sucrose in the organisms was demonstrated chemically and chromatographically. The remaining strains did not take up sucrose or took up less than 1 per cent that of positive strains. Results were not affected by prior growth on 1 per cent nutrient agar. Transport is stereospecific in that strains which took up sucrose did not accumulate lactose under identical conditions. Penetration of sucrose is temperature (Fig. 1) and energy dependent. Inhibition of ATP generation by 1 mM sodium azide or 2,4-dinitrophenol results in reduced permeation of sucrose by 29 and 69 per cent respectively. A time course of uptake of sucrose by strains *suc*-5 and *suc*-10 is presented in Fig. 2. These results are taken to support the enzymatic nature of sucrose transport across the permeability barrier of the cell and the enzyme will be called a permease[14]. Strains which were positive with respect to sucrose permease had little or no β-fructofuranosidase activity apart from the *suc*$^+$ parent which was active in both respects. Prior growth on GNM or 1 per cent sucrose nutrient agar did not affect results. Permease negative strains possessed β-fructofuranosidase although specific activities varied over a wide range (Table 1). This latter phenomenon was investigated by adding extracts of *suc*-7 (low β-fructofuranosidase) to extracts of strain 24 *suc*$^+$ and estimating the invertase activity of the mixture. In other experiments, extracts of *suc*-15 (high β-fructofuranosidase) were substituted for *suc*-7. In all cases the activity of mixtures corresponded to the sum of individual activities. This is an argument

against the presence of soluble inhibitors or stimulators of fructofuranosidase activity in extracts of the two *suc* mutants. All strains were capable of fructose uptake and were also positive for hexokinase, fructokinase, phosphofructokinase and aldolase.

Results of transduction experiments divide the wild organism and *suc* mutants into two distinct linkage groups *P* and *F* (Table 1). Intra-group transductions yield significantly less transductants than combinations where organisms from one group are treated with phage lysates of the other group or with phage derived from the *suc*$^+$ strain. The correspondence in numbers of transductants obtained from the two latter procedures indicates that the two groups are not linked. This experiment was done five times. There was always the same absolute distinction between the two groups but results of intra-group transductions were too variable for use in ordering the sites within loci. There is good correlation between

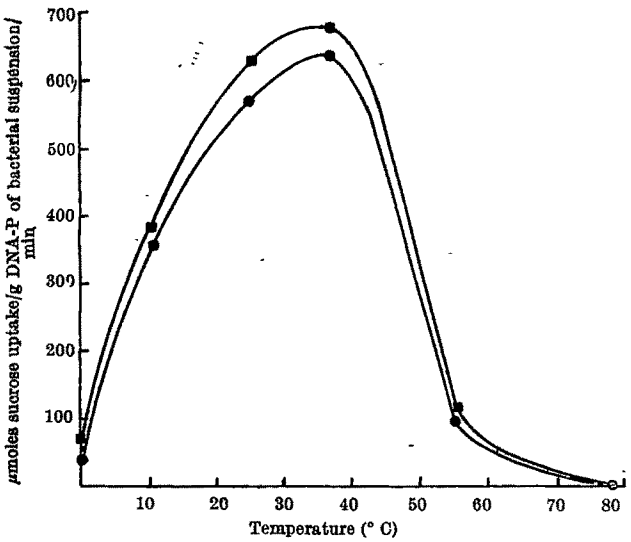


Fig 1. Effect of temperature on the uptake of sucrose by *Proteus mirabilis* mutants. ●, 24 *suc*$^+$; ■, *suc*-10. Assay: bacterial suspensions (5 ml.) and 11 per cent sucrose solution (0·5 ml.) were equilibrated at different temperatures after which the samples were mixed and incubated for 5 min. Free disaccharide was removed by repeated washings and the sucrose concentration measured in disrupted bacterial extracts

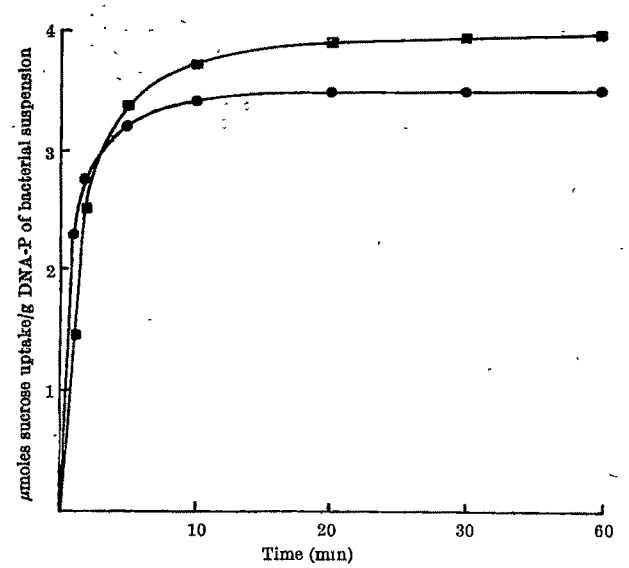


Fig. 2. Uptake of sucrose with time in *Proteus mirabilis* mutants ●, *suc*-5; ■, *suc*-10. Assay: bacterial suspensions (5 ml.) and 11 per cent sucrose solution (0·5 ml.) were equilibrated at 36° C after which they were mixed and incubated for varying intervals. The reaction was terminated by rapid cooling to 0° C. Exogenous sugar was removed by repeated washing and the sucrose concentration in disrupted bacterial extracts estimated

Table 1. SUCROSE PERMEASE AND β-FRUCTOFURANOSIDASE ACTIVITY OF WILD AND MUTANT *Proteus mirabilis* STRAINS

Strain and linkage group	Sucrose permease	β-fructofuranosidase
24 *P*	0	19
24 *suc*$^+$	643	504
suc F-3	667	0
suc F-5	648	1
suc F-6	574	0
suc F-9	603	0
suc F-10	684	1
suc P-1	0	18
suc P-2	0	312
suc P-4	3	6
suc P-7	0	26
suc P-8	4	6
suc P-11	3	16
suc P-12	0	29
suc P-13	4	310
suc P-14	0	378
suc P-15	0	1,132
suc P-16	0	27
suc P-17	0	29
suc P-18	0	8
suc P-19	0	1,152

Sucrose permease activity is expressed as μmoles of sucrose uptake/g DNA-P of bacterial suspension/min.

β-fructofuranosidase activity is expressed as μmoles sucrose hydrolysed/g of protein/min.

Results are the average of three separate experiments.

the genetic and biochemical divisions of *suc* mutants. Thus, members of genetic group *F* all possess strong permease activity and no (or only traces of) β-fructofuranosidase. Members of the genetic group *P* lack permease activity but possess varying degrees of fructofuranosidase activity.

In *Escherichia coli* the lactose permease gene is closely linked to the β-galactosidase structural gene, but the L-arabinose permease locus is unlinked to genes which synthesize enzymes necessary for arabinose utilization[15]. Biochemical definition of *suc* mutants has supplemented genetic data in demonstrating the complexity of the sucrose permease (*P*) gene. Like the gene governing L-ribulokinase activity in *E. coli*, mutations in the *P* linkage group have dual effects[16]. These *P* mutants lack sucrose permease but have increased or decreased constitutive levels of β-fructofuranosidase as compared with the *suc*$^+$ parent (Table 1). The β-fructofuranosidase is constitutive[1] and differences in fructofuranosidase activity due to variability of induction appear to be excluded. The fact that the *P* and *F* loci are not linked makes an explanation on grounds of these genes acting as a "genetic unit of co-ordinate expression"[17] difficult to apply and a regulator mechanism for the *F* locus controlled by the *P* structural gene may have to be postulated. Such a dual function is not unknown and in the classical lactose system of *E. coli* there is evidence that the operator locus may be part of the β-galactosidase structural gene[18].

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PHAGE-DIRECTED SYNTHESIS OF DIPHTHERIAL TOXIN IN NON-TOXINOGENIC *Corynebacterium diphtheriae*

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THE synthesis of diphtherial toxin is controlled by a bacteriophage gene, tox^+ (ref. 1). Although it has been shown that this gene is expressed in lysogenic, toxinogenic *Corynebacterium diphtheriae* (2, 2*a*) and in non-toxinogenic strains being lysed by phage[3], until now no investigations have been made of the kinetics of toxin production and phage production in the latter system, and this has mainly been due to the lack of a stable, virulent phage. Such a mutant phage, $\beta hv64^{tox+}$, has been isolated from a stock of the clear-plaque corynebacteriophage *B* (ref. 2). This phage is obligately lytic and has been used to introduce the tox^+ gene into cells of a population of a non-toxinogenic strain of *C. diphtheriae*, designated $C7_s(-)^{tox-}$ (refs. 3 and 4). By carrying out the adsorption of bacteriophages to bacteria in the presence of chloramphenicol and afterwards ridding the infected bacteria of chloramphenicol, unadsorbed virus and residual toxin present in the phage preparation[3], it has been possible to examine the expression of tox^+ in a single cycle ('one-step growth curve') of corynebacteriophage multiplication in non-toxinogenic diphtheria bacilli.

This article reports data derived from experiments using this system and designed to ascertain: (*a*) the kinetics of the production of toxin and of phage; (*b*) the effect of iron on the synthesis of these two entities; (*c*) the time of appearance of intra- and extra-cellular toxin; (*d*) the immunochemical relationship of the toxin synthesized to 'standard' diphtherial toxin produced by the $PW8_r(Pdi)^{tox+}$ strain[3]. The conditions for these experiments are given in the legends for Figs. 1, 2 and 3.

The production of toxin by various lysogenic strains of *C. diphtheriae* is inhibited by iron[5]. Because little was known about the effect of iron on toxin production during the lytic cycle, all lots of basal media were deferrated[6] and various amounts of iron were added to them depending on the purpose of the experiment.

In the experiments reported here the infected cells were synchronized with respect to the initiation of viral synthesis in the following manner. Bacteriophage and bacteria were combined in the presence of 125 μg/ml. chloramphenicol (a gift of Dr. H. E. Machamer, Parke, Davis and Co.) and adsorption was allowed to proceed for 20 min. This concentration of chloramphenicol was shown to have no apparent effect on phage adsorption but to block completely viral multiplication and toxin production so long as cells were exposed to it. Once chloramphenicol was removed viral multiplication and toxin production began and lysis occurred after the usual latent period. At the end of the adsorption period, the infected cells were centrifuged and washed once to remove chloramphenicol and unadsorbed virus. After resuspension in fresh medium, they were incubated with gentle shaking, and the growth of virus and the production of toxin followed. The samples for assay of phage as plaque-forming units and extracellular toxin as skin-test doses were sterile filtrates. Intracellular toxin production was followed by assaying filtrates derived from samples in which cells had been disrupted in a Ribi cell fractionator. The number of minimum reacting doses per sample was determined by assaying decrements of each sample by injection into the shaven skin of the backs of rabbits.

The results reported would seem to establish three facts regarding the synthesis of diphtherial toxin by non-toxinogenic *C. diphtheriae* infected with corynebacteriophage $\beta hv64^{tox+}$: (1) toxin production and the synthesis of new phage particles appear to be parallel events; (2) intracellular and extracellular toxin begin to increase early in the latent period of viral growth; (3) added iron causes about 90 per cent inhibition of synthesis of toxin without similarly affecting the synthesis of plaque-forming phage particles.

From Fig. 1 it is evident that with one cycle of viral multiplication there is a better than 10,000-fold increase in diphtherial toxin. The first toxin detectable in disrupted cells appears right after the removal of chloramphenicol. The first free phage is liberated at about the 65th

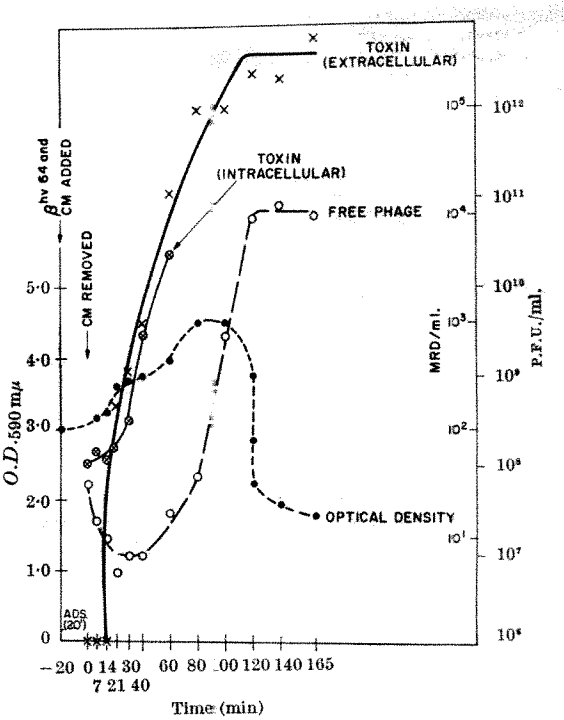


Fig. 1. The synthesis of diphtherial toxin by non-toxinogenic *C. diphtheriae*, strain $C7_s(-)^{tox-}$, infected with hypervirulent corynebacteriophage, $\beta hv64^{tox+}$. Phage and bacteria were combined to yield 288 ml. of a mixture containing: $2{\cdot}9 \times 10^9$ cells of $C7_s(-)^{tox-}$/ml., $2{\cdot}9 \times 10^{12}$ P.F.U. of $\beta hv64^{tox+}$/ml., 5×10^{-3} M calcium chloride and 125 μg/ml. chloramphenicol. Adsorption was allowed to proceed for 20 min at 36° C with gentle shaking. Mixture was then washed in chilled deferrated PGT medium, resuspended in 200 ml. of deferrated PGT medium containing 4 per cent maltose, distributed in 25 ml. portions in 1-l. Erlenmeyer flasks and incubated at 33° C with gentle shaking. Effective multiplicity of infection was 5·2. Samples for the assay of phage and extra-cellular toxin were taken at the intervals indicated, centrifuged and the supernates passed through 'Millipore' filters (*HAWP* 02400). For determining levels of intracellular toxin, 25 ml. samples were iced and centrifuged at 10,300*g* for 10 min at ±4° C; the packed cells were four times washed in chilled *tris*–Mg buffer (0·0 M *tris*, 10 mM magnesium acetate, *p*H 7·4) containing 0·01 per cent mercuhiolate and stored deep frozen. Afterwards, cells from the frozen state were suspended to original volume in *tris* buffer and twice passed through a Ribi cell fractionator at 3,000 p.s.i. at 5°–10° C. The disintegrated cells were twice centrifuged: at 6,000*g* for 20 min and at 10,300*g* for 20 min and the supernatant rendered sterile by passage through a 'Millipore' *HAWP* 02400 filter. CM, Chloromycetin; MRD, minimum skin reacting dose as assayed in the skin of rabbits; P.F.U., plaque forming units; ADS, adsorption period

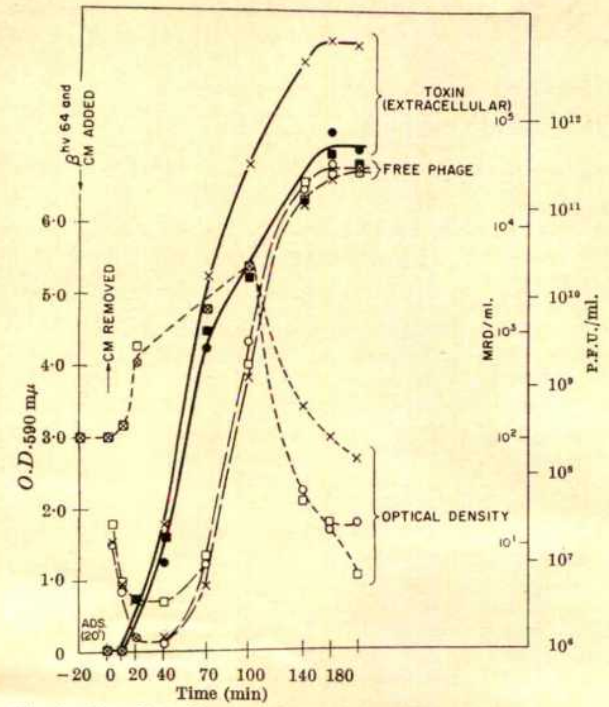


Fig. 2. The effect of iron on the synthesis of diphtherial toxin by *C. diphtheriae*, strain $C7_s(-)^{tox^-}$, infected with hypervirulent corynebacteriophage, $\beta^{hv64tox^+}$. Portions of a culture of $C7_s(-)^{tox^-}$ (grown to an optical density of 4·7 in deferrated medium supplemented with 0·1 μg iron/ml.) were diluted in (1) deferrated medium to which no iron was added and in (2) deferrated medium supplemented with 3·0 μg iron/ml., in both instances to give an optical density of 0·3, and 30 ml. samples of each were incubated in 300 ml. flasks. These 'low iron' and 'high iron' cells were allowed to grow to *O.D.* 2·85 ($3·7 \times 10^9$ cells/ml.); then each was combined with 17·5 ml. of a suspension of β^{hv64} ($4·3 \times 10^{10}$ P.F.U./ml.) Adsorption was allowed to proceed for 20 min in the presence of 5×10^{-3} M calcium chloride and 125 μg chloramphenicol/ml. Infected cells were then washed as described in the legend accompanying Fig. 1 and resuspended as follows: (×) infected 'low iron' cells were resuspended in deferrated medium; (○) a portion of infected 'high iron' cells was resuspended in deferrated medium; (□) a second portion of infected high iron cells was resuspended in medium containing 3·0 μg iron/ml. Samples were taken for optical density, P.F.U. and extracellular toxin at the indicated times. Effective multiplicity of infection was 2·1

minute. Extracellular toxin rises from the 14th to the 20th minute. The plateaux of both phage and toxin are reached at about the same time—a time at which the optical density of the lysing population sharply declines.

In Fig. 2 are plotted data from an experiment in which cells of $C7_s(-)^{tox^-}$ were grown (×) in deferrated medium and (○) in medium containing 3·0 μg iron/ml. The cells grown in deferrated medium eventually showed the lag in division time characteristic of iron-deficient cells. The cells grown in 3·0 μg iron/ml. grew at a normal rate. Such 'high iron' cells, grown as they are in excess iron, if washed, will retain considerable amounts of bound iron[7]. After these 'low iron' and 'high iron' populations had reached an optical density of 2·85, they were infected with $\beta^{hv64tox^+}$ in the presence of chloramphenicol. Following adsorption, the cells were washed. The 'low iron' cells were resuspended in deferrated medium. The 'high iron' cells were treated in two ways: some (○) were resuspended in deferrated medium and others(□) were resuspended in medium containing 3·0 μg iron/ml. The effect of iron on the course of viral infection and on the synthesis of toxin is shown in Fig. 2, where it can be seen that the 'high iron' cells produced less toxin than the 'low iron' cells, whether they were rich in bound iron (○) or, in addition, were supplied exogenous iron in the medium in which they were infected with phage.

In Fig. 3 is shown the immunochemical reactions of toxin produced under the direction of the $\beta^{hv64tox^+}$ gene and of a ten-times crystallized form of diphtheria toxin—kindly supplied by Dr. C. G. Pope[8] of the Wellcome Research Laboratories, Beckenham, Kent. The neat fusion of the antigen-antibody line formed between $\beta^{hv64tox^+}$-toxin and antitoxin with those formed between standard toxin and antitoxin would seem to be reasonable evidence that the two toxins are identical. This conclusion is further strengthened by the fact that standard antitoxin completely neutralizes the toxicity of $\beta^{hv64tox^+}$-toxin *in vivo*.

In a recent review, van Heyningen and Arseculeratne[9] pointed out the differences of opinion which exist regarding the mechanism of diphtherial toxin production. Although it is clear that toxin production depends (*a*) on the genetic information carried in the genome of a bacteriophage, and it has been generally agreed (*b*) that this information is expressed only under conditions in which the bacterial population is iron-starved, the exact relation between these two observations has been difficult to establish. Most of the studies concerned with toxin production so far have been based on the behaviour of a single strain, strain number 8 of Park and Williams[10], which we have designated $PW8_r(Pdi)^{tox^+}$; *r* indicates roughness in the usual sense, and in addition the loss of receptors for corynebacterial phages; *P* indicates that the strain carries a phage, *P*, which while defective (*d*) is inducible (*i*). It is an excellent producer of toxin and for that reason is used throughout the world for the manufacture of diphtherial toxoids and, to a lesser extent, for investigations of the mechanism of toxin production. It differs from most lysogenic, toxinogenic *C. diphtheriae* in that, since it carries a defective phage, there is no way of measuring the number of its cells which succumb to phage-induced lysis. A culture of $PW8_r(Pdi)^{tox^+}$ will, under conditions of iron starvation, produce toxin, but the relation of toxin production to the expected change from prophage to phage cannot be directly assessed. Induction of the vegetative multiplication of *Pdi*-phage by ultra-violet irradiation leads to the prompt production of toxin by $PW8_r(Pdi)^{tox^+}$, but in no greater amount per cell than in a non-irradiated culture allowed to become exhausted of iron. So far, it has not been possible to measure the yield of defective phage in terms of the amount of toxin formed. The situation is further complicated by the fact that the bacterial population appears to be heterogeneous with respect to its response to ultra-violet light.

In the system described here all the non-lysogenic, non-toxinogenic cells of strain $C7_s(-)^{tox^-}$ are brought into

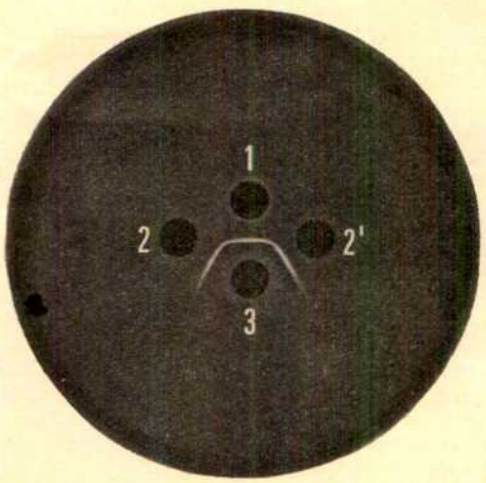


Fig. 3. Fused antigen-antibody bands resulting from diffusion in agar of antitoxin (see below), crystalline diphtherial toxin (Pope) and toxin produced under the direction of corynebacteriophage, $\beta^{hv64tox^+}$, during one cycle of growth in non-toxinogenic *C. diphtheriae*, strain $C7_s(-)^{tox^-}$. The cells labelled 1, 2, 2′, and 3, contained the following reactants in volumes of 0·2 ml.: (1) $\beta^{hv64tox^+}$. $C7_s(-)^{tox^-}$ lysate (24 × concentrated as 0–80 per cent saturated ammonium sulphate fraction); (2) and (2′), 20 Lf of 10 × crystallized diphtherial toxin, Pope. (3), 20 U. of diphtherial antitoxin (rabbit) produced with toxoid derived from crystalline toxin. Contact print was made after 3-days' incubation at 37° C. Prolonged incubation resulted in some thickening of the bands; no spurs were formed

synchrony with respect to toxin synthesis by having the *tox*+ gene injected into them as a part of the genome of an obligately lytic bacteriophage. Once the infected cells are removed from the presence of chloramphenicol (an agent used for establishing synchrony), the biosynthesis of phage and toxin ensues. The two syntheses appear interrelated. Toxin is made early both intra- and extracellularly. Iron does not inhibit the expression of *tox*+ though it does exert a limiting effect on the final yields of toxin. Experiments designed to exploit this lytic system to clarify the relation of toxin to the *tox*+-carrying corynebacteriophage and its host cell are in progress.

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PROLIFERATION OF HUMAN LYMPHOCYTES IN CULTURE

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PHYTOHAEMAGGLUTIN (PHA), bacterial antigens and other foreign proteins cause lymphocytes to undergo an abrupt increase in RNA synthesis, a progressive cell enlargement (transformation), DNA synthesis, and cell division (proliferation)[1–10]. The frequency of cells synthesizing DNA reaches a maximum 60–72 h after exposure to PHA; it is generally assumed that most of the cells involved are synthesizing DNA for the first time in culture[11,12]. Recently, however, Buckton and Pike[13,14], from investigations of the effect of time in culture on chromosome aberrations in lymphocytes obtained from irradiated patients, concluded that the majority of cells in metaphase at 72 h are in at least their second division in culture. Since the interpretation of cytological and biochemical data depends on whether the population of lymphocytes at the time of observation is transforming, proliferating, or both, we have studied the beginning of proliferation in some detail. The results show that not more than 30 per cent of the original mononuclear cells begin DNA synthesis by 72 h, that the transformation of the cells is essentially completed in 24–48 h, that after transformation the cells proliferate with a generation time of 22 h, that the increase in the frequency of cells synthesizing DNA from 48–72 h is due almost entirely to cell proliferation, and that 70–80 per cent of the cells in metaphase at 72 h are in at least their second division in culture. X-ray doses of up to 500 rads given before culture do not delay the onset of the first DNA synthesis, nor do they alter the duration of the G_1 (6 h), S (11 h), G_2 (3 h) and M (2 h) phases of the cell cycle.

Leucocytes were separated from peripheral blood without the aid of PHA and incubated at 37° C in a medium containing 80 per cent *NCTC* 109, 10 per cent autologous plasma, 10 per cent inactivated foetal calf serum, and 0·4 ml. of rehydrated PHA, type *M* (Difco), per 10 ml. of medium. Chromosomes were labelled by exposing cells to tritiated thymidine (specific activity 6·7 c./mmole) at a concentration of 1 μc./ml. The X-ray irradiations were done in whole blood prior to the separation of the leucocytes[15].

In the first set of experiments chromosomes were labelled with tritiated thymidine added to the cultures at various times after the start of incubation. The labelling was terminated by washing the cells with medium containing cold thymidine (100 μg/ml.) and the cells were resuspended in the standard incubation medium supplemented with cold thymidine (30 μg/ml.). After a total of 72 h incubation, the final 1·5 h in the presence of colcemide, the cultures were gathered and processed for autoradiography. The metaphase cells were identified as being in either the first or second division after labelling on the basis of the chromosome labelling pattern: at first division both chromatids are labelled, at second division only one of the two chromatids of the metaphase chromosome is labelled. Fig. 1 represents the results by a plot of the percentage of total cells in metaphase at 72 h that is in the first (solid line) or second (dashed line) division since the addition of tritiated thymidine. From the peak of the dashed curve we conclude that not less than 54 per cent of the metaphase cells are in at least their second division in culture; from the area under the curve we estimate that about 70 per cent of the metaphase cells are in at least their second division.

From the data we also estimate that the duration of the S phase, $T(S)$, is 11 h and that the generation time, T, measured from the mid-points of the two S phases, is about 22 h. Thus about half the cell cycle is occupied by DNA synthesis, the other half being split about equally between G_1 and the sum of G_2 and M.

In the second series of experiments, cells were incubated for 20 h and then treated with colcemide and tritiated thymidine at a final concentration, respectively, of 4·0 μg/ml. and 0·5 μc./ml. The cultures were collected at various times and processed for autoradiography. The labelling index and mitotic index were determined from counts of 1,000–3,000 mononuclear cells. Fig. 2 shows the percentage of labelled mononuclear cells, curve *A*,

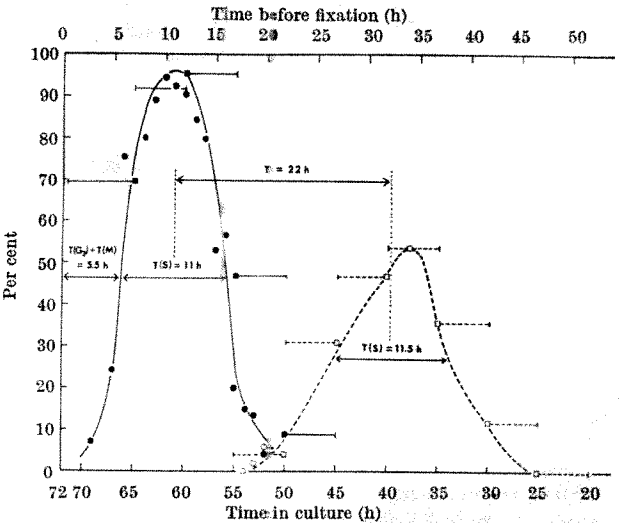


Fig. 1. Percentage of metaphase cells at 72 h in the first (solid line) or second (dashed line) division after labelling. Tritiated thymidine was added for either 5 h (horizontal lines), or 15 min (circles). Cells received 500 rads, circles, or 300 rads, squares

and of metaphase cells, curve B, as a function of time in cultures containing colcemide. Since colcemide does not affect the duration of DNA synthesis or of the cell cycle[16], curves A and B represent, respectively, the entry of cells into the first S phase and arrival at the first metaphase. As can be seen there is no significant difference in the data obtained with irradiated and unirradiated cells; apparently doses as high as 500 rads delivered before exposure to PHA do not cause a significant delay either in the time required for transformation or the time required to reach the first metaphase.

The cells appear in metaphase 14 h after the start of DNA synthesis. Taking this time as the duration of the sum of G_2 and S phases and using the data in Fig. 1 allows us to estimate the duration of each of the four phases in the 22 h cell cycle. The results for G_1, G_2, S and M are 6, 3, 11 and 2 h, respectively, in good agreement with results reported for mammalian cells[17].

If all the cells entering DNA synthesis, curve A, were accumulated in metaphase we would expect curve C (dashed line). The departure of the experimental curve, B, from the expected curve, C, after the 53 h shows that there must be a loss of mitotic figures after this time due to degeneration or escape from arrest.

Curve D was constructed from curve A under the assumption that every cell entering S phase for the first time (curve A) would divide and enter the S phase a second time exactly 22 h later. Since curve D represents reasonably well the experimental data (crosses) obtained with cells labelled in the absence of colcemide, we can conclude that the increase in frequency of cells synthesizing DNA found between 48 and 72 h is due primarily to cell proliferation. The cells in metaphase at 72 h entered DNA synthesis 14 h earlier, 58 h after the start of culture. From the ratio of curves D to curve A we estimate that 69 per cent of the cells are in their second S phase in culture at 58 h, in agreement with the previous estimate for the percentage of metaphase cells in second division at 72 h.

The third set of experiments is based on the observation that the small fraction of acentric chromosomes produced by X-rays that survive cell division appears in the daughter cells as double sets of identical acentric chromosomes. The proportion of cells in at least the second metaphase can be estimated, therefore, from the ratio of the number of cells containing such double sets to the total number of cells with acentric chromosomes. The results of these experiments are shown in Table 1. It can be seen that the fraction of cells in second division at 72 h, when no colcemide is present, averages about 80 per cent. Moreover, this ratio is independent of X-ray dose over the range of 100–600 rads, and this further supports the conclusion that such X-ray doses do not cause significant mitotic delays. Finally, it should be noted that both the frequency of cells with doubled acentric chromosome sets and the frequency of tetraploid cells is reduced almost to zero when the culture is stopped at 50 h; both frequencies are reduced to zero when 4 μg/ml. of colcemide is added to the culture at 20 h and the cells are collected at 50 h.

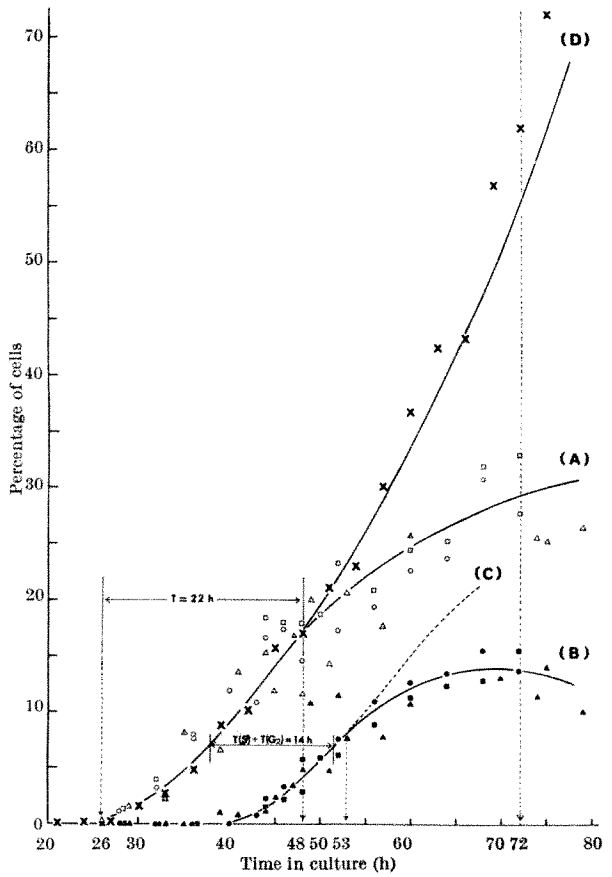


Fig. 2. Percentage of mononuclear cells labelled with tritiated thymidine in the presence (curve A) or absence (curve D, computed) of colcemide; and per cent of mononuclear cells in metaphase (experimental, curve B; expected, curve C). Circles, normal blood; squares, 500 rads *in vitro*; triangles and crosses, blood from irradiated patients. ○, □, △, Labelled mononuclear cells in colcemide; ●, ■, ▲, metaphase cells in colcemide; ×, labelled mononuclear cells (no colcemide)

Table 1. NO. OF CELLS WITH ACENTRIC CHROMOSOMES, N, WITH DOUBLED SETS OF ACENTRIC CHROMOSOMES, N', AND OF TETRAPLOID CELLS, $4n$

X-ray dose (rad)	Time in culture (h)	Colcemide* (μg/ml.)	$\frac{N'}{N}$ Ratio	$\frac{N'}{N}$ Per cent	$4n$ cells per thousand cells
100	72	0	5/7	72	11
200	72	0	32/43	75	12
300	72	0	75/89	84	24
400	72	0	104/121	86	34
500	72	0	39/51	77	56
600	72	0	181/212	85	95
500	50	0	6/43	14	1
500	72	0·05	45/150	30	13
500	72	0·5	15/150	10	25
500	72	1·0	6/110	6	6
500	72	3·0	5/140	4	5
500	72	4·0	2/94	2	7
500	50	4·0	0/179	0	0

* Colcemide added after 20 h in culture.

Our data indicate, thus, that the small lymphocyte finishes its transformation in 24–48 h, then begins a period of cell proliferation with a generation time of 22 h. As a result the majority of cells are in their second division at the end of 72 h, the standard culture time in cytogenetic work with human lymphocytes. For cytogenetic work, therefore, we have adopted the procedure of adding colcemide after 24 h at a concentration of 3–4 μg/ml. and collecting the cells between 48 and 60 h. The yield of cells in metaphase and the quality of chromosome preparations are very similar to those obtained with the standard 72 h cultures.

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HAEMOGLOBIN KÖLN (β–98 VALINE → METHIONINE): AN UNSTABLE PROTEIN CAUSING INCLUSION-BODY ANAEMIA

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IN 1964 an unstable human haemoglobin was described which was associated with Heinz body anaemia[1]. It had been found in a Glasgow family with some German ancestry. Unlike in other haemoglobinopathies, such as sickle-cell anaemia or haemoglobin C disease, this inclusion-body anaemia was not a homozygous condition, nor were the patients doubly heterozygous for two abnormal haemoglobin genes—the major proportion of their circulating haemoglobins was normal adult haemoglobin (haemoglobin A) (Fig. 1). At about this time, a haemoglobin from a German family was investigated which had been considered to be an abnormal haemoglobin A_2 (ref. 2). It had been sent from Cologne and had been denoted as haemoglobin Köln. This haemoglobin seemed to be identical with that from the Glasgow family as regards clinical manifestation, haematological features, tendency to denature on mild heating, electrophoretic behaviour, products of hybridization with canine haemoglobin, and the results of fingerprinting of the soluble peptides after tryptic hydrolysis. The fingerprints were indistinguishable from those of haemoglobin A, and it was concluded that haemoglobin Köln was an abnormal variant of haemoglobin A and not haemoglobin A_2. Because on electrophoresis at alkaline *p*H haemoglobin Köln moved more slowly than haemoglobin A, it was assumed that the part of the molecule carrying the additional positive charge did not appear in the fingerprint of the soluble tryptic peptides, but formed part of the insoluble 'core'—that portion of the haemoglobin molecule which does not go into solution on tryptic digestion. Hybridization showed that the α-chains of haemoglobin Köln formed the normal $\alpha_2^{human}\beta_2^{canine}$ hybrid, but that the β-chains of haemoglobin Köln did not form a hybrid with canine α-chains. It was concluded that the abnormality of haemoglobin Köln rested in its β-chain. Of the 146 residues of the β-chain of human haemoglobin the core comprises residues 83–120, and it was assumed that one of these residues had been substituted in haemoglobin Köln[1,3].

To confirm that the electrophoretic abnormality in haemoglobin Köln was situated in the β-chain, globin of pure haemoglobin Köln was prepared and the α- and β-chains were separated and compared with those of globin from haemoglobin A by electrophoresis at *p*H 8·6 in 8 M urea according to Chernoff and Pettit[4]. It was surprising to find that there was no electrophoretic difference between the α- and β-chains of globin Köln and globin A. Because the polypeptide chains became dissociated in 8 M urea and lost their tertiary structure, a possible explanation was that the difference in charge between the two haemoglobins was dependent on their tertiary structure and not primarily on a difference in charge of one of their amino-acid residues, as had so far been the case with all other abnormal haemoglobins (see Fig. 2).

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The possibility was considered that, on electrophoresis, haemoglobin Köln was moving more slowly than haemoglobin A because due to polymerization it was a larger molecule. However, on ultracentrifugation, the sedimentation constant proved to be the same as that of haemoglobin A.

An analysis of the haemoglobin was undertaken by fingerprinting and also an amino-acid analysis of tryptic peptides using methods recently summarized[5]. Fingerprints of the tryptic peptides were prepared for the whole molecule, including the core which had been made soluble by amino-ethylation of the cysteine residues according to the method of Jones[6]. No difference could be detected in the position of any of the tryptic peptides, which now included βTpX (residues 83–95), βTpXI (96–104), and βTpXII (105–120), that is, the core peptides.

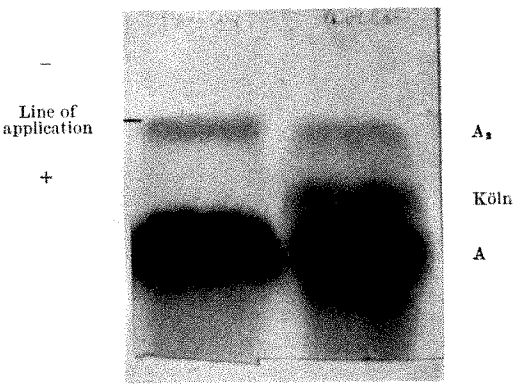


Fig. 1. Paper electrophoresis at *p*H 8·9 of a control (left) and haemoglobin from a patient with haemoglobin Köln disease (right). Electrophoretic separation of haemoglobin Köln from haemoglobin A is clearly shown

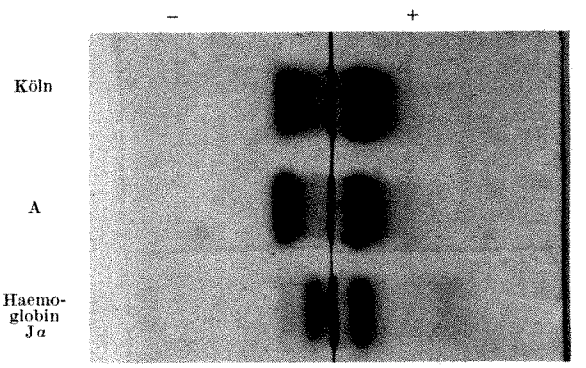


Fig. 2. Starch-gel electrophoresis at *p*H 8·6 in 8 M urea of the globins of haemoglobin Köln, A, and a fast-moving α-chain abnormal haemoglobin (the latter has been included to demonstrate that the method usually shows up electrophoretic differences of abnormal haemoglobins in one of their chains). It will be seen that by this method the separated globin chains of A and Köln show no electrophoretic differences

The α- and β-chains of haemoglobin A and haemoglobin Köln were then examined separately. Chain separation was effected on carboxymethylcellulose columns according to the method of Clegg, Naughton and Weatherall[7]. Samples of the separated chains were then hydrolysed in 6 N hydrochloric acid at 105° C for both 24 h and 48 h. Amino-acid analysis of the hydrolysates showed no difference between the two α-chains, but the β-chains of haemoglobin Köln consistently showed a higher content of methionine. The β-chain of haemoglobin A contains one residue of methionine. In order to confirm the difference in methionine content, both β-chains were oxidized with performic acid which converts methionine to the stable sulphone[8]. The ratio of methionine sulphone from haemoglobin Köln to that of haemoglobin A was exactly 2 : 1. All the other residues were present in near equal amounts, except for valine, which was significantly reduced in haemoglobin Köln. Fingerprints of the amino-ethylated β-chains of haemoglobin Köln and haemoglobin A, both after electrophoresis at *p*H 3·5 and *p*H 6·5, showed no difference in the position of the tryptic peptides (see Fig. 3). This confirmed that there was no difference in charged residues. Colour tests for specific amino-acids were sequentially carried out on the fingerprints as described by Putnam and Easley[9]. Both β^{A}T*p*XI and $\beta^{Köln}$TpXI gave positive reactions on staining for histidine and arginine. On staining for divalent sulphur a positive reaction was obtained in the case of haemoglobin Köln only. This suggested that in haemoglobin Köln a neutral amino-acid of βTpXI of haemoglobin A (residues 96–104 of the β-chain) had been replaced by methionine. From the peptide maps of both β-chains, βTpXI was now isolated using the techniques described by Clegg, Naughton and Weatherall[7]. After hydrolysis for 20 h in 6 N hydrochloric acid at 105° C, amino-acid analysis was carried out. At the same time, a blank area of the paper near βTpXI was eluted and hydrolysed, and on analysis the results were used as a blank correction for the peptide itself. The results are shown in Table 1. It will be seen that in βTpXI of haemoglobin A methionine was absent, and that in haemoglobin Köln methionine replaced a corresponding amount of valine. We consider, therefore, that the sample of haemoglobin Köln from Glasgow is $\alpha_2\beta_2^{98\ Val \rightarrow Met}$.

Residues	96	97	98	99	100	101	102	103	104
β^{A}TpXI	Leu	His	**Val**	Asp	Pro	Glu	Asn	Phe	Arg
$\beta^{Köln}$TpXI	Leu	His	**Met**	Asp	Pro	Glu	Asn	Phe	Arg

From the fact that haemoglobin Köln is highly unstable and denatures on a mild heating which does not affect haemoglobin A, it is concluded that the substitution of methionine for valine in position 98 of the β-chain has a profound effect on the stability of the haemoglobin molecule.

The instability of the haemoglobin Köln molecule *in vivo* and *in vitro* might well be related to a change in tertiary structure in an area which is in close proximity to the haem. The valine in position 98 is present in all the known haemoglobin chains, though in sperm whale myoglobin its counterpart is isoleucine. It is conceivable that valine in this position is an essential requirement for the stability of the haemoglobin molecule.

Table 1. AMINO-ACID ANALYSIS OF βTpXI FROM HAEMOGLOBIN KÖLN AND HAEMOGLOBIN A

	Haemoglobin Köln		Haemoglobin A	
	Moles	Residues	Moles	Residues
Aspartic acid	0·079	1·9	0·034	2·0
Glutamic acid	0·044	1·0	0·019	1·1
Proline	0·040	1·0	0·015	0·9
Valine	0·007	0·2	0·015	0·9
Methionine	0·030	0·7	Nil	Nil.
Leucine	0·043	1·0	0·015	0·9
Phenylaline	0·044	1·0	0·018	1·1
Histidine*	+	1	+	1
Arginine*	+	1	+	1

* A positive specific colour test was considered to represent one residue.

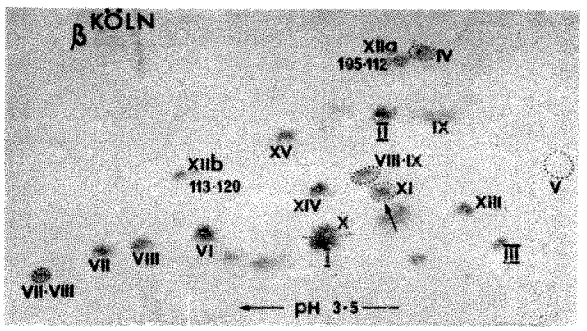


Fig. 3. Fingerprint of the amino-ethylated β-chain of haemoglobin Köln. The various tryptic peptides are indicated. βTpXI (arrowed) is in exactly the same position as it would be in a fingerprint of the amino-ethylated β-chain of haemoglobin A. The only difference is that in the case of haemoglobin A only the colour tests for histidine and arginine are positive, while for haemoglobin Köln there is also a positive test for methionine

There are at least six residues invariantly present in corresponding positions in the polypeptide chains of all functioning haemoglobins and myoglobins which have so far been investigated. They are represented in the β-chain of human haemoglobin by the residues of glycine in position 24, proline 36, phenylalanine 42, leucine 88, histidine 92, and tyrosine 145. Dr. H. C. Watson has pointed out to us that the valine residue in position 98 is in direct contact with the haem, and that there is a continuity of contact between 145tyrosyl—98valyl—haem—42phenylalanyl. It might be expected that replacement of the 98 valine residue by a larger one could interfere with the stability of the tertiary structure of the β-chain. The increased positive charge of the haemoglobin Köln molecule becomes apparent at *p*H 8·6. At this alkaline *p*H, it is possible to exclude a secondary decrease in charge of the neighbouring aspartic acid residue in position 99 (Table 2).

Table 2. THE NEIGHBOURING RESIDUES OF 98-METHIONINE IN THE β-CHAIN OF HAEMOGLOBIN KÖLN

End of helical segment F		Non-helical segment forming corner between segments F and G					Beginning of helical segment G	
92	93	94	95	96	97	98	99	100
His (haem-linked)	Cys[H]	Asp⁻	Lys⁺	Leu	His	Met	Asp⁻	Pro

The observation could, however, be in keeping with an increase in the positive charge of a nearby histidine residue. Dr. M. F. Perutz has suggested to us that it is possible that in the tightly packed area of the molecule around the residue 98, methionine might displace the neighbouring histidine in position 97, thus bringing it nearer to an aspartic acid residue at 99 or possibly 94. The greater proximity of the negative charge would then produce an increase in positive charge of the 97 histidine residue—and such an increase in positive charge would depend, however, on the tertiary structure of the molecule being maintained, which is what, in fact, has been observed.

It still remains to be ascertained that all samples of haemoglobin Köln are identical in their amino-acid constitution. This is of some importance because in addition to the original German family other families with haemoglobin Köln have been observed in Britain.

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PRESENCE IN LIPOPROTEINS FROM HUMAN SERUM OF A CRYSTALLINE BLUE FLUORESCENT SUBSTANCE OF UNKNOWN STRUCTURE ISOLATED FROM BOVINE LIVER

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THE preparation of a bluish fluorescent polypeptide (protein) fraction from bovine liver has previously been described[1–4]. This fraction, which was given the name 'urgocyton', was shown to inhibit the breakdown of bradykinin by kininases[4]. On acid hydrolysis, the fluorescent polypeptide fraction liberated a bluish fluorescent substance of low molecular weight[4]. This substance, which seems to be a novel one, has now been prepared in the crystalline state and the present publication describes its method of preparation together with data of characterization. The same substance has also been isolated after acid hydrolysis of a lipoprotein fraction from human plasma.

To obtain crystalline material, the crude low-molecular-weight fluorescent fraction, prepared after acid hydrolysis of urgocyton by a method described previously[4], was subjected to the following additional purification. The fraction was dissolved in water and the *p*H adjusted to 8·0 with sodium hydroxide. The solution was then passed through a DEAE-'Sephadex *A* 25' coarse grade (3-cm × 41-cm) column previously equilibrated with 0·1 N sodium bicarbonate and washed with water. The column was then treated with 0·005 M sodium bicarbonate until no more material absorbing at 250 mμ was eluted. The bluish fluorescent substance was eluted with 0·05 M sodium bicarbonate solution. The *p*H of the solution was adjusted to 3·5 by the addition of 2 N hydrochloric acid and the solution extracted twice with 90 per cent aqueous phenol. The phenol extracts were combined and added to three times their volume of ether. The aqueous phase was removed and the phenol–ether phase extracted once with water. The two aqueous phases were combined, extracted with ether to remove phenol and concentrated to dryness. The solid was crystallized from water in prismatic needles (see Fig. 1) and had a melting point at 152°–153° (uncorrected). It is soluble in water at room temperature but insoluble in ether. It gives a negative ninhydrin reaction and a pinkish colour with ferric chloride. It was dried at 75° C *in vacuo* over phosphorous pentoxide. Analysis gave its composition as: carbon, 51·14; hydrogen, 4·64; nitrogen, 6·71; and sulphur, 15·16 per cent.

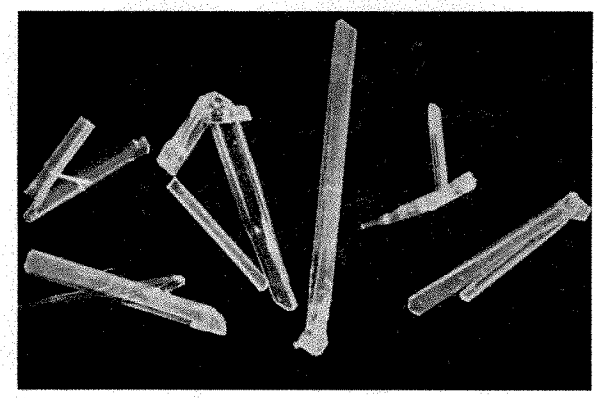


Fig. 1. Crystals of the bluish fluorescent substance photographed under the fluorescent microscope (× 10)

When titrated electrometrically with 0·01 N sodium hydroxide it was shown to possess a dissociating group with a pK'_a of 5·2. Its equivalent weight was calculated to be 212. The results suggest $C_9H_9NSO_3$ as the simplest formula. It is optically active with $[\alpha]_D^{20} = -106{\cdot}2°$ (carbon, 0·18 in water). Its electrophoretic properties were determined on Whatman No. 1 paper at 17 V/cm for 2 h. The buffers used were: 0·2 M acetic acid (*p*H 2·8), pyridine–acetic acid–water (10 : 100 : 90 v/v, *p*H 3·5) and pyridine–acetic acid–water (200 : 8 : 1,800 v/v, *p*H 6·5). At *p*H 6·5 and 3·5 the fluorescent substance moved 5·5 cm and 0·8 cm respectively towards the anode. At *p*H 2·8 it moved 1·2 cm towards the cathode. These results suggest that it exists in aqueous solution with a net negative charge and possesses an acidic group. The *p*H of a 0·025 per cent aqueous solution was 4·1. Furthermore, the nitrogen seems to become protonized at low *p*H.

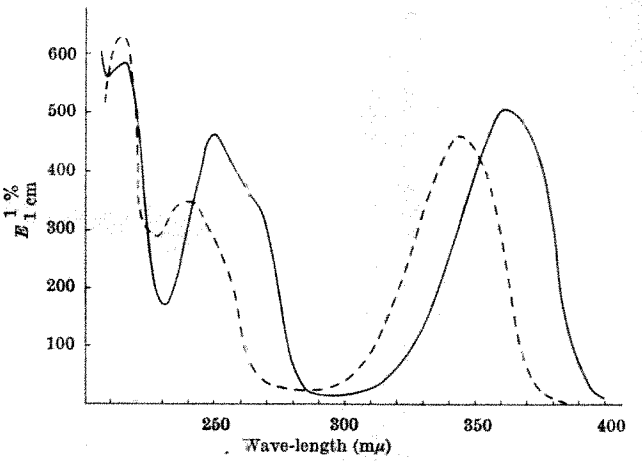


Fig. 2. Ultra-violet absorption spectra of the bluish fluorescent substance in 0·1 N hydrochloric acid (— — —) and 0·1 M phosphate buffer, *p*H 7·6 (——)

The ultra-violet absorption spectra measured in a 'Beckman *DU*' spectrophotometer at *p*H 1·0 and 7·6 are recorded in Fig. 2. The shift in the spectrum when going from *p*H 7·6 to 1·0 is reversible. The three sets of absorption maxima at *p*H 7·6 and 1·0 respectively are 360, 249, 214 mμ and 343, 239, 214 mμ. The absorption spectrum at *p*H 13, which has not been included, has maxima at 360, 248 and 218 mμ and is very similar to that at *p*H 7·6. The near identity of these two spectra suggests that there is no group dissociating between *p*H 7·6 and 13·0. The fluorescence and excitation maxima at *p*H 1·0, 7·4 and 13 were measured in a Farrand fluoro-spectrophotometer

Table 1. FLUORESCENCE AND EXCITATION MAXIMA IN 0·1 N HYDROCHLORIC ACID (*p*H 1·0), 0·1 M PHOSPHATE BUFFER (*p*H 7·4) AND 0·1 N SODIUM HYDROXIDE (*p*H 13) OF THE SUBSTANCE OF LOW MOLECULAR WEIGHT

*p*H	Excitation maximum (mμ)	Fluorescence maximum (mμ)
1·0	340	435
7·4	360	435
13·0	360	435

(uncorrected values with filters 7–54 and 3–73 are shown in Table 1). This instrument (and filters) was used in all subsequent measurements of fluorescence. In all cases, 435 mμ is the maximum of the fluorescence light. Excitation at *p*H 1 occurs at 340 mμ and at *p*H 7·5 and 13 at 360 mμ. The intensity of the fluorescence in water is sufficient to detect 0·1 μg/l. by the naked eye.

The substance is quite stable to acid hydrolysis. 0·076 mg was heated under reflux in 0·6 ml. of 6·3 N hydrochloric acid at 105° C for 20 h. The slightly yellow hydrolysate was concentrated to dryness over potassium hydroxide in vacuum, and the unchanged material was calculated from its ultra-violet absorption (at 360 mμ, *p*H 7·5). 90 per cent of the material remained unchanged.

The presence in ox serum of fluorescent proteins has previously been reported[4]. Some of these proteins in human serum have now been identified. Fig. 3 shows that, when human plasma is filtered on 'Sephadex *G*-200' gel, two fluorescent protein fractions (dotted line) appear in the beginning of the elution diagram. These are designated α_1 and α_2 and exhibited a blue fluorescence which was easily recognizable by the naked eye. A number of sera from healthy donors have also been examined. They all contain the α_1-peak with distribution coefficient 0·11–0·14. In some, peak α_2 with distribution coefficient 0·24–0·28 is much less pronounced and in others almost absent. Both peaks had a neutral *p*H fluorescence maximum at 440 mμ and an excitation maximum at 360 mμ. The large fluorescent peak seen in the last part of the elution diagram in Fig. 3 had a bluish turquoise fluorescence and will be the subject of a subsequent publication by some of us. According to Gelotte, Flodin and Killander[5], the lipoproteins are some of the proteins eluted in the fractions included in peaks α_1 and α_2. Dextran sulphate and calcium chloride added to the serum should precipitate the low-density lipoproteins[6]. However, when examined by agar immuno-electrophoresis[7], the precipitate contained as much high-density lipoprotein as low-density lipoprotein. Small amounts of β_1- and β_2-globulins were also present. When filtering the supernatant through gel after precipitation with dextran sulphate on 'Sephadex *G*-200', the fluorescent α_1-peak was absent and the α_2-peak was substantially reduced. Thus, the results seemed to indicate that the fluorescent substance was part of the lipoproteins. In order to isolate and identify it, the following experiment was carried out.

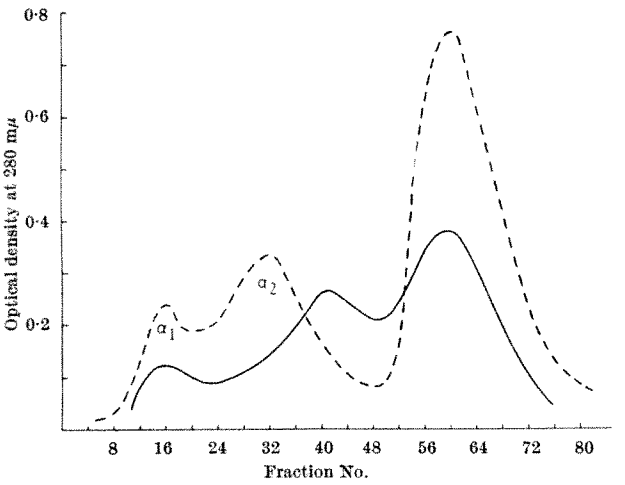


Fig. 3. Gel filtration of 20 ml. serum on 'Sephadex *G*-200' column (6 cm × 60 cm, diam. × height). The blood was drawn (from a fasting, healthy male), left at 20° C for 32 h followed by 6 h at 4° C and then centrifuged at low speed for 30 min at 0° C. The serum was fractionated on the column using as eluant a 0·2 M phosphate buffer (*p*H 7·3) containing 1 M sodium chloride (ref. 5). The flow rate was 40 ml./h and 10 ml. fractions were collected. The optical density (——) of the fractions was measured at 280 mμ after ten-fold dilution with the eluant. The fluorescence (— — —) was measured directly in the fractions at 440 mμ after excitation at 360 mμ. In all fractions beyond fraction No. 48, fluorescence was measured at 460 mμ after excitation at 340 mμ. The fluorescence is given in arbitrary units

For convenience the lipoproteins were isolated by mixing 180 ml. of serum with 7·2 ml. of a 10 per cent solution of dextran sulphate (Nutritional Biochemicals Corporation, clinical grade, molecular weight 16,200) and 18 ml. of a solution of 1 M calcium chloride. The precipitate was purified by dissolving it in 20 ml. of a 5 per cent solution of sodium chloride and reprecipitating it by the addition of 100 ml. of water and 15 ml. of a solution of M calcium chloride. The precipitate was hydrolysed with 5 ml. of 6 N hydrochloric acid at 100° C for 22 h. The filtrate was treated with 0·5 g charcoal. The charcoal was eluted and the eluted fraction[4] purified in water on a polyamide (Woelm) column. The fluorescent fraction was then further purified on thin-layer silica plates (medium *H* according to Stahl. E. Merck *AG*, Darmstadt, Germany) in the solvent system butan-1-ol–acetic acid–water (100 : 22 : 50) and finally in the isopropan-2-ol–butan-1-ol–water (10 : 7 : 10) where it had R_F values of 0·40 and 0·48 respectively. These were identical to those found for the low-molecular-weight fluorescent substances isolated from liver. The fluorescent band was extracted from the silica plates using 50 per cent aqueous ethanol. The final extract (50 per cent ethanol) was concentrated to dryness and the ultra-violet absorption spectra and fluorescence characteristics determined. These were in all respect identical to those of the crystalline substance already described. The characteristic shifts in the ultra-violet absorption and the excitation spectrum when going from neutral to acid solution were exactly the same. When compared electrophoretically at *p*H 2·8, 3·5 and 6·5, the fluorescent substance isolated from the lipoprotein fraction behaved in an identical manner to that of the substance isolated from liver.

Having established the presence of the low-molecular-weight fluorescent substance in the serum-protein fraction, the following less-laborious method for detecting this substance in hydrolysates was used. 100 mg of the material to be analysed was hydrolysed at 100° C for 18 h with 1·5 ml. of 6 N hydrochloric acid. The hydrolysate was diluted with water to 3 ml., filtered and treated with 0·2 g 'Norit' for 2 h at room temperature. The charcoal was washed with water until filtrate was free of chloride ions and eluted at room temperature twice (2 h each time) with ethanol–ammonia (sp. gr. 0·91)–water (48 : 2 : 50), 4 ml. each time. The eluate was concentrated in vacuum and chromatographed on Whatman No. 1 paper in butan-1-ol–ethanol–aqueous ammonia (sp. gr. 0·91)–water (4 : 1 : 2 : 1, by volume) containing 0·2 per cent ammonium chloride. If present, the substance could easily be detected by its blue fluorescence and its approximate R_F of 0·25. The fluorescent band was eluted and its identity confirmed by its fluorescence characteristics, that is, excitation maxima at 360 mμ and 340 mμ at *p*H 7·4 and *p*H 1·0 respectively, and fluorescence maximum at 435 mμ.

To decide whether the lipid part of the lipoprotein contained any of the low-molecular-weight fluorescent substance, the following experiment was carried out. The fluorescent fraction obtained after gel filtration on 'Sephadex *G*-200' was extracted by the method of Scanu, Lewis and Bumpus[8] to remove lipid. The dialysed and freeze-dried lipoprotein fraction was added to 25 ml. of a mixture of ethanol–ether (1 : 1, by volume) and kept at −20° C for 4 h. The precipitated protein was treated with 50 ml. of ether for 24 h at −20° C. The lipid and protein fractions, which weighed 327 mg and 340 mg respectively, were both subjected to the procedure described for detecting the presence of the low-molecular-weight fluorescent substance. A hydrolysate of the lipid contained no such substance, whereas the protein hydrolysate did.

The hydrolytic procedure used in liberating the low-molecular-weight fluorescent substance is fairly drastic, but it is convenient, since proteins are completely broken down to amino-acids. It is conceivable that this hydrolytic procedure could change the fluorescent substance some-

what. There are, however, two reasons why this is probably not the case. When hydrolysing the lipoprotein fraction and urgocyton, respectively, with N-hydrochloric acid at 100° C for 1 h, the same fluorescent substance could be isolated. The fluorescence and excitation maxima (440 and 360 mμ) of the lipoprotein fraction at *p*H 7·3 are very similar to that of the low-molecular-weight fluorescent substance at *p*H 7·6 (fluorescence maximum 430 mμ and excitation maximum 360 mμ).

Certain preliminary conclusions regarding the structure of the fluorescent substance may be drawn. Its strong fluorescence and multi-banded ultra-violet absorption spectrum indicate some unsaturated cyclic structure, an aspect which is also reflected in its low hydrogen content. The presence of one nitrogen molecule per molecular equivalence excluded the presence of purine and pyrimidine ring systems.

The optical activity of the substance makes it more interesting, since it seems unlikely that carbohydrates and amino-acids are present. The presence in it of sulphur is also worth noticing.

What is the biological significance of the bluish fluorescent substance of low molecular weight? Is it required for the proper function of the high-density lipoprotein or is it merely transported by lipoproteins and liberated in the body as such (or attached to peptides) when and where its presence is called for? Furthermore, is it attached to other proteins? What is the relationship between the lipoproteins and the fluorescent polypeptide fraction (urgocyton) isolated from liver?

The biological properties as well as the chemical structure of this substance are under investigation.

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X-Ray Crystal Data on Fluorescent Substance from Bovine Liver

A PRELIMINARY X-ray crystallographic investigation has been carried out on the fluorescent substance of unknown structure described in the preceding communication. Weissenberg diagrams were taken about the *a* and *c* axes. The crystals are orthorhombic, elongated in the *c* direction, with $a = 12{\cdot}60$ Å, $b = 17{\cdot}64$ Å, $c = 8{\cdot}51$ Å (all $\pm 0{\cdot}5$ per cent). The space group is $P\,2_12_12_1$, as reflexions $h00$, $0k0$ and $00l$ are absent for odd values of h, k and l. No other systematic absences occur. By flotation in mixtures of benzene and carbon tetrachloride the density was found to be 1·54 g cm^{-3}. This gives a weight of 439 for the asymmetric unit in the crystals. Two equivalents of weight 211 and one water molecule correspond to a weight of 440, in good agreement with the experimental value. The existence of half a molecule of water per equivalent weight was verified in drying experiments.

The space group indicates that the molecules might be optically active. They were tested directly for this, and strong activity in aqueous solution was found.

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EFFECTS OF PHYTOHAEMAGGLUTININ *IN VITRO* ON CELLS OTHER THAN LYMPHOCYTES

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IN 1960, Nowell demonstrated that phytohaemagglutinin (PHA), an extract of *Phaseolus vulgaris*, known for its haemagglutinating activity[1], possesses the remarkable property of being able to initiate mitoses in normal human lymphocytes *in vitro*[2]. Since this observation was made, a great number of reports from different laboratories have analysed the various facets of this phenomenon. It was found that PHA constantly produces leucoagglutination and that mitoses occur only within the clumps of white blood cells[3]. An increase in both DNA[4–7] and RNA[6–8] synthesis was demonstrated. In addition, the greater content of cytoplasmic ribosomes seen in electron micrographs indicated active protein formation[9,10]. The newly synthesized protein was identified as a γ-globulin[11]. Similarly, after PHA stimulation, the cells had increased pyroninophilia[12], acid phosphatase activity[13] and affinity for periodic acid–Schiff stains (PAS)[14].

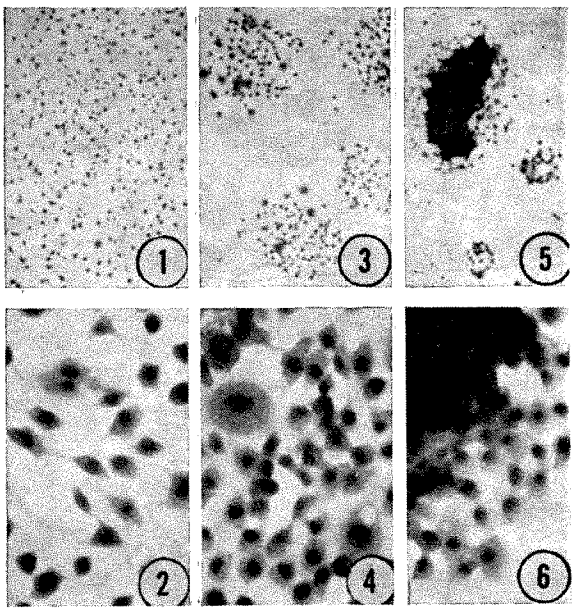
The final result of these cellular alterations is the appearance of a different cell, larger in size, more basophilic, nucleolated and capable of mitosis having the features of a lymphoblast[10,14]. This unusual cellular transformation was interpreted in general as a response to non-specific stimulation produced by PHA[11] and as positive proof for the potentiality of lymphocytes to transform, rejuvenate and divide[15].

* Recipient of Career Scientist Award of the Health Research Council of the City of New York.

No data are available as to what effect, if any, PHA may have on other types of cells in culture.

To investigate the possibility of an effect, PHA, of both types *M* and *P*, with a control of simple diluent, were added to matched subcultures of different cell types and the morphological alterations induced were studied. The types of cultured cells used are shown in Table 1. All were serial, long-term cultures, grown in Petri dishes with a constant flow of 5 per cent carbon dioxide in air, and regularly subcultured. 'Bacto-phytohaemagglutinin *M*' (code 0528, Difco Laboratory, Detroit, Michigan) was used in a concentration of 0·025 ml. per ml. medium. 'Bacto-phytohaemagglutinin *P*' (code 3110, Difco), 0·006 ml. per ml. medium, and 'Bacto-haemagglutination Buffer' (Difco), the reconstituent of lyophilized PHA, 0·025 ml. per ml. medium, were also used. PHA was added to the cultures at different times and observations were made after periods of 24–96 h, with the phase contrast microscope and on preparations fixed and stained with haematoxylin and eosin, Giemsa and PAS.

When PHA was added to cells in suspension immediately after seeding the plates, an apparent and constant agglutination occurred in all types of cultures. PHA-*P* had a more intense and rapid effect than PHA-*M*, inducing formation of larger and denser clumps (Figs. 4, 6). In contrast to control cultures where cells sedimented and attached to the glass in the following hours, in the

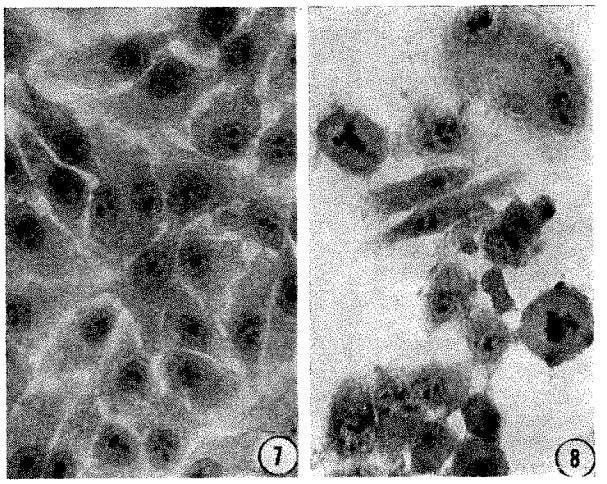


Figs. 1–6. Culture L_{929} with PHA added initially and observed 24 h later. 1, Control culture. Haemagglutination buffer added (× 100); 2, the same (× 360); 3, PHA-*M* added (× 100); 4, the same (× 360); 5, PHA-*P* added (× 100); 6, the same (× 360)

PHA-*P* cultures the cells did not attach and at 48 h remained in clumps floating in the supernatant. However, the agglutinated cells were viable and after 3–4 days formed an incipient monolayer at a time when control cultures had already died, if they had not been subcultured.

The cellular morphology was also altered and, similarly, more intense with PHA-*P* than PHA-*M*. At 24 h the cells in control cultures spread out and displayed characteristic shapes (Figs. 1, 2), whereas in the cultures to which PHA was added all cells were rounded, smaller and more basophilic. In the PHA-*M* cultures the cells remained rounded for a variable time even when attached to the glass, and gradually regained their characteristic features (Figs. 3, 4). The cells in the PHA-*P* cultures remained morphologically altered (Figs. 5, 6).

If PHA was added 8 h later, when most cells were already attached to the glass, and the cultures observed 24 h later, a change in growth pattern was noted. The PHA-treated cultures displayed separate colonies that differed from the uniform monolayers of cells that were formed in the control cultures. These colonies in the PHA-*M* cultures were formed by loosely connected, spread-out cells. In the PHA-*P* cultures the colonies were smaller, formed by closely packed cells that often were piled up in multi-layers. As a result of cytoplasmic condensation the cells were rounded, smaller in size and more basophilic (Figs. 7, 8).



Figs. 7 and 8. Culture D_{98} with PHA added at 8 h and observed 48 h later. 7, Control culture. Haemagglutination buffer added (× 400); 8, PHA-*P* added (× 400)

In a third set of experiments, PHA was added after 24 h when most of the control cultures displayed an organized monolayer. However, even at this time, when PHA was added the growth pattern, as well as the cellular morphology, was altered. The cells, particularly in the PHA-*P* cultures, appeared to be re-arranged in separate or coalescent islands with multilayered areas (Figs. 11–13). A great number of cells in the PHA-treated cultures had lost their characteristic shape, were rounded, with a narrow rim of cytoplasm and more intensely stained. Many cells displayed a granular nucleoplasm and a characteristic chromatin structure. Mitoses in different stages were frequent (Figs. 9–13) and the mitotic indices were higher in the PHA-treated cultures than in the controls (Table 2). PAS-positive material was significantly increased in PHA-treated *HEp* No. 2 cultures.

Table 1. CELL TYPES TESTED FOR PHA EFFECTS

Culture	Animal	Origin: Tissue	Growth pattern	Medium	Mode of subculture
L_{929}*	Mouse	Connective	Fibroblastic	MEM Eagle + 10 per cent calf serum	Trypsinization
HEp No. 2*	Human	Laryngeal carcinoma	Epithelial	,, ,, ,,	Trypsinization
*D98**	Human	Bone marrow	Epithelial-like	,, ,, ,,	,,
J_{111}*	Human	Monocytic leukaemia	Epithelial-like	,, ,, ,,	,,
LT_9†	Rat	Viral lymphoma	Rounded cells in suspension	Puck's + 15 per cent foetal bovine serum	Scrapping
LT_1†	Rat	Viral thymoma	Reticular cells attached + rounded cells in suspension	,, ,, ,,	Scrapping
NRS_2†	Rat	Normal spleen	,, ,, ,, ,,	,, ,, ,,	Scrapping
701 *BJ*†	Mouse	Plasmacytoma	,, ,, ,, ,,	,, ,, ,,	Scrapping

* Obtained from Microscopic Associates, Inc.
† From this laboratory.

Table 2. MITOTIC INDICES FOR ONE OF THE EXPERIMENTS*

Cell type	Time of exposure: Added to cultures 24 h: Diluent	24 h: PHA-*M*	24 h: PHA-*P*	48 h: Diluent	48 h: PHA-*M*	48 h: PHA-*P*	72 h: Diluent	72 h: PHA-*M*	72 h: PHA-*P*
L_{929}	6	9	9	3	6	5	1	2	1
HEp No. 2	4	13	11	9	10	21	8	11	15
D_{98}	12	26	17	10	31	22	3	3	9
J_{111}	0	22	15	6	20	24	8	9	19
LT_9	5	5	4	3	4	8	4	6	7

* Numbers indicate mitotic figures per 500 cells.

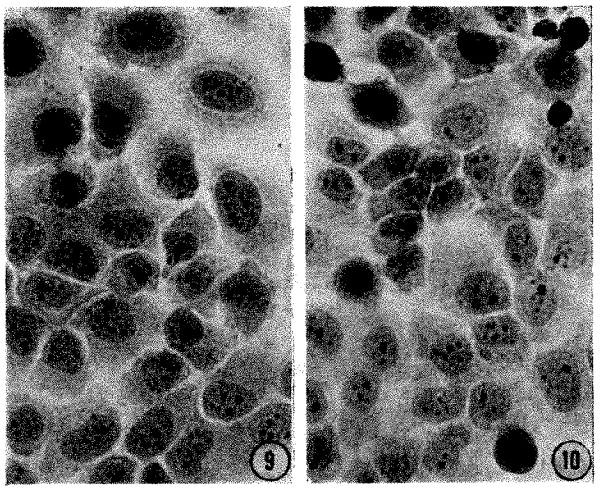
In the LT_9 cultures treated with PHA the changes were different. In contrast to the rounded floating lymphoblasts in control cultures, more cells were immobile and attached to the glass. They were spread out, larger and polyhedral. The cytoplasm appeared more abundant and less basophilic, the nuclei had a granular structure and mitoses were more frequent (Figs. 14, 15).

Specific cellular alterations are known to occur when PHA is added to human lymphocytes *in vitro*. The present experiments demonstrated that PHA induced comparable transformations in other types of cells. These cultures were originated from three different species, of normal and neoplastic, and of epithelial and mesenchymal tissues.

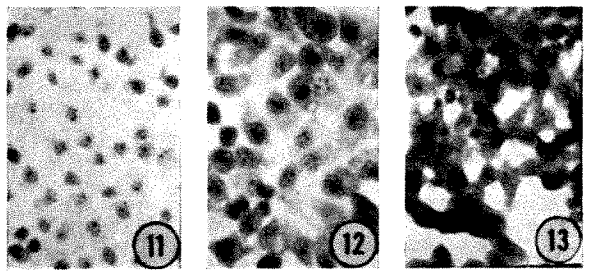
The mechanism by which PHA induces profound metabolic and morphologic changes in cells is not known and a number of explanations have been offered.

Because the effects of this substance were studied exclusively on lymphocytes, the prevalent interpretation is based on an immunological mechanism. According to this interpretation, PHA acts as an antigenic stimulus and elicits an immunologic response[16–18]. Similar alterations occur to lymphocytes from sensitized individuals with the addition to cultures of tuberculin[16], various vaccines[11,19], drugs[20] and by mixing lymphocytes from two unrelated persons[18]. However, while only 5–35 per cent of the lymphocytes in culture were stimulated by these specific antigens, PHA induced alterations in 95 per cent of the total number of cells[11].

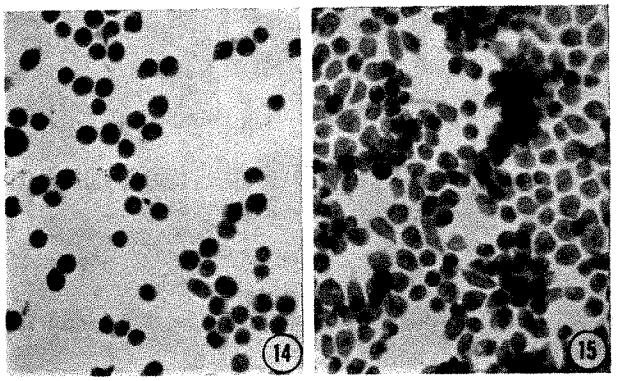
With the demonstration of PHA effects on a variety of cells, most of them unrelated to immunologic phenomena, a mode of action based on a presumed antigenic nature becomes more unlikely. The broad range of cell types affected, as well as the intensity of this effect, indicate



Figs. 9 and 10. Culture J_{111} with PHA added at 24 h and observed 24 h later. 9, Control culture. Haemagglutination buffer added (× 400); 10, PHA-*M* added (× 400)



Figs. 11–13. Culture *HEp* No. 2 with PHA added at 24 h and observed 48 h later. 11, Control culture. Haemagglutination buffer added (× 350); 12, PHA-*M* added (× 350); 13, PHA-*P* added (× 350)



Figs. 14 and 15. Culture LT_9 with PHA added at 24 h and observed 48 h later. 14, Control culture. Haemagglutination buffer added (× 400); 15, PHA-*M* added (× 400)

rather a non-specific nature of this stimulation. A capacity to act on the cell surface as previously suggested[6,11] could possibly explain some of the effects of PHA as cell agglutination and induction of mitoses. This would be in accord with the finding that the leucoagglutinogenic and mitogenic properties of PHA could not be separated by adsorption with leucocytes[21].

Human leukaemic leucocytes in cultures of peripheral blood were reported as non-responsive[22,23] or responsive in a much lower degree[24] to PHA than normal lymphocytes. In the present experiments leukaemic lymphoblasts of rat origin, obtained from long-term cultures, were sensitive to PHA and responded with marked morphologic alterations.

The transformation of lymphocytes under stimulation by PHA to lymphoblastoid forms was interpreted as a cellular activation under immunologic stimuli and used to support the idea of a potentiality of lymphocytes to transform to other types of cells. It seems that some conclusions regarding the rejuvenation of the lymphocyte as well as its metamorphosis to plasmocytes and other types of cells are still questionable.

The leucoagglutinogenic and mitogenic properties of PHA may prove useful as practical laboratory procedures. More work is necessary to understand its mode of action. This appears worth while, as the effects PHA has on cells seem to have implications bearing on some basic problems of cellular biology.

I thank Miss Marlene Sabbath for technical assistance.

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SITE OF LETHAL DAMAGE BY IONIZING RADIATION IN *Escherichia coli B/r* GROWING EXPONENTIALLY IN MINIMAL MEDIUM

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SOME 30 years ago it was shown that the ultra-violet action spectrum for lethal damage to bacteria coincided with the ultra-violet absorption spectrum of nucleic acids. Equally direct evidence that the principal sites of lethal damage by ionizing radiations are also the nucleic acids has not yet been found, although some experimental findings are consistent with this hypothesis[1–5].

Much of the early work on the action of ionizing radiations was centred around the ideas developed by Crowther[6], Lea[7], Zimmer[8], Timoféeff-Ressovsky[8] and others, and which became known as the 'target hypothesis'. Indeed, Lea suggested that lethal damage could be regarded as a particular type of genetic damage, namely a lethal mutation[7]. In recent years, however, it has become apparent that the sensitivity of bacteria to ionizing radiation is dependent on their physiological state before, during and after radiation. It has also been shown that there is more than one possible type of damage in *Escherichia coli* for example[9], and the presence or absence of enzymatic repair systems is known to make an important contribution to observed radiosensitivity in this species[5].

During these developments the target hypothesis has tended to fall into disrepute in bacterial radiobiology because of the view, perhaps mistaken, that observable changes at the genetic level are always induced with the same efficiency irrespective of the physiological state of the cell. There is, however, at least one bacterial system where target hypothesis may be applicable, namely *Escherichia coli B/r* growing exponentially in glucose-salts medium. Brownell[10] found that the survival curve for such cells was sigmoid with an extrapolation number[11] of 4·02. On the assumption that an organism which has two or more similar sensitive sites (targets) will survive if at least one site is undamaged after exposure, Atwood and Norman[12] had previously shown theoretically that the extrapolation number for a population of organisms each containing one, two, three or more equally sensitive targets was equal to the average number of targets per organism. When Brownell observed an average of 3·5 stainable nuclei per organism, he suggested that these nuclei were the radiobiological targets. Munson and Maclean[13] later investigated the survival curves for populations of *E. coli B/r* growing in minimal media in continuous culture and showed that their shapes were correlated with the average length of the bacteria over a wide range of lengths. They concluded that radiosensitive targets were distributed along the length of the organisms at average intervals of between 1 and 1·5μ, while the nuclear bodies shown by staining had an average spacing of 1·2μ in good agreement with the hypothesis that each nucleus contained about one radiosensitive target.

It is now known that each nucleus in an exponentially growing population at 37° C in minimal medium contains a continuously replicating chromosome in which each part of the genome may be represented either once or twice[14,15]. Thus, if lethal damage were genetic in origin it would be expected that the average number of targets per nucleus would lie between one and two; near one when the radiosensitive genes were replicated last and near two when they were replicated first. If all genes were equally sensitive the number of targets per nucleus would be 1/ln2 or 1·44. For genetic damage, a target would therefore be one unreplicated genome containing one copy of every gene which, by a loose analogy with the genetic material of higher organisms, we may call a 'chromatid'.

Besides the multi-target hypothesis of the inactivation of bacteria there are two other likely hypotheses for explaining extrapolation numbers greater than one[5]. They are: (1) the 'multi-hit' model in which n 'hits' anywhere within a hypothetical sensitive volume of the organism results in lethality; (2) the repair model in which enzymatic reactivation mechanisms reduce the slope of the survival curve at low doses but are inhibited or saturated at high doses. The multi-target hypothesis differs from these hypotheses in that it predicts that damaged targets (chromatids ?) will segregate from undamaged targets (chromatids ?) at cell division. This will result in an increase in the proportion of non-viable cells during the first generation time or so of growth after irradiation while the viable targets are segregating (Fig. 1).

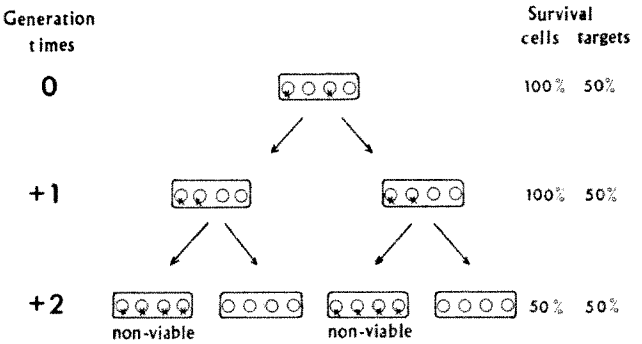


Fig. 1. Illustration of the segregation of viable from non-viable targets from an organism containing four targets of which two are inactivated at zero time. Once segregation is complete the ratio of the number of viable organisms to the number in a similar undamaged clone which has undergone the same number of divisions is equal to the proportion of viable targets at zero time

Segregation of organisms containing viable and non-viable targets. Fig. 2 shows the results of an experiment to test this prediction. A culture of *E. coli B/r* growing exponentially at 37° C in Davis minimal medium[16] was chilled to 0° C and separated into three parts—the average number of nuclei per bacterium at this time being 1·9. Part *A* was irradiated with various doses of cobalt-60 γ-radiation and viable counts on minimal agar plates at 37° C were performed to yield a survival curve. Part *B* was given a dose of 3,500 rads aerobically and together with part *C* (unirradiated) was returned to the 37° C water bath. Viable counts (on pre-warmed minimal agar plates), total counts (using a Hawksley 'Thoma' counting chamber), and nuclear counts (using the acridine orange fluorescence technique[17]) were made on both *B* and *C* at frequent intervals. It can be seen that total and viable counts rose exponentially from zero time in the unirradiated culture (*C*) with a doubling time of 44 min. In the irradiated culture (*B*), the proportion of non-viable organisms at zero time was very small as would be expected from the shape of the survival curve. The total number of organisms rose at about the same rate as in the unirradiated culture for at least one generation time. The

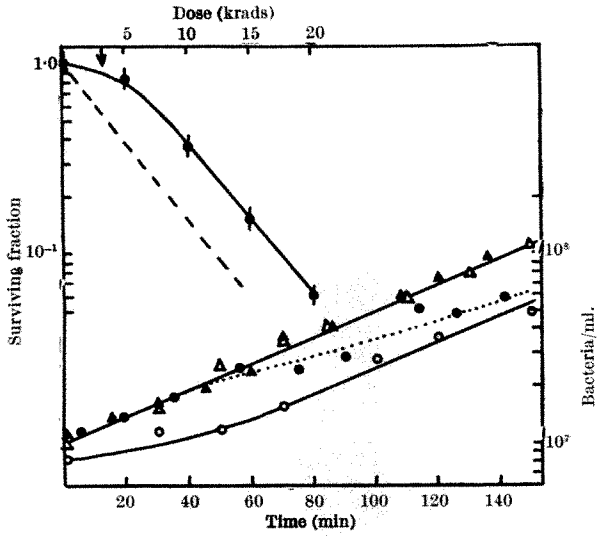


Fig. 2. Post-irradiation segregation of viable and non-viable bacteria (*Escherichia coli B/r*) growing at 37° C after irradiation under oxygen. Uppermost curve indicates survival for total population, broken line is theoretical survival curve for individual targets (chromatids ?). Arrow indicates dose of radiation given for the segregation experiment. Lower curves indicate rise with time of total unirradiated bacteria (▲), viable unirradiated bacteria (△), total irradiated bacteria (●) and viable irradiated bacteria (○). Dotted line is the expected count of total irradiated bacteria (see text)

number of viable cells, however, rose very slowly during the first generation time, indicating that many of the organisms resulting from the first division were non-viable. From between one and two generation times onwards the viable count in the irradiated population rose at the same rate as that in the control culture. This, together with the fact that the total counts in both cultures increased at the same rate during the first generation time, showed that no immediate measurable decrease in growth rate resulted from the dose of 3,500 rads. The total count in the irradiated population rose less steeply than that in the control population after about one generation time—the experimental points being well fitted by a curve (dotted line in Fig. 2) calculated on two assumptions: (*a*) that when a daughter bacterium containing no viable chromatids arises by cell division this daughter undergoes no further division; (*b*) bacteria containing at least one viable chromatid divide at intervals of 44 min.

On the multi-target model, the survival curve for the sensitive targets is a straight line through the origin parallel to the linear portion of the cellular survival curve (broken curve in Fig. 2) and indicates that 56 per cent of the targets should have been viable after a dose of 3,500 rads. When these bacteria had later divided and the undamaged targets had segregated, the viable count should have become equal to 56 per cent of that in a similar unirradiated population. Our observed figure was 54 per cent.

It is possible to perform a number of experiments like that shown in Fig. 2 simultaneously on a suspension divided into aliquots each of which is given a different dose of radiation. This, however, is complicated at all but the lowest doses by unknown irradiation products arising in the medium which have a rapid bactericidal action when the suspension is warmed to 37° C. This difficulty may be overcome by making a ten-fold dilution into fresh warm medium after irradiation. The results of such an experiment are shown in Fig. 3, where the observed surviving fractions before and after segregation are plotted against dose. The former are fitted by a curve computed on a multi-target model (solid line); the latter do not differ significantly from a line through the origin parallel to the limiting slope of the multi-target curve (broken line). The observed and theoretical survival curves for the targets are therefore seen to be in complete agreement.

Thus, *E. coli B/r* organisms growing exponentially in minimal medium appear to contain (on average) more than one target sensitive to ionizing radiation and these targets segregate into independent cells at division. Furthermore, as expression of lethal damage only occurs after cell division in most cases it can be provisionally regarded as recessive lethal damage.

Correlation of targets with chromatids. Earlier workers, as already mentioned, tried to correlate the number of targets with the number of stainable nuclei. In Brownell's experiments there were 1·15 targets per nucleus. In our experiment shown in Fig. 2, analysis by computer of the survival data showed that the extrapolation number of the linear portion of the curve of 'best fit' was 3·05, so there were $3{\cdot}05/1{\cdot}92 = 1{\cdot}59 \pm 0{\cdot}28$ targets per nucleus. Table 1 shows results from eleven recent experiments with four different lines of *E. coli B/r* in which surviving fractions and nuclear numbers were found simultaneously. In all but two experiments the number of targets per nucleus exceeded 1·0, and the average number was $1{\cdot}36 \pm 0{\cdot}15$.

Table 1

Strain	Experiment	Mean nuclear No., *A*	Extrapolation No., *B*	*B/A*	
E. coli B/r WP2 try⁻	1	1·54	2·62	1·70 ± 0·31	
E. coli B/r WP2 try⁻	2	1·70	1·92	1·13 ± 0·47	
E. coli B/r WP2 try⁻	3	1·53	1·72	1·16 ± 0·17	
E. coli B/r RRU 5 meth⁻	4	1·59	2·76	1·74 ± 0·27	
E. coli B/r RRU 14 leu⁻	5	1·60	2·11	1·32 ± 0·23	
E. coli B/r RRU 14 leu⁻	6	1·80	1·69	0·94 ± 0·13	1·36 ± 0·15
E. coli B/r RRU 14 leu⁻	7	2·12	3·93	1·86 ± 0·29	
E. coli B/r	8	2·05	1·62	0·79 ± 0·10	
E. coli B/r	9	1·66	2·29	1·38 ± 0·35	
E. coli B/r	10	2·79	3·71	1·33 ± 1·45	
E. coli B/r	11	1·92	3·05	1·59 ± 0·28	

Ratios of extrapolation number to mean nuclear number for logarithmic phase cultures at 37° C of various strains of *Escherichia coli B/r*. Individual errors of *A* and *B* were not available from the computer print-out but were used to compute the errors shown for *B/A*.

It therefore seems likely that there are more targets than nuclei per cell, in accordance with the hypothesis that inactivation of these cells by ionizing radiation is due to the inactivation of their genetic material and that the minimum amount of this which can organize a living cell (that is, a chromatid) is present on average about 1·4 times in any nucleus.

Survival curve for single mutant chromosomes. A further distinction between the multi-target hypothesis and the 'multi-hit' and 'repair' hypotheses can be made by investigating the survival both of unselected bacteria and of those bacteria which in the same exposure mutate at a particular gene locus. On the 'hit' and 'repair' hypotheses, the chance of survival of an induced mutant would be the same as that of a non-mutant, that is, the survival

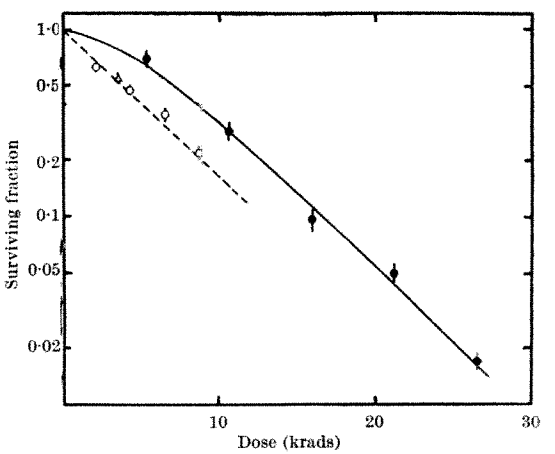


Fig. 3. Surviving fractions of *Escherichia coli B/r* with (○) and without (●) segregation following various doses of γ-radiation under oxygen. The solid line is fitted to the experimental points on a multi-target model. The broken line is the theoretical survival curve for individual targets (chromatids ?). The value after segregation calculated from the experiment of Fig. 2 is also shown (△)

curves of mutants and of the whole population would be identical. On the target hypothesis, the survival of the mutant would depend only on the survival of the single chromatid which contained the mutant gene or of one of the two mutant chromatids in the case of a chromosome in which the particular gene had not replicated at the time of irradiation. The survival curve of mutants should then show a small shoulder corresponding to an extrapolation number between one and two depending on the position of the gene in the replication order.

Survival curves for genetically 'labelled' (mutant) chromosomes of *E. coli* may be obtained by recording the number of mutants induced by various doses of radiation and plotting mutation frequency per nucleus per rad against the radiation dose. This method, which has been used before at low doses to determine by extrapolation the mutation rate under conditions of no killing[18], depends on the assumption that the induced mutation frequency is proportional to dose. Such survival curves have now been extended to higher doses for prototrophic revertants of the tryptophan-requiring strain *E. coli B/r WP2* and compared with those obtained concurrently for the whole population. The linear portions of the survival curves for mutants and for the whole population are parallel, confirming that the induction of mutations is linearly proportional to the dose under these conditions. The survival curve for unselected bacteria had an extrapolation number of 2·62 (average nuclear number 1·54) while the mutant fraction gave a curve with an extrapolation number of not more than two (Fig. 4).

Unfortunately the rather large errors involved in determining mutation frequency and survival at low doses prevented a convincing demonstration of a real difference in the shape of the two curves. Such a difference in shape, if real, could be more readily seen if it were possible to increase the number of chromatids per bacterium. This should have the effect of increasing the extrapolation number for the population as a whole, without affecting that for the mutant fraction. Unfortunately, it has not been possible to increase the number of nuclei per cell and in this way achieve a higher extrapolation number with *E. coli WP2* at 37° C. An alternative approach was suggested by earlier work with this strain which had indicated that at 16° C a large proportion of nuclei contain chromosomes which have replicated but not separated[18,19]. They would, therefore, be in a state analogous to *G2* in higher organisms, each

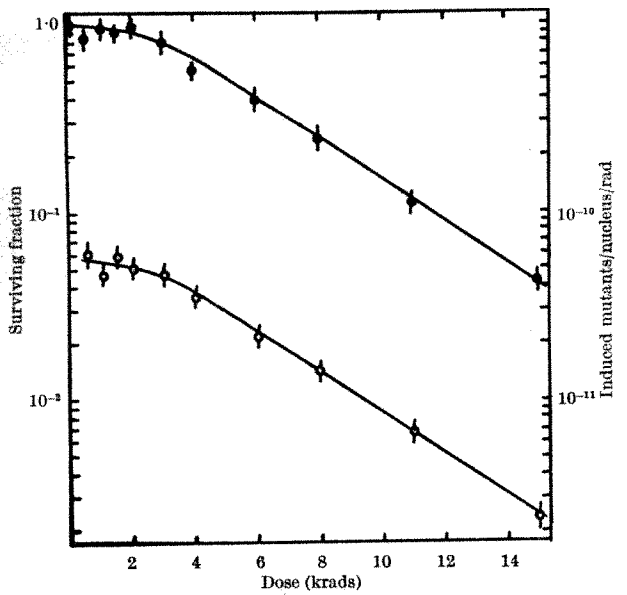


Fig. 4. X-ray survival curves under oxygen for unselected bacteria (●) and newly induced prototrophic revertants (○) of *Escherichia coli B/r WP2 try*⁻ grown at 37° C

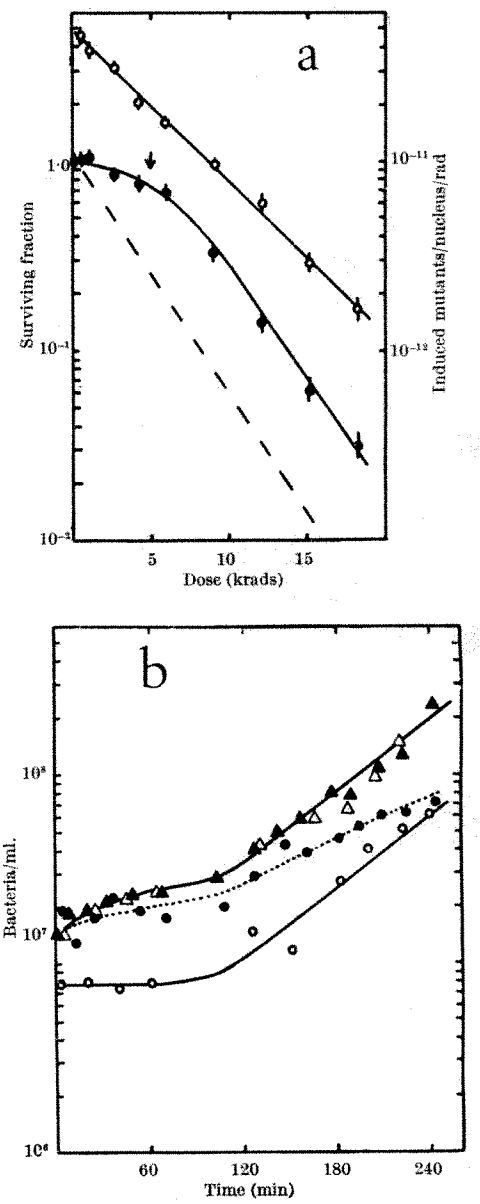


Fig. 5. *a*, Survival curves (γ-radiation under oxygen) for the total population of bacteria (●) and newly induced revertants (○) of *Escherichia coli B/r WP2* grown before irradiation at 16° C; broken line is theoretical survival curve for individual targets (chromatids ?). *b*, Segregation at 37° C of viable and non-viable bacteria (*Escherichia coli B/r WP2*) grown before irradiation at 16° C. Arrow in (*a*) indicates dose of radiation given at zero time. Curves indicate rise with time of total unirradiated bacteria (▲), viable unirradiated bacteria (△), total irradiated bacteria (●, dotted curve, see text) and viable irradiated bacteria (○)

chromosome consisting of two complete chromatids. If this were so, the survival curve for the whole population should have an extrapolation number of nearly twice the nuclear number while that for the mutants should be close to unity. There should also be a greater delay in the segregation of non-viable cells.

These predictions have been verified experimentally (Fig. 5*a*). A population grown at 16° C with an average of 1·82 nuclei per bacterium gave a survival curve with an extrapolation number of 5·00, while the curve for the mutant fraction was exponential. The number of targets per nucleus in this experiment was $5{\cdot}00/1{\cdot}82 = 2{\cdot}75 \pm 0{\cdot}54$. This value may be compared with the number of mutable units of the tryptophan gene per nucleus in a comparable population by two different methods, namely 2·06 and 2·23 (refs. 18 and 19). If targets are to be identified with chromatids and mutable units with single genes on chromatids, numbers per nucleus greater than two may

indicate that some nuclei go through a second replication cycle before separation of the chromatids when bacteria are grown at 16° C.

The rather surprising fact that the linear portions of the two survival curves are not strictly parallel could mean either that mutants (revertants) are more resistant to radiation than the non-mutants (auxotrophs) or that mutation induction is not a linear function of dose for bacteria grown under these rather special conditions.

Total and viable counts after irradiation at 0° C (Fig. 5*b*) show that in these experiments there was a delay in achieving the full growth rate appropriate to 37° C when samples were inoculated into fresh medium at 37° C. Since this delay occurred with irradiated and unirradiated populations and could be reduced by a slower temperature rise between 0° C and 37° C, it was probably a result of temperature shock.

Comparison of the viable counts for the irradiated and unirradiated populations shows that after nuclear segregation the viability of the irradiated population was 29 per cent compared with the value 24 per cent expected from the survival curve. This is consistent with the segregation of undamaged targets or chromatids in bacteria each containing four or five. As with 37° C-grown bacteria, the observed increase in the total count of the irradiated population can be entirely attributed to division of bacteria containing viable chromatids.

In conclusion, we have demonstrated that the inactivation by ionizing radiation of *E. coli B/r* growing exponentially in minimal medium is due to the inactivation of discrete units (targets) within the organism, which segregate from one another at cell division. A bacterium which possesses only inactivated targets is unable to undergo further division. Evidence has been presented that the target is the bacterial chromatid which is itself inactivated with 'one-hit' kinetics. The inactivating lesion must therefore prevent both strands of the DNA from passing on 'viable' information during replication. A similar effectively 'double-stranded' event has also been observed with mutational damage[18,19]. Under the conditions of growth and irradiation used in our experiments the radiosensitivity of *E. coli B/r* is probably at its greatest. Obviously under conditions where radiosensitivity is less, there must be some mechanism for the prevention or repair of lethal chromosomal damage. Under such conditions it is quite conceivable that non-chromosomal damage may make a significant contribution to the observed lethal effect.

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EMPEROR PENGUIN COLONY AT BEAUFORT ISLAND, ROSS SEA, ANTARCTICA

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THE existence of a colony of Emperor Penguins (*Aptenodytes forsteri*) at Beaufort Island was first noted by Harrington[1]. His report was based on a letter from Mr. S. O. Wilson of the U.S. Antarctic Research Programme, who saw the colony from a passing ship on November 5, 1956. The colony was reported to occupy part of a 25-ft. bench on the north side of the island; no further details were given by Harrington.

Beaufort Island (76° 56′ S., 67° 03′ E.) is a small ice-capped island off the mouth of McMurdo Sound in the south-western corner of Ross Sea (Fig. 1). Often beset by pack ice in summer, it is difficult to approach by sea and received little attention from early explorers. A current of up to three knots setting westward between the island and Cape Bird helps to ensure early break-up of fast ice each spring. By mid-November a polynia some 20–30 miles across is usually found to the west of Beaufort Island and Cape Bird, extending southward into McMurdo Sound as far as Cape Royds. There are large colonies of Adelie Penguins (*Pygoscelis adelie*) at Beaufort Island, Cape Bird and Cape Crozier, the three together accommodating nearly half a million birds. A small colony of Emperor Penguins, at present numbering some 1,500 pairs[2], breeds each year on the sea ice below Cape Crozier.

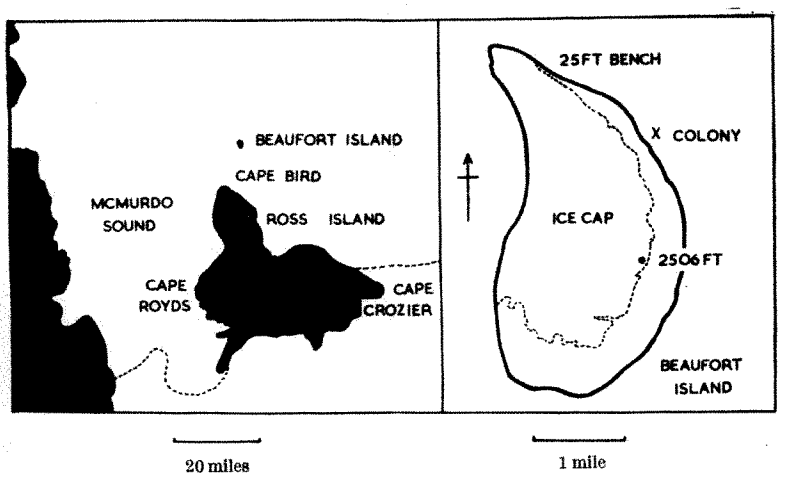


Fig. 1

The Beaufort Island Emperors were first examined and photographed on December 6, 1962, from a U.S. naval aircraft (Fig. 2). The size and situation of the colony were determined, and similar flying visits were made on November 26, 1963, and November 19, 1964, for annual comparisons. This work is part of a long-term conservation investigation sponsored by the University of Canterbury Antarctic Biology Unit.

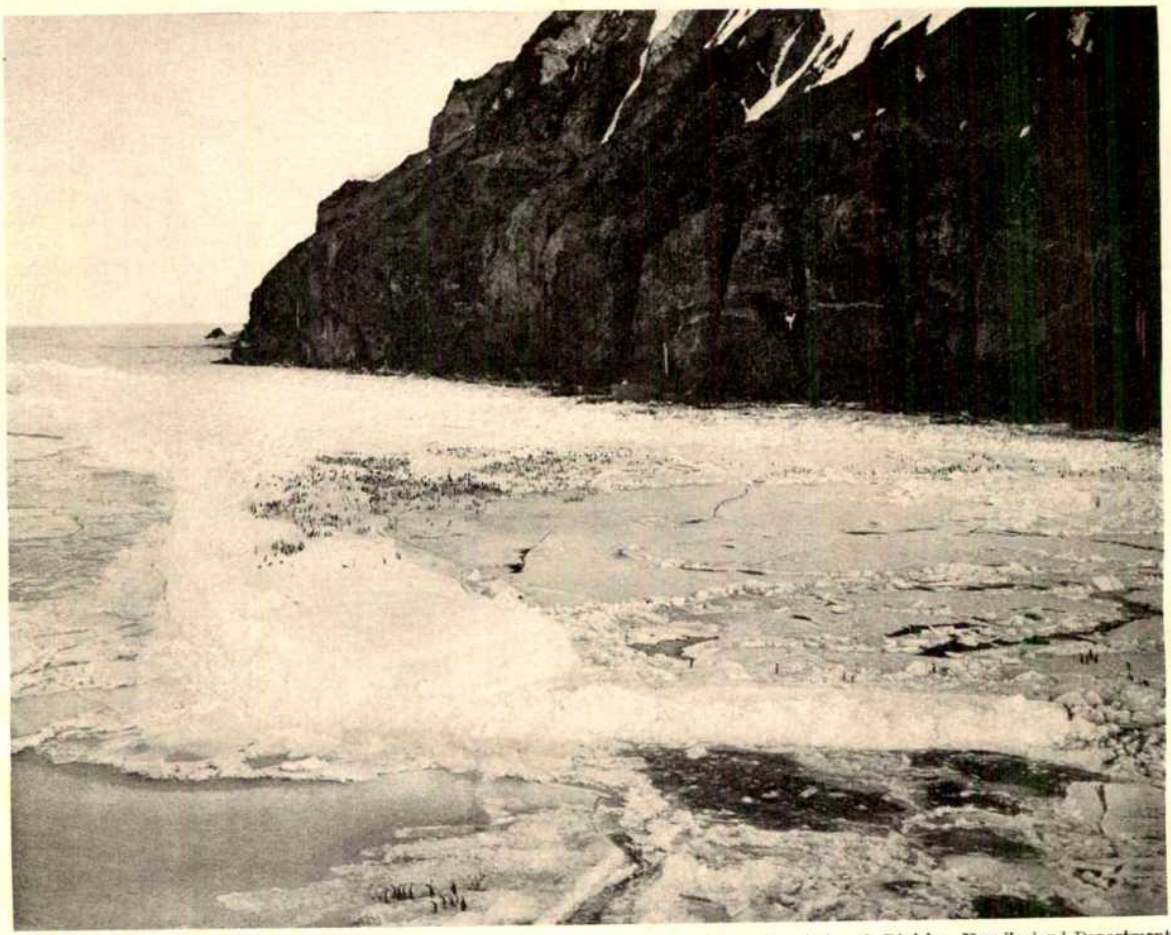


Fig. 2. Emperor Penguin Colony, Beaufort Island, December 6, 1962. (Photo by Guy Mannering, Antarctic Division, New Zealand Department of Scientific and Industrial Research)

The colony has been found each season on sea ice at the foot of a steep, ice-capped cliff off the north-eastern corner of the island, 0·75 mile south and east of the 25-ft. bench. The birds congregate on a narrow platform of winter ice, 100–200 yd. wide, fringing the cliff at this point. Open sea is usually close at hand; in 1962 a quarter-mile of rafted, newly formed ice separated the colony from several square miles of open water, and in 1963 and 1964 smaller pools, in which adults were swimming, lay within a hundred yards of the main group of birds. Extensive open water dotted with floes and frozen patches spread to south and west in all three seasons.

A line of brash ice at the edge of the platform in 1962 and 1964 gave evidence of very recent wave action within a few yards of where the birds were standing. The surface of the platform is usually rough, with many cracks and upheavals, as though subject to pressure and distortion. Frozen-in fragments of berg give protection to the colony; in 1964 a small tabular berg was present nearby, and guano-stains showed that the colony had spent some time in its lee. The roughness of the platform would screen the birds from ships passing more than a few hundred yards from the island.

Adults and chicks were usually assembled in three or four loose groups over an area of a few hundred square yards; the groups were always centred under the same point of cliff, with several large guano-stained areas visible on either side of them. Beaufort Island is a prominent landmark, and birds approaching from any direction would have little difficulty in finding the traditional colony site. Sastrugi suggests that local winds blow strongly along the cliff base, but some shelter would be afforded at ground level by hummocks and depressions in the ice platform.

Close examination from the air showed no evidence that the birds had ever occupied the 25-ft. bench, as the original discoverer of the colony suggested. However, this species is noted for its absence of nest sites and tendency to wander during incubation and chick rearing, and it would not be entirely uncharacteristic if incubation began on land and chick rearing were completed on the sea ice. Flights earlier in the season would help to determine this point. The fringing platform of winter ice, although more stable than that further out to sea, is clearly subject to disturbance during its formation, and might be unsuitable for courtship and laying in May and June.

Photographs taken in 1962 showed 1,069 chicks and 707 adults present in the colony; these figures were means from independent counts by two observers using binocular microscope under varying conditions of light and magnification, and were likely to be accurate within ± 5 per cent. The 1963 and 1964 photographs suggest very similar colony sizes, possibly within 10 per cent of the 1962 figures. Thus the colony is likely to have a breeding population of some 1,200–1,500 pairs, equivalent to or perhaps slightly smaller than that of Cape Crozier 5 miles to the south-east.

I thank Guy Mannering, whose excellent photograph made this work possible, and the Research Committee of the New Zealand University Grants Committee for financial support. The field assistance of *VX* 6 Squadron U.S. Navy, and the good offices of U.S.A.R.P. and Antarctic Division, New Zealand Department of Scientific and Industrial Research, are also acknowledged with thanks. Miss Felicity Cutten helped in checking photo counts and illustrated this paper.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Strength of Cosmic Radio Sources

THE discovery of quasi-stellar objects has brought with it the realization that bremsstrahlung radiation in the radio wave region as generated in a plasma has not been properly understood. The present communication represents an effort to account for the unusually high levels of radio emission from quasars and to explain, in particular, the spectral output from the sub-structures A and B of $3C$-273.

It was suggested earlier[1] that the intense radio-wave emission from quasi-stellar bodies might be explained in terms of radiation from tenuous plasmas. Subsequent calculations[2] based on a stimulated bremsstrahlung mechanism in optically thin plasmas revealed that for $3C$-273 B a self-consistent model confirming the Hubble law deduced location of the source (in the megaparsecs range) could be obtained. Indeed, the flat spectral power output in the neighbourhood of 10^3 Mc/s was reproduced.

The problem remained, however, to account for the power spectrum of $3C$-273 A, the spectral index of which $(-0{\cdot}9)$ had prompted speculation[3] that a synchrotron mechanism was indicated, although it was rather doubtful that sufficiently adequate magnetic fields were present; in point of fact, there was no evidence of strongly polarized radio waves from this portion of $3C$-273.

The following rational explanation is offered as a unified theory not only for parts A and B of $3C$-273 and quasars generally, but possibly for radio galaxies as well. Suppose the number of ions and electrons in a fixed volume be augmented. The power will at first grow as more and more free transition occurs; but soon the inverse bremsstrahlung will tend to cut down the radiative transfer to the surface of the plasma as the black-body limit is approached. In other words, a maximum output of bremsstrahlung radiation prevails at some intermediate plasma density. Presumably part B of $3C$-273 at $n = 10/\text{cm}^3$ lies close to this point with a small optical depth in effect, whereas part A at $n = 10^7/\text{cm}^3$ manifests a larger optical depth, so that self-absorption gives rise to a fall-off of radio power at higher frequencies.

Galaxies at still higher densities are further from the optimum region and are then characterized by varying degrees of power drop-off at high frequencies. Combining this aspect with the fact that quasi-stellar sources have lower temperatures (10^4 °K compared with 10^5 °K or greater), the enhancement factor m_0c^2/kT for the latter provides added cause for quasars to emit proportionately larger amounts of radio waves. In essence, then, the combination of stimulated emission and low opacity for a plasma can give rise to unexpectedly large quantities of emitted electromagnetic radiation, particularly in the longer wave-length region of the spectrum.

It is not surprising that very dense, hot plasmas are notoriously poor sources of steady radio emission. The Sun, for example, exhibits low levels of steady thermal radiation.

The effect of optimal density plasmas should be looked for in the laboratory and more systematically investigated. Theoretical effort is being directed toward characterizing the build-up of radiated power from a plasma as the carrier density runs the gamut from the low- to the high-density region.

This work was supported in part by a grant from the National Aeronautics and Space Administration and is based on a Sigma Xi address delivered at Fordham University on December 15, 1965.

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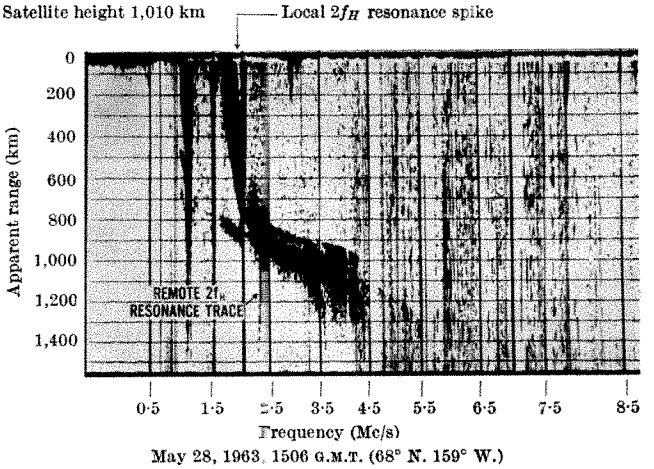
GEOPHYSICS

Remote Cyclotron Resonance Phenomenon observed by the *Alouette* Satellite

A NOVEL cyclotron resonance phenomenon associated with the second harmonic of the electron gyrofrequency $2f_H$ is sometimes observed on *Alouette* topside ionograms. The phenomenon occurs remote from the satellite and will consequently be called the 'remote resonance'. The cyclotron resonances which occur in the vicinity of the satellite, and which are responsible for the cyclotron spikes, have been discussed by previous authors[1,2].

A topside ionogram showing the trace produced by the remote resonance ('resonance trace') is presented in Fig. 1. The presence of substantial range-spreading of the echoes, commonly known as 'spread F', tends to obscure some of the features on this ionogram; for clarity, a somewhat idealized schematic diagram of this same ionogram is presented in Fig. 2. In Fig. 1 the lowest observed frequency of the resonance trace occurs at a delay time corresponding to an apparent range of about 1,450 km. At slightly higher frequencies it appears at rapidly decreasing range and subsequently joins the extraordinary wave trace. The frequency at which the resonance trace meets the extraordinary reflexion trace is defined as the 'cut-off frequency' f_c (see Fig. 2). The second harmonic cyclotron spike ($2f_H$ spike) caused by a local resonance at the satellite is also indicated on the ionogram.

Fig. 3 shows the real height of reflexion of the extraordinary wave (obtained from the ionogram of Fig. 1) plotted as a function of frequency. Also shown is the variation with height of $2f_H$ calculated from the Jensen



May 28, 1963, 1506 G.M.T. (68° N. 159° W.)

Fig. 1. Ionogram recorded by the *Alouette* topside sounder, illustrating the remote resonance trace

and Cain spherical harmonic coefficients[3]. The observed cut-off frequency obtained from the ionogram is found to occur where the extraordinary wave reflexion curve and the $2f_H$ curve intersect. The height at which these curves intersect is defined as h_r.

In Fig. 4, the observed cut-off frequencies f_c from eleven ionograms are plotted against the corresponding calculated values of $2f_H$ at height h_r. These heights (h_r) were obtained from electron number density-height profiles calculated from the ionograms and are indicated in brackets in Fig. 4. For equality between the observed cut-off frequencies and the calculated value of $2f_H$ at h_r the experimental points should fall on the straight line drawn at 45° to the axes of the graph. Uncertainties in the calculated values of $2f_H$, using the Jensen and Cain coefficients[3], are negligible compared with the uncertainties in scaling and real height reduction. The inner error bars indicate the accuracy to which the cut-off frequencies were scaled. The outer bars are an estimate of probable error due to uncertainties in the real height reductions because of spread F on the ionogram traces. Additional errors in the real height reductions due to ionospheric tilts and gradients are also possible. Fig. 4 demonstrates that

$$f_c = 2f_H(h_r) = f_x(h_r) \quad (1)$$

where $f_x(h_r)$ is the frequency of the extraordinary wave reflected at h_r. Since h_r is the extraordinary wave reflexion height for frequency $f_x(h_r)$, the Appleton–Hartree equation for the index of refraction in the ionosphere gives:

$$f_N^2(h_r) = f_x(h_r)\,[f_x(h_r) - f_H(h_r)] \quad (2)$$

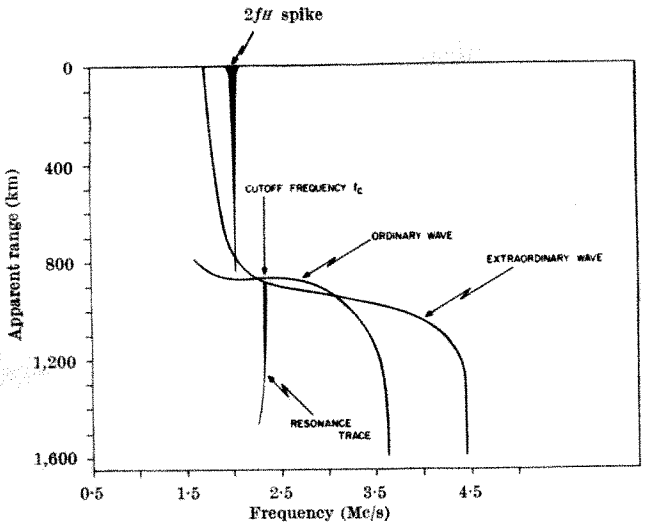


Fig. 2. Schematic diagram of ionogram in Fig. 1

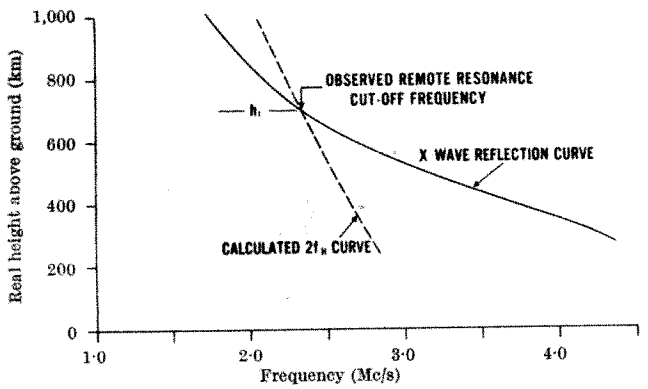


Fig. 3. The relationship of the observed remote resonance cut-off frequency (from the ionogram in Fig. 1) to twice the gyrofrequency and to the extraordinary wave reflexion height

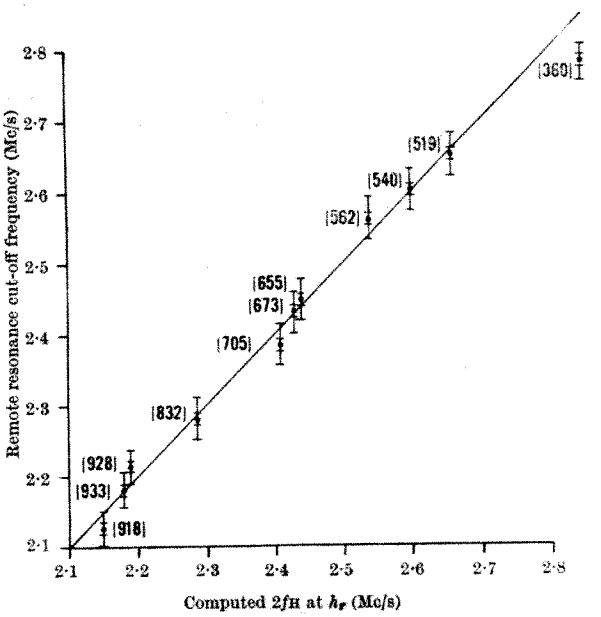


Fig. 4. Comparison of remote resonance cut-off frequencies obtained from ionograms, with computed values of $2f_H$ at height h_r. The numbers in brackets beside the data points are h_r values

where $f_N(h_r)$ is the plasma frequency at h_r. From equations (1) and (2):

$$f_N(h_r) = \sqrt{2}\,f_H(h_r) = \frac{f_c}{\sqrt{2}} \quad (3)$$

The variation of f_H with height is known quite accurately[3] so that h_r can be determined from f_c; from equation (3) the electron density at this height h_r can be obtained. A single measurement of frequency (f_c) determines both the real height and electron density. This method of obtaining the electron density at h_r is completely independent of the usual assumptions made in the calculation of electron number density as a function of height from ionograms.

The remote resonance trace is only observed on high-latitude ionograms which show moderate to extreme spread F and spread echoes at the height of the satellite[4]. About 4,000 high-latitude ionograms (recorded during the period May 2–11, 1963, at the Resolute Bay, North-West Territory, and College, Alaska, telemetry stations) were examined and resonance traces were observed on about 1,050 (26 per cent) of these ionograms. The resonance traces were observed most frequently on ionograms recorded above about 70° N. geomagnetic latitude, although on occasion they were observed down to about 50° N.

Remote resonance traces have not been observed to be associated with the third or higher harmonics of the cyclotron frequency.

Several thousand bottomside ionograms recorded at Baker Lake and Resolute Bay, and at Thule, Greenland, have also been examined; no resonance phenomena associated with twice the gyrofrequency were found on these.

In summary, an ionospheric cyclotron resonance is found to occur remote from the satellite at the height at which the extraordinary wave frequency is equal to $2f_H$. From the resonance cut-off frequency, the plasma frequency at a particular height in the ionosphere can be obtained. This technique is independent of the usual assumptions made in obtaining electron number density profiles from ionograms. The remote resonance trace is a common feature on high-latitude topside ionograms that show substantial spread F.

A more detailed discussion of the remote resonance trace is given by Muldrew and Hagg[5,6].

I thank Mr. D. B. Muldrew for useful discussions.

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Prediction of the Intensity of the Present Sunspot Cycle

King-Hele[1] predicted that the intensity of the present sunspot cycle (beginning about late 1964) may resemble the relatively high activity cycles with maxima in 1870 and 1949 and that the solar activity of the forthcoming maximum would reach the level which prevailed in late 1959. This prediction of a fairly high level of sunspot activity for the present cycle can be supported by circumstantial evidence from three periods A.D. 1100–1203, 1724–98 and 1914–64, which have remarkable similarities in various aspects of solar activity, and in terrestrial climate, glacial activity and tree growth.

The 1100–1203 period apparently had a mean sunspot cycle length of about 10·2 years, an interval less than the average cycle length and one which probably indicated a high level of sunspot activity[2]. The mean sunspot number (Wolf number of '*R*' *sensu* Waldmeier[3]) for the maximum years of the ten cycles from the period 1100–1203 was about 113 (including the maximum year of 1098, presumably the same maximum as the year 1100 in Bray[2]) as calculated from the estimates in Schove[4]. This high mean sunspot maximum number again suggests a fairly high cycle strength during the period.

The 1724–98 period had a cycle length of 10·7 years and a mean yearly sunspot number of 54. The lengthy interval following the sunspot maximum of the last cycle of this period somewhat increased its mean cycle length[2]. The mean sunspot number for the maximum years of the seven cycles from the period 1724–98 was about 117 calculated from the estimates in Schove[4] and 113 from the measurements in Waldmeier[3].

The mean cycle length for the 1914–64 period was 10·2 years and the mean yearly sunspot number was 62. The mean sunspot number for the maximum years of the five cycles from the period 1914–64 was about 116 calculated from the estimates in Schove[4] and 127 from the measurements in Waldmeier[3].

The foregoing results show that the three outlined periods had a relatively high mean yearly sunspot activity and a moderately high to high activity for the cycle maximum. A comparison of these periods with other sequences of high sunspot activity in Schove indicates that the years 1100–1203, 1724–98 and 1914–64 were among the most active of the past millennium. Further evidence of high solar activity in the first two periods (no reliable data for 1914–64) is their below-normal level of carbon-14 activity (1100–1203 = −0·5 per cent; 1724–98 = −0·1 per cent) (ref. 5). Such lower carbon-14 activity may be an indication of higher than average sunspot activity[6,7].

All three of the outlined periods were intervals of warm and apparently comparable terrestrial temperature[6–13] and represent maximum temperature intervals during the past millennium[7,10,14]. These periods were also intervals of agricultural expansion into cooler areas[10,14] and recession or stagnation in volume of glacial and sea ice[2,10,13–19]. During the last two periods, tree growth over the past four centuries was at a maximum in the Canadian Rocky Mountains[2] and in the polar Ural Mountains[20]. If there tend to be warmer terrestrial temperatures and glacial recession during periods of high solar activity[2], then these observations are additional evidence for a relatively high solar activity during the three indicated periods.

If the length of the present high sunspot activity period, which began in 1914, is analogous to the length of the high activity periods of 1100–1203 and 1724–98 (that is, 7–10 cycles), it is conceivable that the present interval of high activity may extend for seven or more cycles. If this occurs and if the present high-activity period can be dated as beginning with the 1914 cycle, then the present cycle which began in 1964 should be of moderately high to high activity. The similarity in solar activity and in terrestrial climate, glacial activity, and tree growth between the 1724–98 and 1914–64 periods suggests that future sunspot cycles of the present period may be similar in pattern to the latter part of the eighteenth century. Mean yearly sunspot number per cycle for 1914–64 has shown the same general tendency to increase with each succeeding cycle as occurred in 1724–98 (Table 1, calculated from Waldmeier[3]). If this tendency continues, the present cycle may be of similar magnitude to the record cycle 1954–64 and may conceivably exceed it in mean yearly sunspot number.

Table 1. Aspects of Sunspot Activity for the Periods 1700–1798 and 1890–1964

Sunspot cycle	Mean yearly sunspot No.	Sunspot cycle	Mean yearly sunspot No.
1700–1711	18	1890–1901	38
1712–1723	28	1902–1913	31
1724–1733	54	1914–1923	45
1734–1744	52	1924–1933	41
1745–1755	40	1934–1944	55
1756–1766	43	1945–1954	75
1767–1775	59	1955–1964	96
1776–1784	69		
1785–1798	60		

The high sunspot activity from the period 1914–64 has been identical in sunspot cycle length (10·2 y) to that estimated for the ten cycles of the twelfth century[2]. The climate and glacial activity for these two periods also correspond. This again suggests that the present period of high sunspot activity may continue for several more cycles. Bray and Struik[17] noted the possibility of the present level of relatively high sunspot activity extending to near the close of the twentieth century.

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Slow-speed Tape Recordings of Seismic Signals

Seismic interpretation at the University of Tasmania has been enhanced by using very-low-speed magnetic tape recordings in conjunction with the normal pen recordings. When played back with a time compression of 250, the outputs from the short period 'Willmore' seismometers become audible. By comparing the tape and

the pen recordings for earthquakes easily identifiable on the pen recordings it has been found, once the various tones are memorized, that it is not only possible to hear seismic activity which is lost in background noise on the pen records, but also to tell approximately the region from which they originated. Since a 2,400 ft. reel of 0·25 in. magnetic tape lasts approximately 3 months if used continuously, this slow-speed recording also affords an economical way of permanently storing the records in an electrical form.

Recording is achieved by passing analogue signals from the Tasmanian telemetered seismic net[1] by way of low-pass filters directly on to the heads of a commercial stereo tape recorder. A recording speed of 0·00375 in./sec, which allows signals in the frequency range 0·1–10 Hz to be recorded with a dynamic range of approximately 40 dB, has been achieved by driving the capstan flywheel with a hinged motor and a rubber driving pulley.

To enable four channels to be recorded at once, the erase head has been replaced by another record head. By means of a six-position switch any two channels may be reproduced in stereo. Timing is achieved by recording speech at normal tape speeds once per day to give the date and time, while hour marks are automatically recorded by saturating the tape for approximately 15 sec on each hour.

Each day the previous day's recordings are listened to at a tape speed of $\frac{15}{16}$ in./sec, so that one hour is analysed in fourteen seconds. Any seismic activity can thus be pinpointed to within two minutes, making a detailed examination of the pen recordings for the relevant time interval feasible.

Table 1, which covers the period, April 24–May 16, 1965, a period of twenty-three days, shows the effectiveness of this method of recording:

Table 1

No. of earthquakes found on tape	88
No. of earthquakes found on pen recorder	59
No. of earthquakes which would otherwise have been missed	14

The discrepancy between the tape and pen recordings (that is 29 events) are events which are indistinguishable from background noise on the pens.

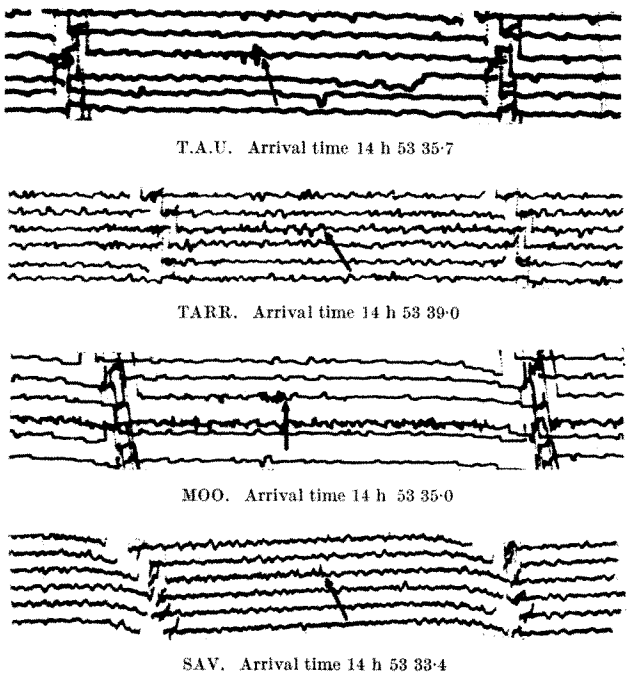


Fig. 1. Pen recordings of an earthquake which occurred south of Fiji Island, lat. 24·1 S., long. 179·5 W., on April 27, 1965. Pen travel is from right on a 1-h per revolution drum recorder. The vertical pen deflexions are minute marks

An example of an event which was first found by listening to the tape and afterwards located on the pen recording is shown in Fig. 1. The reality of this event is shown by the fact that it was recorded on all four stations and its occurrence has also been listed by the U.S. Coast and Geodetic Survey.

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PHYSICS

Spherically Symmetric Motions in General Relativity

I HAVE recently obtained certain exact solutions of Einstein's equations of general relativity which, so far as I can trace, have not been noticed before. They refer to a spherically symmetric mass of material in radial motion which may either be collapsing on to the central point (gravitational collapse) or be exploding from that point (spherical blast). The solutions are determined from three general conditions: (1) from a certain pre-assigned form for the coefficients of the metric; (2) from the existence of co-moving co-ordinates; (3) from the assumption that the stress is spatially isotropic. The results have analogies with the spherical blast waves of classical gas-dynamics[1], but with the additional feature that the gravitational self-attraction of the material is a controlling element of the motion. A by-product of the investigation is the discovery of the class of solutions of Einstein's equations to which belongs a particular case[2] obtained in 1933 by a different method.

The form for the coefficients of the metric is indicated by the formula:

$$ds^2 = y^2 dt^2 - R_0^2 S^2 \{e^{\eta}\, dr^2 + e^{\zeta} f^2 (d\theta^2 + \sin^2\theta\, d\varphi^2)\}/c^2 \qquad (2)$$

where R_0, c are constants, $f(r)$ and $S(t)$ are functions of r and t, respectively, and y, η, ζ are functions of a variable z, which is defined by $z = \ln\{Q(r)/S(t)\}$, Q being a function of r. It then follows from Einstein's equations with zero cosmical constant, namely:

$$R^{ij} - \frac{1}{2} g^{ij} R = -(8\pi G/c^2)\, T^{ij} \qquad (2)$$

that co-moving co-ordinates exist if

$$\zeta = \eta,\ y = 1 - \frac{1}{2}\frac{d\eta}{dz}.$$

Physically this means that the material described by the energy-tensor T^{ij} has a uni-directional motion at each point of space and at each instant of time. The material is moving radially either inwards or outwards. It then also follows from equation (2) that the energy-tensor reduces to its four diagonal components: one of these is the generalized density; the other three components describe the stress. Of these the radial component is different from the two cross-radial components which, however, are equal to one another. The assumption of an isotropic stress, describable by a single pressure-function, yields three ordinary differential equations of the second order which involve two arbitrary constants a and b. Two of the equations determine f and Q in terms of elementary functions of r; the third equation is:

$$\frac{d^2y}{dz^2} + (a - 3 + y)\frac{dy}{dz} + y\,(b + a - 2 - (a - 3)y - y^2) = 0. \qquad (4)$$

It cannot, in general, be integrated in terms of elementary functions and resort must be had to numerical computa-

tions. But if certain relations exist between the values of a and b, particular integrals, involving one constant of integration only, can be found. In addition, it proves to be the case that the result discovered in 1933 arises if $a = 3$ and $b = 0$.

The density and pressure can be expressed in terms of the known functions f, Q, y and η through the appropriate Einstein equations (2). However, the as yet undetermined function $S(t)$, and its first and second derivatives, also occur in the expressions for the density and the pressure. The function can be found by imposing an additional requirement, for example, a boundary condition. Thus if there is a vacuum outside the spherical mass, the pressure must be zero at the outer boundary. The function S can thus be found and it involves, among other constants, the boundary value of the co-moving co-ordinate r. It is, of course, the case that S determines the speed of the motion, the time to reach a given size, and other similar properties. Speeds up to the local velocity of light are possible.

The detailed analysis of special cases based on the particular integrals of equation (4), and which correspond to collapsing configurations, shows that a wide variety of motions is possible. In general, there are pressure and density gradients within the material. In some cases, a spherical surface on which the pressure is infinite moves outwards through the material[3]—rather like a shock wave. In other cases, singularities of this kind are absent. The effective times of collapse tend, on the whole, to be short—by astronomical standards at least. The exploding configurations can be dealt with in a similar manner.

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On the Theory of Layer Structures

Recently, Shinada and Sugano[1] and also Ralph[2] have published theoretical calculations on the optical absorption in layer structures. These calculations are based on a two-dimensional effective mass approximation and the implications are that conclusions drawn from such an approach apply to the actual physical situation met with in layer structures. In our opinion, this view is at variance with the basic principles of quantum mechanics. Indeed, any two-dimensional representation fails to satisfy the requirements of completeness necessary to describe the states of a physical system and, therefore, is liable to miss physically significant facts. Although the electron wave-functions in layer structures may often be represented with sufficient accuracy as products:

$$\varphi(x,y,z) = \chi(x,y)\zeta(z)$$

of two separate components[3,4], this does not imply that the two-dimensional component $\chi(x,y)$ alone covers the whole situation. The proper representation of the system involves both components and they must appear explicitly in the definition of any physical parameter. In particular, we note that the optical properties of layer structures are subject to selection rules which depend on the mutual orthogonality properties of the two components, and which cannot, *a priori*, be ignored. This example illustrates that it is necessary to make a careful assessment of the physical significance of two-dimensional approximations as applied to layer structures.

The implications of the ideas outlined here will be discussed in some detail in a forthcoming paper[5]. Here we add that the discrepancy noted by Ralph between his results and those of Shinada and Sugano is due to Ralph's omission of the energy dependence appearing in the matrix elements of the dipole interaction. Although permissible in isotropic crystals, this omission is no longer justifiable in layer structures.

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Current Transients in Potassium Chloride

A preliminary investigation has been carried out of current transients in potassium chloride at 150° C. The specimens used were single crystals of AnalaR grade, supplied by Hilger and Watts, Ltd.—the slab size being about 2·5 cm square and 0·4 cm thick. Into each slab a flat-bottomed cylindrical recess of 0·5 cm diameter was drilled and the thickness reduced by polishing on a Selvyt cloth moistened with water. Final thicknesses of specimens lay in the range 0·01–0·02 cm. Silver paint was used as electrodes, and the specimen and its spring-loaded contacts were enclosed in a well-insulated thermostatically controlled chamber in which the temperature was maintained at 150° ± 1° C. The pulse generator used provided linearly rising voltage transients of variable rise time and amplitude. The displacement current to the crystal was balanced by adjusting a variable air condenser in parallel with the specimen and feeding the output voltages from the measuring resistor in series with the crystal and from the balancing resistor in series with the condenser to the differential amplifier of a Tektronix type 502*A* oscilloscope. The following is a summary of our observations.

(1) Fig. 1 *A* (lower traces) shows the current in a potassium chloride specimen resulting from the application of two voltage pulses having the form indicated in the upper trace. The second pulse was applied about 2 sec after the first (both are superimposed on the photograph). Before the first pulse was applied, the specimen had not been subjected to high voltage. It can be seen that the magnitude of the first current pulse considerably exceeds the second. At a particular value of the field the current departs from a linear field dependence, increases steeply and then decays.

(2) The second and further successive pulses decrease the magnitude of the current and the position of the decreased current maximum shifts to higher voltages.

(3) The initial high current pulse cannot be repeated by either short-circuiting the specimen after application of voltage or by interchanging the connexions to the electrodes so as to apply the same magnitude pulse in reversed polarity. Regardless of the polarity the high-current pulse is only seen on the first application of voltage.

(4) The initial current can, however, be partially or totally recovered by heating the crystal to a higher temperature (for example, 200° C) and leaving it at this temperature for an hour or two.

(5) The faster the application of voltage the steeper and the higher is the initial current.

(6) If after having applied several pulses of given maximum voltage a pulse of magnitude higher than this series is applied, the current once again shoots up at a threshold voltage exceeding that previously observed, and its magnitude is greater at this higher field. This settles down as before to a very low value on application of further pulses of this magnitude. It is usually found that the residual current at lower fields is now less than before, that is to say, the application of a higher voltage increases

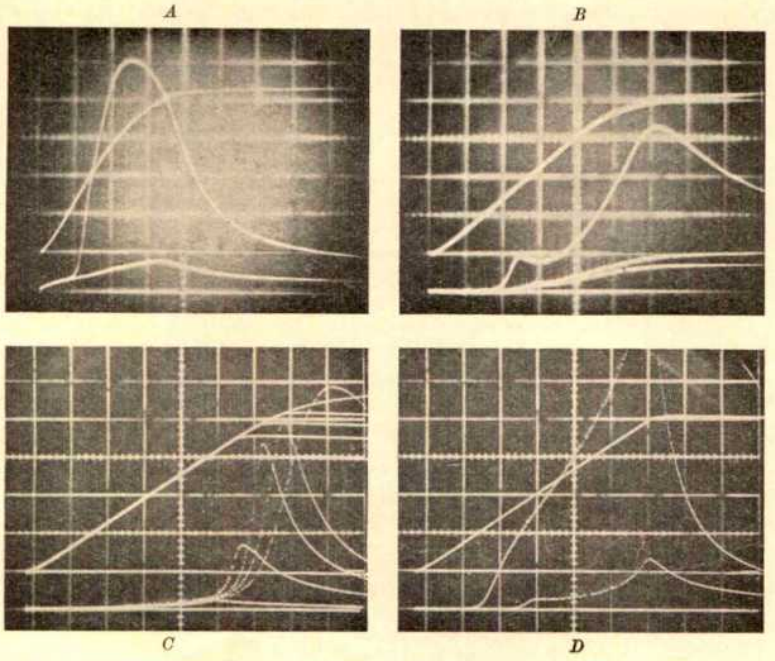


Fig. 1. Transient current in potassium chloride at 150° C (lower traces) and pulse voltage (upper traces). *A*, Voltage/large division = 1 kV; current/large division = 5 μamp; time/large division = 0·5 msec; thickness = 0·013 cm; area = 0·138 cm². *B*, Voltage/large division = 2 kV; current/large division = 10 μamp; time/large division = 0·5 msec; thickness = 0·014 cm; area = 0·196 cm². *C*, Voltage/large division = 2 kV; current/large division = 10 μamp; time/large division = 0·5 msec; spherical recess, minimum thickness 0·0127 cm, diameter = 0·8 cm. *D*, Voltage/large division = 2 kV; current/large division = 10 μamp; time/large division = 0·5 msec; same specimen as *C*

the resistance of the crystal at lower voltages. (See Fig. 1 *C*, in which successive pulses of increasing amplitude were applied.)

(7) It is sometimes found that if the voltage is progressively increased so that the current pulse moves to higher and higher fields, some recovery at lower fields occurs. This can be seen in the double humped oscillogram in Figs. 1 *B* and *D*, second pulse.

(8) It is generally found that the higher the current the shorter the time taken for it to decay.

(9) If a sample is allowed to recover as described in (4), all the foregoing phenomena can be repeated. Differences exist in absolute magnitude of current from sample to sample of the same area and thickness, but the phenomena observed are the same qualitatively.

(10) All the aforementioned characteristics are also observed if the recess is made spherical instead of flat and if evaporated electrodes of silver or aluminium are used.

(11) Figs. 1 *C* and *D* refer to the same specimen. After application of the voltage shown in Fig. 1 *C*, the specimen was allowed to recover and then subjected to two voltage transients of greater amplitude than that mentioned in paragraph (1) (see Fig. 1 *D*). The current due to the first pulse is seen to rise at the low threshold voltage and it continues to rise with increasing voltage until the latter reaches its maximum (the decay of this current can be seen on the right in the oscillogram). The current at fields above the threshold voltage is some 1,000 times that recorded at the same field using direct current.

(12) Breakdown when it occurred was always abrupt.

The foregoing results indicate that the current in potassium chloride at 150° C is dependent on the thermal and electrical history of the specimen and on the rise time of the applied voltage. Similar phenomena have also been found in semiconductors[1]. Von Hippel[2] and Konorova[3] have suggested that high-field conduction is mainly electronic due to field emission from the cathode and that the current decay with time is due to space charge formed by electrons trapped at crystal defects. Our observations can be accounted for on this hypothesis. The sudden increase in current at a threshold voltage can be attributed to electron injection into the conduction band due to field emission from the cathode. These electrons gradually settle into traps and form negative space charge near the cathode, which acts to diminish the field at the cathode, to limit further emission and to slow down electron movement.

An increase in the rate of rise of the voltage will result in greater acceleration of free electrons and in addition will maintain emission from the cathode if the trapping rate—and thus the build-up of space charge at the cathode—is less than, or at least comparable with, the voltage rise. The current under these conditions should be steeper and higher as is observed.

As the initial current may be recovered after some time has elapsed with moderate heating of the crystal, it would seem that shallow traps are involved. It may be that at higher fields electrons can be ejected from these traps resulting in a partial removal of the space charge near the cathode and allowing, on subsequent application of voltage, injection at lower fields. This could then account for the double humped curves seen in Fig. 1 *B*.

The rate of settling into traps will, in part, be determined by the free electron density. This would account for our observation that the decay of current is more rapid the greater the magnitude of the current observed, and also that the resistance of the crystal at low fields is increased by the application of higher voltages.

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Relation between the Poincaré Group and the Exceptional Lie Group G_2

THERE is a correspondence, $L_3 \leftrightarrow P_5$, between the line geometry on a three-manifold M_3 and that of planes on M_5 (ref. 1). Because, essentially, of the role of quaternions and octonions as co-ordinate rings here, this correspondence is unique[2]. It can be expressed in terms of the groups which act on manifolds, with dimensions, respectively, of the following elements:

$$\begin{aligned} &M^2{}_{3(5)}, \text{ lines (planes) restricted to a linear complex} \\ &M^2{}_{4(6)}, \text{ lines (planes)} \\ &R_{5(7)}, \text{ linear complexes of lines (planes)} \end{aligned} \qquad (1)$$

and is (the middle group being in each case the conformal group):

$$(B_1, D_3, B_2) \leftrightarrow (G_2, D_4, B_3) \qquad (2)$$

Thus, instead of B_2 (de Sitter group and, by restriction to four-space, Poincaré group), on the right-hand side one finds the exceptional G_2. Behind this is the fact that B_3, D_4, though classical groups, admit exceptionally an outer automorphism of order three, generally known as 'triality'[3].

In view of the known representations of the electromagnetic field $F = (E,B)$ by quadratic[4] or linear[5] com-

plexes in L_3, it is important to see with what physical interpretation the generalization to P_5 corresponds.

In a constant isotropic medium the radiation field $F = (E,B)$ with $E \cdot B = 0$ is determined by five parameters and can therefore be represented by the linear complex:

$$F_{\mu\nu}\, p^{\mu\nu} = F_{\mu\nu}\, x^{\mu}\, y^{\nu} = \hat{F}^{\mu\nu}\, p_{\mu}\, q_{\nu} = 0 \qquad (3)$$

in L_3 where x^{μ},p_{μ} are homogeneous point and plane co-ordinates on the light cone $M_3{}^2$. In the non-isotropic case, one has a non-trivial constitutive tensor K given by:

$$F^{\mu\nu} = K^{\mu\nu\sigma\varepsilon}\, G_{\sigma\varepsilon} \qquad (4)$$

where $G = (D,H)$. In L_3, K defines a quadratic complex:

$$\Omega = K^{\mu\nu\sigma\varepsilon}\, G_{\mu\nu}\, G_{\sigma\varepsilon} = 0 \qquad (5)$$

of which the singular surface $F_{\mu\nu}\, x^{\nu} = G_{\mu\nu}\, x^{\nu} = 0$ is well known to be the wave surface of geometrical optics. As a local property of space K must, according to the philosophy of general relativity, be a function of the fields used to describe the structure of that space. In the Born–Infeld theory[6] $K_B = K(g_{\mu\nu},F_{\mu\nu})$ and, so far as dependence on F is concerned, can be reduced[7] by a four-parameter Lorentz transformation to the identity times a complex gauge factor $e^{\theta+i\varphi}$. The unitary part of this is a duality rotation[8]:

$$(E + iH)(\varphi) = e^{i\varphi}\,(E + iH)(0) \qquad (6)$$

so that $\hat{F}(0) = F(\pi/2)$. The remainder is a real gauge transformation:

$$e^{\theta} = (F_0{}^2 - F_R{}^2)\, F_0{}^{-2} \qquad (7)$$

where F_R is the remaining, purely magnetic, component of F in the reduced frame, and F_0 is a constant in the Born theory, but in general must depend on the metric like $g^{-1/2} = |-g_{\mu\nu}|^{-1/2}$ times an invariant density ρ. In any case K now depends on seven parameters and this, from (1), indicates the possibility of representation by a linear complex in P_5. Furthermore, since K in general depends on 21 parameters, one expects representation in this case by three complexes which are interchanged by a triality rotation.

One can show this very briefly by taking, for a 'field-with-gauge', $F = g^{1/2}\, F_{\mu\nu}$, $g^{1/2}$, ρ) an octonion with components as homogeneous co-ordinates in R_7. This already implies that F can be represented as a linear complex in P_5 because G_2 is the largest group that leaves invariant the multiplication law of octonions[9]. The scalar part, $S(F_1(F_2F_3))$ say, then of any product of three fields F_1,F_2,F_3 is invariant under the conformal group D_4 (ref. 10) acting on the component fields and also under B_3 acting on the product. It follows then that the twenty-one independent transforms can be taken under the latter as elements of K. Thus, B_3 is the analogue of the Lorentz group in the L_3-theory.

Using the definition:

$$(AX)(A^{*}Y) = A^{**}(XY) \qquad (8)$$

of triality, where $A^{***} = A$, one can define a triality rotation analogous to equation (6). Particular interest then attaches to the form in which this is parametrized by elliptic functions. This is the case if ρ is taken to be a (positive) scalar curvature density; then

$$\frac{\mathrm{d}^2 \log g^{1/2}}{\mathrm{d}s^2} = \rho\, g^{-1/2} \qquad (9)$$

has solution $g^{-1/2} = \mathfrak{p}((u) - e_i)$ in terms of the Weierstrauss elliptic function $\mathfrak{p}(u)$ of a formally complex proper time $s = \rho^{-1/2}\, u$ in the three neighbourhoods $e_i u \sim 0$, where e are the roots of $\mathfrak{p}'(u) = 0$, permuted by triality. Thus, $\varkappa_i = \rho^{1/2}\, e_i$ may be taken as the inverse 'lifetime' (if real) or 'range' (if imaginary) of the fields in each case; especially if $\varkappa_3$ is real, then $\varkappa_1 = -(\varkappa_2 + \varkappa_3) = \varkappa_2$ and F_1,F_2 may be taken conjugate in the sense, $F_2 = \bar{F}^{*}$, so that by (8) $F_1(F_2F_3) = N(F_1)(F_1{}^{**}F_3)$. The corresponding fourteen-parameter constitutive tensor K_G, say, is a generalization of the Born K_B in the sense that it reduces to the ten-parameter form:

$$K_E^{\mu\nu\sigma\varepsilon} = \tfrac{1}{2}\,(g^{\mu\sigma}\, g^{\nu\varepsilon} - g^{\nu\sigma}\, g^{\mu\varepsilon}) \qquad (10)$$

of general relativity[11] instead of equation (7) up to, that is, possibly a triality rotation. The fields $F_1,F_2 = \bar{F}_1^{*}$, then supply a non-trivial metric which suggests, as a particle interpretation, that the gravitational 'vacuum' consists of vector meson pairs, possibly the W-mesons of weak interaction theory.

Finally, each of the groups on the right of equation (2) has an invariant of degree six which is the determinant $|K|$ of K considered as a symmetric tensor on $M_6{}^2$, and is an intrinsic property of the medium. This action of G_2 is to be compared with that of the Poincaré group which leaves invariant the Lagrangian of the Born theory, that is essentially $|g_{\mu\nu} - F_{\mu\nu}|$ which is of degree four.

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Avoidance of Growth-striae in Semiconductor and Metal Crystals grown by Zone-melting Techniques

It has recently been shown[1] that the growth striae found in semiconductor crystals grown by zone-melting techniques are caused by fluctuations in temperature in the melt from which the crystal is being grown. (The term growth-striae is used to describe a banded distribution of impurity revealed on etched crystal sections.) One of us has shown[2] that the temperature fluctuations were a characteristic property of a liquid metal contained in a horizontal boat on which was impressed an axial temperature gradient. At lower temperature gradients, but still above some critical gradient, the temperature varied sinusoidally with time.

Hurle *et al.*[3] carried out a spectral analysis of the temperature fluctuations and have shown that they are composed predominantly of a harmonic series, the fundamental of which is the sinusoidal oscillation obtained at low temperature gradients. Hurle[2] has postulated that the oscillations are an example of the phenomenon of overstability[4,5]—a form of convective instability. Hurle[2] has also shown that the fluctuations can be eliminated by the application of a transverse magnetic field and has proposed the use of a magnetic field during crystal growth as a means of eliminating growth striations.

This communication reports the results of experiments in which tellurium-doped indium antimonide has been zone-melted with the molten zone maintained in a horizontal, transverse magnetic field.

A conventional horizontal zone-melting apparatus was modified to permit the mounting of a water-cooled electromagnet on the carriage containing the zone-heater. The silica envelope was fitted with a small side arm angled at approximately 45° to the tube, which permitted the introduction of a fine chromel-alumel thermocouple into the molten zone. The tip of the thermocouple was protected by a thin coating of boron nitride. The thermocouple was fed, by means of a backing off circuit, to a potentiometric recorder.

The indium antimonide charge was doped to a concentration of 1×10^{18} atoms/c.c. of tellurium and was contained in a vitreous carbon[6] boat of 16 cm length and about semicircular cross-section of diameter 2 cm. Purified argon was flowed through the tube at a rate of 1 l./min.

The carriage was initially positioned so as to melt a zone of about 4 cm length at one end of the ingot. The ingot was positioned with respect to the side arm such that the thermocouple could be inserted into this zone. With zero magnetic field temperature fluctuations of $\sim 5°$ C amplitude peak-to-peak were recorded (Fig. 1*A*). On applying a magnetic field of 2·5 kG the fluctuations disappeared (Fig. 1*B*). The field was then steadily decreased until at a field of 1·96 kG sinusoidal oscillations of frequency 0·5 c/s were observed (Fig. 1*C*). The critical magnetic field at which the oscillations disappeared increased markedly with increasing length of zone. No quantitative measurements, however, of this effect were made.

After the preliminary experiments had been completed, the thermocouple was removed from the molten zone and the zone was passed along the bar at a rate of 4 cm/h. During passage of the zone through the first and last thirds of the ingot the field was zero, but during passage through the middle third, a magnetic field of 2·5 kG was applied. The bar was then removed, sectioned longitudinally and etched in a modified *CP*-4 etchant. Irregularly spaced striations were visible in the regions which had been crystallized without a magnetic field; there were no striations where the magnetic field had been applied.

In another experiment, a field of 1·96 kG, which was just sufficient to produce sinusoidal oscillations, was applied as before. A bar processed as in the foregoing experiment was found to have striae spaced equally at a distance equal to the crystallization velocity divided by the oscillation frequency. Fig. 2 is a micrograph of the crystal section taken at the point where the field was applied.

All the crystals grown were polycrystalline, no attempt having been made to obtain single crystals.

Both the observation that the striae disappear when the temperature fluctuations are suppressed and the correlation between striae spacing and oscillation frequency provide additional proof that the striae are directly due to the temperature fluctuations.

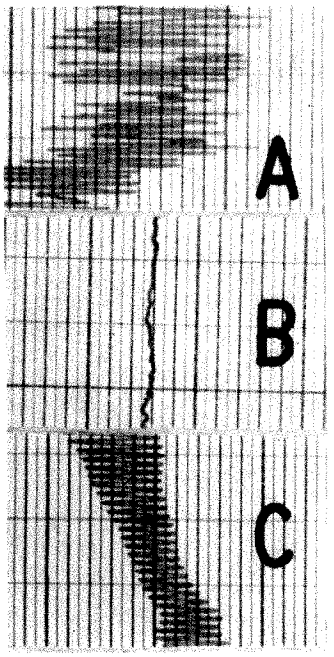


Fig. 1. Thermocouple records: Abscissa, one division = ~0·25° C; ordinate, one division = 15 sec. Magnetic field H: in *A*, 0; in *B*, 2·5 kG; in *C*, 1·96 kG. Frequency, 0·5 c/s



Fig. 2. Photomicrograph of etched crystal section. The region to the left of the arrow was grown with $H = 0$; that to the right with $H = 1{\cdot}96$ kG (×44)

The use of a magnetic field, as described here, for avoiding growth striae has definite advantages over the use of short zones and low-temperature gradients proposed by Müller and Wiehelm[1]. First, the use of low-temperature gradients is likely to result in the occurrence of constitutional supercooling, with its consequent inhomogeneities, in all but very dilute alloys. Secondly, it is technically difficult to maintain short zones. If the heat is applied to the zone externally (as distinct from being generated within the bar by, for example, joule heating), then the zone length cannot be made appreciably smaller than the ingot diameter. On the other hand, the magnetic fields required to suppress the fluctuations are modest and can readily be generated over the necessary volume for crystal growth.

Whether the striae observed in doped oxide crystals[7] are also due to overstable temperature oscillations has yet to be determined. If they are, then the application of a magnetic field may again be useful. However, the electrical conductivity of such melts is somewhat lower than that of liquid metals, and the magnetic fields required are likely to be proportionately higher.

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CRYSTALLOGRAPHY

Ordered Structure of Titanium Oxide

THE TiO phase in titanium–oxygen alloys is known to have an extraordinarily wide range of composition, that is, from $TiO_{0{\cdot}7}$ to $TiO_{1{\cdot}25}$, and its crystal structure has varying proportions of both titanium and oxygen vacancies. Density and X-ray lattice parameter measurements[1,2] have shown that a third of the oxygen sites are vacant in $TiO_{0{\cdot}7}$, a quarter of the titanium sites are vacant in $TiO_{1{\cdot}25}$ and even in stoichiometric TiO about a seventh of both sites are vacant. According to the X-ray examina-

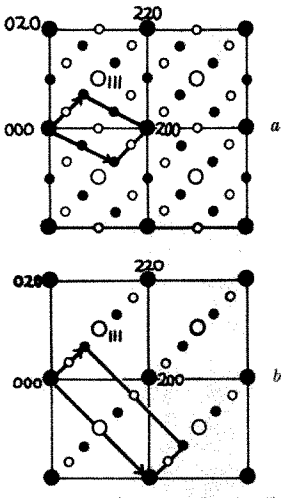


Fig. 1. Reciprocal lattices. Indices refer to the NaCl-type cell. ●, Reflexion on $l=0$ plane; ○, reflexion on $l=1$ plane

tions, these vacancies are arranged randomly at temperatures above the equilibrium temperature, 990° C, giving the diffraction patterns typical of a cubic NaCl-type structure. On the other hand, many weak superlattice lines have been observed on the X-ray powder patterns[3,4] after annealing below 950° C, suggesting the formation of an ordered arrangement of vacancies. However, many attempts to determine this ordered structure in terms of an NaCl-type lattice with a periodic vacancy arrangement have been unsuccessful.

In the present investigation, the ordered structure was examined by transmission electron microscopy/diffraction using alloy foils thinned chemically from bulk. The main advantage of this method of examination over conventional X-ray methods is that it is possible to obtain single crystal diffraction patterns by means of the 'selected-area' diffraction technique. Prior to thinning, all specimens were quenched from above 1,500° C or the melt to retain the cubic NaCl-type structure followed by isothermal annealing below 950° C. Within the composition range $TiO_{0\cdot9}$ to $TiO_{1\cdot1}$ numerous superlattice spots in addition to the fundamental NaCl-type reflexions were observed in the electron diffraction patterns. In the electron microscope images an array of small domains ($\sim 3\mu$ diameter), each containing one set of parallel sub-bands (~ 300–1000 Å wide), was observed. Selected area diffraction patterns of the domains were taken for many crystal orientations, but most of them were actually the superposition of two or more patterns. In extracting diffraction patterns due to single domains from these complex ones, the sub-bands were revealed as twins on a {110} plane of the NaCl-type lattice. A reciprocal lattice was constructed (Fig. 1*a*) from which a monoclinic cell (shown by thick lines) was chosen to describe the ordered structure.

The space group C_{2h}^3-A2/m (No. 12 with A, B interchanged) was obtained from the extinction rules given by the reciprocal lattice. All the observed reflexions are explained by assuming that of the twelve titanium and twelve oxygen sites in the ordered unit cell there are a sixth of each site vacant, that is, two titanium and two oxygen atoms are missing. This assumption is consistent with the previous observations that a seventh of both kinds of atoms are absent at the stoichiometric composition. In the structural model, atoms have been assigned to the following positions:

2 Ti in 2 (*c*): $\frac{1}{2}, 0, 0 : \frac{1}{2}, \frac{1}{2}, \frac{1}{2}$.
2 O in 2 (*b*): $0, \frac{1}{2}, 0 : 0, 0, \frac{1}{2}$.
4 Ti in 4 (*i*): $(0, 0, 0 : 0, \frac{1}{2}, \frac{1}{2}) + x, y, 0 : \bar{x}, \bar{y}, 0$.
With $x = \frac{1}{6}, y = \frac{1}{3}$.
4 Ti in 4 (*i*): with $x = \frac{2}{3}, y = \frac{1}{3}$.
4 O in 4 (*i*): with $x = \frac{1}{3}, y = \frac{1}{6}$.
4 O in 4 (*i*): with $x = \frac{2}{3}, y = \frac{1}{6}$.

At the moment these positions are being refined by computation using a least-squares method on intensity data obtained from X-ray diffractometer measurements on powdered samples.

The structure can be described in terms of vacancy ordering; that is, in every third plane parallel to the (110) plane of the original NaCl-type lattice, half the titanium and half the oxygen atoms are missing alternately (see Fig. 2). The spacing of these (110) planes is slightly larger than that of the other {110} planes. As a result, the symmetry of the original cubic structure is lowered to monoclinic. From previously published X-ray powder data[3,4] and the X-ray diffractometer data obtained in the present investigation, the lattice parameters of $TiO_{1\cdot00}$ have been calculated as follows:

$a = 5\cdot855 \pm 0\cdot003$ Å.
$b = 9\cdot340 \pm 0\cdot005$ Å, $\gamma = 107°\ 32' \pm 3'$.
$c = 4\cdot142 \pm 0\cdot002$ Å.

This proposed structure accounts adequately for all the X-ray powder lines reported previously[3,4].

The structural model described here corresponds to the stoichiometric composition TiO, but the observation that this structure is stable from $TiO_{0\cdot9}$ to $TiO_{1\cdot1}$ implies that a small excess or deficiency of titanium and oxygen vacancies can be tolerated. However, within the composition range $TiO_{0\cdot7}$ to $TiO_{0\cdot9}$ for non-equilibrium conditions of cooling there appears to be a transition structure formed which is different from the structure just described. The superlattice unit cell in the reciprocal lattice is orthorhombic (thick lines in Fig. 1*b*) and from the extinction rules the space group I222 (No. 23) was chosen for the structure. While the orthorhombic unit cell involves six titanium sites and six oxygen sites, it is considered that there are more oxygen vacancies than titanium vacancies in this structure since it has been observed only in the oxygen-deficient alloys. The structure can be described by the ordering of oxygen vacancies with random titanium vacancies such that all the oxygen atoms are missing from every third atomic plane parallel to the (110) plane of the original NaCl-type lattice. This structure model accounts adequately for all the observed superlattice spots obtained on the electron diffraction patterns.

In conclusion, it is to be noted that when the TiO alloys were quenched from above 1,500° C the electron diffraction patterns contained strangely shaped diffuse scattering effects, in addition to the fundamental spots due to the NaCl-type lattice. It seems that these effects are due to short range order in the arrangement of vacancies. Work on this aspect of the ordering reaction is being continued.

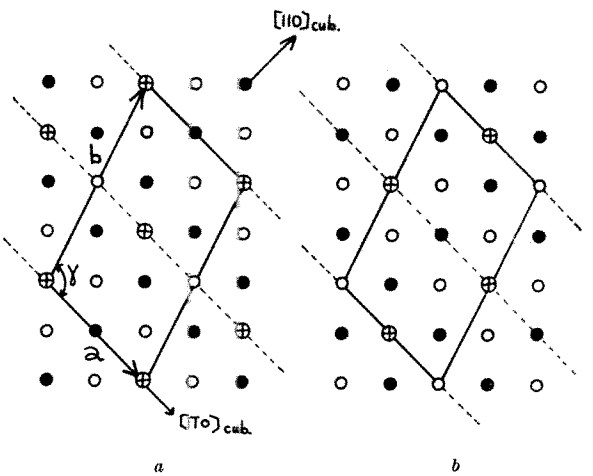


Fig. 2. Structure of ordered TiO. (*a*) Shows $z=0$ plane and (*b*) shows $z=0\cdot5$ plane. Thick lines represent unit cell and dotted lines the planes containing vacancies. ●, Titanium; ○, oxygen; ⊕, vacancy

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Rapid X-ray Diffraction Studies using Image Intensification

A METHOD of quickly making observations by X-ray diffraction is needed for investigation of unstable phases and of structural rearrangements in crystals : it is also desirable in order to expedite the orienting of normal specimens for crystallography. The required direct observation and rapid recording have now been achieved by use of electronic intensification of the image of the diffraction pattern. It has proved possible to correct approximate settings of the crystal by inspection, and transient diffraction patterns have been observed. Exposure times for photographic recording range between 0·5 sec and 5 sec.

Image intensifiers[1] are supplied commercially for medical and industrial radiography. The pattern is visible through a lens which produces an image at infinity. These experiments were made with a unit having an input window 15 cm in diameter. As the protective cover was found to cut the intensity of copper $K\alpha$ radiation by a factor of 10^3, it was replaced by black paper. An 'intensification camera' was constructed incorporating the intensifier instead of a film-holder, a 35 mm camera which could be swung into position when required, provision for a 'vertical circle' for universal orientation of the specimen, and a normal collimator. Provision was made to record reflexions at higher angles, but front reflexions were more useful. Cross-wires placed across the window facilitated adjustment of the crystal. To reduce the exposure of the worker's hands to radiation the goniometer head should be operated through flexible leads or by keys attached to long rods. The device was calibrated by photographing the diffraction pattern from a standardized quartz powder.

The resolution of the intensifier is stated to be 30 lines/cm. This has been found adequate for most purposes. Though the sensitivity is poor for wave-lengths above about 1·5 Å, good Laue and characteristic patterns have been obtained with molybdenum radiation.

When polychromatic radiation is used an important zone axis can be recognized by the way the spots move when the crystal is rotated slightly. Whereas the analysis of conventionally recorded Laue patterns is limited because the wave-length causing each reflexion is not known, here various planes can be brought to the angle at which they diffract the characteristic ($K\alpha$) wave-length. This is recognized by the sudden increase in intensity and confirmed by introducing the β-filter. The low wave-lengths can be determined approximately by reducing the excitation potential on the generator until the reflexion disappears. Similarly, the diffraction pattern given by $K\alpha$ radiation can be quickly recorded in successive photographs with a stationary crystal. As with a diffractometer the diffraction angle is known from the photograph and the crystal orientation is read from a scale on the apparatus; but as in the photographic method several reflexions (for example in different layer lines) can be recorded simultaneously. In this situation the crystal can be set very precisely. In following the progress of a structure change it is an advantage that a representative diffraction pattern of both structures can be observed rather than only one peak as with a diffractometer, and transient effects due to stresses can be detected.

The method has facilitated several investigations. Phase I of rubidium nitrate exists between 284° C and the melting point 310° C. Conventional powder photographs showed it to have essentially the NaCl structure (a fact of interest in relation to melting), but experiments to confirm this by moving film methods remained incomplete because the crystal decomposed during the long exposure. The crystal has to be grown from the melt and does not necessarily appear single and in a convenient orientation. By using this technique it was possible to monitor the growth, remelting as required, and to take a series of photographs. By watching the diffraction pattern while changing the temperature it was seen that there is a progressive and completely reversible fading of some reflexions and change in the relative intensity of others. This indicates a gradual order–disorder change which explains a known specific-heat anomaly in this

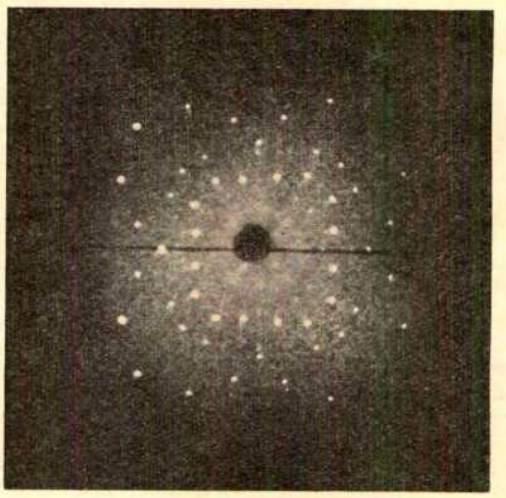


Fig. 1. Intensification photograph of Laue pattern from an ammonium iodide crystal, with [100] about 1° off the beam. Exposure 4 sec

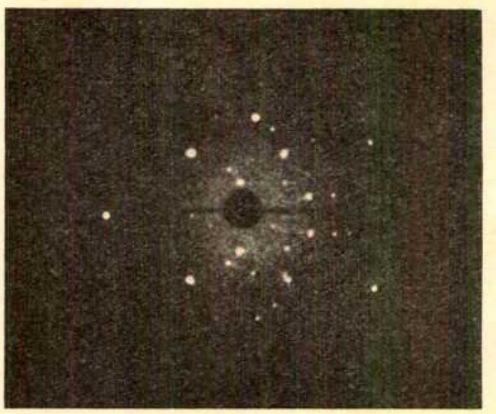


Fig. 2. Pattern showing on the left three intense reflexions of the $K\alpha$ wave-length from a stationary ammonium iodide crystal, [011] vertical, with molybdenum radiation and zirconium filter. Exposure 5 sec, over-exposed for reproduction

phase. When on careful cooling the specimen changed to phase II, a strict orientation relation was maintained in a way which strongly suggested that the general arrangement of cations remained unaltered. This inference about the unknown structure of phase II goes far to rationalize the complex polymorphism of this compound.

Plastic deformation and relaxation are expected to be important in the kinetics and thermodynamics of some types of structure-change[2], but no information has been available on the rate at which stresses are released during and just after transformation. Direct viewing showed that ammonium iodide could be taken through the transformation cycle NaCl-type⇄CsCl-type at about −16° C with maintenance of an apparently definite orientation relation. While strain effects in the low-temperature form were great and the pattern poor, the pattern from the room-temperature structure appeared immediately on reversing the transformation, but annealing continued and reflexions became sharper over a period of 40 min. The rate of annealing on gentle heating, shown by reduction of asterism, was also followed and was initially rapid. By contrast, at the reconstructive transformations III→II at 219° C in rubidium nitrate and IV→III at 32° C in ammonium nitrate, when the diffraction pattern of the first phase disappeared it was replaced initially by the picture due to a very strained and disordered specimen. The pattern of the somewhat strained new structure then appeared gradually over a period of 1 min. Microscopy showed that the newly formed phase II of rubidium nitrate actually recrystallizes. One concludes that consideration of defects and free-energy differences in the treatment of phase growth[2] should include relatively large allowance for temporary disorder not recorded by conventional methods.

Image intensification[3] promises to provide an additional technique for crystallography of value particularly for study of reactions involving structural rearrangements, especially when combined with new techniques for precise measurement at the equilibrium temperature.

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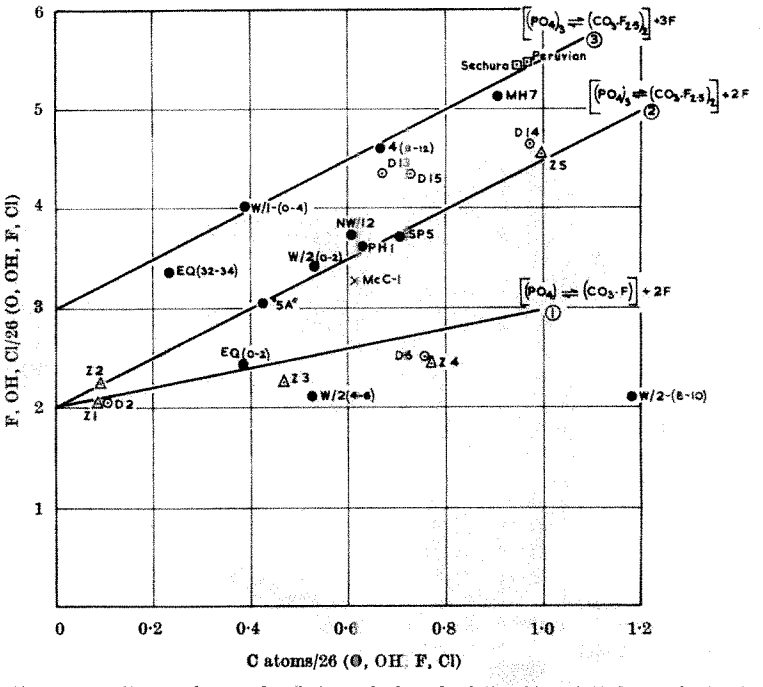


Fig. 1. Apatite samples: carbon/halogen–hydroxyl relationships plotted on a basis of 26 F, O, OH, ions per two formula units. *A*, ●, Analyses of Christmas Island apatite samples. *B*, △, Analyses of carbonate–apatite samples from Deer, Howie and Zussman (ref. 1, p. 328): *Z*1, blue apatite, pegmatite dyke in contact with limestone, Mansjo Mountain, Halsingland, Sweden; *Z*2, colourless apatite, pegmatite dyke, Mansjo Mountain, Halsingland, Sweden; *Z*3, francolite, oolitic ironstone, Robin Hood Quarry, Thorpe-on-the-Hill, Leeds, Yorkshire; *Z*4, francolite, Wheal Franco, Buckland Monachorum, Tavistock, Devon; *Z*5, francolite, altered lava, Annisfontein farm, Richtersveld, Namaqualand, South Africa. *C*, ⊙, Analyses of carbonate–apatite samples from Palache, Berman and Frondel (ref. 3, p. 883): *D*2, fluorapatite, Faraday Township, Ontario; *D*6, carbonatian fluorapatite, Wheal Franco, Devonshire (francolite); *D*13, carbonate–apatite, St. Paul's, Atlantic Ocean; *D*14, carbonatian fluorapatite, Richtersveld, South Africa; *D*15, carbonatian fluorapatite, Milburn, New Zealand. *D*, ×, Analysis of dahllite from McConnell (ref. 5): *McC*–1, post-Wisconsin (upper Pleistocene) fossilized dental enamel from a mastodon Bluffton, Ohio. *E*, ⊡, Analyses of other apatites carried out at the Australian Mineral Development Laboratories: 'Sechura', Sechura Desert, Peru; 'Peruvian', unspecified locality, Peru

MINERALOGY

Substitutions for Phosphate Ions in Apatite

IN the course of recent investigations of the geology and mineralogy of the phosphate deposits of Christmas Island, Indian Ocean, numerous samples of apatite (optical varieties, collophane and dahllite) were analysed chemically. The purity of the samples was established by X-ray diffraction and differential thermal analysis. A typical analysis is shown in Table 1.

The ionic proportions for apatite were computed both on a basis of ten Ca^{+2} etc. ions and 26 (F, O, OH) ions per two formula units, namely, 2 $(Ca_5(PO_4)_3$ (F, OH)). It was assumed that the Ti^{+4}, Al^{+3}, Fe^{+3}, Na^{+1}, K^{+1} and Li^{+1} ions substitute for Ca^{+2} in the same manner as the divalent ions Mg^{+2}, Mn^{+2} and Zn^{+2}. These substitutions, with the exception of Ti^{+4}, have already been demonstrated[1]. The relative proportions of the sesquioxides in samples from all the phosphate deposits of Christmas Island are remarkably constant, the ratio of Al_2O_3/Fe_2O_3 being approximately 2·5. This ratio is also maintained in the crandallite and millisite-bearing overburden.

The sum of the F^{-1} and OH^{-1} ions was plotted against the number of carbon atoms on a basis of 26 (F, O, OH) ions as shown in Fig. 1. In addition to the twelve analyses of apatite from Christmas Island, the complete analyses of carbonate apatite recorded by Deer, Howie and Zussman[1], McConnell[2] and Palache, Berman and Frondel[3] are also included. Two, and possibly three, linear relations

Table 1. TYPICAL APATITE FROM CHRISTMAS ISLAND, INDIAN OCEAN

	Per cent
P_2O_5	37·4
CO_2	2·85
TiO_2	0·05
Al_2O_3	2·60
Fe_2O_3	1·50
CaO	50·5
MgO	0·48
MnO	0·12
ZnO	0·07
Na_2O	0·37
K_2O	0·04
Li_2O	0·06
F	2·00
Cl	0·01
H_2O^+	2·40
H_2O^-	0·46
SiO_2	0·04
Total	101·14
Less O equivalent to F	0·84
	100·3

(Analysis by D. K. Rowley, the Australian Mineral Development Laboratories, Adelaide, South Australia.)

for carbonate and fluorine are indicated. The first accords with the substitution:

$$CO_3 \cdot F \rightleftarrows PO_4 \qquad (1)$$

This conforms with the formula of carbonate apatite proposed by Borneman–Starynkevich and Belov[4], $xCa_{10}P_6O_{24}F_2 + yCa_{10}P_5CO_{23}F_3$. The correspondence of values with this relationship is not close. McConnell[2] has indicated that there is no straightforward relationship between $(CO_3 \cdot OH)$ and PO_4 groups in francolite.

The gradients of the other two relationships in Fig. 1 are parallel and suggest substitutions of the type:

$$2(CO_3 \cdot F_{2 \cdot 5})^{-4 \cdot 5} \rightleftarrows 3(PO_4)^{-3} \qquad (2) \text{ and } (3)$$

Apatites represented by relationship (3) contain 3 (F, OH) ions in the non-carbonate end member. They also contain an average of three times more monovalent metal ions ($Na^{+1} + K^{+1} + Li^{+1}$) than those represented by relationship (2), and show a corresponding decrease in the divalent metal ions. It is likely that as well as substitution of fluorine, hydroxyl and carbonate ions for phosphate ions occurring, fluorine and/or hydroxyl can substitute for oxygen with the attendant replacement of sodium, etc., ions for calcium to balance the charge.

These results are in accordance with the theory advanced by McConnell[5], Simpson[6] and Winand[7] that the carbonate ions, together with fluorine or hydroxyl ions, substitute for PO_4^{-3} ions and do not conform with substitutions of carbonate for the $2F^{-1}$ ions in the apatite structure.

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GEOLOGY

Detection and Location of a New Underwater Volcano

THE irregular appearance of an unusual component of the sea-noise spectrum in the waters to the east of the North Island first attracted attention in 1963. Measurements carried out over a period of several months showed that these signals invariably came from one direction, and led to the conclusion that the source was probably geophysical rather than biological in origin. Cross-bearing measurements ultimately pointed to the source lying some 160 miles east-north-east of Great Barrier Island.

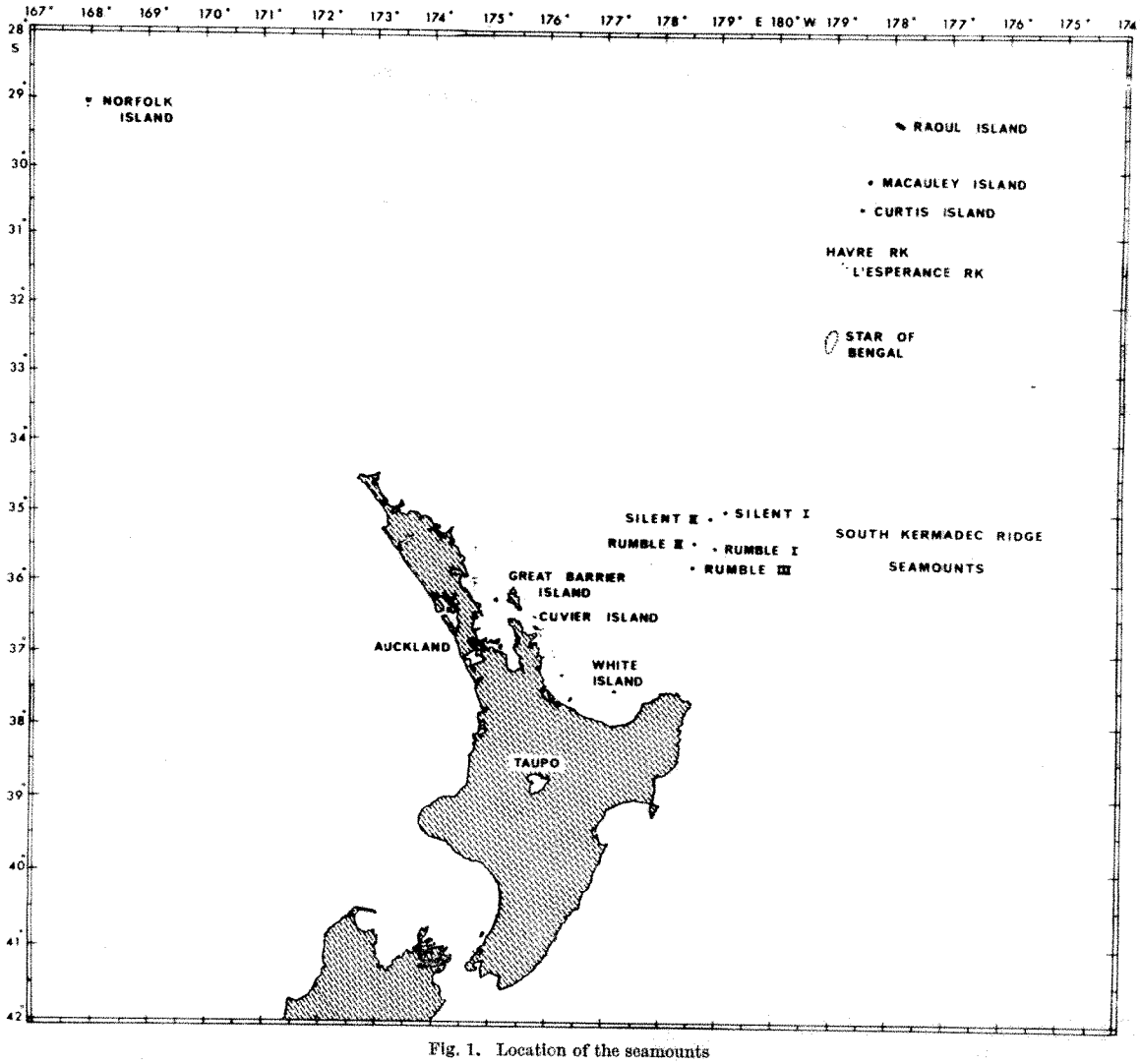


Fig. 1. Location of the seamounts

According to existing charts the sea bed in this area was quite featureless. However, subsequent bathymetric surveys have revealed, so far, six large topographical features rising up to 8,000 ft. from a surrounding sea floor at 1,500 fathoms. Because of the position of these peaks in relation to the Kermadec Ridge they have been called the South Kermadec Ridge Seamounts. The positions of the five most significant peaks are shown in Fig. 1.

Although limited towed nuclear precession magnetometer measurements have suggested that all the seamounts are of volcanic origin, extensive acoustic investigations have pointed finally to Seamount Rumble III, at 35° 42′ S., 178° 29′ E., being the source of the acoustic signals and the site of the present volcanism. The soundings available so far show Rumble III to be a seamount rising to produce a minimum sounding of 120 fathoms from an ocean depth of 1,500 fathoms. It is some 10 miles across at the 1,250-fathom contour, and slightly elongated in the north–south direction.

Only limited dredging has been carried out on the various seamounts, but by far the most significant sample, recovered from Rumble III, confirms the volcanic origin suggested by the magnetic measurements.

Although one series of acoustic measurements has been made close to the seamounts, most of the information relating to the acoustic signals has been obtained from recordings made with a hydrophone at a position close to Great Barrier Island. Several distinct forms of the acoustic signal have been identified, and these indicate that the volcano behaves in several characteristic ways.

By measuring the transmission loss between Great Barrier Island and Seamount Rumble III, it has been possible to establish fairly accurately the charge weight required to produce an equivalent signal level. For some of the strongest activity recorded so far the equivalent charge weight appears to be 10^6 lb. of TNT. Estimates of the total acoustic energy released during prolonged outbursts give values which are higher than those deduced for other volcanic activity[1]. It is felt that, as the transmission loss is accurately known in the present case, the acoustic energy output produced by underwater volcanoes may be much higher than previously estimated.

The occurrence of a region of volcanic activity in the position of the South Kermadec Ridge Seamounts is not surprising considering the geology of the region. Rumble III lies on the line of present volcanic activity passing through Taupo, White Island and Raoul Island. Although Rumble III has been active intermittently for several years, it was marked by a particularly intense outburst in November–December 1964, a period during which Raoul Island erupted violently[2].

The low-frequency rumbling heard in the present case is obviously similar to that reported by others[1,3,4]; but, as Rumble III displays various modes of activity, has been continuously monitored for several years, and its location is known, it has provided an excellent opportunity for studying the acoustic properties of underwater volcanic activity.

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Colour Variations in Bituminous Coal with Thickness of Sections

The colour of bituminous coal has been variously attributed to large polynuclear condensed aromatics[1–3], free radicals[4–6], charge-transfer complexes[3,7] and free π-electrons[8]. The chemical-physical causes of colour remain matters of conjecture and will not be treated here; the optical aspects and simulation of colour in coal will be discussed. Thick sections of coal vitrain are black, or opaque in the visible region of the spectrum. For thin sections, however, the transmitted colour progressively changes from brown to red to yellow with decrease in thickness[9,10]. The yellow colour is characteristic of the thinnest sections that can be successfully made by the normal procedure. The recent success at this laboratory with the grinding of a thin section to form a wedge suggests that, at thicknesses represented by regions near the tip of the wedge, white light is transmitted; that is, the vitrain is clear and colourless[11]. Similar variations in colour with thickness are observed for carbonaceous derivatives such as some heavy oils, asphalts and pitches from coal and petroleum. This communication presents an explanation for these colour changes, and the production of identical colour-changes with thickness is demonstrated with pure compounds that are lightly coloured or colourless.

Published absorption spectra for bituminous coals[4,5,12,13] —usually as thin vitrain sections, Fig. 1*a* (ref. 12)—show that there are no absorption bands, but only general absorption throughout the visible region of the spectrum. This absorption, which is greatest at the short-wavelength limit of the visible region, constitutes the 'wing' of stronger general absorption in the ultra-violet region. Thus vitrain transmits more light at the red end of the spectrum than at the blue. If absorption bands were present in this region little or no colour change would occur with thickness.

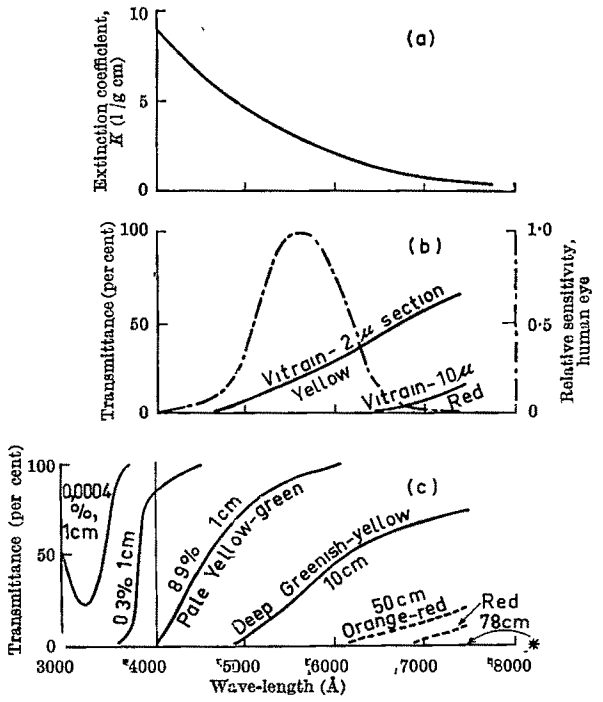


Fig. 1. *a*, Absorption spectrum of Pittsburgh vitrain (84 per cent carbon) determined with ground sections. *b*, Schematic curves for two bituminous vitrain sections, and response curve for the human eye. *c*, Curves for sodium salt of *p*-hydroxyacetophenone. ——, Transmittance curves, and colours for solutions - - —, Calculated curves, and colours observed through long cells. — · —, Response curve for human eye

* For a cell of 191 cm the calculated transmittance at 7500 Å is 0 2 per cent (dark brown to opaque).

The fact that absorption of vitrain thin sections at each wave-length in the visible region obeys Lambert's law of absorption (that is, the optical density is a linear function of the thickness) establishes that the observed colour-changes are of physical rather than chemical origin. In other words, these changes are not due to changes in the

chemical structure or to oxidation of the vitrain induced by grinding.

The observed colour of a substance is a function of the spectral response of the human eye and of the spectral distribution of the light transmitted, or reflected, by the substance[14,15]. The sensitivity of the eye is a maximum near 5550 Å, which is at the yellow end of the green, and it falls rapidly both to shorter and longer wave-lengths with cut-offs near 4000 and 7500 Å (Fig. 1*b*). Thus, for thin sections of vitrain the decreasing sensitivity of the eye towards the blue end of the visible region, below 5550 Å, is coupled with decreasing transmittance for vitrain (Fig. 1*b*); but decreasing sensitivity of the eye towards the red, beyond 5550 Å, is partly compensated by the increasing transmittance of vitrain. Therefore the eye receives greatest stimulus from the red end of the spectrum. To explain the variation with thickness from colourless through yellow and orange to red and brown the thin vitrain sections may be regarded as optical filters with broad shallow transmittance cut-offs at the short wave-length end of the spectrum. For very thin sections this cut-off becomes significant only at some wave-length below 4000 Å in the ultra-violet, and white light is transmitted with negligible attenuation; the section appears colourless. However, as the thickness increases, the absorption edge progressively passes through the visible, and the apparent colour changes from colourless to greenish-yellow to yellow to orange to red and then to brown. This effect is illustrated in Fig. 1*b* by schematic curves for vitrain sections; for 2μ and 10μ sections of a bituminous vitrain the predominant colours would be yellow and red respectively.

The foregoing explanation for the colour change in vitrains receives support from the fact that these changes can be simulated in a simple laboratory experiment using selected pure compounds. The sodium salt of *p*-hydroxyacetophenone, a colourless compound, was selected for this purpose on the basis that it possesses an absorption band with a maximum in the near ultra-violet at 3240 Å and a 'wing' that extends nearly into the visible region. The absorption spectra in the visible region of solutions of this sodium salt were measured with progressively higher concentrations and/or path lengths (for long cells, stainless steel tubes were used) (Fig. 1*c*); changes from colourless to opaque were noted. At the concentrations normally used to obtain accuracy in the measurement of the optical density at peak absorption the absorption in the visible region (Fig. 1*c*) was negligible and the solution was colourless. With increasing concentrations, absorption in the visible region became increasingly significant and progressed towards longer wave-length. In parallel, changes were observed from colourless to yellow-green, through yellow, orange, red and brown to opacity.

Similar colour changes through part of the visible region were observed for solutions of other compounds, both organic and inorganic, having 'wing' absorption in the visible spectrum. Compounds and the colours observed were as follows: 2-methylanthracene (colourless, green-yellow, yellow), picric acid (yellow, orange), sodium picrate and sodium dichromate (orange, red, brown, opacity). Because of low solubilities and steep absorption curves it was not practicable to produce colour changes to complete opacity in all cases.

Spectral absorption data and visual observations of colour must be reconciled. There is some variance in the published absorption spectra of thin vitrain sections. The data of Friedel[12], obtained for Pittsburgh vitrain (84 per cent carbon), using thin sections prepared by grinding, indicated that the transmittance at 6350 Å for a 10μ-thick section was 1·3 per cent (Fig. 1*a*). On the other hand, calculations based on the data for microtomed sections[13] of the same vitrain indicate that for a 10μ section at 6350 Å the transmittance would be only $1{\cdot}3 \times 10^{-11}$ per cent. The former value of 1·3 per cent is more compatible with the observed translucence of a 10μ section.

We thank Dr. G. H. Taylor for helpful discussions.

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CHEMISTRY

Radiation-induced Synthesis of Ammonia from Nitrogen and Water

THIS communication reports on the synthesis of small amounts of ammonia by the irradiation of nitrogen and water either with ultra-violet light below 1800 Å or with gamma-rays.

Since the dissociation energy of nitrogen[1] (9·76 eV) is rather high, high-energy ultra-violet light is necessary to split the nitrogen molecules. Groth *et al.*[2] have shown that the krypton resonance lines (1165 and 1236 Å) which have a quantum energy of 10·03 and 10·64 eV are sufficient for photolysing nitrogen in the gas phase. A new source of ultra-violet light which may also be used for some photochemical processes has recently been developed by Okabe[3]. This lamp, filled with a mixture of 20 per cent nitrogen and 80 per cent argon (total pressure 1 mm of mercury), emits lines at 1495 Å (about 55 per cent) and 1745 Å (100 per cent), which originate from the excited nitrogen atoms. In this case the resonance lines of pure argon at 1048 and 1067 Å disappear.

In my photochemical experiments, a low-pressure mercury lamp filled with argon was used to excite the mercury vapour. Small quantities of nitrogen were present in the lamp. To obtain the lines at 1495 and 1745 Å, and in order to suppress the mercury lines at 2537 and 1849 Å, the lamp was operated at 0° C. Up to 50 per cent of light at 1745 Å is transmitted through quartz glass depending on its degree of purity. In my experiments the ultra-violet lamp was submerged in the solution in a specially designed apparatus. The solutions were prepared using three-times distilled water and reagent-grade chemicals. The cooled aqueous solution was kept saturated with nitrogen, during exposure to the ultra-violet light, by bubbling the gas through it. The irradiated solution was made alkaline and immediately distilled under nitrogen. The ammonia was collected in a vessel containing 0·01 M hydrochloric acid. Nessler's reagent was added to an aliquot and the ammonia was spectrophotometrically determined at 4100 Å (ref. 4). In order to prevent any back reaction of ammonia with hydroxyl radicals, methanol was added as a radical scavenger. Any aldehyde formed in this way was transformed back into methanol by reduction; thus it does not interfere with the

analysis. Thereafter the ammonia was distilled and determined. Blank determinations of unirradiated solutions were made using the same procedure.

Some typical results, representing the mean values of several experiments, are shown in Table 1. No ammonia could be detected after the irradiation of pure water saturated with nitrogen (see exp. 1), but small amounts of ammonia could be found in acidic media. The yield of ammonia was increased by adding ferrous ions. Our experiments showed: (1) that a maximum ammonia yield was reached after irradiation with ultra-violet light for 6 h (see exps. 3–5); (2) that the yield was lower when a 1 : 1 mixture of nitrogen and hydrogen was bubbled through the solution (see exps. 6 and 7); (3) that the yield was higher when methanol was added as a scavenger for the hydroxyl radicals (compare exps. 2 and 8).

Table 1. PHOTOCHEMICAL SYNTHESIS OF AMMONIA FROM NITROGEN GAS AND WATER AT 0° C

Exp. No.	Aqueous solution	pH	Duration of irradiation (h)	Ammonia yield (μmoles/l.)
1	Distilled water	6 7	6	Not detectable
2	10^{-3} M hydrochloric acid	2·9	5	14
3	5×10^{-3} M hydrochloric acid 10^{-2} M ferrous sulphate	2·5	4	16
4	10^{-3} M ferrous sulphate 5×10^{-3} M hydrochloric acid	2 5	6	25
5	10^{-2} M ferrous sulphate 5×10^{-3} M hydrochloric acid	2 5	12	12
6	5×10^{-3} M hydrochloric acid 10^{-3} M ferrous sulphate Nitrogen : hydrogen = 1 . 1	2 5	6	10
7	5×10^{-3} M hydrochloric acid 10^{-2} M ferrous sulphate Nitrogen . hydrogen = 1 1	2·5	6	12
8	5×10^{-3} M hydrochloric acid 10^{-3} M methanol	2·5	5	18

For the formation of ammonia using gamma-rays, a cobalt-60 source (14,000 c.; dose rate, $5{\cdot}5\times10^{19}$ eV/ml./h) was used. The preparation of the solutions and the analysis were carried out as described earlier. Some results are shown in Table 2. Here, too, the yield of ammonia was increased to a G-value of 0·24 by the use of methanol as a scavenger for the hydroxyl radicals.

Table 2. FORMATION OF AMMONIA INDUCED BY γ-RAYS FROM NITROGEN GAS AND WATER AT pH 2·5 AND ABOUT 25° C

Exp. No.	Aqueous solution	Dose (eV/ml.)	Ammonia yield (μmoles/l.)	G-value
1	5×10^{-3} M hydrochloric acid	$4\,1\times10^{19}$	14	0·02
2	5×10^{-3} M hydrochloric acid	$5{\cdot}5\times10^{19}$	6	0 01
3	5×10^{-3} M hydrochloric acid 10^{-3} M methanol	$0\,9\times10^{19}$	36	0·24
4	5×10^{-3} M hydrochloric acid 10^{-3} M methanol	$2{\cdot}8\times10^{19}$	22	0·05

It should be mentioned that some years ago ammonia was synthesized with a G-value of 0·67 by exposing a mixture of gaseous nitrogen and hydrogen to ionizing radiation[5,6]. However, these experiments were apparently not continued. (*Note added in proof.* It was brought to my attention that by irradiating water under a nitrogen pressure of 150 atmospheres, ammonia was formed with $G = 0{\cdot}76$ (Dimitriev, M. T., and Pshezhetskii, S. Ya., *Atomnaya Energiya*, 8, 59; 1960)).

The mechanism of the reaction involved in these syntheses of ammonia in aqueous solutions has not yet been clarified, but some tentative conclusions can be drawn from the results obtained.

When water is photolysed by ultra-violet light at 1849 Å, hydrogen and hydroxyl radicals are produced (quantum yield, $\Phi = 0{\cdot}6$) (ref. 7):

$$H_2O \rightsquigarrow H_2O^* \rightarrow H + OH \qquad (1)$$

This line (1849 Å) supplies an energy of only 6·7 eV and is essentially not absorbed by the nitrogen. The line at 1745 Å (7·1 eV) and at 1495 Å (8·2 eV) cannot excite the nitrogen molecules sufficiently, since there is no continuous absorption by nitrogen in the region from 1050 to 1600 Å with k-value greater than 0·01 cm^{-1} (ref. 12). However, ammonia could then be formed in the presence of hydrogen atoms and excited water molecules. It has been established that ferrous ions[8–10] as well as chloride ions[11] can take part in the formation of solvated electrons (e-aq), on exposure to ultra-violet light. My experimental results indicate that these solvated electrons may also take part in the formation of ammonia.

The mechanism of synthesis of ammonia under the influence of gamma-rays from cobalt-60 seems to be due both to the hydrogen atoms and the solvated electrons which are formed by the radiolysis of the water.

I have only obtained small yields of ammonia, but this is of considerable interest, especially in explaining the formation of organic substances under possible primitive earth conditions. My investigations are still in progress and a complete report will be given elsewhere.

I thank Prof. B. Karlik for her interest in this work, Dr. G. B. Cook for the use of the cobalt-60 source and Mr. A. Fritsch for technical assistance. Part of this work was carried out in the Institute of Chemistry at the Siebersdorf Reactorcentre.

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Hydration in Liquid and Solid Cation Exchangers

THE extraction of water and acids by the liquid cation exchanger dinonylnaphthalene sulphonic acid (HDNNS) dissolved in heptane has recently been investigated for two different concentrations of HDNNS and over a large range of concentrations for nitric, hydrochloric, perchloric and sulphuric acids[1]. The experiments have been performing by shaking samples of HDNNS dissolved in heptane with various water–acid mixtures. After centrifugation and separation of the two phases, both were analysed for acid by titration with alkali and the organic phase analysed for water, using the modification of the Karl Fischer method due to Axel Johansson[2,3].

In Fig. 1 the number of water molecules per sulphonic acid group (Z_{H_2O}) is plotted against $-\log\{H_2O\}$, where $\{H_2O\}$ is the activity of water in the aqueous phase for nitric (□), hydrochloric (■), perchloric (●) and sulphuric (○) acids and 5 per cent (by volume) of HDNNS in heptane. Correction has been made for the small amount of water extracted by the diluent itself and for the co-extraction of some water with nitric acid. From Fig. 1 it is clear that Z_{H_2O} reaches quite large numbers when approaching pure water and slowly tends towards zero with decreasing water activity. It is also clear from Fig. 1 that Z_{H_2O} is a unique function of $\{H_2O\}$ independent of the nature of the strong acid used.

In order to determine the composition of the possible species present, we start to consider the addition of the first water molecule. Application of the equilibrium law to the reaction:

$$HDNNS(org) + H_2O(aq) \rightleftarrows HDNNS{\cdot}H_2O(org) \quad (1)$$

gives, assuming that the organic phase behaves ideally,

$$[HDNNS{\cdot}H_2O] = K_1\,[HDNNS]\,\{H_2O\} \quad (2)$$

where square brackets denote concentration. According to the definition

$$Z_{H_2O} = [HDNNS{\cdot}H_2O]/([HDNNS] + [HDNNS{\cdot}H_2O]) \quad (3)$$

From (2) and (3) we get

$$Z_{H_2O}/(1 - Z_{H_2O}) = K_1\,\{H_2O\} \quad (4)$$

In Fig. 2, $Z_{H_2O}/(1 - Z_{H_2O})$ is plotted against $\{H_2O\}$ for 5 per cent HDNNS in heptane and sulphuric acid–water, the system in which the lowest water activities were obtained. In agreement with (4) a straight line through the origin can be fitted to the data. From the slope of the line we find

$$K_1 = 21 \pm 1 \text{ (dimensionless)} \quad (5)$$

Further analysis along the same lines indicates the possibility of the species $HDNNS(H_2O)_3$, $HDNNS(H_2O)_8$ and a remainder, which can be formally described as $HDNNS(H_2O)_{20}$ in addition to $HDNNS{\cdot}H_2O$.

This sort of treatment is also applicable to the sorption of water by ion exchangers. In Fig 3, Z_{H_2O} is plotted against $-\log\{H_2O\}$ for the sorption of water by sulphonic acid resins in the silver form. The upper curve (○) refers to a 0·5 per cent DVB resin investigated at 0° C by Glueckauf and Kitt[4], while the lower curve (●) refers to a 10 per cent DVB resin studied at 25° C by Gregor *et al.*[5]. From Fig. 3 it is seen that two parallel curves are obtained of a shape very similar to that found for the liquid ion exchanger (of Fig. 1). Finally, in Fig. 4, $Z_{H_2O}/(1-Z_{H_2O})$ is plotted against $\{H_2O\}$ for the lowest water activities investigated by Glueckauf and Kitt. Here also reaction (1) with the equilibrium law formulated according to (4) applies to the data. Further analysis along these lines seems to indicate that both investigations can be described by $AgR(H_2O)_3$ and a remainder $AgR(H_2O)_{10}$ besides $AgR{\cdot}H_2O$, with different constants for the two temperatures.

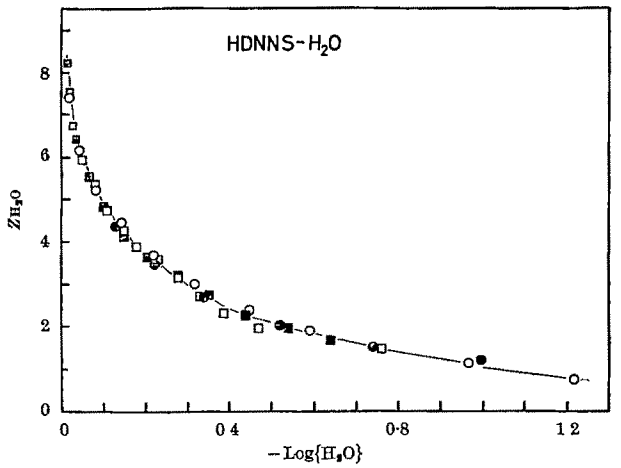


Fig 1. Z_{H_2O} plotted against $-\log\{H_2O\}$ for 5 per cent HDNNS in heptane and the following acid–water systems: □, nitric acid–water; ■, hydrochloric acid–water, ●, perchloric acid–water, ○, sulphuric acid–water

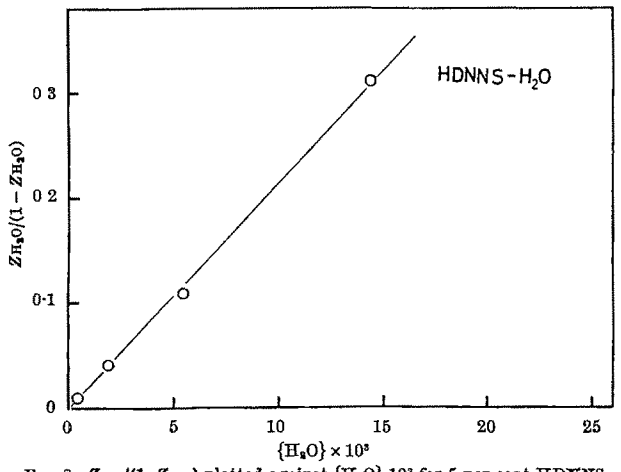


Fig. 2. $Z_{H_2O}/(1-Z_{H_2O})$ plotted against $\{H_2O\}\cdot10^3$ for 5 per cent HDNNS in heptane and sulphuric acid–water

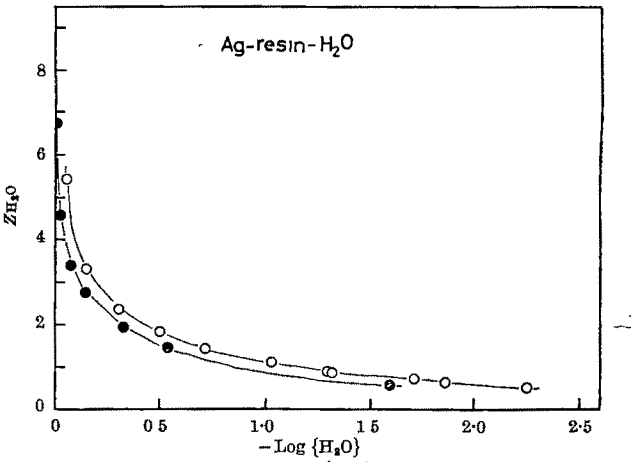


Fig 3 Z_{H_2O} plotted against $-\log\{H_2O\}$ for the systems: ○ AgR (0 5 per cent DVB)–water, $t = 0°$ C (ref 4); ●, AgR (10 per cent DVB)–water, $t = 25°$ C (ref. 5)

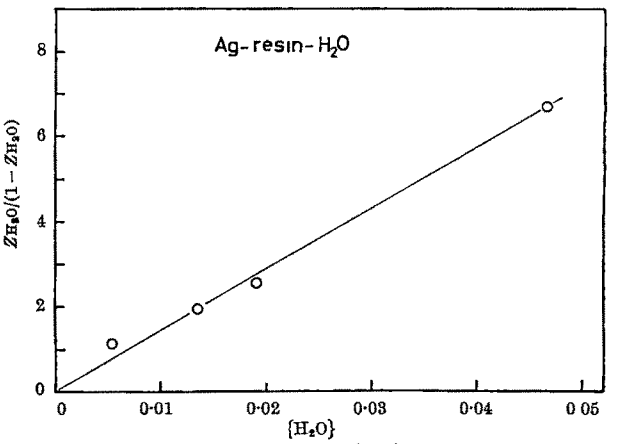


Fig. 4. $Z_{H_2O}/(1-Z_{H_2O})$ plotted against $\{H_2O\}$ for the system AgR (0 5 per cent DVB)–water, $t = 0°$ C

The system presented here offers at low water activities an alternative and simpler description than that given by Glueckauf[4], who suggests that the first water molecule goes to the sulphonic acid group and causes complete dissociation, while all the others go to the cation. Until experimental evidence is obtained in favour of one or the other system, the simpler one is to be preferred. At high water concentrations, dissociation has to be taken into account.

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Yields and Rate Constants of the Precursors of Hydrogen in the Radiolysis of *n*-Propanol

PRESENT-DAY interest in the radiolysis of aliphatic alcohols has been centred on the identification of the precursors of hydrogen. In the case of methanol and ethanol, investigations of pulse radiolysis[1,2] have led to the identification of the solvated electron in agreement with kinetic–mechanistic deductions[3–5]. Furthermore, scavenger studies[3–5] have shown the existence of a second reactive precursor, identified as the hydrogen atom.

It is our purpose to extend these results to higher alcohols in an attempt to correlate differences in precursor yields with the polarity of the medium. In the first of these studies we have investigated the formation of hydrogen in irradiated *n*-propanol in the presence of scavenger.

Propanol was purified by refluxing over 2,4-dinitrophenyl-hydrazine for 24 h to remove aldehydes and ketones followed by fractional distillation. Solutions of acetone (Merck, *pro analysi*) in this material were then degassed by repeated pumping and shaking and transferred to 10 ml. 'Pyrex' ampoules. These ampoules were irradiated for periods varying between 2 and 12 min after which the hydrogen formed was extracted in a modified Van Slyke gas pipette, injected into the carrier gas stream of a gas chromatograph and determined by conventional methods. The acetone concentration of the solutions after degassing was also determined by gas chromatography.

Irradiations were carried out in a nominal 400-c. cobalt-60 source giving a dose rate of $1{\cdot}1 \times 10^{17}$ eV/ml./min as determined by a Fricke dosimeter, taking $G(Fe^{3+})$ as 15·6 ions per 100 eV. The dose rate in propanol was found by correcting this value for the difference in electron density between the alcohol and the dosimeter solution.

Dose-yield curves for pure deaerated propanol were linear over the region investigated, and from the slope $G(H_2)$ was determined as 4·2 molecules/100 eV. In the presence of acetone the characteristic dependence of $G(H_2)$ on scavenger concentration found in the case of ethanol[4,5] was observed as is shown in Fig. 1.

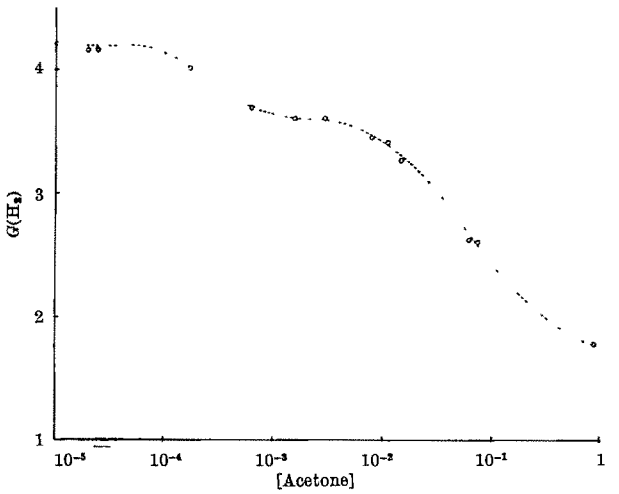


Fig. 1. $G(H_2)$ as a function of added [acetone]

As for ethanol, the initial decrease in $G(H_2)$ is attributed to electron scavenging by acetone. Thus the difference between the initial value, $G(H_2)_0$, and the plateau, $G(H_2)_p$, is equal to $G_{e^-solv.}$: the values determined from Fig. 1 are:

$$G(H_2)_0 = 4{\cdot}2 \text{ and } G(H_2)_p = 3{\cdot}6$$

thus $G_{e^-solv.} = 0{\cdot}6$ molecules/100 eV.

From the foregoing data it is also possible to estimate the rate constant of the reaction of the electron with acetone relative to reaction with propanol. This ratio was found to be:

$$\frac{k(e^- + (CH_3)_2CO)}{k(e^- + C_3H_7OH)} = \frac{k_1}{k_2} = 7 \times 10^4$$

Using the recently determined[6] absolute rate constant of the electron with acetone of $5{\cdot}9 \times 10^1$ l.mole^{-1} sec^{-1} we find:

$$k\,(e^- + C_3H_7OH) = 8{\cdot}5 \times 10^4 \text{ l.mole}^{-1} \text{ sec}^{-1}.$$

This estimation is based on the assumption that the rate constant k_1 is the same in propanol as in water. This is justified by the fact that a recent determination[7] of k_1 in ethanol is in good agreement with k_1 in water; similar observations[1,2] have been made in pulse radiolysis investigations of reactions in water, methanol and ethanol.

The further decrease in $G(H_2)$ can likewise be ascribed to competition between acetone and propanol for hydrogen atoms:

$$H + C_3H_7OH \xrightarrow{k_3} H_2$$

$$H + (CH_3)_2CO \xrightarrow{k_4} \text{products other than } H_2$$

Application of simple competition kinetics leads to the expression:

$$\frac{1}{\Delta G(H_2)} = \frac{1}{G(H_2)_p - G(H_2)} = \frac{1}{G_H} + \frac{k_3[C_3H_7OH]}{k_4[(CH_3)_2CO]G_H} \quad (1)$$

Thus a plot of $\frac{1}{\Delta G(H_2)}$ against $\frac{[C_3H_7OH]}{[(CH_3)_2CO]}$ should be linear with the intercept equal to $\frac{1}{G_H}$. From the slope $\frac{k_3}{k_4}$ may also be estimated. This was applied to the experimental data and the results obtained are shown in Fig. 2.

From Fig. 2 we find $G_H = 1{\cdot}90$ molecules/100 eV and $\frac{k_3}{k_4} = 6{\cdot}63 \times 10^{-3}$.

The remaining non-scavengeable or molecular yield can now be determined as:

$$G^M_{H_2} = G(H_2)_0 - (G_H + G_{e^-solv.})$$
$$= 1{\cdot}7 \text{ molecules/100 eV.}$$

The radical and molecular yields are summarized in Table 1; included are the corresponding yields from methanol and ethanol.

Table 1

Alcohol	$G(H_2)$	G_{e^-solv}	G_H	$G^M_{H_2}$
	Yield in molecules/100 eV			
Methanol (ref. 3)	5·4	1·4	2·4	1·6
Ethanol (ref. 4)	4·8	0·9	2·3	1·65
Propanol	4·2	0·6	1·9	1·70

Two interesting observations may be made from a comparison of these yields with those from methanol and ethanol. First, a systematic decrease in $G(H_2)$ from methanol to propanol, suggesting greater de-activation of excited states in the case of the larger molecules. Secondly, a decrease in the yield of solvated electrons is seen as

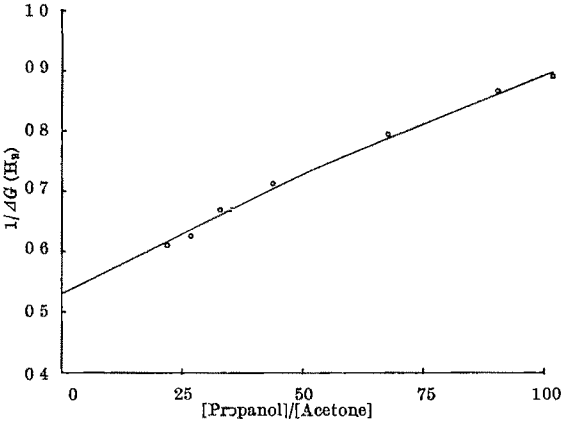


Fig. 2. Plot of equation 1 for hydrogen yields from propanol containing acetone

might be expected from the decreasing polarity of the solvents.

In view of the lack of information on the other products of the radiolysis a discussion of possible reaction mechanisms is not considered appropriate at the present time. A more detailed study of the system is at present being made, the results of which will be communicated at a later date.

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Unusual Co-ordination of α-Phenylbutylamine with Nickel Thiocyanate as a Result of Clathration

NUMEROUS α-arylalkylamines co-ordinate with nickel (II) thiocyanate to form complexes of the type $[Ni(NCS)_2(amine)_n]$, where n is 2 or 4 (ref. 1). The complexes for which n is 2 are light green and are insoluble in most solvents. These properties and their infra-red spectra[2] suggest that they have a polymeric octahedral structure with nickel atoms bridged through the thiocyanato groups. The complexes for which n is 4 are blue and are readily soluble in many solvents, such as methanol or chloroform. However, they are not soluble in water, in which they undergo hydrolysis with formation of nickel hydroxide. Our X-ray diffraction investigations[3], which are still in progress, have shown that these complexes are trans-octahedral with the thiocyanato groups linked through their nitrogen atom.

When nickel thiocyanate is allowed to react with an excess of some α-arylalkylamines, a new complex is obtained in which the nickel atom is apparently co-ordinated with five molecules of amine. The same compound may also be obtained by recrystallization of the tetramine complex in a methanol solution of the amine. With α-phenylbutylamine, a complex of the following composition was thus obtained: nickel, 6·4; thiocyanate, 12·6; α-phenylbutylamine, 80·9 weight per cent. Calculated for $Ni(NCS)_2(amine)_5$: nickel, 6·4; thiocyanate, 12·6; α-phenylbutylamine, 81·0.

Considering the aforementioned composition, this compound may be taken as five-, six- or seven-co-ordinate, according to the following formulations: $[Ni(Am)_5](NCS)_2$, $[Ni(NCS)(Am)_5](NCS)$ and $[Ni(NCS)_2(Am)_5]$. The first two structures can be rejected for several reasons. On one hand, the solubilities of the complex do not account for ionic character. On the other hand, the N—C stretching frequency of the thiocyanate group appears to be the same in the infra-red spectra of both the pentamine and the tetramine complex (2,096 cm^{-1}). It is known that the stretching frequency of the N—C bond is different for ionic and co-ordinated thiocyanate[4]. However, the seven-co-ordinate formula is also improbable as the spatial configuration of the molecule of α-phenylbutylamine should preclude such an arrangement.

Numerous complexes of the type $[Ni(NCS)_2(\alpha\text{-arylalkylamine})_4]$ are able to form clathrates with many aromatic compounds[5]. Those formed by the complex of α-phenylbutylamine are particularly stable and contain exactly one molecule of 'guest' per molecule of complex so that they may be considered as true stoichiometric compounds[6]. However, the association between both components does not involve covalent bonding but more probably charge-transfer interactions through the aromatic nuclei of both the guest and the amine co-ordinated to nickel[7]. In view of the aromatic character of α-phenylbutylamine, it is plausible that it should be clathrated as well as other aromatic compounds of similar shape. This interpretation is strongly supported by X-ray diffraction data. Fig. 1 shows the powder diagrams of (I) the tetramine complex, (II) the pentamine complex, and (III) the clathrate formed by the former with *sec*-butylbenzene. The three diagrams each have many diffraction maxima in common, but the similarity between (II) and (III) is particularly marked and shows that the pentamine complex is clearly a clathrate formed by the octahedral tetramine complex with one additional molecule of amine.

Previous investigations have shown the crystal structure of the clathrates formed by the complex $[Ni(NCS)_2(\alpha\text{-phenylbutylamine})_4]$ to be largely governed by the shape of the enclosed molecules. Thus, for example, the clathrates of *p*-xylene and *p*-dichlorobenzene have a similar structure which differs markedly from that of the original complex[6]. This conclusion is supported by the preceding data but, apparently, the clathration of *sec*-butylbenzene or of α-phenylbutylamine itself does not involve such a radical change in crystalline structure. Moreover, the tetramine complex of α-phenylbutylamine may be obtained as two distinct forms characterized by slightly different diffraction patterns, one of which is diagram (I) of the figure, whereas the second is quite similar to diagram (II). Similarly, the infra-red spectra of both forms are different but the spectrum of the second form is practically identical with that of the pseudo-pentamine complex. Obviously, one form of the tetramine complex has a structure which is not significantly modified

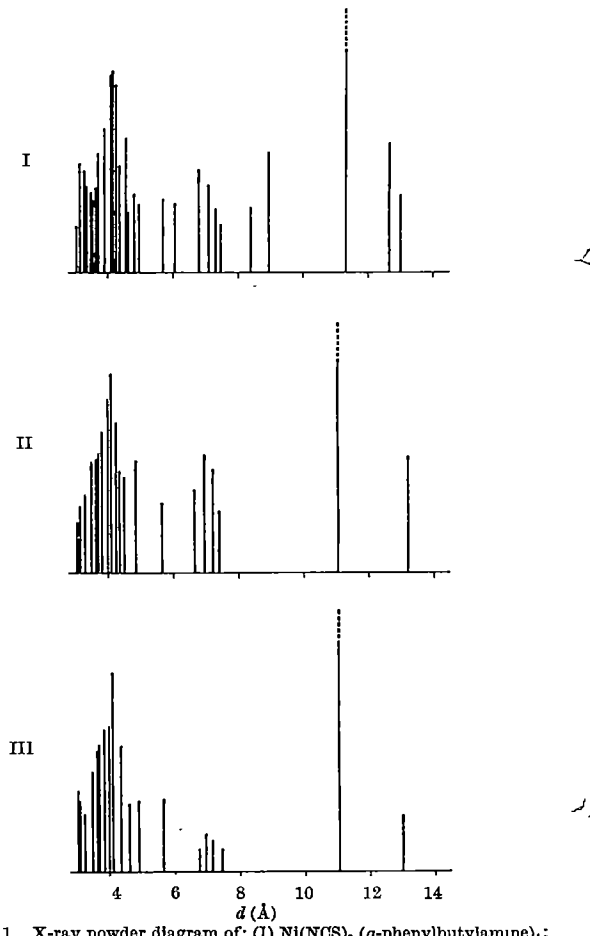


Fig. 1. X-ray powder diagram of: (I) $Ni(NCS)_2(\alpha\text{-phenylbutylamine})_4$; (II) $Ni(NCS)_2(\alpha\text{-phenylbutylamine})_5$; (III) clathrate formed by (I) with *sec*-butylbenzene

by the clathration of a fifth molecule of amine. In this case, clathration appears to be merely the result of filling spaces present in the crystal lattice of the complex.

The preparation of other co-ordination complexes in which nickel (II) is apparently seven-co-ordinate was recently reported[8]. These complexes belong to the type $[NiX_2(\text{methylpyrazine})_5]$, where X is a halogen atom or thiocyanate. Taking into account that complexes of methylpyridines with nickel (II) thiocyanate readily form clathrates with a great variety of aromatic compounds[9], there is little doubt that the abnormal stoichiometry of these methylpyrazine complexes has also to be explained by clathration.

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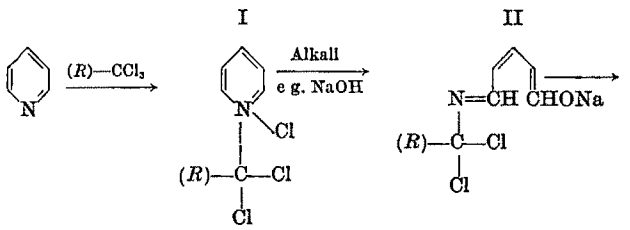
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The Fujiwara Reaction : Some Observations on the Mechanism

WHEN certain 1 : 1 : 1-trichloro-compounds are heated in the presence of pyridine and aqueous caustic alkali, a red colour is imparted to the pyridine layer[1,2]. The reaction is extremely sensitive. The nature of the red material has not been fully elucidated, but the following mechanism has been proposed[3]:



The reaction is used extensively in both toxicological screening[4] and for quantitative work[5–9], and because of this it is surprising to find that little work has been undertaken to elucidate the mechanism.

In this investigation the reaction was conducted by heating for 1 min in a boiling water bath a test-tube containing about 3 ml. aqueous caustic and 3 ml. pyridine, and (if colourless) adding the substance under test. Heating was continued for several minutes, and a positive reaction indicated by a red colour in the pyridine layer.

As a result of the following experiments, we concluded that the red reaction product from all 1 : 1 : 1-trichloro compounds tested (that is, chlorbutol, chloral hydrate, trichloroacetic acid, and chloroform) was identical and independent of the starting material: (1) The pyridine absorbed at 365 and 530 mμ in all cases. (2) The red pyridine, chromatographed by thin-layer chromatography (Merck 'Kieselgel *GF* 254'—solvent ethyl acetate, saturated with water), yielded red spots, after it had been sprayed with aqueous caustic of identical R_F (0·54). (3) In all cases the ethanol eluate of the red spots absorbed at 314 and 410 mμ, which shifted after adding aqueous caustic to 362 and 525 mμ and was reversed by the addition of concentrated hydrochloric acid. Spontaneous decomposition of the material absorbing at 410/525 mμ slowly occurred, leaving the 314/362 mμ material, which persisted.

It appeared likely that chloroform (or dichlorocarbene) was implicated, since hot caustic treatment of chloral hydrate and trichloroacetic acid is known to yield chloroform under these conditions[10] as does chlorbutol, which we have shown to produce acetone (2 : 4-dinitrophenyl hydrazone derivative, m.p. 122°–124° C, mixed m.p. 123°–124° C). By contrast, trichloroethanol does not produce chloroform or formaldehyde (except in trace amounts) but yields mainly glycollic acid (benzoyl derivative; m.p. 118°–121° C, mixed m.p. 118°–121° C) and this does not produce a red colour in the Fujiwara reaction.

Trichloroethylene ($CCl_2=CHCl$) also reacts to give a red colour with different spectrophotometric properties from the 1 : 1 : 1-trichloro derivative, with a different R_F value (0·75), and does not therefore conflict with this theory.

We found that a reaction would also occur in ice-cold conditions, and absorption curves in these cases varied according to the reactant. These are summarized in Table 1. Peaks in cold solution slowly drifted on warming or prolonged standing in most cases.

We propose on the basis of our findings the following mechanism for the Fujiwara reaction. The compound I absorbing at 390–400 mμ is formed initially by the attachment of the trichloro compound to the pyridine ring. This is the 'cold reactor' and has spectrophotometric properties and stability which differ according to the reactants. We are unable to demonstrate this complex under the normal 'hot' conditions except, transitorily, in the case of chlorbutol, and indeed it is possible that under hot conditions it does not form in all cases. It is possible that chloral hydrate and trichloroacetic acid do not directly couple but decompose to chloroform first, which then couples. Such a complex would never reach a measurable level with trichloroacetic acid since the reaction proceeds too slowly. All these 'closed ring' complexes are clearly unstable, and have not been reported hitherto. Ring breakage occurs giving compound II, which absorbs at 530 mμ. Feigl[3] has implied that these open chain compounds differ according to the starting material, but our experiments do not indicate this in the case of the 1 : 1 : 1-trichloro red reactors, since they are indistinguishable one from another. Further heating causes decomposition to glutaconic aldehyde (III). This absorbs at 365 mμ, an observation in agreement with Schwarzenbach *et al.*[11].

As further evidence of this theory, the eluted red spots exhibit a property consistent with a keto-enol structure, in that absorption bands vary with changes in *p*H. We suggest that the 410/525 mμ material is II and the 314/362 mμ material is I–II, both of which co-exist in the normal Fujiwara reaction. We have shown that prolonged standing or heating causes the disappearance of II, which is changed to III. This has been demonstrated both in the alcohol eluate and in the pyridine.

Table 1

Reactant	Absorption peaks under cold conditions (mμ)	Absorption peaks under normal hot conditions (mμ)
Chloroform	365 400 530	365 530
Chloral hydrate	400 530	365 530
Trichloroacetic acid	365 530	365 530
Chlorbutol	390	365 530
Trichloroethylene	365 530→540	365 540
Trichloroethanol	390	365 430

The strikingly different properties of the trichloroethanol reaction lead us to suggest that the whole molecule remains attached after ring breakage: first, because we were unable to obtain chloroform on heating trichloroethanol with aqueous potassium hydroxide; secondly, by analogy with the chlorbutol complex I, which we have shown to be relatively stable.

It is apparent that the reaction as normally conducted leads to a mixture of II and III, and that prolonged heating or standing converts II to III. Friedman and Cooper[8] use the peak at 365 mμ for quantitative measurements, as they find it more stable than the 530 mμ peak, which agrees with our observations. Furthermore, we find a decrease in optical density at 530 mμ produces a relatively small increase at 365 mμ, a fact previously observed[12]. It seems possible, therefore, that optimum conditions for quantitative work are more likely if the reaction is carried to completion, and all measurements of optical density are made on the glutaconic aldehyde. Whatever method is used, however, it is clearly vital in quantitative work to standardize conditions and time of heating, and length of delay before reading.

Although we did not pursue in detail the reaction with trichloroethylene, we did observe a somewhat similar phenomenon under ice-cold conditions, which is shown in Table 1.

The 'cold' reaction, although capable of distinguishing reacting compounds, has little micro-analytical value since 50–100 mg are necessary to demonstrate the phenomenon.

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Some Observations on Ions of Low Mass Number in Hydrocarbon Flames

An A.E.I. *MS2* mass spectrometer has been modified to sample ions from flames burning at atmospheric pressure. The spectrometer is of the 90° sector magnet type. The flame ions after traversing the magnetic field are collected on an electrode plate subsequent to passing through a twelve-stage Allen type electron multiplier. The system enables ion currents at the collector as small as 10^{-15} amp. to be measured.

In essence, the system bears close resemblance to that used by Knewstubb and Sugden[1] and Green and Sugden[2]. The burner used consists of a 6 in. length of capillary 'Pyrex' or silica tube of about 1 mm bore. The flame produced by this system is a fast burning pre-mixed flame of the Bunsen type. The ions are introduced into the mass spectrometer through a small orifice, 0·001–0·002 in. diameter, pierced in 0·002 in. thick platinum foil mounted in a massive water-cooled brass plate. The ions entering the apparatus are focused through a series of differentially pumped chambers, each at a successively reduced pressure, by a system of electrostatic lenses (Fig. 1).

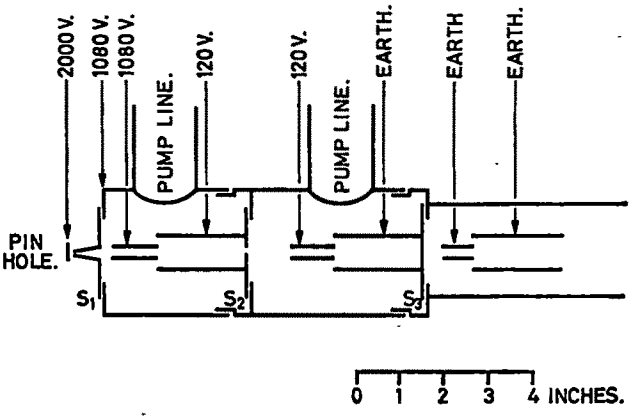


Fig. 1. Schematic diagram of ion collimating systems

The ion collimating system used in this work differs from that used by Knewstubb and Sugden in that the ions enter the pinhole at a potential of 2,000 V and are focused by a series of reducing potentials, finally arriving at the analyser tube at earth potential. Also with this system the ions arrive more quickly in the low-pressure region of the spectrometer, effecting a rapid quenching effect on the ions and reducing the chance of collisions in the ion beam.

Ion spectra can be scanned, either manually, or automatically in conjunction with a chart recorder system, by varying the magnetic field.

A hydrogen/air flame was chosen as the basic flame for the experimental work, small amounts of hydrocarbon additive being added either from a direct supply of commercially bottled gas or in the case of liquid additives by the use of a concentric jet atomizer. A wide range of hydrocarbon additives have been studied including methane, ethylene, acetylene, benzene, kerosene and petroleum.

A direct study of the flame by Schlieren photography has revealed that the flames are quite laminar within the sampling zone, which was between the reaction zone and about 1 in. downstream. The Schlieren work has also clearly shown the presence of the boundary layer on the sampling surface reported by Green and Sugden[2], and has given some indication of its size.

Several interesting features have been observed which have not been reported previously[1–4]. Experiments have shown the presence of H^+, H_2^+ and H_3^+ ions in hydrogen/air/hydrocarbon flames. The identity of these ions has been confirmed using deuterium in place of hydrogen. The H_2^+ and H_3^+ ions are present in about equal concentrations, producing ion currents of around 10^{-15} amp. These ions reach a maximum concentration in the reaction zone and persist downstream into the burnt gas region.

Results show that the concentration of the H_2^+ ion relative to the total ionization was dependent to a considerable extent on the size of the input orifice in the platinum foil. However, the concentration of the H_3^+ ion was not so dependent suggesting that this is a 'true' flame ion and that the H_2^+ ion is most probably formed within the sampling system.

The proton is present to the extent of 1/500th of the concentration of the most abundant ion, H_3O^+, the latter producing an ion current on the collector plate of about 10^{-11} amp. The *MS2* in standard form is capable of detecting ions at mass 2, but not at mass 1. Thus in order to establish whether or not an ion of mass 1 existed in the flame, the spectrometer had to be modified. For this purpose there are two alternatives—namely, reducing the current to the magnet coil or increasing the accelerating voltage. In our case the former modification was the

simpler. Unfortunately, the instability of the magnetic field at low magnet coil currents prevented an accurate profile of the H^+ ion along the flame from being obtained.

However, using deuterium instead of hydrogen it was possible to obtain an axial profile of the D^+ ion in a deuterium/air/acetylene flame. This is shown in Fig. 2, which also shows a similar profile for the D_3O^+ ion taken at the same positions in the same flame. The similarity between the two profiles suggests the possibility of an equilibrium between the proton and the hydroxonium ion, and accounts for the presence of the protons.

$$H^+ + H_2O \rightleftharpoons H_3O^+$$

A further interesting feature of this work has been the observation of the presence of detectable ion currents in the mass number region 7–11 in hydrocarbon flames. A well-defined peak occurring at about mass number 10 has not so far been clearly assigned. Using deuterium it was observed that the peak shift is about 2 mass numbers.

Addition of lithium chloride to the flame does not enhance the ion currents observed in this region due to the formation of ions of lithium hydride. It is therefore concluded that the ions are not due to the presence of lithium impurities either in the flame or from the 'Pyrex' burner.

The shape of the peak at mass number 10 does not suggest the presence of a meta-stable ion—a possibility which is unlikely due to the apparent absence of higher molecular weight hydrocarbon peaks, these usually being associated with the formation of meta-stable ions.

The presence of the doubly charged ions H_3O^{++} and H_4O^{++} at mass numbers $9\frac{1}{2}$ and 10 respectively cannot be ruled out and this possibility is receiving further consideration.

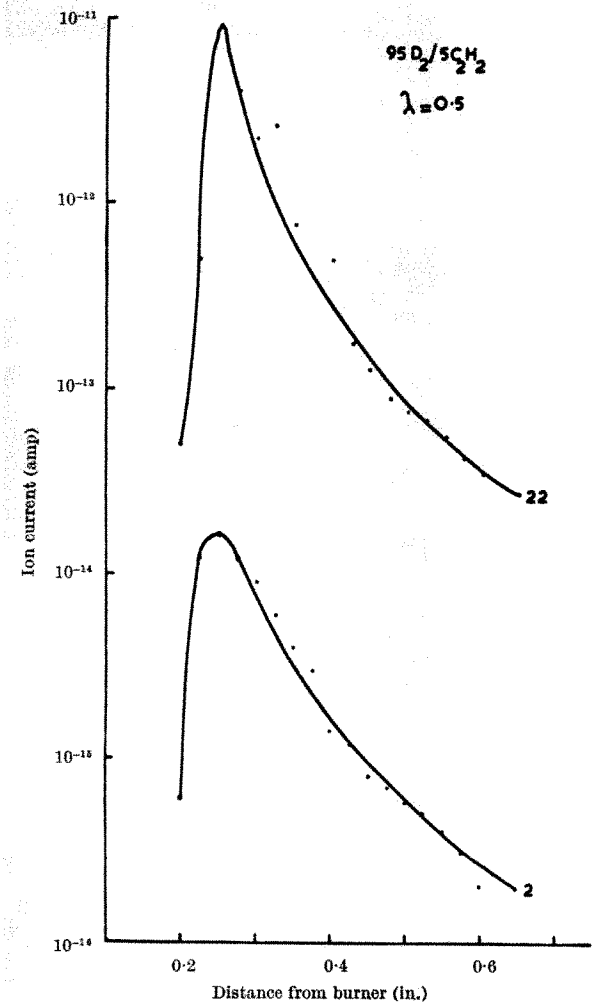


Fig. 2. Profiles of ions of mass numbers 2 and 22 in a deuterium/acetylene/air flame

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BIOCHEMISTRY

Enzymatic Synthesis of Labelled Cytidine-5′-Diphosphate Ethanolamine

The chemical methods available for preparing cytidine diphosphate ethanolamine (CDP-ethanolamine) either give a poor yield of the product[1] or require rather elaborate procedures[2,3]. In the method described here, use has been made of the enzyme CTP : ethanolamine-phosphate cytidylyltransferase (*EC*. 2.7.7.14) from rat liver, to prepare cytidine-5′-diphosphate-(2-^{14}C)-ethanolamine and cytidine-5′-(β-^{32}P)-diphosphate ethanolamine in high yield. This enzyme, which was originally described by Kennedy and Weiss[4], is present exclusively in the particle-free supernatant from a tissue homogenate[5,6].

A 20 per cent homogenate of adult rat liver (4 g) in 0·3 M sucrose was centrifuged at 100,000*g* for 1 h. The supernatant (10 ml.) was applied to a column (2 × 20 cm) of 'Sephadex *G*-25' (or *G*-50), particle size 20–80μ and eluted with 0·01 M phosphate buffer, *p*H 7·6. Collection was commenced when the effluent became reddish in colour, and was stopped when the effluent became colourless. The red effluent (10 ml.), containing the bulk of the cytoplasmic protein, could be stored at 0° C, or in a frozen state, for 3–4 days with little loss of cytidylyltransferase activity.

Phosphorylethanolamine, labelled with phosphorus-32 or carbon-14, was prepared using a method similar to that of Riley[7], by heating equimolar amounts of ethanolamine and orthophosphoric acid at 155°–160° C for 4 h at a pressure of 10 mm of mercury. Phosphorylethanolamine was isolated from the reaction mixture using column chromatography on 'Dowex 1 × 2 (formate)[8]. The fraction containing phosphorylethanolamine was evaporated to dryness, dissolved in water and the *p*H adjusted to about 7 with N sodium hydroxide.

To the cytidylyltransferase preparation (5 ml., containing 15 mg protein per ml.) was added 0·1 M potassium *ortho*phosphate–sodium hydroxide buffer, *p*H 7·6 (4·5 ml.), M magnesium chloride (0·5 ml.), 0·001 M phosphorylethanolamine (10 ml.), and 0·04 M cytidine triphosphate disodium salt (Schwarz BioResearch, Inc.) (2·0 ml.). After incubating at 37° C for 2 h, the reaction mixture was heated at 100° C for 2 min to precipitate the protein, cooled to 0° C, diluted to 50 ml. with water and the *p*H adjusted to 8–9 using N sodium hydroxide (1·0 ml.).

The cloudy fluid was centrifuged, and the supernatant and subsequent washings (80 ml.) passed through a 'Dowex 1 × 2' (formate) column (1 × 15 cm), which was then washed with 50 ml. of water. Elution of the column was carried out in a manner similar to that of Kennedy[1], by linearly increasing the concentration of formic acid. The linear gradient was obtained by running 0·04 M formic acid into the mixing chamber, which originally contained water (500 ml.). When collecting fractions of 10 ml., CDP-ethanolamine appeared in fractions 21–24; it was preceded by a peak of unreacted phosphorylethanolamine

(fractions 10–14) and succeeded by a small peak of cytidine-5′-monophosphate (fractions 27–31). The CDP-ethanolamine was evaporated to dryness at about 40° C in a rotary evaporator.

On analysis, the product gave the correct ratio for cytidine : phosphorus and was homogeneous when examined by paper chromatography in isopropanol : water : trichloroacetic acid : 18 N ammonium hydroxide (75 : 25 : 5 : 0·3, v/v/w/v)[8]. Hydrolysis with N hydrochloric acid at 100° C for 1 h yielded only cytidine monophosphate and phosphorylethanolamine. The product was as effective as the chemically synthesized nucleotide in transferring phosphorylethanolamine to phospholipid precursors in brain and liver homogenates.

Using a molar ratio of phosphorylethanolamine to cytidine triphosphate of 1 : 8 in the reaction mixture, yields of CDP-ethanolamine, based on phosphorylethanolamine, were 40–60 per cent. A yield of about 30 per cent was obtained with a molar ratio of 1 : 2. The original liver homogenate cannot be used efficiently for the biosynthesis, since the yield of CDP-ethanolamine is reduced both by hydrolytic enzymes and by the presence of CDP-ethanolamine : 1,2-diglyceride ethanolamine phosphotransferase. Though liver supernatant alone was as active in the reaction as the effluent from 'Sephadex', this step removed compounds of low molecular weight, including endogenous nucleotides, which would otherwise have been difficult to remove from the final product.

This procedure can be recommended for a rapid, efficient and inexpensive synthesis of labelled CDP-ethanolamine.

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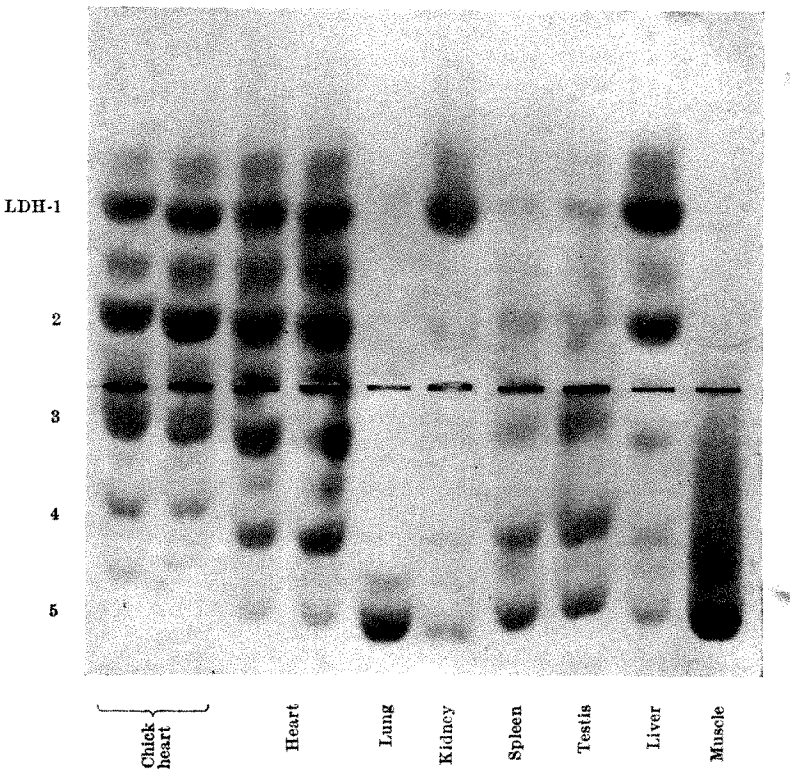


Fig. 1. Photograph of a starch-gel zymogram of penguin isozyme patterns. Note that each organ shows a characteristic pattern. The chick heart patterns are slightly different from the adults, though nearing completion of their ontogeny. The five major isozymes are numbered; the intercalated bands probably represent permutations of the major five though still showing some specificity in tissue distribution. The muscle tissue was from the pectoralis major

Stability of Lactate Dehydrogenase Isozyme Patterns in Penguins

PENGUINS were chosen for this investigation because of their highly specialized adaptation to an aquatic life and because of the extreme physiological conditions they are subjected to during the breeding season. One of the most abundant species is the Adelie penguin, *Pygoscelis adeliae*, which has a circumpolar distribution south of the Antarctic pack-ice. Unlike other birds this species endures a prolonged fast averaging 6 weeks during incubation[1] and another fast of shorter duration when it moults. At both times they may lose more than 40 per cent of their body-weight. Though flightless, their pectoralis muscles are well developed for extensive swimming and diving at sea.

Material from sixteen Adelie penguins and one African penguin, *Spheniscus demersus*, was examined. The African penguin was a captive bird which had died from aspergillosis. The Adelies were collected at the Cape Crozier rookery on Ross Island (77° S., 169° E.) at the end of the season in February 1964, when most of the adults and the chicks were leaving for their winter sojourn in the pack-ice. Four physiological conditions were represented in the specimens:

(i) *Parents* (six males and two females). Birds that were either arriving with full stomachs or about to depart to sea having regurgitated semidigested food to their offspring. The weight of these birds varied from 3·2 kg to 4·7 kg and their blubber (also an indication of their state of nourishment) from 2 mm to 4 mm thick. It is believed that a small amount of food would be digested, but most of it remains in the stomach for feeding the chicks.

(ii) *Pre-moult adults* (three males) could have been non-breeders, unsuccessful breeders, or parents after chick-rearing[2]. Before the moult Adelies gorge with food at sea and rapidly gain weight, accumulating large deposits of fat. Our three birds weighed 6·0, 6·3 and 6·5 kg, respectively, and their blubber thickness was 10–12 mm.

(iii) *Post-moult adults* (two males). These Adelies had fasted on land for no less than 2 weeks during the moult. Most of their fat reserve would have been metabolized. Our two specimens weighed 2·9 kg and 3·4 kg and their blubber was 2 mm or less in thickness.

(iv) *The chicks* (two males and one female) had been deserted by their parents and were ready for their first swim at sea. Two of our samples were dying from starvation, though fully developed. The other was strong and capable of survival at sea. Their weights were 1·5, 1·8, and 2·1 kg.

Thus, these four physiological states represented extreme emaciation and inactivity (starving chicks and post-moult adults) for comparison with parents which had been swimming and living at sea, and with pre-moult birds which had accumulated a great store of fat before the prolonged fast and inactivity away from sea during the forthcoming moult.

Seven of the Adelie penguins were dissected soon after being killed, the tissues frozen within 2 h and kept in this state until analysed. The rest of the birds were frozen whole in the Antarctic, thawed for dissection in the United States and the material refrozen immediately.

The penguin tissues were assayed for their content of lactate dehydrogenase (LDH) isozymes by the usual procedures of starch-gel electrophoresis[3]. Tissues were homogenized in phosphate buffer *p*H 7·2 and electrophoresed about 24 h at 4° C. A photograph of a starch-gel zymogram is shown in Fig. 1. Like all other vertebrates studied, each tissue of the penguin shows a specific pattern in terms of the relative abundance of the different isozymes. The patterns in penguins are unusual in that the net charge on the isozymes is more positive than is true for mammals or other birds that have been investigated. These observations were also true for the African penguin, although it is distinguishable from the Adelie in its isozyme pattern. Moreover, the pattern in the Adelie heart tissue is remarkable in showing large amounts of all the isozymes, not just those at the LDH-1 end of the spectrum as is true for other warm-blooded animals. At least nine bands of LDH activity are evident in heart homogenates. By analogy with mammalian systems five major isozymes can be identified. These are presumably the five tetramers of *A* and *B* sub-units. The four additional minor isozymes, conspicuous in heart tissues, but not so evident in other tissues, constitute an unexplained deviation from the simple patterns seen in avian and mammalian tissues[4]. A similar type of pattern in heart tissues has, however, been found in a fish (*Merluccius bilinearis*[3]) and analysis of this pattern in the fish heart indicates that the extra four bands intercalated among the major five are probably permutations or modified versions of them. The specific molecular responsibility for their electrophoretic behaviour has not been determined.

The characteristic tissue specific patterns of isozymes strongly imply that each enzyme plays a specific physiological part, and because we now know that at least two genes are involved in the synthesis of LDH isozymes the different patterns pose a problem in differential gene function—or gene regulation. Much experimentation has been directed towards changing isozyme patterns by altering the cellular environment of cells in tissue culture[5] or attempting to correlate the dramatic changes evident during ontogeny with physiological conditions. These efforts have so far failed.

Since the penguin is probably subjected to greater extremes of physiological stress than any other bird, its tissues might be expected to reflect these conditions in changed isozyme patterns. However, our results show that in the adult the isozyme content of tissues is highly constant. Some slight indication of terminal ontogenetic changes in heart patterns was evident in the penguin chicks (Fig. 1), but they were already fully developed and ready for departure to sea. Only the liver zymograms showed some differences among individuals, but the differences could not be correlated with any particular physiological state or method of collection. The conclusion from our analyses must therefore be that tissue content of isozymes is highly characteristic of a state of cellular differentiation but is quite refractory to change in the face of the most extreme forms of environmental or physiological stress. It is difficult to reconcile this stability in the adult, subject to extreme physiological changes, with the extensive changes that occur during embryonic life. It would seem that the molecular events related to the functioning of LDH are divorced from the mechanisms regulating the genes which control the patterns of isozyme synthesis.

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Distribution of Selenium in the Tissues of the Eye

FOR the past few years we have been investigating the presence and distribution of selenium in the normal and dystrophic state[1,2]. The biological role of selenium is as yet unknown; however, the physical and chemical properties of the element are well established. One of the outstanding characteristics of selenium is that it behaves differently in the light and in the dark. This fact is used to advantage in a number of modern instrumentations, such as the photoelectric colorimeter, the xerographic process, and the so-called 'electric eye'. Consequently, we were interested in determining whether selenium is a constituent of the eye in various animal species as well as in man.

Eyes of the lamb, calf and pig were collected directly from Charles Miller's slaughter house in North Bergen, New Jersey. They were kept in dry containers surrounded by wet ice and were processed immediately on arrival at the laboratory. Each whole eye was weighed, freeze-dried, and selenium was determined by the method of Taussky *et al.*[3,4].

The data in Table 1 represent the arithmetical mean of six animals of each species ranging from six months to one year. The age of this group is significant, because it has previously been observed[2] that selenium shows a tendency to increase in the tissues of chickens as they grow older. The Manhattan Eye Bank provided the human eyes. This group ranged in age from sixty to eighty years, which may explain the high value of their selenium content.

Our next step was to investigate the distribution of selenium in the different tissues of the eye, because we expected to find the greatest concentration in those parts

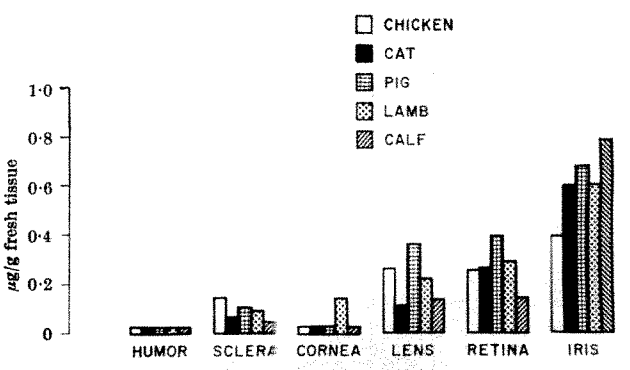


Fig. 1. Distribution of selenium in the tissues of the eye of five species ♀♂

Table 1. SELENIUM CONTENT OF THE WHOLE EYE

Species	Wet weight (g)	Selenium (μg)
Baby chicks	0·4–0·6	0·04
Chicken	1·5–2·5	0·19
Cat	3·0–4·0	0·20
Pig	6·0–7·0	0·39
Lamb	11·0–12·0	0·85
Calf	18·0–19·0	0·73
Human	6·0–8·0	5·98

Table 2. SELENIUM CONTENT OF THE TISSUES OF THE EYE

Tissue	Chicken		Cat		Pig	
	Wet wt. (g)	Selenium (μg)	Wet wt. (g)	Selenium (μg)	Wet wt. (g)	Selenium (μg)
Humour	1·30	0·01	1·72	0·01	3·86	0·02
Sclera	0·14	0·03	0·38	0·04	1·24	0·12
Cornea	0·02	Trace	0·20	Trace	0·18	Trace
Lens	0·08	0·02	0·55	0·06	0·18	0·06
Retina	0·30	0·07	0·45	0·08	0·37	0·14
Iris	0·04	0·02	0·05	0·03	0·03	0·02

Table 3. SELENIUM CONTENT OF THE TISSUES OF THE EYE

Tissue	Lamb		Calf		Human	
	Wet wt. (g)	Selenium (μg)	Wet wt. (g)	Selenium (μg)	Wet wt. (g)	Selenium (μg)
Humour	6·80	0·03	12·63	0·02	4·92	2·20
Sclera	2·46	0·20	3·02	0·15	1·27	2·20
Cornea	0·22	0·03	0·40	0·01	0·11	0·09
Lens	0·88	0·19	1·10	0·15	0·17	0·07
Retina	0·76	0·22	1·15	0·16	0·38	1·29
Iris	0·41	0·25	0·42	0·32	0·16	0·14

concerned with the regulation of light. We investigated six tissues: the humour, sclera, cornea, lens, retina, and iris (Fig. 1). Whereas the aqueous and vitreous humour, the sclera, and the cornea show only traces of selenium, the lens and retina have larger amounts, and the iris has the greatest accumulation. These results are expressed as concentrations of selenium; the actual amount of selenium per tissue is shown in Tables 2 and 3.

Investigations of selenium in the eye of additional species, as well as of man in younger age groups, are in progress.

We thank Messrs. Albert and Gordon Miller for contributing the eyes to our investigations. We also thank Mrs. Cornelius E. Rhoads, director of the Manhattan Eye Bank, for her collaboration. The work was supported in part by a grant from Muscular Dystrophy Associations of America, Inc.

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Lysozyme and Serum Bactericidal Action

NORMAL human serum kills Gram-negative bacteria by the antibody-complement system[1–4]. Killed bacteria are converted to spheroplasts by lysozyme[5–8]. Subsequent loss of intracellular contents by spheroplasts leaves the empty cytoplasmic membranes or ghosts[5,8]. Serum action proceeds as follows[8], using R for viable bacterial rod, R^* for killed rod, S for spheroplast, and G for ghost:

$$R \xrightarrow[\text{Complement}]{\text{Antibody}} R^* \xrightarrow{\text{Lysozyme}} S \longrightarrow G$$

However, Inoue *et al.*[6] also reported that a high concentration of lysozyme enhanced the killing or bactericidal action of serum. This is at variance with the generally accepted concept that cell wall damage by the antibody-complement system makes these bacteria susceptible to lysozyme[5,7,8].

The influence of a high concentration of lysozyme on serum action was therefore studied by kinetic analysis which allows a correlation of the bactericidal action of serum with morphological changes in bacteria[8]. Smooth *Escherichia coli* 0:127 in rapid growth phase from brain-heart infusion broth culture were collected by centrifugation and suspended in 0·15 sodium chloride at an optical density of 0·40. Optical density was measured spectrophotometrically at 650 mμ with the suspension in 16 × 150 mm tubes. Numbers of viable bacteria were determined by growth assay[9]. Differential counts of bacterial rods, spheroplasts, and ghosts were made on samples fixed in 2 per cent formalin using a phase-contrast microscope. Two experiments were done at the same time on bacteria from one broth culture. One tube contained 0·25 ml. normal human serum, 2·0 ml. bacteria, and sufficient saline to make 3·0 ml. The second tube contained similar volumes of serum and bacterial suspension with egg white lysozyme added to a concentration of 100 μg/ml. in a total volume of 2 ml. The bacterial suspensions were chilled rapidly in an ice bath, the other reagents added, and both tubes were then incubated in a water bath at 37° C. Samples were taken frequently for the growth assay and microscopic counts.

The results of these experiments are illustrated in Figs. 1 and 2. Normal serum killed the bacteria and transformed the bacterial rods to spheroplasts and ghosts. Added

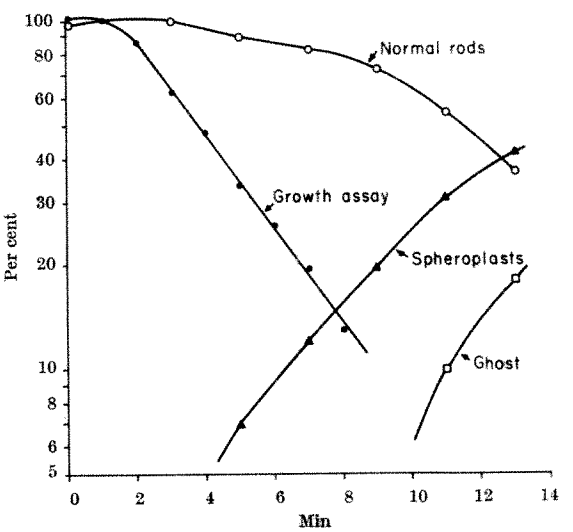


Fig. 1. Killing of *E. coli* and formation of spheroplasts and ghosts by normal human serum

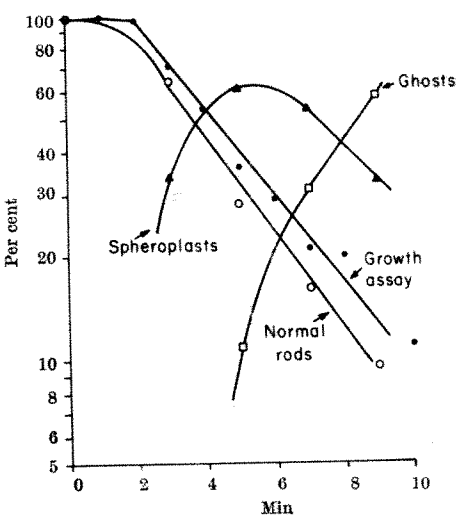


Fig. 2. Killing of *E. coli* and formation of spheroplasts and ghosts by serum with added 100 μg/ml. lysozyme

egg white lysozyme had no demonstrable effect on the rate of serum killing as determined by growth assay, but did increase the rate of transformation of killed rods to spheroplasts. Similar experiments, using colony counts instead of the growth assay, also failed to detect any influence of the added lysozyme on serum killing.

Egg white lysozyme at 100 μg/ml. had no effect on this *E. coli* in the absence of serum. Killed bacterial rods, prepared by exposing the *E. coli* to bentonite-absorbed serum[6], were promptly converted to spheroplasts by lysozyme. Spheroplasts, prepared with serum[8] and stabilized by 0·006 M calcium chloride, were not affected by lysozyme.

These investigations support the concept that the role of lysozyme in serum action is limited to the conversion of killed bacteria to spheroplasts.

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Actomyosin of Human Vascular Smooth Muscle

Actomyosin of vascular smooth muscle appears to be similar in certain properties to actomyosin of uterine smooth muscle[1]. These properties include solubility, viscosity (ATP sensitivity), and ATPase activity. Needham[2] first observed that uterine actomyosin showed an elevated ATPase activity when the potassium chloride concentration was raised from 0·1 M to 0·5 M. Filo *et al.*[3] also observed this to be true for hog carotid artery actomyosin, and we have observed the potassium chloride-induced elevation of ATPase activity of actomyosin of cow carotid artery[1]. In extending our investigations of contractile proteins of vascular smooth muscle to human blood vessels, we find that in the properties of solubility and viscosity behaviour (ATP sensitivity) the actomyosin of human umbilical artery is similar to the contractile proteins of other vascular smooth muscles. However, with respect to the potassium chloride induced elevation of the ATPase activity, the actomyosin of the human umbilical artery differs from the contractile proteins of other vascular smooth muscles. This report presents data to show that, unlike the actomyosin ATPase enzymes of those smooth muscles studied to date, that of the human umbilical artery is not stimulated by high concentrations of potassium chloride (0·5–0·6 M).

Umbilical cords, obtained from the hospital delivery room, were collected over a 24 h period and maintained in Ringer's solution at 4° C. The arteries were separated from the cords, rinsed in saline, minced, and homogenized using three volumes of the Weber–Edsall medium consisting of 0·6 M potassium chloride, 0·01 M sodium carbonate, 0·04 M sodium bicarbonate, and fortified with 5×10^{-4} M ATP (final concentration). The whole homogenate was stirred overnight at 4° C and the actomyosin was extracted as previously described[1]. The final yield of human umbilical artery actomyosin was 4 mg/g of wet weight. ATPase assays and viscosity runs for ATP sensitivity were carried out as previously described[1].

Table 1 demonstrates that artery actomyosin, either from the cow or the human, shows essentially the same degree of viscosity change in response to added ATP. This parameter, the ATP sensitivity, is a useful property in comparing various contractile proteins. For example, skeletal muscle actomyosin and cardiac actomyosin show ATP sensitivities in the neighbourhood of 150. Uterine and artery actomyosins are usually well below this, generally in the range of 30–60 (refs. 2 and 4).

It can be seen by the data in Table 2 that the actomyosin of the human umbilical artery has ATPase activity which is not stimulated by elevated concentrations of potassium chloride. In this respect the actomyosin of human umbilical artery differs from the actomyosins of cow artery, hog artery, and uterine smooth muscle[1–3]. Control studies with actomyosin of skeletal muscle showed that enhanced enzyme activity induced by high concentrations of potassium chloride was specific for the smooth muscle proteins. The ATPase activity of human artery actomyosin, like those of all actomyosins studied so far, is activated by either calcium or magnesium provided that the potassium chloride concentration is of a low ionic strength (final concentration 0·05–0·1 M). In the presence of a high ionic strength concentration of potassium chloride (0·5–0·6 M) magnesium depresses the ATPase activity.

Table 1. ATP Sensitivity of Artery Actomyosin (Human Umbilical)

Preparation No.	ATP sensitivity*
1	66
2	51
3	59
4	51
5	53

Conducted at 25° C using an Ostwald viscometer (3·0 ml.). Preparation 5 is actomyosin derived from cow carotid artery. All protein concentrations were between 4 and 5 mg/ml. in 0·5 M potassium chloride, 0·1 M *tris* pH 7·2, and 0·002 M ATP (final concentration).

* ATP sensitivity in per cent $= \frac{\log \eta_{rel} \log \eta_{rel\ ATP}}{\log \eta_{rel\ ATP}} \times 100$

Log η_{rel} = logarithm relative viscosity.

Table 2. The Effect of High and Low Concentration of Potassium Chloride on ATPase Activity of Actomyosin of Human Artery

Preparation No.	Specific activity* High KCl	Low KCl
11	0·8	0·9
13	1·2	1·5
14	1·3	2·0
15	0·7	0·9
16	0·6	0·6
17	0·6	1·1
18	1·3	1·3

Conditions: actomyosin of different preparations of human umbilical artery, 1–2 mg protein/ml. in 0·02 M imidazole buffer, *p*H 7·2, 0·005 M calcium chloride and either 0·05 M potassium (low KCl) or 0·5 M potassium chloride (high KCl), ATP (final conc. 0·0025 M) was added to start the reaction. The reaction was run at room temperature for 10 min.

* Specific activity equals micromoles inorganic phosphorus liberated per mg protein per min.

Another difference between the protein of the umbilical artery and the other smooth muscle proteins was observed: whereas the alkali earth metal cations of barium chloride, calcium chloride, and strontium chloride stimulated the ATPase activity of contractile proteins of cow aorta and cow carotid artery, barium chloride and strontium chloride had no such effect on the ATPase activity of the umbilical artery protein. (The effect of these ions on the contractile protein of the uterus was not investigated.)

These results indicate that the stimulation of actomyosin ATPase activity by high concentrations of potassium chloride is not a general effect observed in all smooth muscle contractile proteins, and that umbilical artery is distinct in this respect from uterine smooth muscle and from vascular smooth muscles such as hog and bovine carotid artery and bovine aorta. Whether this is a species difference or a reflexion of the anomalous nature of the smooth muscle of the umbilical artery remains to be investigated.

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Cystine Content of Silk Fibroin (*Bombyx mori*)

UNTIL recent years silk fibroin was thought to be free from the sulphur-containing amino-acids, and in a critical assessment of many amino-acid analyses in 1958 (ref. 1) it was concluded that there was insufficient evidence to support the theory that cystine was present in fibroin. Since that time there have been several reports of the presence of cystine in this protein.

Schroeder and Kay[2] had already estimated cystine in 1954 during their amino-acid analysis of fibroin, though they did not regard its presence as proved. They used the method of Schram, Moore and Bigwood[3], in which the protein was oxidized with performic acid and hydrolysed; the cysteic acid formed was estimated on a column of 'Dowex 2' and indicated 0·18 per cent of cystine in fibroin. Zuber, Ziegler and Zahn[4] found a content of 0·12 per cent by estimating the cysteic acid in hydrolysates of performic acid-oxidized fibroin by high-voltage electrophoresis and reaction with ninhydrin.

Earland and Raven[5] converted the amino-acids in a fibroin hydrolysate into their 2 : 4-dinitrophenyl derivatives, oxidized these with performic acid, and separated the strongly acidic DNP-cysteic acid from the other DNP-amino-acids by high voltage electrophoresis in 33 per cent acetic acid. They were able to obtain 35 mg of the compound and thus add elementary analyses to the chromatographic evidence of its identity. The amount of DNP-cysteic acid isolated corresponded to a cystine content of 0·13 per cent.

More recent evidence for the presence of cystine was given by Gustus[6], who showed that treatment of fibroin with liquid ammonia gave the blue colour characteristic of proteins containing fully combined cystine residues. Thus the presence of cystine in fibroin now seems to be well established.

In view of the importance of this amino-acid in relation to the structure of the fibroin molecule, it is desirable to have an accurate assessment of the cystine content. To this end determinations have been made by two independent methods, due to Goa[7] and Moore[8]. The first method involves heating with hydrazine to convert cystine to hydrogen sulphide, which is measured colorimetrically after absorption in a bismuth nitrate reagent. In the second method the protein is oxidized by performic acid, to convert cystine residues to cysteic acid, and afterwards hydrolysed; the cysteic acid is then determined on an amino-acid analyser.

These methods were applied to two types of fibroin, namely, that taken directly from the glands of silkworms and that prepared from raw silk after removal of sericin by degumming. Gland fibroin was prepared from glands removed from silkworms as they were about to spin. After a brief rinse in water, the glands were coagulated in 25 per cent (v/v) alcohol : water for about an hour. The outer layers of cells and sericin were then removed and the core of fibroin was cut into small pieces, washed in turn with water, alcohol and ether, and then dried *in vacuo*. Raw Japanese silk was degummed in soap solution and washed as described by Lucas, Shaw and Smith[9]. A sample of raw silk was also degummed by the method of Dunn *et al.*[10], which involves the treatment with water under pressure at 120° C for 10 h.

The analyses were made on samples that had been dried to constant weight over phosphorus pentoxide *in vacuo*. The procedures of the original authors were followed, except for a few modifications that were made because the small cystine content necessitated the use of relatively large samples of protein. In Goa's method, 25–100 mg samples of fibroin were heated with 1 ml. hydrazine hydrate at 140° C in sealed tubes for 18 h; even with this prolonged treatment traces of insoluble material remained in the digests. The contents of the tubes were transferred to an all-glass apparatus similar to that of Goa, and acidified with 3 ml. of 18 N sulphuric acid. The hydrogen sulphide liberated was carried by nitrogen into 4 ml. of the bismuth nitrate reagent and the optical density of the brown colour produced was measured at 400 mμ in a 1-cm cuvette on a 'Unicam *SP* 600' spectrophotometer against the reagent as blank. Standard solutions of cystine containing 20–150 μg, subjected to the same procedure, gave the relationship: $C = 20{\cdot}2\,E + 1{\cdot}89$ between the amount of cystine (C, μg) and optical density (E) and from this the quantity of cystine in each sample of fibroin was calculated.

Moore's method was used unchanged on 30–90 mg samples of fibroin. Portions of the hydrolysates, corresponding to two-fifths of each sample, were analysed for cysteic acid on the 150-cm columns of a Phoenix model 8000 amino-acid analyser by the method of Spackman, Stein and Moore[11]. The amounts of cysteic acid were calculated from the constant for aspartic acid assuming a recovery of 94 per cent.

Table 1. DETERMINATION OF CYSTINE IN VARIOUS PREPARATIONS OF SILK FIBROIN

	Cystine content			
	Goa's method		Moore's method	
Type of fibroin	Per cent	N/N per cent*	Per cent	N/N per cent*
Gland	0·23	0·15	0·24	0·15
Soap-degummed	0·21	0·13	0·19	0·12
Water-degummed at 120° C†	0·16	0·10	—	—

* Cystine nitrogen per 100 g fibroin nitrogen.
† Dunn *et al.* (ref. 10).

The results in Table 1 show good agreement between the two methods and, as these are based on quite different reactions, this gives confidence in them. While both procedures would determine cystine and cysteine, the experiments of Gustus[6] suggest that most of the amino-acid is present in the oxidized form.

We may conclude, therefore, that intact fibroin has a cystine content of 0·23 per cent, and that about 13 per cent of this cystine is destroyed during soap-degumming and about 30 per cent during degumming with superheated water.

The importance of the cystine content in our knowledge of the fibroin molecule can only be conjectured until more is known about the intact molecule. Most determinations of molecular weight[12] and *N*-terminal residues[13] have been made on fibroin prepared by degumming and re-dissolution and, in view of the possibilities of degradation involved in these processes, it is not surprising that a wide range of values has been found. A preliminary study of gland fibroin in the ultracentrifuge in these laboratories[14] indicates that the molecular weight is of the order of 4×10^5 and the cystine content now found therefore corresponds to about four cystine residues per molecule. Preliminary determination of the N-terminal residues of gland fibroin[14] by Sanger's method have shown that glycine, serine and aspartic acid are present in about equimolecular amounts, with a small amount of alanine. It is likely, therefore, that there are at least four chains in the fibroin molecule, and the cystine present could provide inter-chain disulphide links for such a molecule of a molecular weight about 4×10^5. However, the possibility of intra-chain disulphide links cannot as yet be excluded.

Further progress in our knowledge of the structure of fibroin will depend on attempts to establish unequivocally the existence of inter-chain bonds and to break these and isolate the component chains of the molecule.

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BIOPHYSICS

Dry Thermal Inactivation of Trypsin and Ribonuclease

IN the course of some recent work, it became necessary to obtain information on the dry thermal inactivation of trypsin and ribonuclease. A search of the literature indicated that very little work had been done on the dry inactivation of enzymes. In the present experiments, dry samples of these two enzymes were heated *in vacuo* at temperatures ranging from 160° to 200° C. The inactivation process was found to be irreversible. The values of the thermodynamic parameters ΔH and ΔS were also calculated from the survival data.

The vacuum heating chambers used in these experiments consisted of six small brass cups with thin (0·6-mm) copper bottoms. The cups were connected to a mechanical forepump through a vacuum manifold. With this arrangement, the pressure could be maintained at 40μ of mercury during the course of an experiment.

The enzyme samples were prepared by pipetting 0·02 ml. of a stock solution (1 mg/ml.) of the enzyme in distilled water on to a stainless steel disk. These disks were then placed in a desiccator and dried by fast vacuum pumping. After this operation, the disks were placed in the vacuum chambers and the chambers were evacuated.

The heating procedure consisted of immersing the cups in a constant-temperature bath for a known length of time. After the heating period, the cups were cooled by placing them in a cold-water bath (20° C). The temperature was measured by means of a thermocouple placed in the bath. A number of runs were made with a second thermocouple placed on the inside bottom of the cups. The purpose of these runs was to determine the time necessary for the temperature in the cup to rise to the bath temperature. This rise time was found to be short (the maximum observed was 200 sec at 210° C) and all heating times were corrected for it.

The activity of heated trypsin samples, with respect to a non-heated control, was determined by the usual casein digestion assay[1]. The protein from two disks was dissolved in 2 ml. of acetate buffer at *p*H 5·5. 1 ml. of this solution (20 μg/ml.) was added to the casein solution (10 mg/ml.) and incubated for 24 min at 37° C. The assay was standard in all other respects.

In the case of ribonuclease, only one disk was added to 2 ml. of acetate buffer, also at *p*H 5·5. 1 ml. of this solution (10 μg/ml.) was incubated with 3 ml. of a 1-mg/ml. solution of ribonucleic acid (RNA), in the same buffer, for 10 min at 25° C. The reaction was stopped by adding 1 ml. of a 25 per cent perchloric acid–uranyl acetate solution. The assay of Anfinsen was carried out on the supernatant liquid[2]. The enzymes and the RNA were commercial products obtained from General Biochemicals, Inc. The distilled water, used in making all solutions, was injection-grade water supplied by Baxter Laboratories, Inc.

Within the limits of experimental error, the survival ratio at any fixed temperature was found to be an exponential function of the heating time. This function had the form $A = A_0 \exp -kt$. The value of k, the rate of thermal inactivation, was obtained from the slope of the inactivation plots (on a semi-logarithmic scale) and the value of ΔF was calculated according to the theory of absolute reaction rates[3]. These data are summarized in Table 1.

Table 1

Enzyme	Temperature (° C)	k (sec^{-1})	ΔF (cal/mole)
Ribonuclease	161	$1{\cdot}04 \times 10^{-4}$	33,700
	170	$1{\cdot}94 \times 10^{-4}$	34,200
	192	$6{\cdot}00 \times 10^{-4}$	34,500
	204	$1{\cdot}04 \times 10^{-3}$	34,900
Trypsin	160	$1{\cdot}58 \times 10^{-4}$	33,300
	175	$2{\cdot}98 \times 10^{-4}$	33,800
	184	$5{\cdot}20 \times 10^{-4}$	34,100
	195	$8{\cdot}75 \times 10^{-4}$	34,300
	205	$1{\cdot}50 \times 10^{-3}$	34,500

As can be seen from Table 1, ΔF is a slowly increasing function of temperature. ΔF also satisfies the relation $\Delta F = \Delta H - T\Delta S$. Thus for trypsin, $\Delta H = 22{,}500$ cal/mole; $\Delta S = -25$ cal/mole degree and, for ribonuclease, $\Delta H = 22{,}400$ cal/mole and $\Delta S = -24$ cal/mole degree. Zero or negative values of ΔS have been observed in the past for the dry heating of biological materials[4]. Since the meaning of this behaviour is obscure, no physical significance has been attached to the values of ΔH and ΔS found in this work. They are considered as constants which characterize a given reaction.

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Influence of Electric Field on the Capacity of Phospholipid Membranes

VARIOUS bimolecular models of the cell membrane have already been made[1–3]. Such bimolecular films separating two aqueous solutions are formed by applying a solution of phospholipids to the hole in a 'Teflon' cup.

In the present investigation, we have attempted, using artificial phospholipid membranes, to investigate the electromechanical properties which apparently play an essential part in the activity of the cell membrane. Indeed, there is a potential difference of the order of 0·1 V across the resting excitable cell membrane. This produces substantial mechanical forces which compress the membrane. In the course of excitation, the electric field across the membrane decreases and the mechanical compression also falls. This, in turn, could lead to enlargement of the radii of the pores and an increase in the permeability of the cell membrane. In fact, the pores in cell membranes do enlarge in the course of excitation[4]. With the use of a phospholipid model, it became possible

to investigate whether the electric field can change the essential configuration of the membrane.

If the electric field is capable of compressing the cell membranes it might be expected that the application of voltages to bimolecular phospholipid models would increase their capacity, which would decrease with the removal of the voltage. An effect of this kind has, in fact, been observed. The capacity of the membrane was measured by the bridge method (5,000 c.p.s.), using platinum electrodes, and was found to be $0{\cdot}37 \pm 0{\cdot}01$ F/cm². The constant polarizing voltage was measured with separate non-polarizing electrodes. The change in the capacity of an artificial bimolecular membrane under the influence of direct and alternating voltages is shown in Fig. 1. Fig. 1 also shows that the capacity increases on the application of either direct or alternating voltages. After removal of the voltage, the capacity reverts to its previous value. The potential difference (of the order of 0·1 V) which normally exists across the excitable cell membrane substantially changes the capacity of bimolecular membranes. This effect does not depend on the direction of the applied electric field. ΔC increases approximately in proportion to the square of the applied voltage.

If the change of capacity is connected with the mechanical compression of the membrane, it might be expected that the effect of alternating field would be proportional to the value of the effective voltage. This effect has been shown to occur. In all cases the value ΔC was a constant function of the sum of the effective alternating and constant voltages applied.

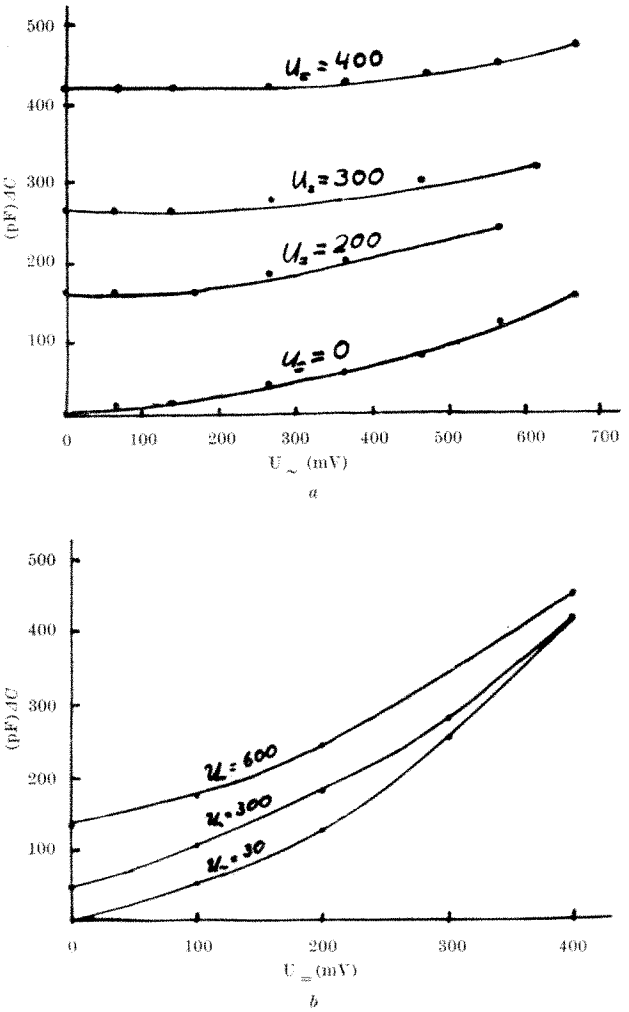


Fig. 1. The dependence of capacity change on voltage: (*a*) alternating, (*b*) constant. Membrane capacity at $U_{=} = 0$ and $U_{\sim} = 30$ mV is equal to 6,200 pF

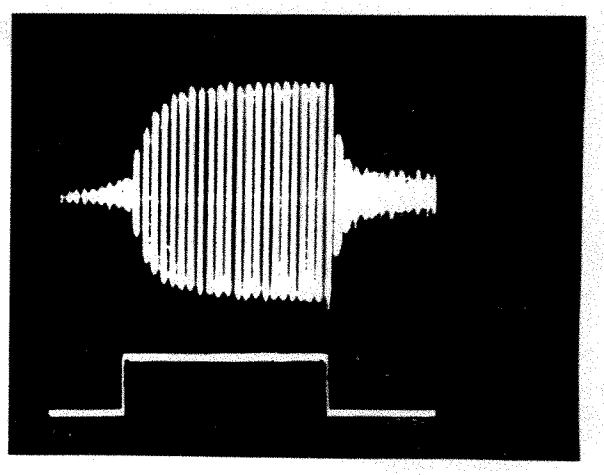


Fig. 2. A bridge imbalance due to the increase of capacity after the application of constant voltage. The amplitude of the rectangular impulse is 200 mV. Time marks at 50 msec intervals

When the electric field is applied, the capacity does not instantly assume a value appropriate to the voltage: the time constant of the delay is approximately 0·3 sec. A bridge imbalancement due to an increase of capacity after the application of constant voltage is shown in Fig. 2.

This rise of capacity as a result of the application of voltage may be associated either with a decrease in the thickness of the bimolecular film or with the extension of its surface as a result of the transformation of the edge of the phospholipid ring into a bimolecular film. In order to find out which of the two effects is present, the thickness of the bimolecular layer was found by measuring the intensity of the light reflected from the membrane. A narrow ray of monochromatic light was used, which was focused on the bimolecular part of the membrane, so that the intensity of the reflected light was not influenced by any changes in the thickness of the lipid at the edges. The refractive index was obtained by measuring Brewster's angle[2]. The intensity of the reflected light was registered on a photo-electronic multiplier. The thickness of the bimolecular membrane was found to equal 74 ± 15 Å, and the error is determined mainly by an error in the measurement of the refractive index. The method of measurement of the change in thickness was accurate to within 1 per cent. Experiments showed that the intensity of the reflected light did not vary with the applied alternating voltage up to amplitudes of 1 V. This means that the change in thickness does not exceed 1 per cent, although the capacity undergoes a change of up to 3 per cent. It might be expected that the thickness variation would be compensated by the simultaneous change of the refractive index of the film, but the intensity of the reflected light measured at different polarizations of the incident light showed that this effect is absent.

The change of capacity is evidently connected with the reversible transformation of the edge of the membrane ring into a bimolecular layer. In fact, optical measurements have shown such effects at the border of the bimolecular membrane after the application of the voltage. In some cases the bimolecular film is not continuous, as a result of impurities in phospholipid solution, and small light spots corresponding to thicker sites in the membrane can be observed on the film. A distinct optical effect is seen in this case: light-spots darken with the application of the field and become visible again when the field is removed. The effect of changes in capacity is far greater in this case. It may be mentioned that constant and alternating voltages of amplitude greater than 0·1 V can be seen to accelerate the formation of a bimolecular film from coloured membrane.

It might be asked whether an effect of this kind has significance for the activity of cell membranes *in vivo*.

Such membranes are not homogeneous; the phospholipid layer in the region of the pores is unlikely to be a simple bimolecular film. It is in just this area that it is logical to expect the electric field to have substantial mechanical influence.

The transformation of the non-bimolecular part of the membrane into a bimolecular one on the border and in other regions of non-homogeneity under the influence of electric fields apparently takes place as the result of the work of the electric field against surface tension. We therefore investigated the correlation between the value of the surface tension coefficient and the effect of applied voltage.

The surface tension coefficient was found from the analysis of membrane surface variations as a function of the differences in hydrostatic pressure at its sides. The coefficient of surface tension was calculated by extrapolation of the function to zero difference of pressure and was equal to $2{\cdot}3 \pm 0{\cdot}1$ dyne/cm.

The dependence of capacity change on the voltage and the coefficient of surface tension calculated for a non-compressible bimolecular membrane surrounded by a non-bimolecular ring, gives the following relation:

$$\frac{\Delta C}{C_0} = K \frac{(\Delta V)^2}{\sigma}$$

where C_0 is the capacity of the membrane in the absence of applied voltage; ΔC, the change in capacity as a result of an applied voltage; σ, the coefficient of surface tension; and K is a constant depending on the geometric parameters of the membrane and the ring.

The value of σ calculated from this relation is consistent in order of magnitude with that found experimentally. Preliminary results also showed a correlation between the value of the coefficient of surface tension and the change in capacity under the influence of the applied voltage.

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PHYSIOLOGY

Uptake of Norepinephrine in Periglomerular Cells of the Olfactory Bulb of the Mouse

A HISTOCHEMICAL investigation, using the fluorescence method of Falck[2], has shown that the uptake of exogenous catecholamines in reserpine-depleted, normally catecholamine-containing neurones is limited within the mouse brain to a rather small number of nerve cell groups[4]. The majority of these nerve cells are situated in regions which also show catecholamine-containing nerve cells in untreated animals. In this communication evidence is presented that norepinephrine can be taken up in numerous periglomerular cells in the olfactory bulb. No catecholamine-containing cells have so far been observed in the olfactory bulb in normal animals.

The experiments reported here were performed on twenty male albino mice (20–25 g). The amine stores were first depleted by reserpine (15 mg/kg subcutaneously). 3 h later, nialamide (100 mg/kg) was injected intraperitoneally in order to inhibit rapid metabolism of the administered catecholamine. 15 min later, azapetine phosphate (10 mg/kg) was given intraperitoneally, followed after another 15 min by the intraperitoneal administration of either L-norepinephrine (16–24 mg/kg, ten animals), L-epinephrine (10 mg/kg, two animals) or L-3,4-dihydroxyphenylalanine (DOPA; 50 mg/kg without azapetine, two animals). Doses are given for the free base. Five animals served as controls, receiving reserpine, nialamide and azapetine. One animal was given 500 mg/kg nialamide only and was killed 6 h later. The other mice were decapitated about 5 h after reserpine, that is, 1–2 h after administration of the catecholamine. The brain was rapidly excised and thin brain slices were frozen in *iso*pentane cooled by liquid nitrogen, 2 min after decapitation of the animal. The tissue pieces were then freeze-dried for 6 days and subsequently exposed to formaldehyde gas using the method of Falck[2,3]. During this treatment, catecholamines, DOPA and 5-hydroxytryptamine condense with formaldehyde to form products which exhibit a green fluorescence in the case of the catecholamines and DOPA. The fluorescence colour of the condensation product formed by 5-hydroxytryptamine is yellow, with the combination of filters used[2,4]. After infiltration of the tissue pieces with paraffin *in vacuo*, 10μ serial sections were cut in a frontal or sagittal plane and mounted in 'Entellan' (or in liquid paraffin in the case of epinephrine). Some sections were stained with toluidine blue after fluorescence microscopy. The frontal sections comprised olfactory bulb, olfactory peduncle and olfactory tubercle, whereas the sagittal sections reached from the olfactory bulb to the medulla oblongata. The same regions were also investigated in untreated animals.

In the olfactory bulb of normal mice, no green fluorescent cells were observed. The same is true for the animal which received 500 mg/kg nialamide. No green fluorescence at all (neither cell bodies nor nerve endings) could be detected in the brain of the control animals. After 16–24 mg/kg norepinephrine a varying number of green fluorescent cells showed up in the layer of periglomerular nerve cells (or lamina granularis externa)[1,6] of the olfactory bulb (Fig. 1). The cells were small, round to oval, and exhibited a weak to moderate, seldom strong, fluorescence. The fluorescence intensity varied greatly from one cell to the other. Only in rare cases could the proximal parts of processes be seen fluorescing for a short distance, mostly directed towards a glomerulus. In sections stained with toluidine blue the green fluorescent cells were iden-



Fig. 1. Frontal section through the olfactory bulb of a mouse. After 24 mg/kg norepinephrine administered under the conditions described in the text, a green fluorescence of varying intensity shows up in the small periglomerular cells situated around two glomeruli. The ventromedial surface of the bulb is seen at left; the outer plexiform layer appears at right (×300)

tified after fluorescence microscopy as periglomerular cells. Fluorescence was only found in part of the periglomerular cells. They were distributed regularly about the glomeruli. They were most numerous and their fluorescence was most intense in the posterior portion of the olfactory bulb, especially in its ventral half. Close to the posterior end of the bulb, a few fluorescent cells were always present within the ventromedial sector of the superficial fibre layer, medial to the pars medialis of the anterior olfactory nucleus. They may belong to caudally situated glomeruli. Towards the anterior part of the bulb, the periglomerular cells generally showed a weaker fluorescence and the fluorescent cells became less numerous, being mostly confined to the ventromedial edge of the bulb. The number of fluorescent periglomerular cells changed somewhat from one experiment to the other, and in three of the ten cases very few fluorescent cells were observed. No other green fluorescent structures were found within the bulb, nor was green fluorescence seen in the anterior olfactory nucleus. After 50 mg/kg DOPA a weak green fluorescence developed in a number of periglomerular cells, whereas after administration of 10 mg/kg epinephrine no green fluorescence was found in this brain region.

As no green fluorescence was seen in the controls, the appearance of green fluorescence in the periglomerular cells after administration of norepinephrine and—less intense—after DOPA indicates an uptake of norepinephrine and DOPA by these cells. This DOPA is probably transformed in part to the related amines. Hence the question arises whether the periglomerular cells of normal animals contain a catecholamine. In experiments carried out under the aforementioned conditions, green fluorescence was found in nerve cell bodies in the arcuate and periventricular nuclei of the hypothalamus, in the area retrochiasmatica, area postrema and in the olfactory bulb, as well as in nerve endings in the external layer of the median eminence. Green fluorescence was also observed in the endothelial cells after administration of DOPA and, sometimes, in the choroid plexuses after norepinephrine[4]. With the exception of the retrochiasmatic area and the olfactory bulb, all these nuclei also show catecholamine-containing nerve cells under normal conditions. However, the fluorescence intensity was greatly increased in these cells after administration of norepinephrine as compared to that in normal animals, and more green fluorescent nerve cells became visible. It therefore seems possible that the periglomerular cells normally contain a catecholamine, but in such small quantities that no fluorescence can be observed. No conclusion can be drawn from the uptake of norepinephrine as to the type of catecholamine present in the cells.

The olfactory bulb is not known to belong to the regions situated outside the blood-brain barrier[5]. As the other groups of nerve cells showing uptake of catecholamines are all localized in such regions or are—at least possibly—connected with them, the uptake of norepinephrine by periglomerular cells is a somewhat surprising finding. Since the dendrites and axons of periglomerular cells end in glomeruli[1], it must be assumed that norepinephrine was taken up from the circulation within the bulb.

The function of the periglomerular cells is as yet poorly understood. It is therefore difficult to understand the significance of the possible catecholamine content of such cells.

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Effect of Vasopressor Substances on the Thoracic Duct Lymph Flow

PREVIOUS observations on the effect of vasopressor substances on the thoracic duct lymph flow have been summarized by Haynes[1] and Ruscynyák *et al.*[2]. However, there is still a certain amount of disagreement in the literature and previous investigations have only shown changes in lymph flow rate without recording blood pressure. Recently adrenaline[3,4] and noradrenaline[3] were observed to stimulate the thoracic duct lymph flow in dogs. An increase in lymph flow rate following administration of angiotensin was also demonstrated in our laboratory[5]. The mechanism underlying the lymph production due to vasopressor substances, however, is not completely understood. The present communication reports experiments carried out on rats in which the effects of various vasopressor substances on thoracic duct lymph flow were studied and compared.

Experiments were performed on heparinized (1,000 U./kg) male Wistar rats weighing 150–250 g, which had been anaesthetized by intraperitoneal administration of 25 per cent urethane solution (5 ml./kg). The subdiaphragmatic thoracic duct cannulation[6] and measurements of lymph flow were identical with those previously described[5]. The lymph flow rate was determined by measuring the volume for 5-min periods. Arterial blood pressure was recorded with a mercury manometer from the common carotid artery. After a steady flow rate had been established for 20 min as a control period, vasopressor substances in 0·2 ml. saline solution were injected intravenously for 90 sec. A total of thirty-nine tests were performed on thirty-two rats.

The thoracic duct lymph flow rate and its response to vasopressor substances showed considerable individual variation. Initial blood pressure ranged from 80 to 120 mm mercury and initial lymph flow rate ranged from 0·015 ml./5 min to 0·040 ml./5 min. In each test the mean value obtained in the control period was taken to be the 100 per cent value and all measurements concerning either blood pressure or lymph flow rate were expressed as a percentage of the control. Mean responses of blood pressure and lymph flow rate to various pressor substances are shown in Fig. 1.

Various grades of response in lymph flow rate were accompanied by variable pressor responses to the vasopressor substances, that is, 0·02 U. and 0·1 U. vasopressin (lysin-8-vasopressin, Sandoz), 0·5 μg noradrenaline, 0·5 μg α-angiotensin (α-L-asp[1]-angiotensin II-amide, 'Hypertensin', Ciba) and 0·5 μg β-angiotensin (β-L-asp[1]-angiotensin II). The increase in lymph flow rate lasted 25 min or more, while the rise in blood pressure passed more temporarily, especially within 5 min after noradrenaline and angiotensin administration. Magnitude and length of time of the increase in lymph flow rate were different among the substances. The mean percentage value and standard error for the total increase in lymph flow within 25 min after the injection was 44·0 ± 43·2 with 0·02 U. vasopressin, 58·2 ± 36·0 with 0·1 U. vasopressin, 173·0 ± 55·5 with 5 μg noradrenaline, 102·1 ± 64·8 with 0·5 μg α-angiotensin and 182·0 ± 101·0 with 0·5 μg β-angiotensin. Although vasopressin caused the greatest rise in blood pressure, the total increase in lymph flow following 0·1 U. vasopressin was significantly smaller ($P > 0·05$) than that after 5 μg noradrenaline or 0·5 μg α-angiotensin. Noradrenaline, which showed the same pressor effect on arterial blood pressure as α-angiotensin, caused a significantly greater increase in total lymph flow than angiotensin ($P > 0·05$). 0·5 μg β-angiotensin seemed to induce a greater increase in lymph flow than 0·5 μg α-angiotensin. However, the difference is statistically not significant because of large individual variations in the former group.

According to Bollman *et al.*[6], about 80 per cent of the thoracic duct lymph is contributed by intestinal lymph

and about 20 per cent of it is derived from the liver in resting rats. The most probable factor responsible for the overproduction of lymph in intestine and liver following the administration of vasopressor substances is considered to be a temporary increase in capillary blood pressure in these organs, which is also accompanied by other haemodynamic changes. Intestinal motility has been observed to have no definite effect on lymph flow rate[7]. It has long been known[8,9] that elevated venous pressure increases capillary pressure and lymph formation more than similar changes of arterial blood pressure[10,11]. However, attention should be paid to the fact that the draining vein is the portal vein for the intestine while for the liver it is the hepatic vein. Noradrenaline is known to increase either caval or portal venous pressure[12,13], while angiotensin has been observed to induce a slight increase in caval venous pressure but no change[14] or a decrease[15] in portal venous pressure. Our explorative experiments, however, showed an elevation of portal venous pressure of about 50 mm water for 5 min following the intravenous administration of 0·5 μg angiotensin to 12 rats (unpublished data). The equipressor dose of noradrenaline has been shown to cause a greater increase in caval venous pressure than angiotensin[13]. None of these findings contradict the fact that the equipressor dose of noradrenaline causes a greater increase in total lymph flow than does angiotensin. As Fig. 1 shows, lymph flow responses following the administration of vasopressor substances lasted much longer than did the changes in blood pressure. It could not be ascertained, however, whether or not any other factor (such as an increased capillary permeability) plays a part in the sustained lymph flow rate. An initial decrease following administration of vasopressin is considered to be due to a sudden fall in portal blood pressure after vasopressin[16–18].

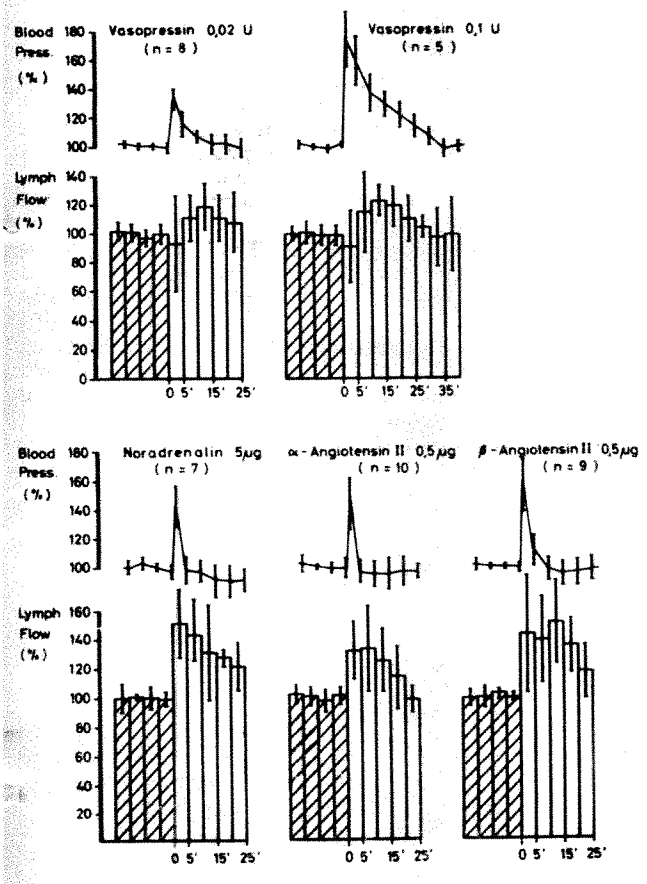


Fig. 1. Blood pressure and lymph flow before (shaded column) and after (white column) the administration of various vasopressor substances. For details see text

Vasopressin was recently observed to cause an accentuated and sustained constriction of venous blood vessels, but only a moderate constriction of arterial blood vessels using hind leg preparation of cats[19]. If the abdominal blood vessels were to respond similarly to vasopressin, the latter increase in lymph flow rate following vasopressin could also be explained by a rise in capillary blood pressure.

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α-Methyldihydroxyphenylalanine and Release of Noradrenaline

IT is now generally accepted that α-methyldihydroxyphenylalanine (α-methyldopa) is an effective means of lowering the blood pressure in cases of hypertension. The mechanism of its action, however, is by no means clear. One of the known actions of α-methyldopa is the inhibition of the enzyme dopadecarboxylase, and, as this enzyme is involved in the synthesis of noradrenaline, it was thought that its inhibition could explain the lowering of the blood pressure. It was found[1,2] that α-methyldopa caused depletion of noradrenaline in the brain and heart, and at first this seemed to support the ideas about its hypotensive action. The duration of tissue noradrenaline depletion was, however, far greater than the duration of the dopadecarboxylase inhibition and the lowering of blood pressure. Furthermore, stronger decarboxylase inhibitors than α-methyldopa do not cause a lowering of blood pressure in hypertensive patients[3]. Animal experiment has shown[4] that, despite noradrenaline depletion of tissues, α-methyldopa does not interfere with the effects of sympathetic nerve stimulation.

In view of the uncertainty about the action of α-methyldopa, experiments were designed to see if this substance affected the release of the chemical transmitter of the sympathetic nervous system.

Cats were anaesthetized with chloralose (80 mg/kg) given intravenously after induction with ethyl chloride and ether. The release of noradrenaline by the postganglionic sympathetic nerve fibres was measured in the spleen, by the method of Brown and Gillespie[5]. They found a relationship between the output of noradrenaline from the spleen and the frequency of stimulation of the splenic nerves. At a frequency of 30/sec the mean output is 980 pg/stimulus and at 10/sec it is 160 pg/stimulus.

In the present work, the output of noradrenaline has been measured after both chronic and acute treatment with α-methyldopa. In chronic experiments, the cats were given intraperitoneal injections of α-methyldopa (100 mg/kg) between 5 and 16 h before the experiment. In some experiments, in which treatment was started up to 30 h before the acute experiment, a second injection of α-methyldopa was given. In the acute experiments, the α-methyldopa was given intravenously (100 mg/kg) or by close arterial injection (10–15 mg) into the spleen by way of the hepatic artery. In all experiments, the output of noradrenaline from the spleen in response to splenic nerve stimulation at frequencies of 30 and 10/sec was normal, regardless of the method or duration of the treatment with α-methyldopa.

It was shown, further, that in normal anaesthetized cats the output of noradrenaline at a stimulus frequency of 10/sec is raised by giving the adrenergic blocking agent phenoxybenzamine, while that at 30/sec shows little alteration. It is thought that in the presence of phenoxybenzamine the output of noradrenaline as a result of nerve stimulation is a good indication of the actual release by the nerve endings. Treatment with α-methyldopa did not cause any alteration in the action of phenoxybenzamine. It is concluded, therefore, that α-methyldopa has no effect on the release of noradrenaline by the postganglionic sympathetic splenic nerves.

In some experiments the blood pressure responses of cats treated with α-methyldopa were investigated. The pressor responses to clipping the common carotid arteries and to splanchnic nerve stimulation were the same before and after intravenous α-methyldopa. In cats which had been pretreated with α-methyldopa, the pressor responses were no different from those in control animals. These findings are consistent with observations on the effect of α-methyldopa on the cardiac contractility response to stellate ganglion stimulation[6] and on the cardiovascular responses to central vagal stimulation[7].

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PHARMACOLOGY

'Thalidomide' Syndrome in Baboons

NUMEROUS attempts to reproduce the malformations which occurred in human babies from 'Thalidomide'-treated mothers have met with only limited success[1–3]. Although many representatives of aves and mammalian experimental species have been investigated for this purpose, the results fall short of paralleling the effect of the drug on the human foetus[4]. Sub-human primates have been utilized on two occasions[5,6]. Lucey and Behrmann[5] showed that treating rhesus monkeys with 'Thalidomide' before implantation resulted in the inhibition of implantation. Delahunt and Lassen[6] induced typically malformed foetuses in four of seven pregnant *Cynomolgus* monkeys which were treated after implantation had already occurred (days 32–42). Although the embryology of the baboon has not been investigated as extensively as that of some other sub-human primates, developmental processes paralleling those in the human have been found[7]. Further investigation of the embryology is in progress.



Fig. 1. A normal foetus (9 weeks)

Our experiments reveal that 'Thalidomide', given in relatively low dosage (5 mg/kg) *per os*[8] at the time of conception, does not prevent nidation in the baboon (in contrast to rhesus) and that distinct deformities are induced in the baboon embryo and foetus which are associated with the 'Thalidomide' syndrome as observed in man[9].

Ten female baboons were used in the present investigation. They were placed with a male for a 2-day period except in two cases (*A*-331 and *A*-336) in which mating occurred for 5 days. The time of mating was based on perineal turgescence, vaginal smears and the interpretation of previous cycles[10]. Mating for a 2–12 h period was successful in at least ten cases in which embryos were delivered in early stages of development. Mating over long periods of time has not been found necessary when these criteria were used and a history of the menstrual cycle was known.

Four female baboons were given 'Thalidomide' (5 mg/kg) orally from the time of ovulation (day 0) until the animals recycled (no conception) or until day 30. Pregnancy was confirmed in one of the four females (*A*-336) on the twentieth day by digital palpation and is currently in the sixth week of gestation.

Five female baboons (*A*-74, *A*-303, *A*-331, *A*-466 and *N*-20) received 'Thalidomide' (5 mg/kg) starting between days 18 and 20 of gestation, except for female *N*-20, which received the first dosage on the twenty-second day. The duration of the treatment was varied but ranged between 12 and 22 days. Table 1 summarizes the results of the experiment.

Female *A*-466 aborted during the ninth week of gestation and only the placenta was recovered. Caesarean

Table 1

Animal No.	Start of treatment (days gestation)	Duration of treatment (days)	Gross observation
A-336	0	30	Pregnant, sixth week
A-74	18	14	Amelia, spina bifida cystica. Slight malformation of ear
A-303	18	12	Amelia, posterior limb. Phocomelia, anterior limb. Bilateral cleft lip. Agnathia. Retarded growth
A-331	18	18	Resorbing
A-466	18	18	Spontaneous abortion, foetus not recovered
N-20	22	22	Oedema, single umbilical artery
A-167	30	10	Normal



Fig. 2. A malformed baboon foetus (9 weeks). Arrows indicate the deformity of the limbs, spina bifida and slight malformation of the ear

sections were performed during the ninth week of gestation on females *A*-74, *A*-303, *A*-331 and *N*-20. The foetus of *A*-74 was grossly malformed (Fig. 2). All four limbs were severely retarded in growth (amelia) (verification of this malformation is being sought histologically); spina bifida cystica and shortening of the tail were also apparent. The ears showed a slight malformation, having rotated slightly more posteriorly than normal. The foetus of *A*-303 showed a marked retardation in development, being equivalent to a normal 40-day baboon embryo in overall size. The foetus was probably dead but the overall composition appeared quite normal; the diameters of the chorion and amnion were equivalent to other 9-week foetuses, but the extra-embryonic fluid had a very definite yellow tinge as did the fluid of the other animals in the experiment. The gross malformations were amelia of the posterior limbs, phocomelia of the anterior limbs, bilateral cleft lip and agnathia.

Female *A*-331 showed blood on the external genitalia, just before surgery, and was in the process of aborting judging by coloration and foetal–maternal tissue relationships. The foetus was almost entirely resorbed and appeared as a white stringy mucous mass within the normal-appearing amnion and chorion. The overall dimensions of the entire embryonic sac were considerably smaller than normal.

The foetus of *N*-20 was characterized by extreme oedema and a single umbilical artery. Since oedema is often associated with venous complications, additional cardiovascular anomalies are strongly suggestive and will be studied histologically.

To test the effects of 'Thalidomide' during the second month of pregnancy, female *A*-167 received 100 mg/kg for 10 days starting with day 30. A normal foetus, as observed by gross examination, was delivered by Caesarean section at three and one-half months.

Based on the present findings, the hypothesis advanced by Lucey and Behrmann[5] that 'Thalidomide' kills the embryo prior to implantation cannot be supported. In the baboon, at least, it is suggested that a close relationship in the early reproductive physiological processes exists between man and the baboon. Experimental work on armadillo[11,12] and rabbit[13] embryos suggests that 'Thalidomide' damages only the embryoblastic cells, leaving the trophoblastic cells uninjured and capable of implantation. This is also borne out by our experiments, as exemplified by the foetuses of *A*-303 (retardation of development, gross anomaly and apparent foetal death) and *A*-331 (remnant of a resorbing embryo), and yet both these embryos were supported by normal membranes.

The foetal malformations described for the baboon are identical in type to most of those described in the newborn infants of women receiving 'Thalidomide'[8]. The deformities are distinct and occurred in all animals treated at minimum dosages. The effect of this drug on developmental processes in the baboon and the parallelism to the human is striking.

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Lysosomes and Gout

IN recent years there has been much interest in the role of crystals in causing acute attacks of gout. Faires and his colleagues at Philadelphia[1] and Seegmiller *et al.* at Bethesda[2] were able to produce such attacks by injecting microcrystalline sodium monourate into gouty and non-gouty volunteers. With this experiment in mind, investigations were undertaken, at cellular and sub-cellular level, of events that may be important in initiating the inflammatory phenomenon in acute gout.

Microcrystals of sodium monourate were prepared by the method of Seegmiller *et al.*[2]. Fresh human polymorphs were obtained by sedimenting fresh blood in heparin with 1/10th volume of 6 per cent dextran in saline. Most of the white cells were in the upper plasma layer which was sampled for cell counts; 0·1 ml. of the cell suspension in the patients' own plasma was then placed on an ordinary 3 in. × 1 in. glass slide and covered with a 19 mm × 38 mm cover-slip, three sides of which were sealed with 'Vaseline'. Crystals ranging in length from 0·5μ to 8μ were suspended in normal saline and a drop of the suspension was introduced into the preparation through the unsealed side.

The slide, resting on a pad of wet cotton wool, was enclosed in a Petri dish and incubated at 37° C in a sealed incubator periodically flushed with 5 per cent carbon dioxide in air.

The polymorphs readily ingested the smaller crystals and rapidly became degranulated, the process being completed within a few minutes (Fig. 1). The dissolution of the granules was readily observed in living cells vitally stained with acridine orange and examined by phase contrast. The cells showed a different type of response to crystals longer than 8μ or to clumps of small crystals; they were unable to engulf such large bodies, and instead clustered around them to form rosettes (Fig. 2), which were also rapidly degranulated.

The effect of colchicine on polymorphs exposed to crystals of sodium monourate was investigated; the cultures were observed at 5-min intervals up to 60 min. In the presence of the drug, some cells failed to phagocytose crystals, while others ingested crystals but were not degranulated (Fig. 3). It was thought that these phenomena indicated a stabilizing action of colchicine on the

membrane systems of the cell and particularly on the membranes of the cytoplasmic granules.

Recent work (by Novikoff)[3] has shown that the characteristic granules of polymorphs are lysosomal in nature and contain acid hydrolases in a bound form. Preliminary experiments were carried out to see whether colchicine would have a direct stabilizing action on the isolated granules *in vitro* and inhibit the release of their enzymes. Granules were isolated from polymorphs by the technique of Cohn and Hirsch[4], treated *in vitro* with the drug at concentrations of 5–50 μg/ml., and incubated for 60 min at 37° C. After incubation the suspension was spun at 10,000*g* for 20 min and the proteolytic activity of the supernatant was measured by the method of Anson[5] with 4 per cent (weight/volume) haemoglobin in 0·1 M acetate buffer at *p*H 4 as the substrate. Activity was expressed either as μg of acid-soluble tyrosine released by 1 ml. of suspension after 20 min incubation at 37° C or as the percentage of the total activity of the sample measured after the release of the protease by the non-ionic detergent 0·1 per cent *BRIJ* 35. Preliminary results showed that 75 per cent of the total proteolytic activity of the normal 'granular-mitochondrial' fraction was recovered from the supernatant. When the granule fraction was pretreated with colchicine, however, only 50 per cent of the activity was released. Thus it would appear that colchicine reduced the titration of bound enzyme.

The results of this investigation suggest that in acute gout, the polymorphonuclear leucocytes either ingest or adhere to microcrystals of sodium monourate, become degranulated and release lysosomal enzymes into the tissue; this reaction may be partly responsible for the subsequent inflammatory changes. If so, then colchicine may produce its therapeutic effects by stabilizing the lysosomal membranes and thus preventing degranulation

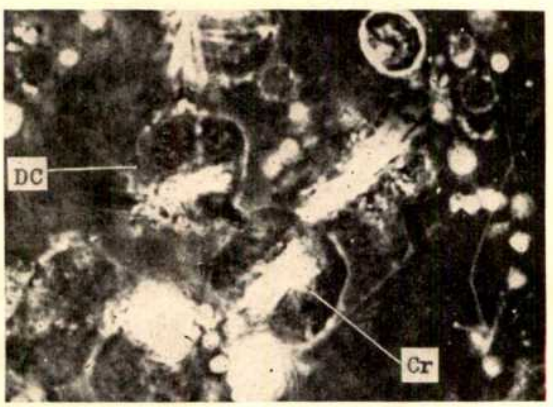


Fig. 1. *DC*, Degranulated cell; *Cr*, crystals. Stained with acridine orange: phase contrast (× 750)

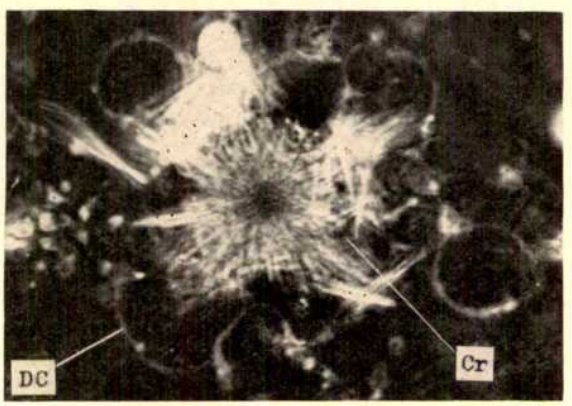


Fig. 2. Rosette formation. Stained with acridine orange: phase contrast (× 750)

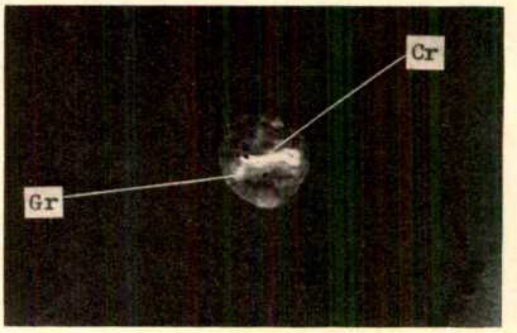


Fig. 3. Cell treated with colchicine. *Gr*, granules. Stained with acridine orange: phase contrast (× 750)

with consequent liberation of hydrolases; in this way, by limiting degranulation following phagocytosis, the drug may break the vicious circle of inflammation.

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IMMUNOLOGY

Species Distribution of Gastrointestinal Antigens

IMMUNOFLUORESCENCE provides a simple means of studying the species distribution of antigenic components of animal tissues[1]. The availability of gastrointestinal-specific antisera as immunohistological reagents prompted us to explore the distribution of gastrointestinal antigens in mammalian and other vertebrate fauna of Australia. Two types of sera were used. One, prepared in rabbits by immunization with microsomal material from fresh surgical specimens of human colonic mucosa, had been previously shown to react with an acid mucopolysaccharide component of human and some other mammalian gastrointestinal mucosal cells and with no other tissue in the body[2]. The other, obtained from patients with pernicious anaemia, contains naturally occurring autoantibody to human gastric parietal cells; this antibody has also been shown not to be species-specific[3].

The investigation was conducted on separate tissue blocks from stomach, small intestine and large intestine taken usually within 1 h of death from a large variety of mammals, as listed in Table 1, and other vertebrate species. Blocks, snap frozen in liquid nitrogen–isopentane freezing mixture at about −160° C or, less satisfactorily, in containers cooled to −76° C in a dry ice–alcohol mixture, were stored at −70° C until they were sectioned at 6μ in a cryotome at −20° C; sections were air-dried at 0°–2° C. In the case of the gastrointestinal-specific rabbit serum, staining was normally carried out on sections fixed for 2 min in 95 per cent ethanol at 0°–2° C by direct immunofluorescence using a conjugate with lissamine rhodamine *B* (*RB* 200); sandwich staining with conjugated anti-rabbit

globulin was also used in some confirmatory experiments. With the pernicious anaemia sera, unfixed sections of gastric fundus were stained by the sandwich method using *RB* 200-conjugated anti-human globulin. Stained slides were examined by brightground ultra-violet-blue fluorescence microscopy. Details of the technical methods are described elsewhere[4].

Specificity of the immunofluorescence reactions of the two antisera was demonstrated by failure to stain tissues other than gastrointestinal mucosa or gastric parietal cells, respectively; these were themselves not stained by labelled normal pre-immunization rabbit serum or by normal human serum. Confirmatory information was obtained by cross-absorptions of the antisera with homogenates of colon, stomach fundus or liver of various animals.

The results are summarized in Table 1. With the rabbit antiserum, staining was obtained in all the mammalian colons, but, except for a positive reaction with the human foetus, staining of small intestine was seen only in the adult placentals and two marsupials (koala and marsupial mouse); gastric staining was confined to man, monkey and pig. The reaction was strongest in colon, less intense in small intestine and least in stomach, where it was patchily distributed with the mucous cells. The most intense staining was given with the homologous (human) mucosa, while a strong but less-intense reaction was obtained with all other placentals examined except the rodents, in which it was least and, moreover, not regularly obtained. Marsupial gastrointestinal tract either gave similar staining intensity to the rodents or a little better, and gastric staining was not seen. In the monotremes, colonic staining only was found, but this was as intense (Fig. 1) as that of any of the placentals other than man.

With the pernicious anaemia serum, staining was limited to gastric parietal cells. It was most intense in the placentals among which no conspicuous difference in brightness was seen, and was uniformly of lesser degree in the marsupials (Fig. 2); no reaction was seen with the monotreme stomachs. Positive staining was invariably obtained where there were gastric parietal cells as recognized by conventional histological methods; these cells were never detected in specimens giving negative immunofluorescence reactions.

No staining was observed in animals from any other vertebrate class. These included seven bird species (domestic fowl (2), hawk, kingfisher, kookaburra (3), magpie, seagull (2) and swamp hen), three reptile species (blue-tongued lizard (2), red-bellied black snake and tortoise), two amphibians (frog and toad), and thirteen fish species (including dogfish, shark, flathead, snapper, trevally, whiting, goldfish, tench, brown trout and rainbow trout). One invertebrate alimentary organ, from the squid, also gave negative results.

Table 1. IMMUNOFLUORESCENT STAINING OF MAMMALIAN GASTROINTESTINAL TRACT

	Number tested	Mucous cell staining by rabbit antiserum: Stomach*	Small intestine	Colon	Gastric parietal cell staining by human pernicious anaemia serum
Placentals					
Human—adult	Numerous	+	+ +	+ + +	+ + +
foetus	Several	+	+	+ +	+ + +
Monkey	2	+	+	+ +	+ + +
Dog—adult	2	−	+	+ +	+ + +
pup	2	−	−	−	+ + +
Cat—adult	2	−	+	+ +	+ + +
kitten	3	−	−	−	+ + +
Pig	3	+	+	+ +	+ + +
Horse	1	−	+	+ +	+ + +
Cow	2	−	+	+ +	+ + +
Sheep—adult	3	−	+	+ +	+ + +
lamb	2	−	−	+	+ + +
Rabbit	4	−	+(3)	+(3)	+ + +
Guinea-pig	4	−	+(3)	+(3)	+ + +
Rat	9	−	(+)(6)	+(7)	+ + +
Mouse (six strain)	19	−	(+)(17)	+(17)	+ + +
Marsupials					
Kangaroo (*Macropus*)	3	−	−	+	+ +
Rat kangaroo	1	−	−	+	+ +
Wombat	3	−	−	+(+)	+ +
Koala	4	−	+	+(+)	+ +
Ringtail possum	1	−	−	+	+ +
Bushtail possum	2	−	−	+	+ +
Marsupial mouse	1	−	+	+	+ +
Long-nosed bandicoot	1	−	−	+	+ +
Monotremes					
Echidna	3	−	−	+ +	−
Platypus	1	−	−	+ +	−

* Staining patchy where positive. (+) Staining weak. +(+) Staining intermediate between + and + +. Rodent figures in brackets indicate number with positive staining.

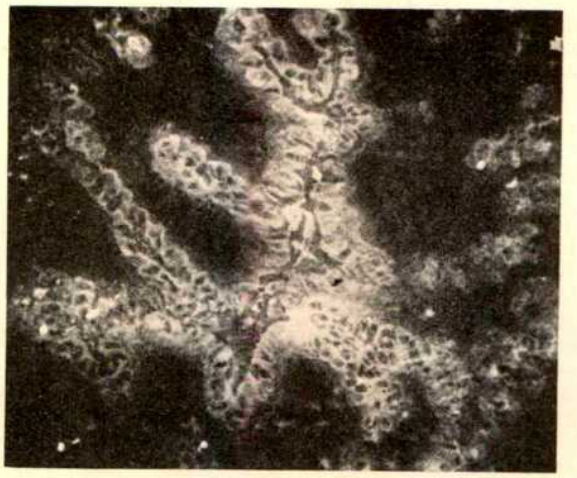


Fig. 1. Fresh frozen section of echidna colon treated first with rabbit anti-human-colon serum and then with *RB* 200-conjugated goat anti-rabbit-globulin. Bright fluorescent staining of the mucosal cells. (×90)

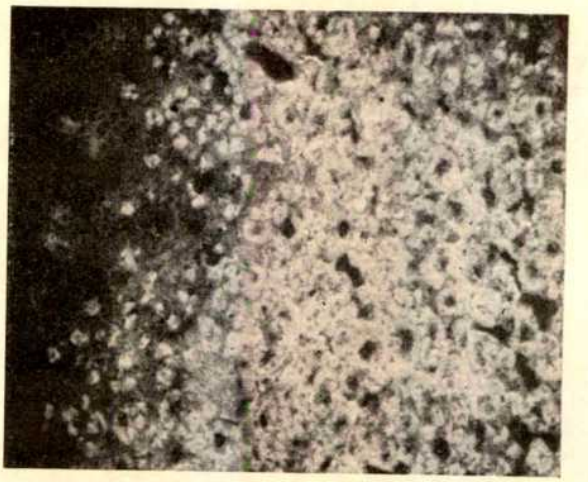


Fig. 2. Fresh frozen section of koala stomach fundus treated first with human pernicious anaemia serum and then with *RB* 200-conjugated goat anti-human-globulin. Staining limited to the parietal cells. (×90)

The cross-antigenicity in the gastrointestinal tracts of the mammals was confirmed by the serum absorption studies. Neither serum showed any inhibition of staining from absorption by non-mammalian gastrointestinal homogenates or by liver homogenate of any species. With rabbit-serum reactions, complete inhibition of staining was achieved only by absorption with human colon homogenate. Colon homogenates from other species were effective in inhibiting staining of that species, but only partially so in inhibiting staining of human colon; in this respect species which gave the least staining had the least inhibitory power.

With the pernicious anaemia serum, less exhaustive absorption studies were carried out, but they suggest greater antigenic identity between the mammalian gastric parietal cells. Just as staining intensity did not vary materially among the placentals or among the marsupials, absorption inhibition of staining appeared to depend only on the concentration of parietal cells in the stomach from which the absorbing homogenate was derived; when these

were abundant, complete inhibition was achieved. Rat gastric fundus was, for example, completely effective as an absorbing agent in inhibiting staining of human gastric parietal cells by the pernicious anaemia serum.

The relatively poor and somewhat inconstant staining of rodent gastrointestinal mucosa by the rabbit antiserum is worthy of comment. As the serum had been prepared in a rabbit, its lesser reaction with this and other rodents can perhaps be attributed to a partial failure to develop gastrointestinal autoimmunity or immunity against too closely related gastrointestinal antigens of the other rodents. In earlier investigations of the properties of the antiserum[2], staining of rodent gastrointestinal mucosa was not observed in the few animals examined. The present positive results are presumably the outcome of working with different animal strains in larger numbers and perhaps of the introduction of liquid nitrogen snap-freezing as a technical improvement in preserving tissue antigens for immunohistology. The last-mentioned point may also explain the present failure to confirm our previous tentative suggestion that there might be systematic differences in the anatomical distribution of gastrointestinal staining by the rabbit serum between ruminants and carnivores. The negative findings in some young animals (pup and kitten) reflect some species variation when compared with the positive results in human foetuses and in the lamb.

Perhaps the most interesting observation from the present investigation is the demonstration of closer antigenic similarity between placental and monotreme colonic mucosa than the marsupial, in contrast with the total absence of gastric parietal cell antigen in the monotremes, though abundant in placentals and marsupials. The limitation of the gastrointestinal antigens to the mammals also differs from the distributional behaviour of a kidney-specific material demonstrated in various vertebrates including mammals, birds and fish[1].

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Growth of *L1210* Leukaemia Cells

THERE is evidence that the number or size of the cell inoculum, the site of inoculation, the host of origin or the conditioning of the host[1] can alter the strain specificity of neoplasms and that some neoplasms can be transplanted from strain of origin and grow progressively in homologous strains with the animals dying of the neoplastic disease. Greene[2] reported the successful transplantation of heterologous neoplasms into the brains of rats and mice and suggested that the brain might be exempt from any immunological response.

The present communication concerns investigations of the progressive growth of leukaemia *L1210* implanted intracerebrally at various log-dose inocula into homologous strains of mice which grew (*BDF1*, *CDBA*) or regressed (*C3H*, *C57BL/6*) when an intraperitoneal inoculation of 5×10^6 cells was used.

Leukaemia *L1210* cells were collected from a *DBA/2* host which had been inoculated intraperitoneally 6 days previously. A cell count was carried out and the stock was diluted with cold Earle's balanced salt solution (EBSS) so that the cell inoculum was injected intracerebrally in 0·025 ml. using a microlitre syringe (Hamilton Co.) by penetrating the skull with a 27-gauge needle. Ten mice of each strain (*BDF1*, *CDBA*, *C3H* and *C57BL/6*) were used at each dose-level. The animals were observed daily for survival and subsequent death due to tumour growth.

The results (Table 1) indicate that the intracerebrally implanted *L1210* was lethal to compatible hosts (BDF_1 and *CDBA*) with an inoculation of ten cells, whereas the number of cells necessary to kill more than 50 per cent of the mice was greater than ten cells for *C3H* mice and greater than 1,000 cells for *C57BL/6* mice.

The data reveal that, at the higher cell inocula, *L1210* grows progressively in the brain probably because of its ability to overcome an immunological response, whereas at lower cell inocula the immune mechanism exists but is not as effective as the response elicited by the intraperitoneal inoculation of the leukaemia cells.

It would appear that each mouse strain may have its own threshold level for growth of incompatible neoplasms when brain is used as the site of inoculation. This may, in turn, be an expression of the antigenicity of each tumour or the natural resistance of the host.

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Table 1. MEAN SURVIVAL TIMES OF MICE INOCULATED WITH LEUKAEMIA *L1210* INTRACEREBRALLY

Strain	Cell inoculum 10^6 MST ± *S.D.**	*T/I*†	10^5 MST ± *S.D.*	*T/I*	10^4 MST ± *S.D.*	*T/I*	10^3 MST ± *S.D.*	*T/I*	10^2 MST ± *S.D.*	*T/I*	10^1 MST ± *S.D.*	*T/I*
BDF1	6·0 ± 0·0	10/10	7·3 ± 0·3	10/10	8·4 ± 0·3	10/10	10·5 ± 1·0	10/10	12·1 ± 0·7	10/10	13·5 ± 1·0	10/10
CDBA	7·1 ± 0·6	10/10	8·4 ± 1·3	10/10	10·2 ± 0·7	10/10	10·8 ± 0·7	10/10	12·0 ± 0·7	10/10	13·3 ± 1·0	9/10
C3H	7·7 ± 1·7	9/10	9·0 ± 1·3	9/10	11·5 ± 2·8	8/10	12·3 ± 1·4	8/10	16·5 ± 4·4	7/10	> 23·0	5/10
C57BL/6	6·9 ± 0·7	10/10	8·0 ± 0·7	10/10	10·0 ± 1·7	9/10	> 30·0	3/10	> 30·0	4/10	> 30·0	0/10

* MST ± *S.D.*, mean survival time (days) ± standard deviation.
† *T/I*, No. of tumour deaths/No. of mice inoculated.

PATHOLOGY

Carcinogenic Action of *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine

N-Methyl-*N'*-nitro-*N*-nitrosoguanidine (NG) is known to be a potent mutagenic substance for *Escherichia coli*[1], *Salmonella typhimurium*[2], and *Chlamydomonas reinhardi*[3]. Induction of chromosomal aberrations in the cells of root meristems of *Vicia faba* by NG has been reported[4]. It is claimed that NG is the most potent mutagen yet discovered, since NG induced at least one mutation per cell of *Escherichia coli* treated at the optimal condition permitting 50 per cent survival[5]. The production of a number of multi-site mutants resulting from the accumulation of single-site mutations has been noticed[2,5].

This report deals with the carcinogenic activity of NG. NG was purchased from K and K Laboratories, Inc., New York, and was dissolved in warmed sterile saline at a concentration of 5 mg per ml. Wistar strain male rats,

Initially 6 weeks old, were maintained on commercial CE-2 animal diet and water *ad libitum*. Either 0·2 ml. or 0·5 ml. of the NG solution was subcutaneously injected into the backs of these animals once a week as near as possible in the same site; the total doses of NG per animal were 10 or 25 mg. Any tumours which formed were removed when an animal died or when they reached an area of 2 × 3 cm. Tumours were histologically examined and subcutaneously transplanted into young normal rats by the trocar method. During the period of application of NG, ulcerations occurred in some of the animals, but they gradually subsided after the last injection.

Table 1. TUMOURS IN RATS INJECTED WITH NG AND THEIR TRANSPLANTABILITY

Rat No.	Total carcinogen injected (mg)	Survival after the first injection (days)	Tumour produced	Transplantability at the first generation (takes/total rats)
1	10	303	—	
2	10	330	—	
3	10	365	—	
4	10	365	—	
5	10	301	Fibrosarcoma	1/5
6	10	330	Fibrosarcoma	Not tried
7	10	365	—	
8	10	361	Fibrosarcoma	2/5
9	10	331	Fibrosarcoma	Not tried
10	10	365	—	
11	10	365	—	
12	10	365	—	
13	25	174	Myxomatous fibrosarcoma	2/5
14	25	365	—	
15	25	333	Medullary type fibrosarcoma	Not tried
16	25	170	Fibrosarcoma	Not tried
17	25	365	—	
18	25	365	—	
19	25	294	Fibrosarcoma	1/5
20	25	294	Fibrosarcoma with giant cells	2/3
21	25	333	Rhabdomyosarcoma	2/5
22	25	365	—	
23	25	365		
24	25	365		

Twenty-four rats survived for a period of 5 months after the first injection and the observations were continued for a further 7 months. The results are summarized in Table 1. The tumour incidences were 33 and 50 per cent in animal groups which received total doses of 10 and 25 mg, respectively. Most of the tumours were typical fibrosarcoma, but there were single cases of myxomatous fibrosarcoma, medullary-type fibrosarcoma, fibrosarcoma with giant cell, and rhabdomyosarcoma. The tumour transplantability is also recorded in Table 1. The rate of takes of transplants ranged from 20 per cent to 66 per cent in the first generation.

The mechanism of this carcinogenicity is obscure, but the alkylation of nucleic acids and/or proteins may be involved in the process as in the case of dimethylnitrosoamine, revealed by Magee *et al.*[6]. Although it was reported that NG yields neither nitrous acid nor diazomethane in neutral condition[5], enzymes may exist which catalyse this reaction *in vivo*.

We are now exploring various ways of applying NG with the hope of producing different types of tumour in different organs.

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MICROBIOLOGY

Methaemoglobin Formation by *Mycoplasma gallisepticum*: the Role of Hydrogen Peroxide

Mycoplasma gallisepticum, the agent responsible for common respiratory disease of turkeys, has been shown to agglutinate turkey erythrocytes[1]. During the course of investigations of a neurotropic strain of this organism (*S6*) which produces encephalitis associated with cerebral arteritis[2], it was observed that incubation of a suspension of *M. gallisepticum* with washed turkey erythrocytes at 37° C caused the cells to become dark brown. Washed mycoplasmas enclosed in dialysis tubing also caused browning of erythrocyte suspensions and converted turkey and human haemoglobin to a brown solution which exhibited the spectral characteristics of methaemoglobin.

Methaemoglobin formation was studied in a system consisting of 5×10^9 mycoplasmas, washed three times, suspended in 0·5 ml. of buffered Ringer's solution and enclosed in dialysis tubing which was submerged in 5 ml. of a 0·16 per cent haemoglobin solution. The addition of galactose, mannose or glucose, in concentrations of 25 mg/ml., resulted in the appearance of methaemoglobin after 15 min of incubation at 37° C. In the absence of a sugar the same process required 1–2 h. Mycoplasmas incubated at 4° C, with or without added sugar, and organisms killed by heating at 50° C for 30 min, or disrupted by ten cycles of rapid freezing and thawing, failed to produce methaemoglobin.

The browning of intact turkey erythrocytes by mycoplasmas was also temperature-dependent, accelerated by the addition of simple sugars, and not produced by heat-killed or disrupted mycoplasmas. The addition of 10^9 mycoplasmas to 2 ml. of a 2 per cent suspension of erythrocytes containing galactose or mannose resulted in methaemoglobin formation after incubation for 15 min at 37° C. Although methaemoglobin was not formed by mycoplasmas at 4° C, the organisms rapidly became attached to the surface of erythrocytes at this temperature, and Giemsa-stained films showed individual erythrocytes to be coated with numerous mycoplasmas. When such cells were washed three times in cold Ringer's solution, using low-speed centrifugation to eliminate unattached mycoplasmas, the majority of erythrocytes were still coated with ten or more organisms. The washed, coated cells were resuspended in buffered Ringer's solution to a 2 per cent concentration, and incubated at 37° C. In the presence of mannose or galactose, the cells became dark brown within 15 min; without added sugar the reaction required 2 h or longer. It thus appeared that the presence of actively metabolizing mycoplasmas on the surface of erythrocytes facilitated methaemoglobin formation. It was estimated that the number of cell-attached mycoplasmas required for browning of erythrocytes was less than 0·25 per cent of the number needed when the organisms were separated from the erythrocytes by dialysis membranes.

In view of the known capacity of hydrogen peroxide to form methaemoglobin, and the reported absence of detectable catalase in several strains of mycoplasma[3], it seemed possible that the dialysable factor produced by *M. gallisepticum* might be hydrogen peroxide. No catalase activity could be detected in sonically disrupted *M. gallisepticum* in this laboratory. Accordingly, catalase and peroxidase were tested for their ability to prevent methaemoglobin formation by mycoplasmas.

Horseradish peroxidase in combination with ascorbic acid, protected turkey haemoglobin against the action of mycoplasmas contained in dialysis sacs (Table 1). As little as 40 μg of enzyme per ml. prevented the appearance of methaemoglobin.

Catalase had a similar protective effect. With mycoplasmas contained in dialysis sacs surrounded by hae-

moglobin, the presence of 40 μg of the enzyme per ml. prevented the formation of methaemoglobin (Table 2). Bovine serum albumin, at a protein concentration 2·5 times that of catalase, afforded no protection. When a 'moat' was created between the mycoplasmas and haemoglobin, by a system comprised of a dialysis sac of mycoplasmas and one of haemoglobin, both suspended freely in a solution of buffer containing catalase, as little as 1 μg per ml. of enzyme prevented the formation of methaemoglobin (Table 3). In this system, the lowest molar concentration of catalase haem groups which afforded protection was less than 0·1 per cent of the protected haemoglobin haem groups, indicating that the catalase protection was enzymatic rather than competitive.

By using an excess of horseradish peroxidase in combination with 4-methoxy-α-naphthol[4], it was possible to detect and measure peroxide in high speed supernatants prepared from mycoplasma suspensions (Table 4). No peroxide was detectable in washed suspensions of mycoplasmas held at 4° C. In contrast, portions of 5×10^9 organisms incubated in the presence of mannose elaborated hydrogen peroxide in amounts of $6{\cdot}3 \times 10^{-5}$ M during 10 min at 37° C. The kinetics of peroxide production and its stimulation by sugars closely paralleled the kinetics of methaemoglobin formation by *M. gallisepticum*.

Colonies of *M. gallisepticum*, growing at the surface of PPLO-agar[5], produce sharply delineated areas of α-haemolysis when the agar is covered with a new layer of agar containing 4 per cent turkey or human blood. The incorporation of catalase in the blood agar overlay prevented the development of haemolysis (Table 5). The appearance of plates in the presence and absence of catalase is illustrated in Fig. 1.

It is not known whether the observations reported here apply in general to other mycoplasma species. We have also observed methaemoglobin formation by a dialysable factor elaborated by suspensions of *M. pneumoniae* and *M. neurolyticum*. In both instances the reaction was inhibited by the presence of catalase.

Table 1. EFFECT OF PEROXIDASE ON MYCOPLASMA-INDUCED METHAEMOGLOBIN FORMATION

Time (min)	Mycoplasma peroxidase	Mycoplasma no peroxidase	No mycoplasma peroxidase
0	−	−	−
30	−	+	−

Reaction mixtures were incubated at 37° C and contained 8×10^9 mycoplasmas (*M. gallisepticum*) in 0·8 ml. of Ringer's solution in dialysis tubing, turkey haemoglobin (0·157 per cent), potassium phosphate buffer, *p*H 7·2 (0·05 M), ascorbate (5×10^{-3} M) and horseradish peroxidase (Sigma) 40 γ/ml. The presence of methaemoglobin is indicated by '+'.

Table 2. EFFECT OF CATALASE ON MYCOPLASMA-INDUCED METHAEMOGLOBIN FORMATION

Time	Catalase	Albumin	No additive
0	−	−	−
30	−	+	+

Reaction mixtures were incubated at 37° C and contained mycoplasmas, haemoglobin and buffer as in Table 1, mannose (25 mg/ml.), beef liver catalase (Sigma) 40 γ/ml. or bovine serum albumin (0·1 mg/ml.) as indicated.

Table 3. EFFECT OF VARYING CATALASE CONCENTRATIONS ON MYCOPLASMA-INDUCED METHAEMOGLOBIN FORMATION

Incubation time	Catalase concentration (γ/ml.) 8	4	2	1	0
0	−	−	−	−	−
1·5 h	−	−	−	−	+

Reaction mixtures resembled those in Table 2 except that 1 ml. of turkey haemoglobin (0·167 per cent) in Ringer's solution was contained in a dialysis sac which was separated from the similarly enclosed mycoplasma suspension by buffer, mannose and catalase at the concentrations shown.

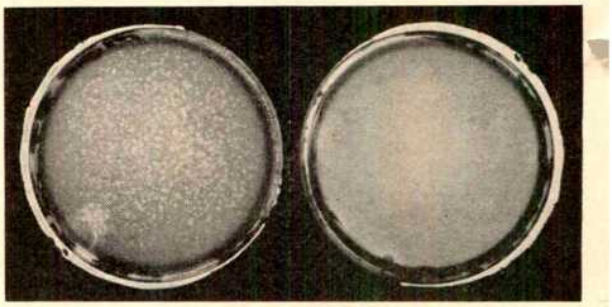
Table 4. PEROXIDE PRODUCTION BY *M. gallisepticum*

Conditions of incubation	0° C 1 h	Heat inactivated* mycoplasmas	37° C 5 sec	37° C 10 min
Peroxide detected	None	None	None	$6{\cdot}3 \times 10^{-5}$ molar

Reaction mixtures contained a suspension of 5×10^9 mycoplasmas in mannose (25 mg/ml.) and phosphate buffer *p*H 7·2 (0·05 M) in a 1 ml. volume. Following incubation the mycoplasmas were sedimented at 18,000*g* for 15 min and the supernatant was treated with peroxidase (4 u./ml.) and 4-methoxy-α-naphthol (2×10^{-4} M). The *O.D.* at 620 mμ was recorded on the Gilford spectrophotometer.
* 70° C for 15 min.

Table 5. INHIBITION BY CATALASE OF HAEMOLYSIS AROUND COLONIES OF *M. gallisepticum* IN BLOOD AGAR

	Concentration of catalase in overlay 60 γ/ml.	15 γ/ml.	1·5 γ/ml.	0
No. of haemolytic colonies	0	200	500	>1,000
Av. diam. of haemolysed areas	−	0·1 mm	0·5 mm	1·0 mm



A *B*

Fig. 1. Inhibition by catalase of *M. gallisepticum* haemolysis. *A*, *M. gallisepticum* grown on PPLO-agar, overlaid with turkey blood agar, showing haemolysis around colonies; *B*, same, with 0·060 mg/ml. catalase incorporated in blood agar

This work was supported by a contract from the U.S. Army Medical Research and Development Command Department of the Army.

Note added in proof. Following the submission of this paper, Somerson *et al.*[6] reported that the haemolysis produced by *M. pneumoniae* on blood agar plates is inhibited by catalase and peroxidase, and suggested that hydrogen peroxide was responsible.

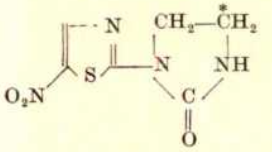
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Selective Uptake of an Antibilharzial Nitrothiazole Compound by *Schistosoma mansoni*

REPORTS on the uptake of schistomicidal drugs in the parasite are few and so far have been limited to ^{124}Sb-labelled antimonials[1,2]. Following the description of a potent nitrothiazole schistosomicide, 1-(5-nitro-2-thiazolyl)-2-imidazolidinone (Ciba 32,644-*Ba*)[3,4], the distribution of this compound was studied in mice infected with *Schistosoma mansoni*. The ^{14}C-labelled nitrothiazole was found to be highly concentrated in both adult worms and embryonated eggs.

Ciba 32,644-*Ba*, labelled with ^{14}C in position 4 of the imidazolidinone ring (I) (specific activity 2·3 μc./mg) (ref. 5), was administered orally to two female mice in a dose of 1 g/kg.



I

The animals, which had been infected with cercariae 7 weeks before treatment, were bled 6 h and 24 h after receiving the drug. Various tissues were fixed in phosphate-buffered 10 per cent formalin and embedded in paraffin. Adult worms were removed from the mesenteric veins and were carefully washed in saline before histological processing; segments of the intestine were treated in the same way. Microautoradiographs were prepared by covering 5μ sections with '*AR* 10' stripping

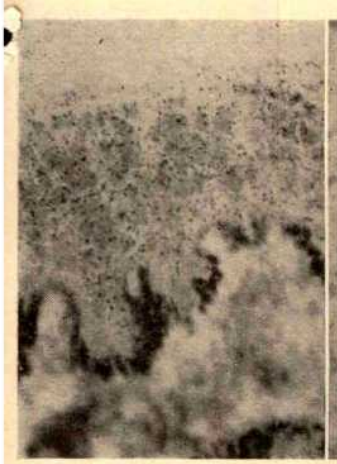


Fig. 1

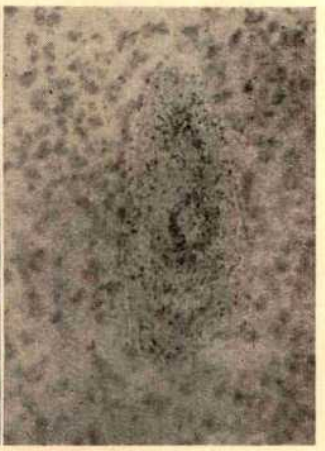


Fig. 2

Fig. 1. Autoradiograph of female *S. mansoni* shows high concentration of activity in vitellogenous cells, 24 h after treatment of the host with ^{14}C-labelled 32,644-*Ba*. Intestinal cells are covered with pigment deposits (×540)

Fig. 2. Heavy labelling of embryonated ovum in the liver tissue of an infected mouse, 24 h after treatment with ^{14}C-labelled nitrothiazole. Cells composing the granuloma contain little activity (×540)

film and exposing for 8 weeks at 4° C. Grain counts were made over 20 random areas of 10^{-4} mm^3 in each tissue examined.

The distribution of ^{14}C in the autoradiographs is given in Table 1. Female worms (Fig. 1) were found to incorporate the label to a larger extent than the males and the concentration in the deposited ova (Fig. 2) was greater than that in any other tissue except gastric mucosa. Eggs within the liver showed the same level of radioactivity as eggs deposited in the intestinal wall, despite the fact that the former were more densely surrounded by fibrous granulomata.

Table 1. AUTORADIOGRAPHIC DISTRIBUTION* OF ^{14}C IN *Schistosoma* AND IN TISSUES OF INFECTED MICE TREATED WITH A SINGLE ORAL DOSE OF 1 G/KG CIBA 32,644-*Ba* LABELLED WITH ^{14}C

Tissue	Time after administration 6 h	24 h	Significance of difference P†
S. mansoni, male			
Muscle cells	7·6 ± 2·1	21·1 ± 3·2	< 0·001
Gonads	6·2 ± 1·9	22·9 ± 3·2	< 0·001
S. mansoni, female			
Vitellogenous cells	14·8 ± 3·2	37·7 ± 5·8	< 0·001
Gonads	9·6 ± 1·6	22·5 ± 3·2	< 0·001
S. mansoni eggs (liver)	22·1 ± 7·8	40·9 ± 4·2	< 0·001
Liver granuloma	11·4 ± 4·3	13·0 ± 2·9	N.S.
Liver epithelium	10·4 ± 2·4	11·4 ± 2·9	N.S.
Gastric mucosa	57·5 ± 6·4	50·8 ± 5·6	≃ 0·001
Ileum mucosa	14·6 ± 4·9	9·4 ± 2·4	< 0·01
Colon mucosa	7·4 ± 2·1	15·0 ± 3·2	< 0·001
Kidney cortex	10·3 ± 2·4	13·2 ± 3·5	< 0·01
Heart muscle	7·9 ± 2·1	9·4 ± 3·5	N.S.
Lung	4·1 ± 2·7	7·7 ± 2·1	< 0·001
Spleen	5·6 ± 2·4	8·6 ± 3·5	< 0·02
Ovary, corpus luteum	—	12·0 ± 4·3	—
Ovary, follicular cells	—	7·3 ± 1·6	—

* Grain number per unit area ($n = 20$), mean ± standard deviation.
† P ascertained with t-test.
N.S., not significant.

In the majority of the host tissues, radioactivity levels were fairly uniform and there was little or no increase with time. The high concentration found in the intestinal mucosa appears to be consistent with prolonged absorption of the orally administered compound.

Studies in species other than the mouse[5] have indicated that the absorption of ^{14}C-labelled 32,644-*Ba* proceeds slowly over several hours following a single oral dose. During absorption, the plasma concentration of the unchanged product is maintained at a low level (about 0·5 μg/ml.) since rapid metabolic conversion of the compound occurs in the tissues. This leads to an increase in the plasma level of the metabolites. The latter are excreted slowly in the urine, as well in the bile and faeces.

In order to exclude the possibility that the accumulation of ^{14}C in the parasite was due to the uptake of metabolites, an *in vitro* incubation technique[4] was used.

Pairs of adult worms were incubated for 24 h at 37° C either in rabbit plasma to which was added ^{14}C-labelled 32,644-*Ba* or in metabolite-containing plasma obtained from rabbits pretreated 48 h previously with 100 mg/kg of the compound. The parasites (about twenty pairs of approximately 15 mg wet weight) were then ashed and their activity was determined by liquid scintillation spectrometry. The results (calculated with the aid of a ^{14}C–32,644-*Ba* standard) are given in Table 2. The data indicate that only the non-metabolized nitrothiazole was incorporated by the parasite. The rate of incorporation was independent of the concentration of 32,644-*Ba* in the medium at low levels. The small amount of label which was taken up by parasites from metabolite-containing rabbit plasma was due presumably to the presence of traces of unchanged nitrothiazole. By means of thin-layer chromatography of lyophilized homogenate of worms on silicic acid (solvent system: chloroform-dimethylformamide, 9 : 1) it was shown further that treated parasites contained only metabolites of 32,644-*Ba*.

Table 2. *In vitro* UPTAKE OF ^{14}C-LABELLED CIBA 32,644-*Ba* AND ITS METABOLITES BY *S. mansoni*

Addition to incubation medium	Concentration in Plasma (μg/ml.)	Parasites (μg/g)	Incorporation index
32,644-*Ba*	0·58	34	59
32,644-*Ba*	1·65	98	59
32,644-*Ba*	12·9	121	9
Metabolites*	9·1	1·4	0·15

For conditions of experiment see text. The lowest plasma concentration used (0·58 μg/ml.) corresponds to the level of Ciba 32,644-*Ba* attained *in vivo*.
* Containing traces of non-metabolized nitrothiazole (approx. 0·005 μg/ml.).

The present results allow the conclusion that unchanged Ciba 32,644-*Ba* is taken up from body fluids both by adult *Schistosoma mansoni* parasites and by embryonated eggs over a prolonged period of time and that metabolites accumulate in organs of the parasite. Rapid metabolic conversion of the nitrothiazole by the parasite appears to be responsible for the ensuing tissue damage, possibly by causing overloading of a particular enzyme system which, in the light of preliminary data, might be a nitro-reductase. The concentration of radioactivity was highest in tissues which can be shown to suffer early morphological changes (that is vitellogenous cells of females and gonads of both sexes)[6].

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BIOLOGY

Observations on the Opah, *Lampris regius* (Bonnaterre)

AN opah, *Lampris regius* (Bonnaterre), was captured on February 11, 1964, by the Japanese research vessel *Shoyo Maru* at approximately 3° 15·8′ S. and 128° 18·0′ W. in the South Pacific ocean. This individual, weighing 64 kg and measuring 119 cm (fork length), was caught at a depth of approximately 100 m on long-line gear used for fishing for tunas and billfishes[1].

The body of the fish was covered with scars, as is usual for this species. Bell and Kask[2] have shown that some of the scars are caused by lampreys, *Lampetra tridentata*, but that others are of unknown origin. My experience with *L. regius* in the waters of the South

Pacific leads me to conclude that many of the scars result from bites by squid. The outline of the scars, which is often oval rather than round, or were like two overlapping circles or ovals, is very similar to some of the wounds and scars observed by Guitart[3] on *Xiphias gladius*. Such shapes could be expected as a result of a wound inflicted by the beak of a squid. It is unlikely that such scars could be a result of such parasites as *Pennella*. I have examined twenty-seven specimens of *L. regius* from the South Pacific, caught by the R.V. *Shoyo Maru* during her twelfth voyage[4], and none of these has had any external parasites, with the exception of one individual which was infested around the eye with a few minute copepods *Clavellistes shoyoae*[6]. *L. regius* is caught on long-line gear in many parts of the Pacific Ocean[1,4,5]. Persons engaged in long-lining for tunas have informed me that *Pennella* is not found on opah.

This 64 kg individual was a running-ripe female. The eggs, which oozed out when pressure was applied to the sides of the body, were spherical, colourless, and transparent, with a diameter of 2·30 mm. When placed in sea-water the eggs sank. Neither oil globules nor any pattern on the surface of the eggs were visible with the naked eye. The eggs were kept in sea-water for about half an hour prior to preservation in 5 per cent formalin, at which time they became whitish and opaque although their diameter did not change. The membrane of the preserved eggs appears to be transparent, but under the magnification of a dissecting microscope the presence of very minute, linearly arranged rugosites can be detected. There is a well-delimited perivitelline-like space in the preserved eggs.

The fish was dissected and its ovaries examined. The two ovaries together weighed 980 g; a considerable portion of the eggs was, however, lost while bringing the fish aboard the vessel. The ovarian eggs were in various stages of maturation, the immature eggs being opaque. Two squid beaks were the only material found in the stomach.

I thank the Nankai Regional Fisheries Research Laboratory for permission to participate in a portion of Voyage 13 of the *Shoyo Maru*.

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Nest-structure of a Social Wasp varying with Siting of Leaves

THE nest-building habits of a social wasp, *Ropalidia variegata* (Smith) were closely observed at the Indian Statistical Institute, Calcutta, from November 1963 to July 1965. Each nest is constructed largely on the lower side of a leaf, to which it is attached by a stiff and rather brittle pedicel, which is continuous with the first cell. The remaining cells of the nest, arranged in vertical rows[1,2], hang almost vertically from the first cell, the second cell being placed about 45° to the right or left of the line of gravity of the first cell (Fig. 1).

Forty-seven nests, comprising eleven colonies, were built on leaves of the cultivated banana (*Musa sapientum*), and fifty-three nests (five colonies) on leaves of the dicotyledon, *Diospyros discolor*. It was found that the number of vertical rows of cells differed in the nests on the two types of leaves, and this is thought to have been due to the pronounced differences in the morphology of the leaves. That the shape of the nest of some species of *Ropalidia*, notably *R. fasciata*, depends to some extent on that of the object to which it is attached has been reported[1]. This means that nests suspended from an elongate object, such as a stalk or a palm leaflet, tend to be more elongate than those hanging from a leaf with a broad surface.

A banana leaf is very large, its lamina measuring about 180 × 60 cm. Its petiole is very stout and grooved, extending into the lamina region as the prominent mid-rib. The lower part of the petiole always remains vertical as it partially wraps the erect pseudo-stem. The long and broad lamina is thus adapted for upward or downward movement. Even when the lamina is blown horizontally, its mid-rib generally maintains the usual angle of inclination. The wasp nests only on the lower surface of the lamina and invariably along the raised mid-rib (Fig. 1*a*). On account of this position and since the banana leaf generally moves in a vertical plane, the hanging nests seldom lose their balance.

A leaf of *Diospyros discolor* measures about 20 × 6 cm and the plane and position of its lamina often change, since it easily responds to wind. The smaller size of the leaf (compared to that of the banana), the weaker petiole and the flexible nature of the shoots on which the leaves are borne are factors that tend to produce frequent changes in the position of the lamina.

59·6 per cent of nests of the eleven colonies on the banana plants possessed only two rows of cells each, and this figure differs significantly even at the 1 per cent level (χ^2 test with one degree of freedom) from the corresponding one for the nests on *Diospyros* (34 per cent).

In order to test how far the structure of the nests on the two plant species differed, tests for homogeneity of parallel samples[3] were performed after condensing the data as shown in Table 2, where group *A* represents nests on *Diospyros* and *B* on *Musa*.

The χ^2 test showed that this difference is significant at the 5 per cent level. The difference between the mean number of rows of cells per nest on banana and *Diospyros* (Table 1) was significant at the 5 per cent level ('*t*'-test).

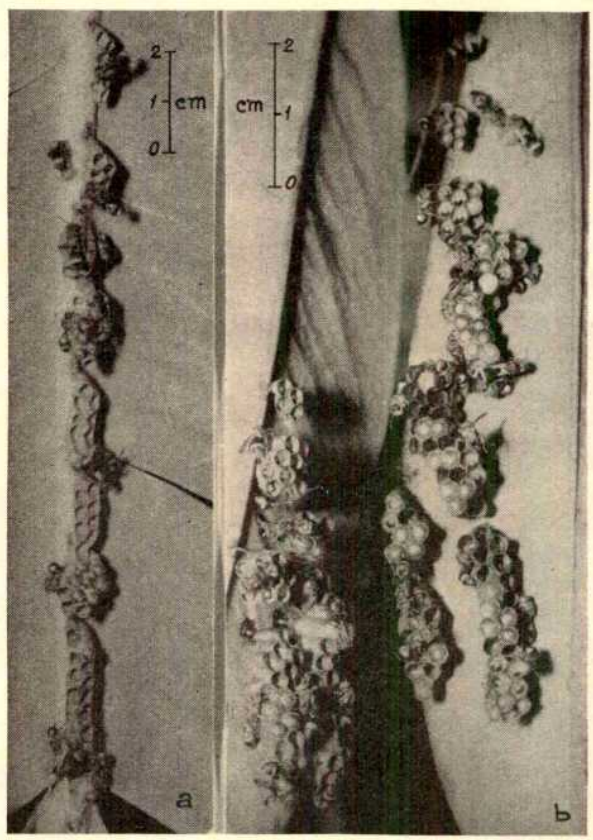


Fig. 1. Colonies of *Ropalidia variegata* (Smith) on the leaves of *Musa sapientum* (*a*) and *Diospyros discolor* (*b*)

Table 1. NUMBER OF ROWS OF CELLS PER NEST OF *Ropalidia variegata*

No. of colonies	No. of nests	Plant	No. of vertical rows per nest 1	2	3	4	5	6	7	Mean
5	53	*Diospyros*	1	18	20	9	4	0	1	3·0189
11	47	*Musa*	1	28	11	7	0	0	0	2·5106

Table 2

Group	Rows of cells per nest <3	3	>3	Total
A	19	20	14	53
B	29	11	7	47
A+*B*	48	31	21	100

As the nests always hang from their first cells, they are unable to withstand permanent deviations from their initial vertical postures that may be caused by wind. When the position of a leaf bearing these nests is slightly altered, the stability of the tilted nest is restored by adding new cells or rows of cells along the side nearer to the line of gravity. A colony on a leaf lasts for a few months, and during this period the leaf may be subjected to further deflections in different directions. Thus, the number of cell-rows in a nest increases in course of time, and the nests show vast variations. In many nests, the early formed cells follow a two-row pattern. But, with time, more and more cells are added at various positions of the nest to combat the disturbances caused by the wind.

One of the nests of a colony, which had two rows of cells, slipped from its attachment and was refixed by a paper clip. Obviously the repair was not approved by the wasps, for they forthwith added new cells, forming a further five vertical rows of two cells each, thereby restoring the equilibrium of the nest[4]. The biological significance of this phenomenon is important. In changing the structure of the nest to suit different sites and trees, the wasp demonstrates adaptability to ensure survival under varying conditions.

I thank Prof. van der Vecht, Rijksmuseum van Natuurlijke Historie, Leiden, for identifying the wasp.

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Infection of *Culex fatigans* with a Microsporidian

BIOLOGICAL control is assuming an ever-increasing importance as a means of augmentation of, or substitution for, the use of pesticides in the control of both medically and economically important arthropods. This applies in particular to *Culex fatigans*, the main vector of *Wuchereria bancrofti* throughout most of the tropics and subtropics. Control of this mosquito is complicated by the widespread occurrence of resistance to most insecticides, together with the complexities of larval control by sanitation or other non-insecticidal methods.

In the course of attempts to infect *C. fatigans* with various pathogens and parasites, infections with a microsporidian provisionally identified as *Plistophora culicis* Weiser[1,2] were established in both adult and larval stages of this mosquito. The spores of this microsporidian were obtained from Prof. Meir Yoeli, of New York, who found it to be the cause of high mortality in his colonies of *Anopheles stephensi*. The infection originated in wild-caught *Anopheles dureni* from the Congo[3].

The infections were obtained in the adults by suspending a cotton-wool swab moistened with a fresh suspension of spores in a cage containing newly emerged mosquitoes. The swab was moistened morning and evening with distilled water. No alternative food supply was provided for the first week, then sugar water was made available.

Larvae were infected by hatching *C. fatigans* eggs in a bowl to which fresh spores had been added. Once again no food was added for the first week during which time

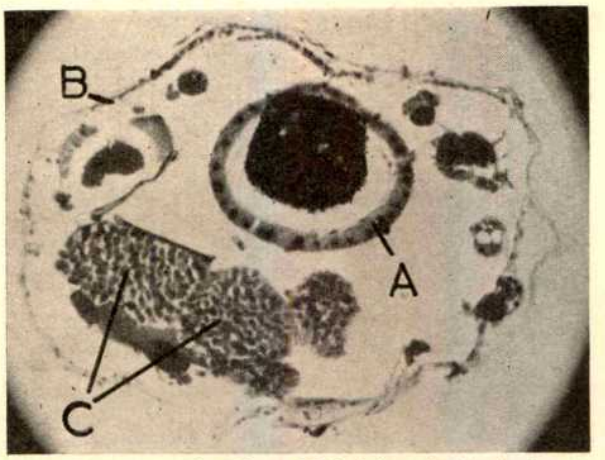


Fig. 1. Transverse section of larva of *Culex fatigans* showing developmental stages of *Plistophora culicis* in fat body. *A*, Gut wall; *B*, body wall; *C*, infected fat body

mortality was high. Thirty-three larvae remained alive at this stage. From these a total of three adults, two pupae and two larvae were found to be infected.

No external signs of infection were noted in the parasitized larvae, pupae, or adults, all infections being found on microscopic examination. Larvae, on sectioning, showed considerable involvement of the fat bodies (Fig. 1). The infection did not prevent larvae pupating or adults emerging from these pupae; larval life was prolonged, however, up to 56 days in one case.

Further experiments are being carried out using spores stored in distilled water at 0° C for 2–4 months and spores stored in liquid nitrogen, either dry or in glycerol, for 3–12 months. Infections have been established in *Anopheles stephensi* using spores stored dry in liquid nitrogen for 3 months.

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Air Movement and Termite Behaviour

TERMITES show great activity around even a small breach in their nest and soon begin to build to repair the damage. In laboratory vessels, termites seal off any gaps to the outside air, even around the edge of an apparently close-fitting stopper. The stimulus inducing this behaviour has most often been considered to be a fall or gradient of humidity (see, for example, Grassé and Noirot[1], Stuart[2]).

I have investigated the nest-building behaviour of the damp-wood termites *Zootermopsis angusticollis* and *Z. nevadensis* (Hagen). In addition to the behaviour already described, these insects build crusts or ledges of material over their living quarters when they are kept in laboratory vessels. This material usually consists of faecal or wood particles cemented together with a watery secretion exuded from the anus. In large specimen tubes that contain a central partition made of wood or cardboard and sawdust for building material at the bottom of the tube, the termites tend to build on upward-facing edges and ultimately build a ledge from the top of the partition across to the walls of the tube. Any gaps in the partition above a certain level are filled in. It was found, however, that whereas building continued at a rapid and fairly constant rate at a relative humidity of 92 per cent, at lower relative humidities (56 per cent and 35 per cent) the rate of building was initially lower and building ceased almost totally after the first 24 h of the experiment, when the overall activity of the insects was greatly

reduced. It seems unlikely, then, that a fall in humidity is a stimulus to nest-building activity. In fact, Ernst[3] showed that *Z. nevadensis* takes 5–6 h to react in a humidity gradient; some other species took several days.

In further tests, termites were put into an open 100 × 30 mm glass tube with a centre partition 15 cm high coming out of the tube. The termites immediately began to climb the partition, but at certain levels showed an 'avoidance reaction' and turned back. The avoidance reaction began 2–3 cm above the bottom of the tube and occurred with gradually increasing frequency towards the open end. It must be assumed that this is a response to air movement, since within the relatively few seconds taken to set up the experiment there was no time for gradients of, for example, humidity or carbon dioxide to be established. After a brief phase of apparent exploratory behaviour the insects began to cement building particles on to the partition. From the combined results of a number of experiments with *Z. nevadensis* a direct correlation was found between the distribution of building particles and the 'distribution' of avoidance reactions ($r = 0{\cdot}87$, $P < 0{\cdot}01$). The air movement in an open tube was measured by recording the movement of a very fine cotton hair under a microscope, and the amplitude of movement was found to increase logarithmically towards the open end of the tube. The amplitude of air movement and the number of avoidance reactions at different levels were also found to be closely correlated ($r = 0{\cdot}97$, $P < 0{\cdot}0001$ for *Z. augusticollis*; $r = 0{\cdot}93$, $P < 0{\cdot}001$ for *Z. nevadensis*).

These results show that termites have an extremely high sensitivity to air movement. This sensitivity varies continuously, and the absence of a clear threshold is in accordance with the general theory of sensory discrimination of Duncan and Sheppard[4]. The evidence obtained here shows that a very small proportion of termites can detect air movements of the order of one thousandth of those in a closed room. The sense organs concerned are believed to be located on the antennae, since (*a*) termites with the distal two thirds of their antennae amputated do not show an avoidance reaction in a gradient of moving air, and (*b*) responses to light puffs of air on the antennae have been recorded from electrophysiological preparations with electrodes in the antennal nerve.

This very high sensitivity to air movement clearly helps to explain how termites are attracted to even minute openings of their nest to the exterior, and could explain how some dry-wood termites are able to leave a paper-thin shell of wood between themselves and the outside air. It also suggests that great caution is necessary in the design of experiments with termites. Lüscher[5] has shown that there is a continuous circulation of air in the large mound nests of *Macrotermes natalensis* (Haviland). In Ivory Coast nests hot air rises from the fungus gardens and nursery area and is cooled in large descending ducts below the surface of the mound. In Uganda nests the hot air diffuses outwards from chambers at the top of the mound and cool air enters below. It is very difficult to suggest how such complex nests come to be built, but it may well be worth considering that termites ensure a circulation by building around air currents that are set up in the developing nest.

Most of the work described here was done during the tenure of a North Atlantic Treaty Organization research fellowship of the Department of Scientific and Industrial Research, held in the department of Prof. M. Lüscher (Berne), to whom I am indebted for his hospitality and assistance.

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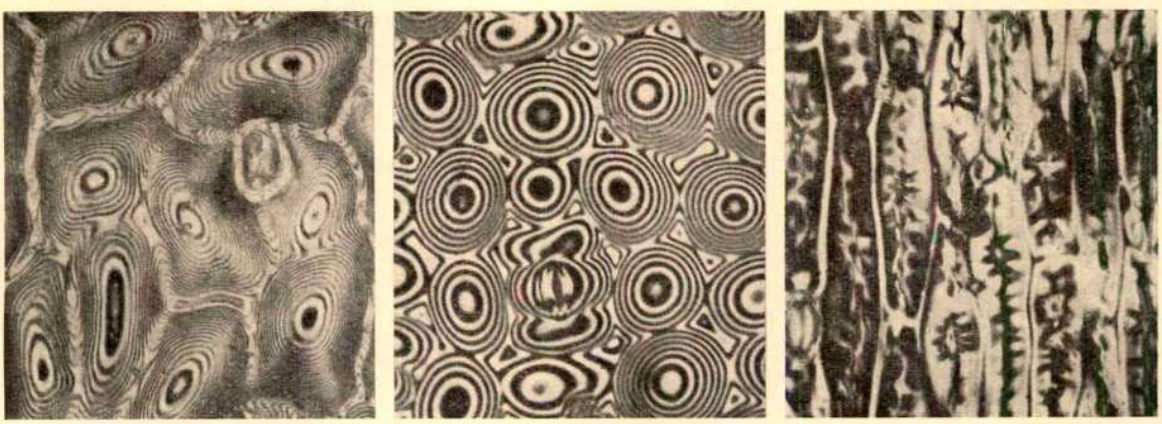
Interference Microscopy of the Pattern of Leaf Surfaces

INTERFERENCE microscopy with reflected light is generally used for surface control of ball-bearings, but the technique can be adapted for investigations on the nature of plant surfaces.

As plant surfaces are generally dull and reflect little light, it is necessary to prepare several replicas. Plastic film (0·07 mm thick) is softened with acetone and pressed on to the leaf surface. After 3–5 min the replica is sufficiently hard and is stripped off and placed in the so-called 'Zehender chamber' (a mirrored surface with a cover slip); it is then viewed under the microscope.

Depending on the nature of the surface, one obtains interference pictures with lines of constant distance on the embedding medium. In air the line distance is 0·54μ, in water 1·5μ, and in certain oil mixtures 3μ and 5μ, respectively. In Fig. 1*a*, *b* and *c*, replicas with contour lines enable one to draw profile lines along any direction. In this way a picture of the actual relief of the surface can be gained.

This technique can be used to check changes in the pattern of the surface of a leaf as it develops and the influence of various physiological conditions on the



a *b* *c*

Fig. 1. Interference pictures of leaf surfaces (×270) using a surface interference microscope (A. Kohaut). A thallium lamp was used for illumination. *a*, Pattern of the upper surface of the leaf of *Bryophyllum daigremontianum*; plastic replica in air; line distance 0·54μ. *b*, Pattern of the lower surface of the leaf of *Zebrina purpursii*; plastic replica in water; line distance 1·5μ. *c*, Pattern of the leaf of *Haemanthus albiflos*, upper surface; gelatine replica in oil; line distance 3μ

epidermal surface. It can also be used to obtain information regarding the after-effects of pesticides on plant surfaces.

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A Second Gibberellin-like Substance in Immature Barley Seed

IN an earlier paper it was shown that gibberellic acid occurs in the immature seed of barley, and it was mentioned that a second gibberellin-like substance was found in the extracts[1]. This second substance was eluted from a charcoal : celite column with 90 per cent aqueous acetone. When chromatogrammed on paper using *n*-butanol–1·5 N ammonia (3 : 1) or benzene–glacial acetic acid–water (4 : 2 : 1) it had the same R_F as gibberellins A_4 and A_7.

These preliminary experiments were carried out with samples gathered one week and four weeks after anthesis. There appeared to be more activity in the younger sample, so the following year a large batch was gathered about ten days after anthesis. Extraction of the bulk of this yielded only gibberellic acid, but a small part of the sample was extracted separately by a slightly different procedure, and both substances were found on this occasion. Bioassay of the second substance by dwarf pea seedlings[2] indicated an activity of 0·5 μg gibberellic acid equivalent per kg fresh weight of seed, whereas the activity shown by the cucumber hypocotyl assay[3] was 24 μg gibberellin A_9 equivalent per kg. Part of the sample was further purified by elution from a silica : celite column (1 : 2 w/w) with varying proportions of ethyl acetate and chloroform. Most of the activity was found in those fractions in which gibberellins A_4 and A_7 would be expected. Part of this repurified sample was tested by the lettuce seedling assay[4], in which only a trace of activity was found, and part was tested on dwarf maize seedling[5] (cultivar d_1), no significant activity being found.

The high activity in the cucumber assay, the low activity in the pea seedling test, and the R_F evidence all suggested that the substance could be gibberellin A_4 or A_7, but the lettuce assay result appeared to eliminate gibberellin A_7, which is highly active in this assay, while the activity seemed too low even for gibberellin A_4. Both gibberellins A_4 and A_7 are as active as gibberellic acid in the d_1 maize assay, so this assay also failed to confirm the identity of the unknown substance.

Thin-layer chromatography of the sample was carried out by the method previously described, but no fluorescent spot could be detected after spraying with sulphuric acid. It is possible that some fluorescence may have been masked by impurities, but the extracts appeared to be fairly highly purified at this stage. Most and Vlitos[6] have reported the extraction from sugar cane of a group of substances with similar properties, that is, they are biologically active especially in the cucumber assay but do not fluoresce on thin-layer chromatograms. This lack of ability to fluoresce after reacting with sulphuric acid may imply that these substances do not have a gibbane skeleton, but it is not yet known whether all gibbanes fluoresce, and these substances may be gibberellins in structure as well as activity.

I thank Dr. D. F. Jones for the thin-layer chromatography.

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RADIOBIOLOGY

Previous Reproductive History and the Susceptibility to X-ray-induced Congenital Anomalies

THE suspicion that primiparous mice are more apt to produce anomalous offspring, following foetal X-irradiation, than are multiparous mice, has led to the necessity of determining the normal incidence of such anomalies among large numbers of control mice of both categories. This investigation deals with the normal primiparous and multiparous *CF*1 mice of exactly the same age (7–9 months) and the analysis of the condition of their offspring. In direct contrast with this is presented a parallel study of pregnant primiparous and multiparous *CF*1 mice X-irradiated at a specific time and to a specific dose to determine whether a single variable (primiparity or multiparity) might alter the incidence of anomalies induced by X-rays. This paper reports data from 225 litters (2,700 implantations).

The strain of mouse used was the *CF*1 which we have found to be satisfactory for many years. All mice for this experiment were received at one time from the source, and were kept under identical conditions of temperature, humidity, food and water for the duration of the experiment. All females used were from 7 to 9 months of age (the range being due to the fact that pregnancies for the multiparous mice could not always be immediately achieved). The multiparous females were kept pregnant from 2 months of age, and every one had been pregnant four times followed by a 2-week period of rest before the experimental pregnancy.

Pregnancies were achieved by exposing the females to males of the same strain for a period of 2 h (8–10 a.m.) and females found to have vaginal plugs were segregated and labelled as to the time of conception. At 18 days gestation each pregnant mouse (controls or irradiated) was dissected and the contents of the uterus exposed and analysed, and the sex of each foetus determined.

For X-irradiation, paired tubes were used in a cross-fire of rays, with the pregnant mice placed equidistant between the tubes so as to be exposed uniformly to the rays. The dose was calculated as the dose delivered to the foetuses within the gravid uterus. (The physical factors of irradiation were 184 kVp. and 30 m.amp; half value layer, 0·6 mm copper, filtration 0·28 copper and 0·50 aluminium, distance from the tubes to the uterus 72 cm; and dose rate 50 r./min). A single exposure-level of 150 r. was chosen and delivered at exactly 8 days gestation because previous experience had shown that this stage and dose would definitely provide a wide gamut of congenital anomalies[1–3].

The anomalies observed besides resorption and death were: exencephaly, cephalic blisters, microcephaly, brain haemorrhage, eye and jaw defects, persistent exteriorized intestines, and stunting. There was no attempt to determine inner histological alterations.

The experimental data involve 125 control and 100 experimental pregnancies, giving 1,484 control implantations and 1,216 irradiated implantations. There appeared to be little difference in the condition of the foetuses among the controls except that there was a higher (3·3 per cent) incidence of anomalies among the primiparous than among the multiparous females (1·5 per cent). There were a few more dead foetuses among the multiparous controls (1·8 per cent) than among the primiparous controls (0·6 per cent), but the percentage of apparently normal foetuses was quite similar.

The mouse embryo at 8 days gestation is just beginning massive differentiation of almost all its tissues so that it is quite susceptible to the insult of X-rays[4]. An exposure of 150 r. at this time will invariably produce anomalies, so that similar treatment of the primiparous and the multiparous females should indicate whether previous

Table 1. *CF*1 WHITE MICE AT 7–9 MONTHS OF AGE

		No. of litters	No. of implant	Litter Av.	Normals Tot.	%	Dead Tot.	%	Resorptions Tot.	%	Anomalies Tot.	%	Sex ratio ♂	♀
Primiparous	Controls	61	687	11·26	579	84·3	4	0·6	81	11·8	23	3·3	52	48·9
	8-day gestation + 150 r.	50	596	11·92	233	39·1	28	4·7	128	21·5	207	34·7	57·1	42·9
Multiparous	Controls	64	797	12·45	669	83·9	14	1·8	102	12·8	12	1·5	52·6	47·4
	8-day gestation + 150 r.	50	620	12·4	271	43·7	34	5·5	132	21·3	183	29·5	58·3	41·7

Table 2. FEMALE MICE AT 7–9 MONTHS OF AGE X-IRRADIATED TO 150 R. AT 8 DAYS GESTATION. ANOMALY DATA (PER CENT)

	Primipara		Multipara	
	Males (249)	Females (191)	Males (249)	Females (205)
Normals	53·42	52·36	63·47	55·12
Exencephalies	28·11	31·94	23·29	28·78
Ceph. blisters	3·21	1·57	2·41	2·44
Hydrocephaly	0·0	0·0	0·40	0·0
Microcephaly	0·0	0·52	0·40	0·49
Brain haemorrhage	0·40	0·52	0·40	0·97
Eye defects	9·24	4·19	5·22	6·83
Prot. intestines	0·0	1·05	0·40	0·0
Ear deformity	0·0	0·0	0·40	0·49
Deformed jaw/head	0·80	0·0	0·0	0·0
Short tail	0·0	0·0	0·0	0·49
Stunted	4·82	7·85	3·61	4·39
	100%	100%	100%	100%

reproductive experience altered in any way the reaction of the embryos and foetuses to ionizing radiations.

The primiparous females, even though of the same age as the multiparous females, produced more anomalies (34·7 per cent) than did the multiparous females (29·5 per cent). While these percentage differences do not seem great, they involve a total of more than 1,200 foetuses examined and are indeed significant.

With regard to sex ratios, it was found that among unirradiated foetuses there were 52 per cent males to 48 per cent females, but among both X-irradiated groups the percentage of males was significantly higher (57·1 per cent and 58·3 per cent). This seems to suggest a differential mortality among the presumptive females. This is supported by a recent investigation[5] in which radiotherapy increased the frequency of human male births.

The anomalies are reported in Table 2. It should be noted that stunting occurs even among the controls (primiparous females 2·8 per cent and multipara 1·4 per cent). It has not been determined whether this is due to nutritional deficiency which could be rectified after birth, or to a congenital anomaly. Exencephaly rarely occurred among the controls, and is very greatly increased among both the irradiated groups—somewhat more so among the primiparous females. In addition there were other anomalies which occurred among those X-rayed, particularly eye defects which were found more frequently among the foetuses of our primiparous females. The overall incidence of anomalies appears to be slightly higher among those females which had never before been pregnant than among the multiparous females. Among the multiparous pregnancies, the males showed a lower incidence of anomalies than did the females. The explanation of this is not available, since there did not appear a commensurate depression in the implantation frequencies.

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Liberation of 5-Hydroxytryptamine in the Brain Stem of Adrenalectomized Rats after X-irradiation

EXPERIMENTAL results reported in previous papers have suggested that rat brain liberates 5-hydroxytryptamine (5-HT) after X-irradiation[1,2]. The biochemical and physiological investigation of irradiated experimental animals is complicated by non-specific neuroendocrine reactions which play a part in the adaptation syndrome. In order to exclude this factor, we carried out experiments on adrenalectomized rats, which are also more sensitive to X-irradiation. The main changes in the content of 5-hydroxyindoles after X-irradiation were observed in the brain stem[1], which contains the centres which regulate and co-ordinate the endocrine system.

In this paper we examined total, basic and acidic 5-O*R* indoles (where *R* is a hydrogen atom or an aryl or alkyl group) in the brain stem of adrenalectomized rats after X-irradiation (900 r.). The basic 5-O*R* indoles are mainly similar to the 5-HT and the acidic ones to 5-hydroxyindoleacetic acid (5-HIAA), the main metabolite of 5-HT. By the simultaneous determination of all these compounds it is possible to investigate the rate of biosynthesis, release and metabolism of 5-HT. The content of 5-HIAA can be used to measure 5-HT release because the main part of the 5-HT liberated is metabolized to 5-HIAA.

Albino rats of both sexes weighing 150–180 g were used. Bilateral adrenalectomy was performed under ether anaesthesia. The adrenalectomized rats were maintained on 1 per cent saline throughout the experiment. The dose (900 r.) was measured in an ionizing chamber over the period of exposure. The dose rate was 83 r./min. The radiation factors were 220 kV and 15 m.amp. A 0·5-mm copper and a 1·0-mm aluminium filter were used, and the distance of the target from the skin was 40 cm. Immediately after irradiation the animals were killed and the brains were isolated. Four brain stems were pooled for each determination.

Total and acidic 5-O*R* indoles were estimated according to the method of Ashcroft and Sharman[3] and basic compounds by the method described by Bogdanski *et al.*[4], which was slightly modified. Fluorimetric measurements were carried out using a 'Locarte' semifluorescence spectrofluorimeter in concentrated hydrochloric acid at 550 mμ and with an activating wave-length of 295 mμ.

Data showing the influence of X-irradiation on the content of total, basic and acidic 5-O*R* indoles in the rat brain stem are given in Tables 1 and 2.

Irradiation of controls produces a significant increase of total 5-O*R* indoles, as we have already reported[1]. Sham-operated animals behave in the same way as the controls.

In adrenalectomized rats there was an increase of total and basic 5-O*R* indoles on the first day after operation. After irradiation (900 r.) such animals showed a statistically significant increase of total and acidic 5-O*R* indoles in comparison with adrenalectomized and non-irradiated ones (Table 1).

On the seventh day after operation, an increase of total 5-O*R* indoles only was observed in the brain stem of adrenalectomized rats. Irradiation of these animals has a similar effect on 5-O*R* indoles as in rats which were irradiated on the first day after operation (Table 2).

West *et al.* have postulated that glucocorticoids regulate the level of 5-HT and histamine in tissue stores[5,6]. In adrenalectomized rats the content of 5-HT is increased in many tissues[7]. De Maio[8] observed an increase of 5-HT in the brain of adrenalectomized and hypophysectomized rats 24 h after operation. This increase was greatest in the basal part of the brain. Other workers have not obtained the same results[9,10]. From the aforementioned results it seems that the content of 5-HT in the brain of adrenalectomized rats depends on different intervals after operation. Adrenalectomy does not affect brain monoamine oxidase activity[10]. Therefore, increase of the 5-HT content of the brain of adrenalectomized rats is probably a consequence of increased 5-HT biosynthesis.

Table 1. 5-O*R* INDOLES IN BRAIN STEM OF ADRENALECTOMIZED RATS ON THE FIRST DAY AFTER OPERATION AND IMMEDIATELY AFTER X-IRRADIATION (900 r.)

		No. of results	Total 5-O*R* indoles (μg/g tissue)	Basic 5-O*R* indoles (5-HT) (μg/g tissue)	Acidic 5-O*R* indoles (5-HIAA) (μg/g tissue)
I	Control	6	1·234 ± 0·046	0·619 ± 0·073	0·577 ± 0·047
II	Irradiated (900 r.)	6	1·914 ± 0·112	0·563 ± 0·053	0·624 ± 0·043
III	Sham operated	5	1·103 ± 0·068	0·531 ± 0·032	0·402 ± 0·066
IV	Sham operated + irradiated (900 r.)	5	1·664 ± 0·061	0·541 ± 0·031	0·505 ± 0·047
V	Adrenalectomized	6	1·728 ± 0·099	0·889 ± 0·144	0·482 ± 0·032
VI	Adrenalectomized + irradiated (900 r.)	6	2·283 ± 0·039	0·918 ± 0·046	0·712 ± 0·025
		I : II	< 0·001	N.S.	N.S.
	t-test	I : III	N.S.	N.S.	N.S.
	P-values	II : IV	N.S.	N.S.	N.S.
		III : V	< 0·001	< 0·05	N.S.
		III : VI	< 0·001	< 0·001	< 0·01
		IV : VI	< 0·001	< 0·001	< 0·01
		V : VI	< 0·001	N.S.	< 0·01

Table 2. 5-O*R* INDOLES IN BRAIN STEM OF ADRENALECTOMIZED RATS ON THE SEVENTH DAY AFTER OPERATION AND IMMEDIATELY AFTER X-IRRADIATION (900 r.)

		No. of results	Total 5-O*R* indoles (μg/g tissue)	Basic 5-O*R* indoles (5-HT) (μg/g tissue)	Acidic 5-O*R* indoles (5-HIAA) (μg/g tissue)
I	Control	6	1·234 ± 0·046	0·619 ± 0·073	0·577 ± 0·047
II	Irradiated (900 r.)	6	1·914 ± 0·112	0·565 ± 0·053	0·624 ± 0·043
III	Sham operated	5	1·157 ± 0·039	0·559 ± 0·075	0·460 ± 0·050
IV	Sham operated + irradiated (900 r.)	5	1·699 ± 0·050	0·655 ± 0·055	0·581 ± 0·045
V	Adrenalectomized	6	1·684 ± 0·044	0·744 ± 0·134	0·453 ± 0·036
VI	Adrenalectomized + irradiated (900 r.)	6	2·226 ± 0·114	0·869 ± 0·082	0·930 ± 0·078
		I : II	< 0·001	N.S.	N.S.
	t-test	I : III	N.S.	N.S.	N.S.
	P-values	II : IV	N.S.	N.S.	N.S.
		III : V	< 0·001	N.S.	N.S.
		III : VI	< 0·001	< 0·02	< 0·001
		IV : VI	< 0·002	N.S.	< 0·01
		V : VI	< 0·01	N.S.	< 0·001

Ionizing radiation stimulates the hypothalamic centres[11] and activates many of the neuroendocrine reactions. One of the first reactions is the release of adrenocorticotrophic hormone (ACTH)[12]. Moussatché and Pereira[13] have suggested that 5-HT is a chemical mediator of ACTH release. It is possible that the liberation of 5-HT by the brain stem after irradiation is involved in this process.

Many of the early phenomena of radiation damage are caused by the freeing of neurohormones (including 5-HT) (ref. 14). Our results suggest that there is an intensive liberation of 5-HT in the brain stem after X-irradiation in adrenalectomized rats. This explains the increase of 5-HIAA. The increase of total 5-O*R* indoles after irradiation is much greater than the increases of 5-HT and 5-HIAA put together. This is probably due to the presence of other metabolites of liberated 5-HT in the brain stem not at present identified[1]. The liberation seems to be in dynamic equilibrium with biosynthesis, that is, greater liberation causes greater biosynthesis. Therefore, the content of 5-HT does not change in adrenalectomized rats after irradiation.

The next stage of our work will be the investigation of the mechanism by which 5-HT is liberated.

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Effects of Gamma Radiation on Freeze-dehydrated Apples (*Pyrus malus*)

BIOCHEMICAL and physiological effects of gamma-irradiated fresh fruits and vegetables have been reported from our laboratories[1–9] in an attempt to extend the storage life and maintain wholesomeness. Because they are almost weightless, and because of their high nutritive value, freeze-dehydrated food are convenient for consumption by astronauts during space flights. High-frequency short-wave rays may be encountered in space flights and/or subsequent landing on the Moon or other planets. The experiment reported here was to elucidate some chemical and histological effects of gamma-radiation doses on freeze-dehydrated apple slices.

Fresh 'Golden Delicious' apples were peeled, cored, sliced and freeze dehydrated as discussed in a previous paper[10]. The freeze-dehydrated apples were irradiated with caesium-137 gamma-rays at 0 (control), 1×10^5, 3×10^5, 6×10^5 and $1{\cdot}8 \times 10^6$ rads doses at a rate of $2{\cdot}5 \times 10^4$ rads/h. The irradiation temperature was 25° C and nitrogen gas was flushed over the samples during the process of irradiation.

Irradiated and non-irradiated products were analysed for volatile substances responsible for flavour and aroma and for nutritional aspects as reducing sugars and ascorbic acid. Methanol extracts of the products were used for colour determination. Rehydration rates were measured at 40° C. Histological studies were also conducted (Figs. 1–3).

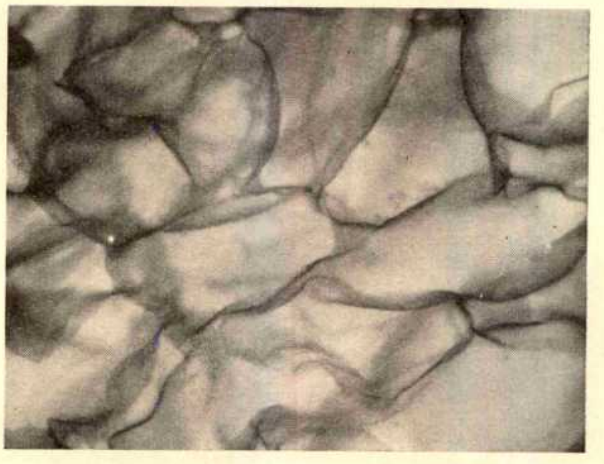


Fig. 1. Photomicrograph of parenchyma cells in freeze-dehydrated apple (control)

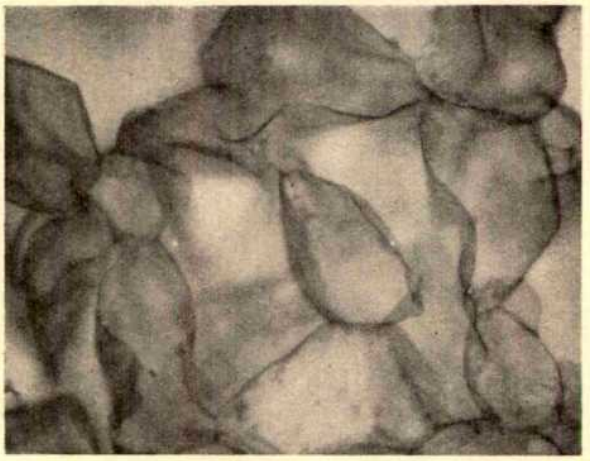


Fig. 2. Photomicrograph of parenchyma cells in freeze-dehydrated and irradiated apple at 3×10^5 rads doses

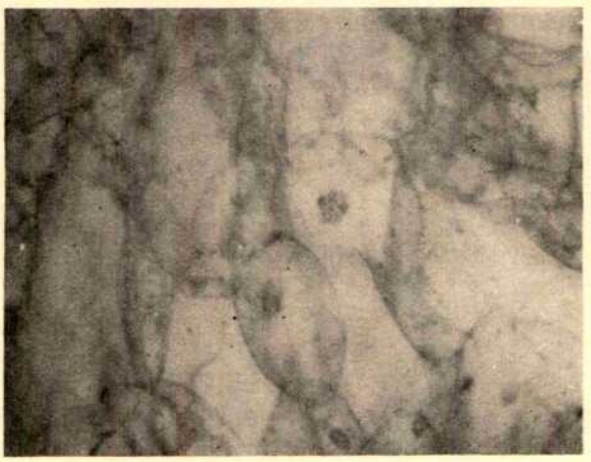


Fig. 3. Photomicrograph of parenchyma cells in freeze-dehydrated and irradiated apple at $1{\cdot}8 \times 10^6$ rads doses

Progressive browning and softening of the products were observed during irradiation as the doses increased beyond 1×10^5 rads. Chemical analyses showed (Table 1) that volatile compounds were reduced by low doses of radiation, but increased by doses of more than 3×10^5 rads. This may be due to the original volatile compounds being destroyed during exposure to 1×10^5 rads, while depolymerization of non-volatiles produced at high doses causes an increase in total volatiles. Free reducing sugars were increased almost imperceptibly as doses increased, while ascorbic acid was decreased. Optical densities at 420 μm and rehydration rates were also increased at high doses. Microscopic examination of the cell structure showed that some parenchyma cells were separated with radiation doses of 3×10^5 rads (Fig. 2). Some ruptured and thin cell walls were observed at doses of $1{\cdot}8 \times 10^6$ rads and coagulation of certain cellular materials was also obvious (Fig. 3). It is believed that freeze-dehydrated fruits are more stable than fresh fruits when both are similarly irradiated. The difference may be due to the active enzyme systems in fresh products.

Table 1. Effects of γ Radiation on Freeze-dehydrated Apples

Doses (rads)	Volatile reducing substances (μequiv./100 g)*	Reducing sugars (%)	Ascorbic acids (loss %)	Optical density at 420 μm	Rehydration ratio†
0×10^5 (control)	2,464	53·56	0	0·158	4·25
1×10^5	770	54·08	6	0·208	4·28
3×10^5	1,078	54·60	16	0·212	4·46
6×10^5	1,232	58·24	27	0·218	4·58
$1{\cdot}8 \times 10^6$	2,433	59·80	38	0·220	4·91

* Microequivalent of potassium permanganate/100 g.
† Ratio of wet weight to dry weight after 3 min.

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ANATOMY

Intestinal Villi in Rhesus Monkeys

Literature on villus pattern in the rhesus monkey is scarce. Lineback[1] noted, using ordinary histological methods, that villi were flange-shaped in the duodenum and that towards the lower end of the ileum they attained a more typical finger-like form. It is, however, difficult to decide firmly about the shape of villi without dissection microscopy. Booth *et al.*[2] pointed out that leaf-like villi may appear finger-like in a histological section depending on its plane. This communication reports on the configuration of intestinal villi in apparently normal rhesus monkeys utilizing the technique of dissection microscopy.

In this investigation, twenty-two animals, which had been trapped 10–20 days previously, were autopsied after sodium pentothal anaesthesia. Their body weight varied from 1·50 to 8·50 kg with an average of 2·80 kg. The animals were fed on a mixed diet of bananas, soaked gram and wheat bread for 1–5 days before they were killed. In a concurrent investigation of 101 animals, including ten used in the present experiments, the faecal fat content varied from 1 to 4 g per cent with an average of 1·8 per cent[3]. The small intestine had a normal gross appearance in these animals.

Eighteen small pieces from the duodenum, forty-six from the proximal 4 in. of the jejunum and twenty-four from the terminal ileum were investigated under the dissection microscope in a fresh state. These blocks were subsequently embedded in paraffin and sections were studied histologically after staining with haematoxylin–eosin.

Three types of villi were commonly encountered on dissection microscopy. Classical finger-like and tongue- or leaf-like villi constituted the first two categories (Figs. 1

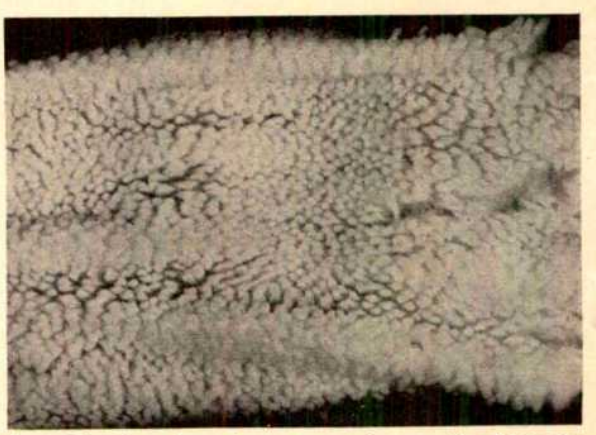


Fig. 1. Dissection microscopic appearance of mucosa of small intestine of rhesus monkey. The entire area is studded with slender finger-like villi which appear as small knobs. These were found in jejunum and ileum

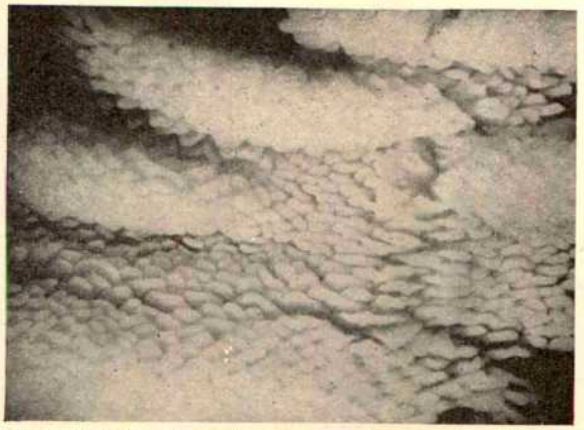


Fig. 2. Dissection microscopic appearance of mucosa of small intestine of rhesus monkey. The entire area shows broad leaf-like or tongue-shaped villi which were found mostly in jejunum and ileum

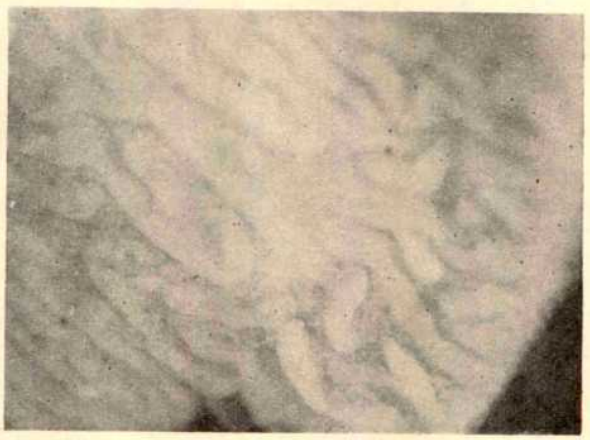


Fig. 3. Dissection microscopic appearance of mucosa of small intestine of rhesus monkey. There are extra-broad, branching and interconnected villi. These were mostly found in the duodenum

and 2). Extra broad leaves curved in places, and irregularly arranged leaves branching occasionally or apparently interconnected, formed a distinct third group (Fig. 3).

It was observed that ten specimens in the duodenum showed the third type of villi, seven revealed leaf-like villi arranged regularly, and one specimen failed to show villi because of excess mucus.

Out of forty-six specimens taken from the jejunum, thirty-three showed leaf-like villi, three finger-like, seven mixed finger- and leaf-like and two showed extra-broad curved villi. One specimen did not show any villi, the surface being denuded mechanically.

Out of twenty-four specimens taken from the terminal ileum, sixteen showed classical leaves, three finger-like villi, three mixed pattern and two showed extra-broad curved villi.

Table 1 shows the overall distribution of different types of villi in the small intestine of the rhesus monkey.

Histological examination showed that the ratio of villus height to crypt varied between 6 : 1 and 3 : 1 depending on the type of villi, usually showing a high value in the case of finger-like villi and a lower value with leaf-like or extra-broad curved villi. The margin was well formed and showed both columnar and mucous cells. The corium of the villi usually contained a number of cells enmeshed in thin connective tissue. These cells were usually mononuclears or lymphocytes and occasionally plasma cells.

Table 1

Type of villus	Number	Percentage
Leaf	56	65
Finger	6	7
Mixed	10	12
Extra-broad curved	14	16

The duodenum in rhesus monkey showed a distinctive villus pattern with little more than half showing broad curving, branched villi. Finger-like villi were not found in the duodenum. The jejunum and ileum showed a broadly comparable pattern with a predominance of leaf-like villi. Lineback[1], who investigated intestinal villi, also found that the majority of villi in the jejunum had leaf-like and tongue shapes. We cannot, however, agree with his observation that the villi have a more and more typical finger-like form the more closely the ileum is approached.

We have classified villi on the basis of clear-cut extreme patterns, but many intermediary stages connecting one to the other were discernible. Between a finger-shaped and a tongue-shaped villus the difference was one of flattening, which in some cases was found only at the base of the villus while its top still retained a tubular configuration. Between a tongue-shaped and a leaf-shaped villus the difference was only one of width and was well bridged by intermediary sizes. Moderately broad and narrow leaf-like villi could be seen in the same piece. It appeared that there was a wide spectrum of varying shapes of villi with finger-like villus at one end, typical leaf-like shapes somewhere in the centre and broad curved, branching interconnected villi at the other end, the gaps in between being bridged by intermediary stages.

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GENETICS

Spontaneous Reduction of Somatic Chromosomes in *Haplopappus gracilis*

THE occurrence of spontaneous[1], as well as induced[2], haploidy has been observed in several plant genera. Haploidy has been reported in cultures from secondary phloem tissue of carrot[3], and haploidy and somatic pairing have also been noted in cultured stem tissue of *Haplopappus gracilis*[4]. Recently, haploid cells were observed to occur spontaneously in leaf primordia of young seedlings of *Haplopappus gracilis*[5]. The purpose of this communication is to report spontaneous reduction in the number of somatic chromosomes in the root meristem of *Haplopappus gracilis*.

Aceto–orcein squash preparations were made of root tips from 2-day-old seedlings, which had been pretreated with a 1 : 1 mixture of 8-hydroxyquinoline and naphthalene monobromide (alpha) according to the technique of Mitra[6]. The normal karyotype, consisting of four chromosomes, one longer submedian pair and one subterminal pair with satellites on the short arms, was observed in all of the root tips examined (Fig. 1). In one root tip, however, three metaphase configurations with two chromosomes, one member of each pair, were seen (Figs. 2 and 3). The chromosomes in Figs. 2 and 3 may represent haploid complements. This interpretation is based on comparative measurements of the width of the individual chromosomes in Figs. 1–3. An alternative view is that chromosomal configurations in these cells represent instances of somatic pairing similar to that observed by Mitra and Steward[4]. In order to evaluate these interpretations, microspectrophotometric analysis of Feulgen-stained somatic cells having the reduced number of chromo-



Fig. 1. Metaphase chromosomes of *Haplopappus gracilis* ($2n = 4$) root tip cells (× 1,800)

Figs. 2 and 3. Metaphase chromosomes in haploid root tip cells of *Haplopappus gracilis* (× 1,800)

somes is required. The observed reduction in chromosome number was probably not induced by the pretreatment, since no other cases of somatic reduction were observed in several hundred root tips pretreated in a similar fashion.

The observation of Lima-De-Faria[5] seems to indicate that the presence of the haploid set of chromosomes is enough to produce well-differentiated tissues in leaf primordia of *Haplopappus gracilis*. However, since only a few 'haploid' cells were seen in one of many root tips examined, and no 'haploid' sectors observed, it would be premature to state that two chromosomes are sufficient to produce normal tissue in the root meristem.

Successful clonal isolation of *Haplopappus gracilis* cells grown in culture has been reported[7]. The isolation and study of haploid clones would be of great genetic interest. If diploidy could be induced in such haploid clones, a homozygous strain of cells would be available for investigation.

This work was aided by grants from the National Institutes of Health, United States Public Health Service, the Saul Singer Foundation, and the Charles H. Silver Fund.

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MISCELLANEOUS

Presenting the Results

IN the recent article by Prof. Robert B. Grieves and Dibakar Bhattacharyya[1], Fig. 2 shows a relation between two variables with the statement ". . . The residual DSS concentrations are related in Fig. 2 to the ratio of DSS to trivalent iron in the feed. As x_t/z_t was increased, x_r remained relatively constant and then rose sharply above a feed ratio of approximately 1·0.". In view of the close attention given to statistical aspects of data analysis in other parts of the article, the statement about the data of Fig. 2 appeared somewhat questionable. Accordingly, I read off the numbers from the graph as best I could with a piece of superimposed graph paper and replotted the data on log–log co-ordinates. The data suggested a relation of the form $x_r = \sqrt{2x_t/z_t}$, so I prepared a graph as shown in Fig. 1. Within the variability of the data, it does not appear to me that the data can be considered approximately constant below x_t/z_t of 1, but rather that a regular increase is observed over the full range of the values. Certainly the variability of the data does not warrant a definite conclusion on the relationship.

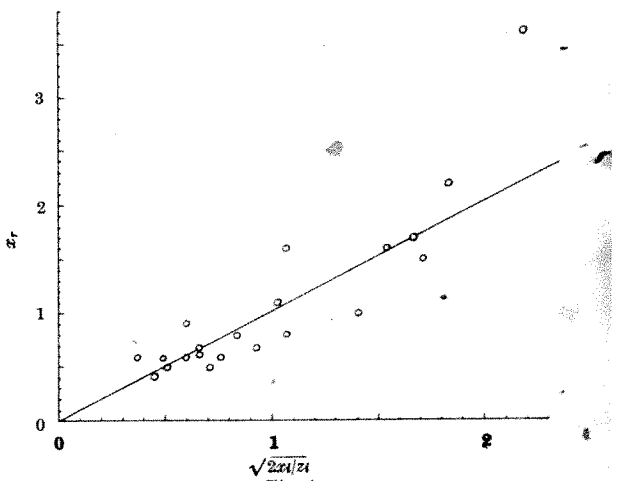


Fig. 1

The foregoing comments are submitted partly to make the point that the use of figures to present data and arguments makes it difficult for the reader to examine the data independently and test the conclusion offered by the author. I realize that limitations of space make such tabular presentations difficult, but I am disturbed at the quantity of literature which is produced without tabulation of the supporting values in a form where related calculations can easily be made. As a result, there is often a tendency to accept the author's statements because of the difficulty of examining the data separately.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Wednesday, June 1

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. M. H. P. Bott and Dr. G. A. L. Johnson: "The Controlling Mechanism of Carboniferous Cyclic Sedimentation".

ROYAL SOCIETY OF MEDICINE, HISTORY OF MEDICINE SECTION (at 1 Wimpole Street, London, W.1), at 5.30 p.m.—Dr. Richard Hunter. "J. Casaubon The Journal of a 17th Century Kentish Practitioner".

Thursday, June 2

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 10.30 a.m.—Discussion Meeting on "The Chemistry and Biology of Immunoglobulins", organized by Prof. R. R. Porter, F.R.S.

GEOLOGICAL SOCIETY OF LONDON, VOLCANIC STUDIES GROUP (at Burlington House, Piccadilly, London, W.1), from 2 p.m. to 7 p.m.—Colloquium on "Alkali Basalts and their Associates".

Monday, June 6

UNIVERSITY OF LONDON (in the Botany Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr Michael Sela (Rehovot). "Molecular Basis Antigenicity".*

Monday, June 6—Wednesday, June 8

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2)—Conference on "Design and Construction of Large Steerable Aerials"

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER IN PURE OR APPLIED MATHEMATICS—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (May 31).

LECTURER IN APPLIED MATHEMATICS—The Registrar, The University, Canterbury, Kent (May 31)

LECTURER OR ASSISTANT LECTURER (preferably with a medical degree or an honours degree in physiology and research experience) IN THE DEPARTMENT OF PHYSIOLOGY—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (May 31).

ASSISTANT LECTURER IN THE DEPARTMENT OF METALLURGY of the Faculty of Science—The Registrar, The University, Manchester, 13 (June 1).

RESEARCH FELLOW IN THE INSTITUTE OF SOUND AND VIBRATION RESEARCH for the fundamental study of the noise from high performance, high speed, direct injection diesel engines—The Deputy Secretary, The University, Southampton (June 1).

ASSISTANT DEPARTMENTAL LIBRARIAN (preferably with a degree) IN THE DEPARTMENT OF GEOGRAPHY—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (June 2)

LECTURER (honours graduate interested in general and social psychology) IN PSYCHOLOGY—The Principal, Lanchester College of Technology, Priory Street, Coventry (June 2).

TERMINABLE LECTURER (science or medical graduate) IN EMBRYOLOGY (Developmental Biology) IN THE DEPARTMENT OF ANATOMY—The Secretary, The University, Aberdeen (June 4).

ASSISTANT LECTURER IN SOCIOLOGY—The Secretary, The University, Northcote House, The Queen's Drive, Exeter, Devonshire (June 6).

LECTURER OR ASSISTANT LECTURER IN PURE MATHEMATICS—The Registrar, The University, Leeds, 2 (June 6).

RESEARCH ASSISTANT (graduate with research experience or expecting to graduate in Summer 1966) to Prof Monica M. Cole for work in vegetation/soil/geomorphology relationships in the Southern Pennines over a three-year period—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (June 6).

UNIVERSITY DEMONSTRATOR IN THE DEPARTMENT OF PHYSICS—The Registrar, University of Nottingham, University Park, Nottingham (June 6).

RESEARCH ASSISTANT (with special qualifications in educational psychology) IN EDUCATION—The Registrar (Room 39, O.R.B.), The University, Reading (June 7)

ASSISTANT LECTURER (perferably with an interest in ecology, experimental taxonomy, cytotaxonomy, algology, or quaternary palaeobotany) in the Department of Botany—The Secretary of the University Court, The University, Glasgow (June 8).

LECTURER OR ASSISTANT LECTURER (graduate in medicine, veterinary science, dentistry or biology) IN THE DEPARTMENT OF HISTOLOGY—The Registrar, The University, Liverpool, quoting ref. CV/60 (June 8).

PROFESSOR OF COMPUTER SCIENCE and DIRECTOR OF THE UNIVERSITY COMPUTER UNIT WITHIN THE SCHOOL OF MATHEMATICAL STUDIES—The Principal, Brunel College, Woodlands Avenue, Acton, London, W.3 (June 10).

LECTURER OR ASSISTANT LECTURER (chemical engineer) IN THE DEPARTMENT OF POLYMER AND FIBRE SCIENCE, to work in the field of polymer technology—The Registrar, University of Manchester Institute of Science and Technology, Sackville Street, Manchester, 1 (June 11).

LECTURERS (2) (with experience either in (*a*) control engineering applied to process control or servo systems or (*b*) synthesis and design of logical systems and computer circuits) IN ELECTRICAL ENGINEERING—The Secretary, The Queen's University, Belfast, Northern Ireland (June 11).

RESEARCH ASSISTANT (with a degree in zoology or in forestry and preferably previous experience in research involving field studies) to work with the LECTURER IN FOREST ZOOLOGY on investigations into the ecology and biological control of forest insects—The Secretary, The University, Aberdeen (June 11).

LECTURER or ASSISTANT LECTURER IN PHILOSOPHY—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (June 12).

LECTURER IN COMPUTER SCIENCE WITH SPECIAL REFERENCE TO NUMERICAL ANALYSIS—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (June 13).

LECTURER or ASSISTANT LECTURER (with an interest in electrochemistry or thermodynamics) IN CHEMISTRY—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (June 13).

LECTURER (with interests in the theoretical or experimental aspects of modern control techniques) IN CONTROL ENGINEERING IN THE SCHOOL OF ENGINEERING SCIENCE—The Registrar, University College of North Wales, Bangor, North Wales (June 13).

LECTURER (preferably interested in biometrical and/or natural population genetics of angiosperms) IN THE DEPARTMENT OF GENETICS—Dr. Sydney Smith, Department of Zoology, University of Cambridge, Downing Street, Cambridge (June 14).

ASSISTANT LECTURER (preferably with special interests in locational studies) IN THE FIELD OF ECONOMIC GEOGRAPHY—Dr. W. W. Black, Sedgwick Museum, The University, Cambridge (June 15).

DIRECTOR OF THE COMPUTATION LABORATORY—The Registrar, The University, Hull (June 15).

LECTURER or ASSISTANT LECTURER (with research experience in low temperature physics) IN PHYSICS—The Registrar, The University, Nottingham (June 15).

ASSISTANT IN THE FRUIT-GROWING SECTION OF THE DEPARTMENT OF HORTICULTURE, Massey University of Manawatu, Palmerston North, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or the Registrar of the University (June 18).

ASSISTANT LECTURER IN VETERINARY ANATOMY—The Registrar and Secretary, The University, Bristol (June 18).

RESEARCH ASSISTANT (preferably with some research experience in an appropriate field) IN THE DEPARTMENT OF ZOOLOGY to participate in work concerned with the effect of antimetabolites on embryos—Dr. F. Billett, Department of Zoology, University of Southampton, Southampton (June 18).

RESEARCH SCIENTIST or SENIOR RESEARCH SCIENTIST (with a Ph.D. degree in physics or mathematics or appropriate postgraduate research experience of equivalent standard and duration supported by satisfactory evidence of research ability, and preferably a previous interest in atmospheric radiation) IN THE DIVISION OF METEOROLOGICAL PHYSICS, Commonwealth Scientific and Industrial Research Organization, Aspendale, Melbourne, Victoria, Australia, to take charge of the Division's research activities in the field of atmospheric radiation—The Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 420/215 (June 18)

UNIVERSITY DEMONSTRATOR (with an honours degree in an appropriate pure science, for example, biochemistry or physiology or agricultural science, and preferably some teaching and/or research experience) IN ANIMAL NUTRITION—The Secretary, School of Agriculture, University of Cambridge, Downing Street, Cambridge (June 18).

ASSISTANTS IN GEOLOGY (one without restriction on special interests, the other preferably with emphasis on geophysics) IN GEOLOGY—The Secretary to the University Court, The University, Glasgow (June 20)

RESEARCH FELLOW (engineer or mathematician) IN STRUCTURAL THEORY for research on structural containers at postgraduate level—The Administrator, Department of Engineering Science, University of Oxford, Oxford (June 20).

TEMPORARY LECTURER or ASSISTANT LECTURER IN PSYCHOLOGY—Prof H. Kay, Department of Psychology, The University, Sheffield (June 20).

ASSISTANT LECTURER (medical, veterinary or biology graduate, with an interest in research on insecticides for a higher degree) IN MEDICAL ENTOMOLOGY—Prof. D. Bertram, Department of Entomology, London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1 (June 22)

DEMONSTRATOR (preferably with some postgraduate experience) IN BIOCHEMISTRY AND AGRICULTURAL BIOCHEMISTRY—The Registrar, University College of Wales, Aberystwyth (June 25).

AGRICULTURIST (with a degree or diploma in agriculture and experience of planning field experiments) FOR THE FIELD EXPERIMENTS SECTION—The Secretary, Rothamsted Experimental Station, Harpenden, Herts, quoting ref. 1052/85 (June 30).

BIOLOGIST to assist with research on bee behaviour—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (June 30).

BIOLOGIST (with at least a good honours degree) FOR RESEARCH ON MICROBIAL PATHOGENS OF INSECTS OF AGRICULTURAL IMPORTANCE—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (June 30).

LECTURER IN THE DEPARTMENT OF PURE MATHEMATICS OR DEPARTMENT OF STATISTICS, University of New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, June 30).

LECTURER/SENIOR LECTURER IN BIOLOGY in the School of Biological Sciences, University of Sydney, Australia, mainly for administrative duties—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, June 30)

PROFESSOR OF TEXTILE PHYSICS at the University of New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, June 30).

SENIOR LECTURER or LECTURER IN MECHANICAL ENGINEERING at the University of Canterbury, Christchurch, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, June 30).

SENIOR LECTURER or LECTURER IN PLANT SCIENCE (Entomology), a SENIOR LECTURER and LECTURER IN CHEMISTRY; a SENIOR LECTURER and LECTURER IN PHYSICS, and a SENIOR LECTURER and LECTURER IN MATHEMATICS at Njala University College, Sierra Leone—The Inter-University Council, 33 Bedford Place, London, W.C.1 (June 30).

SENIOR LECTURER or LECTURER IN BIOCHEMISTRY at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, July 1).

READER IN APPLIED MATHEMATICS in the School of General Studies, Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, July 4).

LECTURER or SENIOR LECTURER IN GEOGRAPHY at the University of Tasmania—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, July 8)

VETERINARY OFFICERS, Grade II (at least 23, member of the Royal College of Veterinary Surgeons, and one year's appropriate postgraduate experience) with the Ministry of Agriculture, Fisheries and Food—The Civil Service Commission, 23 Savile Row, London, W.1 (August 1).

ASSISTANT LECTURER (graduate, or with a professional qualification and some industrial experience) IN CHEMISTRY, Grade B, to teach primarily in courses for chemical technicians and O.N.C. Sciences—The Principal, Medway College of Technology, Horsted, Maidstone Road, Chatham, Kent.

JUNIOR RESEARCH OFFICER IN GEOLOGY—The Registrar, University College of Wales, Aberystwyth.

LABORATORY INSTRUCTORS (with a good honours degree in chemistry, preferably with some graduate work, and interested in teaching modern laboratory techniques to undergraduates)—The Departmental Secretary, Department of Chemistry, The University of British Columbia, Vancouver, 8, B.C., Canada.

LECTURER and an ASSISTANT LECTURER (with a good honours degree in electrical engineering or an honours diploma in technology) IN THE DEPARTMENT OF ELECTRICAL AND ELECTRONIC ENGINEERING—The Academic Registrar, Northampton College of Advanced Technology, St. John Street, London, E.C.1, quoting Ref EE.

MASTER to teach Chemistry to "A" level—The Headmaster, Malvern College, Worcestershire.

PHYSICIST/PHYSICAL CHEMIST (experienced graduate, preferably with some knowledge of chemistry) to take charge of research projects on instrumentation methods for the control of manufacturing processes in the food industry—The Director of Research, British Food Manufacturing Industries Research Association, Randalls Road, Leatherhead, Surrey.

POSTDOCTORAL BIOCHEMIST for work on problems of brain metabolism, including study of control and transport phenomena and energy metabolism in slices and isolated cells—The Director, Metabolic Reactions Research Unit, M.R.C., Biochemistry Department, Imperial College, London, S.W.7.

RESEARCH ASSISTANT (with, or about to submit for the Ph.D. degree, and preferably experience of chemical kinetics or spectroscopy) IN THE DEPARTMENT OF CHEMISTRY, to work with Dr. B. P. Levitt on studies of chemical kinetics of electronic excitation by measurement of light emission from shock-heated gases—Dr. P. B. Levitt, Chemistry Department, Imperial College, London, S.W.7.

SENIOR LECTURER IN PSYCHOLOGY—The Secretary, Wolverhampton College of Technology, Wolverhampton, quoting Ref. MB. 15/1.

REPORTS and other PUBLICATIONS

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IS SCIENCE REALLY INTERNATIONAL?

RECEIVED doctrine holds that science is in some sense supranational. Ever since Davy and Faraday went jaunting in Europe at the height of the Napoleonic Wars, it has been permissible to pad out public speeches with declarations that "science knows no frontiers" or that "the laws of physics are the same the whole world over". As a result, the idealism of young people has been harnessed to the view that science is potentially a substitute for diplomacy. Teach enough people enough physics, and international tensions will melt away in a flood of reason. That is how the theory goes. It is a puzzle to know how it can be so widely held when there is so much evidence to contradict it.

The fact that the laws of Nature are invariant under geographical displacement is, of course, a truism, but a memorable one. For one thing, it is a rallying-cry in the defence of the cultural integrity of the conceptual structure of science. The same problems simultaneously confront people in different parts of the world, and the fact that their solution can be helped by the purposeful but independent efforts of the professional community as a whole is one of the reasons why science is at once stimulating and moving.

There are also important practical consequences of this principle. It is hard for groups of people to pursue eccentric lines of argument for long once their eccentricity has been shown to entail internal contradiction. It is hard for Ruritanians, say, to believe their part of the Earth is flat when the rest of it is known to be spherical. The same intellectual unity of science has also led to the recent sensible awareness that there are limits to the extent to which secrecy can be imposed on scientific discoveries. Then the common problems of science bring people together; they travel, frequently as indifferent to local disturbances as Davy and Faraday were, and they strike up close relationships with each other.

With such an auspicious foundation, it is natural to expect that science will be a great co-operative endeavour, and this expectation is frequently supposed to be a fact. Unfortunately, however, it is not a truism but, at best, a half-truth. Reality does not match the expectation. Science may be the same everywhere, but the institutions of science are not organized for a common attack on the common problems. On the contrary, international rivalry, even chauvinism, is rife. Nobel prizes, like Olympic gold medals, are regarded as feathers in national caps. For various reasons, not all of them pleasant, international collaboration as it exists is much less real, and much less effective, than it could be.

Some things, of course, are being done. There is plenty of talk. There are bilateral agreements between nations which extend programmes of cultural exchange into science—and which serve chiefly as reminders that things were recently even worse. There are international groups under the umbrella of organizations such as the United Nations which perform valuable tasks. (There is also, as always, Unesco.) There are practical and powerful institutions for tackling common scientific problems, of which CERN remains the prototype and shining example. (Because the justification of apparently comparable organizations, such as the European Launcher Development Organization, is political as well as technical, it is not surprising that they are less effective.) Then there are valuable co-ordinating bodies like the OECD, and invaluable means of sharing ideas and efforts such as those which have grown up between Britain and the United States in academic research, because of the tight cultural links between the two countries.

This list is impressive but full of gaps. It would be surprising if it affected directly as much as 10 per cent of the activity in research and development of any advanced nation. For every triumph there is at least one sad case where collaboration could be made more effective. The deficiencies of present arrangements are particularly glaring in Western Europe, where—despite OECD and its good works—the gallop towards economic integration and possibly towards political integration as well is not yet matched by parallel tendencies in science and technology. Yet the opportunities and the benefits are potentially so great that collaboration in science in Western Europe deserves urgent special treatment.

In Western Europe there are many ways quickly to multiply the benefits of present collaboration. Thus a great deal could be done to get more from what is spent on academic research in Western Europe. There would be rapid rewards for arrangements making it easier for people, and especially students embarking on research, to move from one country to another. The training of young research people is the way in which universities and institutes traditionally re-invest their intellectual capital. If Western Europe is indeed one cultural entity, it is a great waste if the right students do not find their way to the right institutes of research, irrespective of nationality. In other words, there is a case for asking that postgraduate awards by, say, the British Science Research Council should be tenable anywhere in Europe.

There is also a need for fuller consultation between European grant-giving agencies. At present the pattern of academic research in European countries derives from the independent decisions of public and private grant-givers, tinged occasionally with pointless mimicry. In time, of course, individual scientists ensure by their applications for new grants that good ideas are widely spread, but that sometimes takes time.

Another obvious field in which European collaboration needs to be strengthened is in co-operative industrial research. In the larger nations co-operative research organizations are now flourishing. Their growth has been rapid in the past few decades, and has come about because well-established industries such as steel and textiles have been persuaded that a common attack on common problems need not prejudice the independence or the competitiveness of individual companies. But what is true of one nation's industry is now true for the whole of Europe. In other words, there is a case for a thoroughgoing integration of co-operative research in Europe.

These are important questions, but they are also untidy. All kinds of considerations apart from science and technology are involved. Because they raise the possibility that nations or industries might limit their freedom to determine the whole of their own affairs for the sake of a more effective attack on common problems, matters of

sovereignty are involved. Their difficulty is no reason for not tackling them.

Where the organization of European science is concerned, the ministerial meetings which the OECD has sponsored promise to be valuable, even if they have not yet done more than enable officials to know the names of those to whom they correspond in other countries. There is also a need for a more particular discussion of the common needs of Western Europe, either within OECD or at Strasbourg, in the Council of Europe. Then it was good to learn, last week, that Sir Harrie Massey, the chairman of the British Council on Scientific Policy, is to take personal responsibility for the international implications of British science policy. It is to be hoped that he will give himself a wide brief, and that he will urge his European colleagues to put stuffing into the old and most empty boast that science is already international.

MONEY TO SPEND

ONE of the cheerful parts of the report of the Council on Scientific Policy, published last week, is the statistical evidence it provides of the rapidly changing pattern of spending on research and development in Britain. There are a number of striking tendencies—some of them, at least, in the right direction. The declining emphasis on defence research and expenditure is the most heartening item of all. Over a decade, the proportion of the country's research and development expenditure provided by the defence agencies has declined from 59·1 per cent to 33·7 per cent (in 1964–65). In the past few years the defence departments of the Government have provided money at the roughly constant rate of £250 millions a year within a steadily rising budget which had grown to £756 millions in 1964–65, and the chances are that an increasing proportion of the defence money is anyway being spent on civil work. To welcome this is not, of course, to take a stand against defence research as such—that would be silly, for no country of importance manages to do without it. But there is no question that a decade ago the pattern of defence expenditure in Britain was lopsided. Now it is less so.

Naturally the universities and industry have benefited from this development. Over the past decade the value of research at universities has increased from £14 millions to £56 millions a year, and the value of industrial research and development from £200 millions to £508 millions a year. The change in the pattern of industrial research and development is especially to be welcomed because the proportion of it now financed by industry itself has increased over the decade from 39 per cent to 64 per cent. On the principle that the one who pays the piper calls the tune, this suggests that industrial research and development are now being directed more effectively towards the goals which should concern British industry—the commercial health and future of itself.

Where, then, are the snags? The sombre tendency in the statistics is that which shows that expenditure on research and development has become a static 2·6 per cent of the Gross National Product of the United Kingdom. Is this enough, or should the proportion be greater? This, it will be recognized, is a chicken and egg problem—which is the offspring, research and development or national prosperity? There will have to be a running battle on this issue in the years ahead, and the sooner it begins the better.

"BORN UNDER ONE LAW, TO ANOTHER BOUND"

Man Adapting

By Prof. René Dubos. Pp. xxii + 527. (New Haven and London: Yale University Press, 1965.) 10 dollars; 72*s*. net.

THE distinction between species, at the present time, is sometimes uncertain; the specific distinction between successive evolutionary stages is nebulous. This is not simply because of lack of evidence but because of the gradualness of the changes in the various factors that are used as criteria for a species. By all criteria our species is young, but our physiology and nutritional requirements probably retain many features that are well adapted because we share them with our predecessors. Dubos's basic thesis is that a person's genetic constitution defines potentialities; what actually happens depends on the environment and on nurture. He discusses in detail the extent to which our behaviour indicates incomplete adaptation.

Man Adapting starts, logically, with some examples of influences *in utero* and the effects of maternal care, or the lack of it, in infancy. There is then a section, in the Hippocratic tradition, on "Airs, Waters and Places" and one on rhythms. The interesting point is made that we are now exposed to an unprecedented amount of light and many of us to periodic inversions of the circadian rhythm. Animal behaviour is often modified by light and there is no reason to think that our own is not influenced also.

Primitive man lived an active life and had to tolerate a wide range of foodstuffs as well as intermittent privation. Modern man is sedentary and chooses to be more conservative and regular. Nutritional research has in the past concentrated attention on the needs of children or well-fed people leading active lives. In several places Dubos regrets the low esteem in which trophology is held and argues strongly for more work on novel foods, especially protein-rich foods, and on the needs of the sedentary and ageing. He goes so far as to suggest that contemporary forms of agriculture may become hobbies, like trout-fishing or cabinet-making, and be replaced by intensive methods in which the crop is the raw material for biochemical engineering. In studying nutrition particular attention should be given to the microbiota of the gut. Many experiments are quoted showing that not only the action, but the very physical structure, of the normal gut depends on the organisms growing in it. It may well be that the process of adaptation to a novel food depends primarily on microbial changes.

This discussion leads naturally to a discussion of pathogenicity and here Dubos is in his element. He quotes many examples of ubiquitous organisms that become pathogens when the host is stressed by crowding, amino-acid deficiency, or in other ways. He accepts specific aetiology, or the germ theory of disease, as "the most powerful single force in the development of medicine during the past century", but points out the enormous improvements in health brought about by people who did not know of it or even, like Ozanam and Florence Nightingale, rejected it. In the view of Dubos, people and animals adapt to changes in their environment, and the result is called disease when the attempt is unsuccessful. He devotes a chapter to the idea that some pathogens could be exterminated, but regards this as unlikely.

To an increasing degree, and throughout an increasing area, we have, in the past few centuries, been changing our physical and psychological environment in an unprecedented way. Dubos argues that we have not had time to adapt to these changes and they now come so fast that we probably never will have. He discusses the new chemicals to which we are now deliberately or inadvertently exposed. A blanket prohibition would be both impracticable and unreasonable, for traditional food-

stuffs contain many potentially harmful constituents and the search has, so far, been superficial. Instead, he suggests that a new drug, insecticide, food additive or process, which seems to be harmless and to be advantageous to the public as well as the manufacturer, should be used tentatively with close supervision. To circumvent the mania for secrecy in commercial and official circles, the supervision would have to be done by an outside body such as the St. Louis Committee for Nuclear Information, which Dubos singles out for praise. The conclusion, naturally, would not have the form "This is safe"; nothing effective is ever safe. Instead, as with vaccination, the conclusion would be semiquantitative: "This procedure will probably injure ten people per million exposed, but seems to have more than compensatory advantages".

There is in this book no Rousseauesque back-to-nature appeal and it will give little satisfaction to the noisy mystical faddists who advocate similar restraint. It should give even less satisfaction to those who direct medical research, for they are roundly criticized for their preoccupation with fashionable topics. A phrase may be quoted: "Any activity, however trivial, that deals with submicroscopic particles or subcelluar chemical phenomena is labelled 'fundamental', whereas efforts to formulate, investigate, or teach the phenomena of life as experienced by the whole organism are considered scientifically unsophisticated".

Dubos has written many informative and iconoclastic books. This is his best. It is compulsive reading and is enlivened by many quotations of historical and contemporary interest. Praise must, however, by tempered by a protest at the abominable method used for citing references; titles are given, otherwise one is left to choose more or less at random among the group attached to each chapter.

N. W. PIRIE

INDICATOR GEOBOTANY

Plant Indicators of Soils, Rocks and Sub-surface Waters
Edited by A. G. Chikishev. Authorized translation from the Russian. Pp. xi+210. (New York: Consultants Bureau, 1965.) 27.50 dollars.

THE close relationship between vegetation distributions and the geographical environment has long been accepted in ecological work. The use of this relationship in applied biogeography and geobotany, however, has developed only since 1945 with the desire to open up new lands in the U.S.S.R., Australia and Africa and to find new sources of scarce minerals. The impetus has come from the geographical sciences, particularly in the U.S.S.R., where indicator geobotany has originated as a special branch of biogeography and has become an effective tool in mineral exploration and in geological and hydrogeological surveys concerned with the search for underground water, with highway construction and the development of industrial sites. The publication in English translation of *Plant Indicators of Soils, Rocks and Subsurface Waters*, edited by A. G. Chikeshev, brings a summary of this work to the English reader.

The book comprises the collection of papers presented at the 1961 conference on "Indicator Geobotany", which was one of a series held since 1956 to bring together the scientists working in this field at the All-Union Research Institute of Hydrogeology and Engineering Geology, where a special laboratory dealing with indicator geobotany research has been established, the Institute of Geography of the U.S.S.R. Academy of Sciences, the Laboratory of Aerial Methods at the All-Union Aerogeological Trust and other institutions within the country. The papers cover a wide range of topics, and record the Russian achievements under widely differing geographical conditions in the different parts of the U.S.S.R. Several papers describe the use of geobotanical methods in hydrogeological surveys in the arid steppe where plant indicators assist in the location of underground water, its depth and mineral content. Others evaluate the relationship between the present vegetation and the structure of the upper layers of peat deposits which has an important bearing on suitability for highway construction. One paper discusses the procedural features of indicator research in different types of country and, in forest zones, emphasizes the need for careful analysis of floral lists since even slight variations in the abundance of a given species may reflect important habitat changes. Several papers demonstrate the value of plant communities in the recognition of soil types and of the geomorphological elements of river valleys. One details the ease with which, both from air photographs and ground survey, the dense growth of large larch trees set in an undergrowth of alder enables kimberlite pipes to be distinguished from the surrounding carbonate rocks carrying a sparser vegetation and so assists the search for diamonds. Another concerned with the search for saline domes in oil and gas exploration underlines the close link between vegetation distribution and the nature and intensity of surface geochemical phenomena determined by geology and geomorphology; and shows that whereas halophytic communities reveal the presence of a dome even where the saline nucleus is beneath a cover of 5–20 metres, a quite different vegetation occurs if the dome produces sufficiently marked relief to promote leaching of the more soluble salts thereby causing a lower salinity over the area of the dome than in surrounding areas.

Thirty-three papers clearly and simply demonstrate the value of indicator geobotany in numerous different areas each of limited extent. To the practising biographer, B. V. Vinogradov's paper on "Ecological Compensation and Replaceability and the Extrapolation of Plant Indicators" is a refreshing reminder of the complexity of relationships of vegetation with environment and the care needed in the use of plant indicators. After distinguishing between climatic, edapho-climatic and climatic influences, he emphasizes that relatively poor soils in a hot climate may provide the same nutritional condition as potentially richer soils in a cold climate, that moderate or inadequate soil moisture in the humid zone is compensated for by surplus soil moisture under arid conditions, etc. In consequence a species like *Carex appropinquata* Schum, which occurs under conditions of moderate moisture in the forest zone, may indicate substantial moisture in the steppe zone. With climatic-geochemical compensation, similar plant groupings may develop on normal soils with an unfavourable water or thermal régime in the steppe or tundra zone and also on soils with a high content of toxic metallic compounds in the forest zone. Thus *Festuca sulcata*, *Stipa* spp. steppe, the normal vegetation of the dry steppes, occurs on the serpentinites of the forest zone of the humid Southern Urals. I have observed a similar phenomenon in the Kalgoorlie district of Australia. Both instances recall Duvigneaud's findings in the Katanga where some species characteristic of cupriferous ground occur elsewhere only in extreme habitats such as ill-drained dambos and arid rock outcrops.

Vinogradov illustrates further the effects of climatic geochemical compensation by reference to kimberlite pipes which may be distinguished by a dense tree cover in humid areas and by the absence of trees in warm dry areas—the differences doubtless arising from the effects of the different concentrations of mineral salts under different climates.

In order to exclude the phenomena of edaptic compensation and indicator replaceability extrapolation within geographical landscapes characterized by a consistent vegetation, moisture, soils, relief, geology relationship is essential. Examples, accompanied by maps, of such landscapes are given. An admirable paper presenting the diagrammatic scheme of succession of indicator inter-

pretations of a geobotanical map follows; this is illustrated by a series of interpretational maps of salinization, age and genesis of soil-forming rocks, depth of bedding and level of mineralization of the first water-carrying horizon, and immediate prospects for agricultural use from the geobotanical map of the north Caspian region.

This collection of papers portrays the Russian achievements in the field of indicator geobotany or, as I should prefer to call it, applied biogeography. They reveal the potential of this field of study and support my contention that, particularly in the southern continents, applied biogeography can make a valuable contribution in land assessment for agriculture, forestry and other uses and in mineral exploration. These vast areas of savannas are undeveloped, sparsely settled, frequently by poverty-stricken people. There much basic biogeographical work has already been undertaken by lone individuals. It is to be hoped that the publication, in English, of the Russian work will provide support for those in Britain ready to assist the emergent African countries, eager to promote the development of the Australian outback and anxious to aid the Latin-American countries. Britain cannot afford to neglect the development of co-ordinated biogeographical research which would assist friendly nations and at the same time help to resolve some of its own problems, notably those concerned with disease incidence. The publication of *Plant Indicators of Soils, Rocks and Sub-surface Waters* is at once timely and challenging.

MONICA M. COLE

COMPONENTS OF ROLLING MILLS

Rolling Mills

By A. I. Tselikov and V. V. Smirnov. Translated from the Russian by M. H. T. Alford. Translation edited by W. J. McG. Tegart. Pp. ix + 405. (London and New York: Pergamon Press, Ltd., 1965.) 160*s*. net.

TSELIKOV is well known for his writings on rolling and rolling mills, and *Rolling Mills*, translated from the Russian, is a collection of his principal works on the mechanical plant requirements for the hot and cold rolling of typical steelworks products, including tubes. Much of the text was originally written more than 12 years ago, and to that extent some of the descriptions are dated, but the co-author Smirnov has been responsible for some revision of the text and illustrations.

The book runs to four hundred pages, is well illustrated, and is complete with a good index and bibliography extending to two hundred references, although most of the latter are to Russian literature that has not been translated. The standard of translation of the text is good and the terminology chosen is that in regular use in the West. Units are in the c.g.s. system, but there is frequent reference to Russian standards. These are used, for example, to denote the sizes of some rolled sections, for specifying the material of some plant components, and for describing fits of components. Without the key to the standards some value is lost. There are the occasional misprints, but to most readers these will be obvious and are not likely to cause confusion.

The book is restricted to mechanical plant requirements, however, and does not deal with either rolling theory or rolling practice, other than where necessary to explain the function of the plant. For some items only a simple description of the plant is given, but for many others the basic theory required for designing is given, and it is in these cases that the book is of most interest and likely to be of most value.

The work is divided into six parts, the first of which gives a background of mill terminology and mill classifications and is supported by detailed layouts of typical installations covering the range from primary mills to re-rolling mills. Typical planning parameters are listed. This is standard treatment of the subject but a necessary introduction.

Part 2 which follows with consideration of the component parts such as rolls and their adjustment and changing, bearings, guides and housings, introduces the theory of design in many cases. Inevitably some parts are covered in much more detail than others. Repeaters, for example, receive sparse mention, and although references are given these are in Russian. Part 3 on transmission equipment covers the design of spindles and couplings and gives a description of typical gearing. Fly-wheel theory is included, but there is no mention here or elsewhere of the characteristics or the control of the driving motors.

Special purpose mills is the subject of Part 4 and this includes description of tube and wheel mills. Mills with vertical rolls are also included in this section, which is one illustration of the dating of this book, because such mills are scarcely 'special purpose' in modern plant. Nevertheless, there is a useful description of the transmission of drive to this type of mill.

Auxiliary machines associated with rolling mills are covered in Part 5. In the chapter on shears there is a useful treatment of both resistance to cutting and the shear forces arising with these machines. Similarly, saws receive detailed treatment. Straightening machines are also well covered and methods of estimating power requirements are given for coilers. The section concludes with descriptions of tables, manipulators, chariots, transfers and cooling beds including methods of estimating required size for specified cooling conditions. The book concludes, in Part 6, with the elements of lubricating systems and methods of calculating their capacity.

The inclusion of the theory of design with some of the description of plant should make this a suitable text-book both for students and for practising designers. There are very few books that cover this branch of engineering adequately and this publication is a notable exception. It should be a worth-while acquisition for anyone concerned with the engineering of rolling mills, and although the plant descriptions are already dating, the basic approach to the design of some of the component parts should be of value for some time to come.

R. GREEN

LANGUAGE MECHANISMS

Speech Disorders

Aphasia, Apraxia and Agnosia. By Lord Brain. Second edition. Pp. ix + 201. (London: Butterworth and Co. (Publishers), Ltd., 1965.) 47*s*. 6*d*.

THE correct use of language depends on the integrity of certain areas of the brain, and in particular of its largest, most recently evolved part, the cerebral hemisphere. In right-handed people, damage to the left, but not to the right, cerebral hemisphere is apt to impair comprehension of speech, and the formulation of meaningful utterances, as well as ability to read and write. Nor do lesions in the various parts of the hemisphere affect all language functions equally. The further back the lesion, the more difficulty in comprehension; the nearer the front, the more trouble in expression. The nearer the lesion is to the visual receiving area, the more likely is reading to be affected. The nearer to the auditory receiving area, the more is comprehension of spoken speech involved. Damage to a certain area, the temporal lobe, disproportionately affects memory for verbal information. Nor does speech expression deteriorate all of a piece. Some patients have outstanding difficulty in naming—finding the precise word to suit the case. They are given to circumlocution. Others experience maximal difficulty in producing syntactically correct sentences. Their speech is telegrammatic in style and sparse in amount. Others are verbose to a degree, perhaps in private jargon, grossly mutilated

language which can nevertheless be to some extent deciphered by the attentive listener.

The varieties of aphasic manifestations are fascinating to observe, and indeed to read about. The theoretical issues raised are important. What brain mechanisms are involved in language function? Where are they located? How do they work? Here are major scientific problems which cannot be resolved by normal studies. To what extent can they be resolved in the light of existing knowledge of the affects of brain damage in man?

Speech Disorders appears opportunely at a cross-roads in the investigation of language disorders.

In spite of periodic pessimistic pronouncements by individuals unacquainted with the literature, the effect on language skills of damage to various parts of the brain is unusually well documented. Generations of clinicians have recorded verbal behaviour of such patients in the utmost detail. Some of the conclusions drawn go well beyond the data which was usually collected in an uncontrolled fashion. Criteria for the acceptance of hypotheses have become more stringent since psychology took its place among the life sciences, and concepts that had previously found ready acceptance now come under close and suspicious scrutiny. These include generalizations by clinicians about correlations between anatomical site of damage and nature of deficit and conclusions drawn from these as to the manner in which the brain functions. However, it is unfair to criticize the older clinicians for failing to use methods developed at a later date; it is foolish to ignore their observations, as this leads merely to redundant researches. The modern investigator, armed with efficient methodology, does well to note the older results, regard them as pilot studies, and use them to form hypotheses which can be put to the experimental test. He should not necessarily be disappointed if some of them prove to be correct.

Lord Brain's book is the ideal guide for this purpose. Written in a concise and exact manner, it succeeds remarkably in guiding the reader through a highly complex literature, each point being amply documented. The book is exceptionally well organized for easy reference; the author's judgments are balanced and never dogmatic. His own concept of the 'word scheme' is a valuable aid to this exposition, and is consistent with recently proposed models of brain function. With its full discussion of classical findings, and its ample consideration also of more recent results, this book will contribute greatly to the pooling of resources from a variety of sciences for use in the investigation of aphasia, which Lord Brain has himself so effectively advocated. M. KINSBOURNE

INFORMATION AND INVENTION

Third Annual Meeting of the Committee for International Co-operation in Information Retrieval Among Examining Patent Offices (ICIREPAT), at the Patent Office of the Federal Republic of Austria, Vienna, September 17th through 26th, 1963

Edited by Harold Pfeffer. Pp. v+347. (Baltimore, Md.: Spartan Books, Inc; London: Macmillan and Co., Ltd., 1964.) 110*s*. net.

FEW information retrieval systems face so severe a test as patent classifications, and few information specialists have as much experience in searching as patent examiners. This volume is therefore doubly welcome, since it represents a progress report on several years of work, on both operational and research levels, about which all too little has been available hitherto. The Committee for International Co-operation in Information Retrieval among Examining Patent Offices (ICIREPAT) is an informal committee of experts from eighteen countries, formed in 1961 on the initiative of the U.S. Patent Office. It has held several meetings, and set up a research associates programme under which members of one office can be seconded to another for extensive periods of research.

The book contains something of a mixture of papers: progress reports from several offices and research associates, technical reports on systems for arrangement, storage and testing, and a number of research memoranda presented but not read to the 1963 conference. Those that were read, being delivered to a relatively small and known audience, are plainly set out and much more readable than most work in this field. There is a great deal of data, especially in the research reports, and no one concerned with the design or testing of information retrieval systems can afford to neglect them. The methodologies are also of prime interest, in the comparative tests for example, since they are based, not on maximum retrieval (as in most such tests), but on the two chief factors in actual patent searching: speed, and the discovery of even a single anticipation. In his report on project "Carburettors", G. J. Koelewijn sets out a scale of values by which retrieved patents may be comparatively evaluated; in an interesting proposal for combining machine and manual (peephole) systems, Otto Schon suggests four 'specifications' against which the performance of information retrieval systems may be judged. Throughout, indeed, the emphasis is on practical results, and other important factors receiving attention include the layout of specifications, coding of chemical compounds (the Dyson–International Union for Pure and Applied Chemistry system was preferred), and the need for controlling terminology, not merely in obvious ways like synonyms, but also in settling fundamental questions such as the detail into which a compound term should be analysed.

Contrary to the statement from Leon Rolling that to-day "classifications are gradually replaced by systems of independent keywords", one finds repeated emphasis on the need for a structured vocabulary. Several offices, including the British, Japanese, Dutch, and apparently the Russian, make use of facet analysis in establishing basic categories for terms, pointing to the role of hierarchical linkage coupled with freedom to combine terms from different categories. The Japanese report that the ratio of specific search to generic search is roughly fifty–fifty. The research associate from the British office, D. C. Snow, names six fundamental categories: operations, matter, articles and apparatus, energy, attributes and conditions, signals; J. H. Callow, principal examiner at the British office, makes a strong case for a universal classification scheme, in which each field "should be developed in great detail with a faceted classification to enable maximum use to be made of mechanised searching". The spirit of international co-operation is everywhere in evidence, and while the difficulties are many and various, a considerable effort in this direction is clearly being made.

From its nature, this book will be used more for consultation than as a descriptive account of information retrieval in general, and as such it has much to offer. But though the aim is so specific, and the concentrations of data make tough going most of the way, several contributions examine basic questions as well, and deserve the attention of all who are faced with the problems of organizing information, even though they may not be directly concerned with patents. It is a pity that such a useful work should suffer from two noticeable defects: a liberal sprinkling of misprints and the lack of an index. The latter is surely relevant to the needs of examiners also, and a detailed contents table, with descriptive titles, makes some amends, but gives no hint of the discussions of steroids, nuclear reactors, glass technology, and the other important subjects concealed under "Progress Reports". Perhaps this might turn out to be an advantage, though, if it makes readers work through the whole book instead of concentrating only on what is relevant to their own specialized interests.

D. J. FOSKETT

Physics

Second edition. Pp. xvii+686. 47*s*. 6*d*.

Physics—Laboratory Guide

Second edition. Pp. vii+86. 12*s*. 6*d*.

(Physical Science Study Committee.) (Boston, Mass.: D. C. Heath and Company; London: George G. Harrap and Co., Ltd., 1965.)

THE profound influence of the physics course elaborated by the Physical Science Study Committee in the United States on the practice of physics teaching in secondary schools the world over is a remarkable example of the present-day revolution in thinking about education. For perhaps the first time, teachers of physics in schools were presented with a thoroughly tested package of teaching resources—modern texts, teachers' guides, films, apparatus, laboratory guides, problems, examinations, in-service training courses. The contents of the package were guaranteed, for its progenitors included such men as Francis Friedman, Philip Morrison, Eric Rogers and Jerrold Zacharias: and their teams included many distinguished school teachers. The course placed its emphasis on the fundamental principles of physics (with dynamics, wave motion and electromagnetism contributing to the basic dialogue between the continuous and the particulate). This was achieved with a minimum of formal mathematics and with no more than an occasional reference to the applications of physics.

Now, almost ten years since the early discussions, the 'feedback' from some six thousand teachers who have taught the course to some six hundred thousand students in American senior high schools has been used most skilfully to produce this second edition. In those years the course has been exported widely, translated into many languages (including Russian—but unofficially) and has markedly influenced the thinking which has informed the work in Scotland on the 'Alternative O Grade' course and in England and Wales on the Nuffield Foundation physics courses.

The exposition of the material in this new edition has been considerably improved; new end-of-chapter problems have been added and additional excellent photographs provided. Major changes are to be found in the chapters on the magnetic field, electro-magnetic induction and electro-magnetic radiation, while the revision of the work on vectors has clarified the concepts discussed. The small section on electrical circuits has been separated from the treatment of the motion of charges in the electric field, while the section on optical instruments has been deleted. Some new experiments, including a version of the Millikan experiment, are described.

The reputation of this unique course can only be enhanced by this new edition, for the treatment can now withstand the most searching criticism of the scientific community. E. J. WENHAM

Light-sensitive Systems

Chemistry and Application of Nonsilver Halide Photographic Processes. By Dr. Jaromir Kosar. (Wiley Series on Photographic Science and Technology and the Graphic Arts.) Pp. xv+473. (New York and London: John Wiley and Sons, Inc., 1965.) 115*s*.

THE term 'photography' is usually applied to the recording of a lens image by the use of a film coated with silver halide. Yet the first known photograph was produced ten years before the birth of silver halide photography by utilizing the hardening action of light on a bitumen layer. It is therefore fitting that in *Light-sensitive Systems*, the first book of a new series on photographic science and technology, Dr. Kosar deals with processes in which light acts to produce a chemical change in substances other than silver halide. Because these light-sensitive materials have much lower overall sensitivity than the silver halide process (the first photograph required an exposure of twelve hours), they have traditionally been used mainly as steps in the photographic production of matrices for printing operations, in which it is possible to make the exposure to a light source of high intensity. During the past two decades there has been a large increase in the use of non-silver products for the duplication of engineering drawings and for the reproduction of documents in offices.

Dr. Kosar discusses the action of light on inorganic compounds, particularly ferric salts, colloids containing dichromate, unsaturated organic compounds and diazonium salts: he also considers the formation or destruction of dyes and light-initiated polymerizations. The final chapter describes the process of thermography in which a record is made on heat-sensitive paper.

For each topic the literature has been successfully reviewed up to the end of 1964: much of the information has been obtained from patents. Some description is also given of technological applications, but detailed information must be sought elsewhere. The present work will be invaluable to research workers requiring ready access to previous work which hitherto has been widely scattered. The organization of each chapter into numbered sub-headings makes reference easy.

Proof reading has been well done and errors are few. A minor criticism is the lack of consistency in the representation of a benzene ring in structural formulae. In the majority a plain hexagon is used, but occasionally, often for one formula out of several on a page, Dr. Kosar has followed the recent fashion of inserting the double bonds of one Kekulé form in order to make clear the distinction from a *cyclo*hexane ring. D. J. FRY

Stationary Phase in Paper and Thin-Layer Chromatography

Edited by K. Macek and I. M. Hais. (Proceedings of the 2nd Symposium held at Liblice, June 10–12, 1964.) Pp. 258. (Amsterdam, London and New York: Elsevier Publishing Company, 1965.) 85*s*.

DURING the three-day symposium on which this book is based, some fifty papers were presented on various aspects of stationary phase effects in paper and thin-layer chromatography; gas chromatography was specifically excluded although its use crept into K. Macek's investigation of aqueous two-phase systems. Twenty-one of the contributions are given in English, while the remainder, together with the greater part of the discussion periods, are printed in German.

Only five of the papers are more than ten pages long, most being covered in two to seven pages, which scarcely allows for full treatment of any subject. Useful contributions include a review of the use of liquid ion-exchangers in paper chromatography by E. Cerrai, M. Brenner's article on polyzonal thin-layer chromatography, R. Consden on the cellulose–water complex and a description of the influence of the activity of aluminium oxide on the chromatographic properties of aromatic hydrocarbons by J. Fohl and N. Kucharczyk.

The greatest emphasis throughout is on theoretical aspects of biochemical applications and nearly half the lectures were of an 'introductory' or review nature. On the whole there is little material here that is not available elsewhere scattered through the literature. However, the assembly of this information in one book provides a useful service for those interested in this somewhat limited but basic aspect of chromatography. The inclusion of the discussion periods following the various sections of the symposium is also of value, bringing out views not usually expressed in published papers. The message that comes across through all these papers is that it is not feasible to isolate the influence of the stationary phase from the effects of the other components of the chromatographic system. D. C. ABBOTT

FIREBALLS ASSOCIATED WITH THE BARWELL METEORITE

By H. G. MILES
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AND
DR. A. J. MEADOWS
Astronomy Department, University of Leicester

ON December 24, 1965, at about 1612 h U.T., meteoritic fragments fell in a fairly restricted area in and about the village of Barwell, Leicestershire (long. 1° 20′.2 W., lat. 52° 33′.9 N.). The sighting of a very bright fireball and the associated sonic effects were recorded over a wide area, particularly in the counties of Warwickshire and Leicestershire.

Eye-witness accounts have been collected by the University of Leicester (Prof. P. C. Sylvester-Bradley), the British Astronomical Association (H. G. M.) and by one of us on a B.B.C. television programme, "Sky at Night", devoted to this event (H. G. M.). Analysis has shown the existence of three fireballs over the Midlands, all travelling in an approximately north-north-easterly direction. It has also shown that two of these were the result of a single object exploding over a point near the Gloucestershire–Warwickshire boundary. One of these resulted in the fall at Barwell. The meteorite fragments have been classified as olivine-hypersthene chondrites. No evidence has yet been found for meteoritic falls associated with the other two fireballs.

Meteorological Conditions

The Meteorological Station at Birmingham Airport reported that at 1600 h G.M.T. on December 24, 1965, the cloud cover at Coventry was 8/8th cloud at 6,000 m with much cloud at various lower levels. Reports from Coventry and the surrounding districts indicated that the cloud distribution was very irregular, resulting in one observer reporting a completely overcast sky and yet another only a few kilometres away reporting a relatively clear sky. Many observers also reported the presence of extensive cirrostratus. The general picture for the area was summed up to be:

cirrus/cirrostratus
↑
altostratus
↑
stratus

with varying amounts of each in any one locality.

From information obtained from the Meteorological Office, Bracknell, the height of the tropopause in the Midland area was estimated to be about 9,800 m at 1200 h G.M.T.

Track of Fireballs

Because of the irregular cloud distribution, people who saw a fireball tended to be clustered in reasonably well-defined areas; this left large regions from which no observations were received. Furthermore, because of the restricted visibility, most observers saw only one fireball. Six people, however, saw two objects and several others could not correlate the direction of the flash which attracted their attention with the direction in which they later saw the fireball.

An analysis of the reports has shown that three separate fireballs were observed from the Coventry area. One of the main difficulties of the analysis has been the sorting of the observations, as few of the reports came from people with astronomical knowledge or with training in reporting this type of phenomenon. In spite of this, the overall standard of the reports was high and, although possibly not given in the best way, much information could be extracted. Most of the observers in the Coventry area were interviewed by one of us (H. G. M.) and the accuracy of their reports assessed.

To clarify our discussion of the fireballs, we shall assign numbers to the tracks corresponding to the order in which they became evident during the analysis:

Track 1—travelling in an approximately north-north-easterly direction, passing over the eastern boundary of Coventry.

Track 2—travelling in an approximately north-north-easterly direction passing near Rugby.

Track 3—travelling in an approximately north-north-easterly direction passing over the western boundary of Coventry.

Track A—the track of the original fireball which produced fireballs 1 and 3.

Fireball 1. Thirty-four observations were obtained of this object. Preliminary investigation showed that the track, when extended, passed through Barwell; subsequent analysis has been based on the assumption that this fireball was responsible for the meteoritic fall in that area. The correctness of this assumption has been strengthened by an investigation of the distribution of the meteoritic fragments which suggests an approach from a direction slightly west of south.

Because of thick cloud immediately to the east of Coventry, most of the observations of this fireball were made from positions to the west of its track. Only one of the five made from the eastern side could be assessed as reasonably accurate. Several observers, however, reported the passage of an object overhead, and each gave an indication of whether it was slightly to the east or to the west. The direction of the track was thus found to be 023° ± 0·5° east of north. A high percentage of the observing positions were relatively near to the track and consequently the height of the bolide could not be determined very accurately. The angle of descent was found to be 20° ± 2° to the horizontal. Ten people who were outdoors at the time, 9–14 km from the impact point, but close to the track, reported no visual effects, but some heard a noise like thunder. This helps to confirm an extinction point, seen by two observers, at a height of 9·5 km (± 1 km) in a position within 1 km of 1° 26′.2 W., 52° 26′.3 N.

Fireball 3. The existence of this bolide became apparent when some observers in Coventry reported seeing an object to the west of the city. It was also indicated by the reports from the Nuneaton and Loughborough areas. Unfortunately, the quality of many of the twenty-eight observations of this fireball was not so high as in the

previous case, but from the few accurate reports it was found possible to determine the track as 020° E. of north with an accuracy of ±2°. The object travelled across the west side of Coventry, passing over a point within 0·5 km of long. 1° 31′.2 W., lat. 52° 25′.5 N. The angle of descent could not be determined accurately, but a value of 13° to the horizontal and an extinction height of 10 km at a position within 3 km of 1° 18′ W., 52° 50′ N., are the most probable values.

Fireball A. If tracks 1 and 3 are produced backwards they converge on a point in the region of long 1° 49′ W., lat. 51° 56′ N. Using the angles of descent already given, a convergence of heights occurs in about the same region. This indicated the possibility of a break-up of a single fireball in the vicinity of the position quoted. The validity of this possibility was strengthened by an observation made from the Birmingham area in which the break-up of a fireball was witnessed. The observer reported that one component descended at an angle a few degrees greater than the original angle of descent and the other at an angle slightly less. He said that the two components were visible for only a short time after separation; however, he was unable to say whether the loss of sight was due to a natural extinction or to interference by cloud. Because of the extreme scarcity of observations in the Birmingham area the latter explanation is more likely to be the correct one.

Fireball 2. From the outset of the investigation, it was apparent that observers in the Coventry area who saw a fireball in the eastern part of the sky could not all have seen the same object. The subsequent analysis was hindered by the absence of reports from eastern and north-eastern Warwickshire due to considerable low cloud. Observations from the Rugby area clarified the situation. One observer saw the flash and trail of an object directly overhead and almost immediately saw another object crossing the sky in the Coventry direction at a much lower altitude. The existence of two objects was further verified by observers situated to the west of Rugby who saw a fireball either to the west or to the east.

The thirty-six observations of this fireball were distributed evenly to the east and west of the track, although most of those from the western side came from a very restricted area. The bolide travelled in a direction roughly parallel to fireballs 1 and 3—the most probable value being 023° east of north with a possible error of ±2°. The track passed to the west of Rugby and ended at a point of extinction above the position long. 1° 11′.5 W., lat. 52° 32′.2 N.; however, there may be an error in a lateral direction of 1·5 km. The angle of descent could not be determined accurately as the bolide was only observed over a relatively short track, but the most probable value is within a few degrees of 20°.

Observations outside the Warwickshire–Leicestershire area.

(i) An observer from Cirencester reported an object in the east travelling in a north-north-easterly direction. This was most likely fireball 2.

(ii) An observer from Harwell (Berkshire) reported an object in the north-west at an altitude of 40°. If this object had been fireball *A*, the altitude would have been lower. If track 2 is extended backwards, the height obtained is similar to that of fireball *A* in the corresponding position. Because the bolide was followed over a considerable angle in the sky and because no separation was reported, it is concluded that the object was fireball 2.

(iii) An observer from north of Worcester reported seeing a single object low over the Malvern Hills.

(iv) An observer from Oswestry (Shropshire) reported seeing two fireballs travelling on parallel tracks low down in the south-south-east.

(v) Eight observations from the Staffordshire area reported objects in the east and south-east. It is thought that they refer to fireball 3, but the accuracy of the reports was such that this could not be confirmed.

(vi) Six observations from the Reading–Aylesbury area reported a fireball to the north which dropped vertically to the horizon. All reported a single object. If the object seen was one of the aforementioned fireballs, no explanation can be put forward to explain why the others were not seen.

Fig. 1 shows the probable tracks of the various fireballs. Fireballs *A* and 2 are shown with parallel tracks. The probable errors involved in deriving these tracks would not contradict the supposition that these two bolides derived from a single body separating, say, at the top of the stratosphere. There is a slight possibility, however, that they may already have been separated in space. (For a recent discussion of meteorite swarms, see Nininger[1].)

Observations not included in the foregoing. Nine observations were received reporting an object with a long tail travelling in a north-east to south-westerly direction. These reports are reasonably consistent and suggest a track passing near Grantham (Lincolnshire), Rugby, and Stroud (Gloucestershire). No attempt has been made to explain these reports.

Visual Appearance of the Fireballs

The main bodies of all three bolides were reported to have been spherical in shape. The reports conflict somewhat as to the presence, or absence, of tails. Fireball 3 definitely developed a noticeable tail, yellow in colour. Fireball 2 possibly developed a small tail. Fireball 1 probably formed a tail during the last part of its flight. Both 1 and 2 left white dust trails behind them. The trail left by 2—at least during the latter part of its flight—was definitely corkscrew in shape. Because of the extensive cloud cover, it is difficult to say precisely how long these trails lasted. That from fireball 2, however, certainly endured for several minutes. There is general agreement among observers that the central regions of

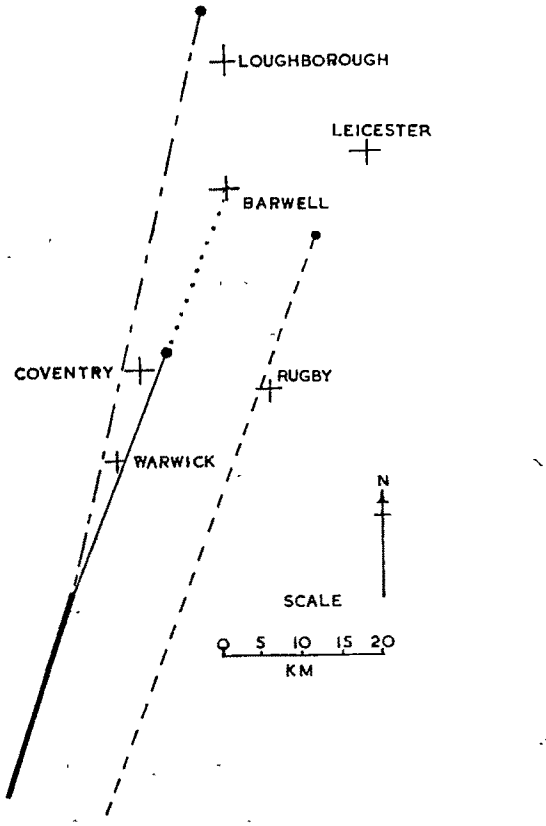


Fig. 1 Probable tracks of the fireballs: ———, Track 1; – – – –, track 2; — — — — —, track 3; ▬▬, track *A*. ●, extinction point

both 1 and 2 were orange-red in colour towards the end of their flight. Reports from earlier along their tracks suggest that they may then have had a whitish tinge. Both 1 and 2 were noted by a few observers to shed sparks.

Fireball 2 is reported by several witnesses to have blown up at the point which is recorded in Fig. 1 as its "extinction point". The reports indicate that four major fragments resulted from the explosion. One piece was shot upwards, the other three downwards. No such explosion was observed for fireball 1.

From the tracks of fireballs 2 and 3 and their estimated final extinction points, it is possible to make a tentative guess at the areas where they finally fell. If either of these bolides produced enough solid material to reach the ground in appreciable quantities (which is by no means certain), then fireball 2 would probably have deposited material somewhere around Leicester and fireball 3 somewhere around Nottingham.

Acoustic Phenomena

Numerous reports of acoustic phenomena have been received from along the tracks of the fireballs. Many people, of course, detected the sound effects without observing the related visual phenomena. The reports are mainly concentrated in a region extending from Wiltshire in the south to Leicestershire in the north. The area covered is about 150 km long (in a south-south-west–north-north-easterly direction) by 50 km wide. This elongated shape for the acoustic field is typical for a bolide trajectory with a low angle of inclination to the Earth's surface[2]. The sound effects seem to have been particularly intense south of Rugby and in the Coventry area. In the latter region breakages (for example, of electric light bulbs) were reported. There also seem to have been zones where less noise was received than would be expected. A particularly obvious 'silent' zone was in the region between Coventry and Barwell.

The reports of sound effects, as might be expected, are considerably more confused than the visual sightings. There are, for example, several reports from observers in the Coventry area who noted the passage of a fireball and then recorded the length of time before any noise was heard. Even over this very small area, the time intervals reported vary from a few seconds to more than 2 min. Apart from the normal subjective errors in recording the passage of time, there are two other possible causes contributing to this scatter. The first is that both fireballs 1 and 2 seem to have produced acoustic phenomena. It seems possible that noise from both fireballs would be audible in Coventry, and at other points along their tracks, if the conditions were suitable. Therefore, observers in Coventry may have been detecting noises from two different sources. This suggestion receives some support from one witness in Coventry who heard two separate sets of acoustic phenomena spaced about a minute apart. It is further supported by the fact that the Coventry observations can, to some extent, be separated into two groups—one with a delay time of half a minute or less, and the other with a delay time of a minute or more. The second possible cause of scatter lies in the differences between the types of sound which were reported. Some witnesses have reported only a bang, others heard a bang preceded by a prolonged rumble, others again heard a bang followed by a rumble. These delay times can also be reconciled in a different way: by assuming that fireball 1 was subsonic in the Coventry region and that the noises came from fireballs 2 and 3 which were still supersonic. There is, in fact, only one direct report of a sonic boom from fireball 3, although several reports of 'rushing' noises have been received from along its track.

Several reports from south of Warwick indicate that three distinct sonic bangs were heard there. These were probably connected with fireball 1. It is also certain that fireball 2 produced at least two sonic bangs and possibly more. It should be added that most meteorite falls are said to produce three detonations during their passage through the atmosphere[3].

Besides numerous reports of normal sonic phenomena, there have been a few reliable reports of electrophonic noises[4]. (These are hissing noises heard well before any other sonic effects.) One observer, for example, reported hearing noises of this type before he saw the bolide break through the cloud cover towards him.

Witnesses in Barwell itself have given consistent accounts of the actual sounds heard at impact. These are typically described as starting with a 'swishing' noise and ending with a succession of dull thuds.

Details of the Fall at Barwell

The final angle of descent can be estimated fairly accurately for one of the pieces that fell at Barwell, because it penetrated the roof of a factory and then a floor below. It struck from a generally south-easterly direction at an angle of somewhat less than 80°. The positions of some of the other fragments which are near buildings or fences indicate that they, too, were falling almost vertically just before impact. It should be noted that the final direction of fall of a meteorite fragment is not necessarily related to the direction of the parent bolide[5].

There are considerable disparities between the depths of penetration of the various meteorite fragments. Thus one piece was found lying on the ground, whereas another piece of similar size and falling on to a similar surface was buried to a depth of more than half a metre.

The meteorite fragments so far recovered from Barwell have been found over an approximately circular area (Fig. 2). There is no obvious graduation in the size of the fragments across this area, although an extended swarm of smaller fragments lies on the southern side. This latter feature would suggest that the meteorite approached from a generally southerly direction, since typically the larger meteorite fragments carry further. Previous meteorite falls which have been investigated in detail have tended to give an appreciably elongated distribution extending over several kilometres, for example, the Brudenheim meteorite[6]. The different distribution of the Barwell meteorite may be due to a final break-up late in flight. This is supported by the lack of secondary burning on the surfaces of the fragments, and by the fact that several pieces which come from various places in the distribution area can be fitted together very accurately. The final break-up must therefore have occurred after the passage of the meteorite through the region of retardation (that is, after it reached a subsonic velocity). The region

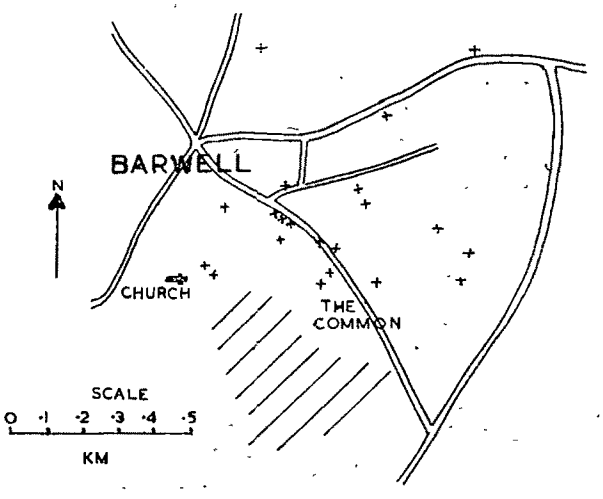


Fig. 2. Barwell. +, Principal falls. Hatched area, small fragments

of retardation may be taken to coincide with the extinction point, which, for the fireballs considered here, coincided with the tropopause. The final fragmentation must therefore have occurred at a height of less than 10 km. It should be added that the meteorite fragments are somewhat friable and do not appear to possess an unusual compressive strength.

One fragment of the meteorite was picked up immediately after impact. It is reported to have been warm to the touch initially, cooling to the ambient temperature within 25 min.

[1] Nininger, H. H., in *The Solar System*, edit. by Middlehurst, B. M., and Kuiper, G. P., 4 (Univ. Chicago Press, 1963).
[2] Krinov, E. L., *Principles Meteorites*, 69 (Pergamon Press, London, 1960).
[3] Krinov, E. L., op. cit., p. 73.
[4] Krinov, E. L., op. cit., p. 67.
[5] Krinov, E. L., op. cit., p. 84.
[6] Follnsbee, R. E., and Bayrock, L. A., *J. Roy. Astro. Soc. Canad.*, 55, 218 (1961).

PHOTOPERIODIC TIMING MECHANISMS IN INSECTS

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RESPONSES to seasonal changes in day length have now been described in a wide range of insects[1]. Several morphogenetic processes are controlled by this factor, but the arrest of growth (diapause), so often found in hibernating or aestivating insects, and seasonal changes in form (polymorphism), are two of the most familiar. Although they vary in detail, the response curves of most species show a very sharp transition when the daily photoperiod is of intermediate or critical length. Recent work on the green vetch aphid *Megoura viciae* provides a typical example. Form determination in this insect is controlled maternally. Parent viviparae that have developed in long days give birth to similar viviparous, parthenogenetic daughters, while parents from short-day conditions produce egg-laying females (the oviparae). In the clone under investigation the critical photoperiod is close to 14 h 45 min. These are the conditions under which approximately half the individuals develop as vivipara-producers and half as ovipara-producers. But this is a point of considerable instability since the photoperiod has only to be lengthened or shortened by 20 min in order to shift the response to completion in either direction.

Results of this kind indicate that insects possess an intrinsic timing mechanism of considerable accuracy. However, there is at present little agreement as to the type of biological clock which may be involved. One view[2] is that the mechanism has analogies with the hour-glass or interval timer and, as such, is concerned in measuring the length of the dark component in the 24-h cycle. Some additional evidence which favours this concept will be presented in the present article. The second hypothesis is based on the belief that the clock embodies an oscillator which is closely coupled to the endogenous circadian systems that govern a wide range of daily rhythms. The latter idea, in one of its forms, has been advocated particularly by Bünning[3] and has been supported experimentally by the work of Bünning and Joerrens[4] on the photoperiodic control of pupal diapause in the lepidopteran *Pieris brassicae*. According to Bünning, time measurement depends on an endogenous 24-h rhythm of photosensitivity which is itself initiated and phased by light. After the rhythm has been phase-set by the onset of light in the first cycle, the period of the rhythm is assumed to remain constant at 24 h even if the external conditions (such as the cycle length) are changed. The rhythm is regarded as first passing through a photophile half-cycle of 12 h when any incident light will be diapause-promoting; this is followed by a second (scotophile) half-cycle when light will be diapause-inhibiting.

Bünning's model is consistent with the obvious effects of normal short and long day cycles, since diapause tends to be induced if the daily photoperiod is 12 h or less, while it is prevented if the photoperiod lengthens to 13–14 h and therefore extends into the scotophile. Bünning and Joerrens tested the hypothesis further by introducing short light "breaks" into inductive long dark periods. Their theory requires that there should be one peak of light sensitivity during the course of the dark half-cycle. This was found, but unfortunately the light interruptions were not continued to the end of the dark period. Work on other Lepidoptera, including *Ostrinia nubilalis*[5], *Pieris rapae*[6] and *Pectinophora gossypiella*[7], as well as in the aphid *Megoura*[8], has shown that in all these species there are two peaks of sensitivity to such interruptions, one at the beginning and the other towards the end of the dark period.

It is also noticeable that the evidence for the photopromotion of diapause is much less satisfactory than the evidence for photoinhibition. For demonstrating the former, Bünning and Joerrens used 24-h cycles containing dark periods of more than 12 h which therefore extended into the photophile. Although diapause was slightly enhanced by light interruptions that fell within this half-cycle, it is evident from the normal response curve that uninterrupted long night cycles of comparable length tend to produce a response which lies near the threshold and is therefore highly variable. The use of very long dark periods in 24-h cycles may also be misleading for another reason. If the dark period exceeds the critical length by an appreciable amount, sub-terminal light breaks may still leave an uninterrupted dark period of more than critical length and an extra "inflexion" in the response curve may be generated.

Pittendrigh[9] and Minis[10] have offered an alternative hypothesis, similarly based on the belief that circadian rhythms are intimately involved in photoperiodic time measurement, but one which also takes into account the bimodal response to light interruptions. Their "coincidence model" rests on the assumption that diapause inhibition in *Pectinophora* depends on the temporal relationship between a light-induced enzyme response and an independently phased substrate oscillation. The hypothetical substrate rhythm in the larva is regarded as having the properties of other circadian processes in the same insect. One such system is the oviposition rhythm of the parent moth. With 24-h cycles containing two separate light periods (one a pulse and the other a more extended photoperiod), the moth responds as if to a single continuous photoperiod. The degree of separation of the two components then decides whether the light pulse is accepted as "dawn" or "dusk" and whether the ensuing phase shift is an advance or a delay. If comparable phase shifts were to occur in the substrate rhythm in response to early and late light interruptions, the peak of the entrained rhythm might be brought to coincide either with the end of the main photoperiod or with the light break itself. Diapause would then be inhibited. On the other hand, "coincidence" might not be achieved in the transitional "resetting zone" and diapause would occur. So far, this model has not been fully tested.

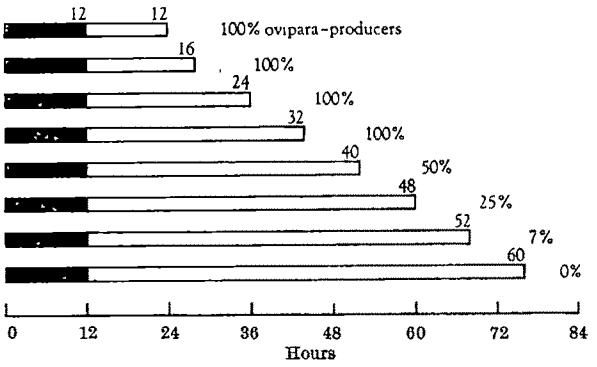


Fig. 1. Response of the aphid *Megoura* to cycles of illumination containing a continuous dark phase (12 h) of more than critical length

Observations on the aphid *Megoura* have led to an entirely different concept, namely, that the photoperiodic clock is an interval timer, apparently unconnected with the circadian system. The dark period is of central importance in time measurement. This is seen if very long intervals of light are alternated with dark periods of more than critical length (Fig. 1). Even if the photoperiods now far exceed the normal "long day", the parent aphids still develop as ovipara-producers; indeed, Fig. 1 shows that one long 12-h dark period every 2 days is sufficient to annul the effects, if any, of the longer light phases.

At the same time, light break experiments demonstrate that the "dark reaction" involves more than the simple interconversion of two chemical substances. The effects of 1-h interruptions of low intensity light (30 ft.-candles) are illustrated in Fig. 2. The length of the dark period (10·5 h) has been chosen so that it exceeds the critical length (9·75 h) by only a small margin. The bimodal response referred to previously can clearly be seen: the light breaks are highly effective at the beginning and during the last two-thirds of the dark period, no oviparae being formed. But during the intermediate phase the response becomes exceedingly refractory to light. Even high intensity white light (5,000 ft.-candles) is usually without effect if the aphids are irradiated 4 h after the beginning of darkness.

Although sensitivity changes produce striking inflexions in the response curve, it would be unsafe to conclude that these are necessarily the manifestations of an endogenous rhythm. Two considerations suggest otherwise.

(1) The response to dark-period interruptions has proved to be independent of the length of the "main" light period, at least within very wide limits. In Fig. 2*A*, the main photoperiod was 13·5 h in length. Figs. 2*B* and *C* show the response to 1-h light break scanning a 10·5-h dark period, when the main light phase has been extended by 12 h to 25·5 h, or reduced by 5·5 h to 8 h. Obviously, the form of the response is almost unchanged.

These results cannot be accounted for by Bünning's hypothesis In several of the cycles shown in Fig. 1 the long light period extends into Bünning's scotophile phase, yet does not annul the inductive effect of the 12-h dark period. The outcome is the same with the cycle of 25·5 h light, 10·5 h dark (Fig. 2*B*), in which the main light period entirely occupies the first scotophile phase. Further, the ensuing dark period with its scanning light interruptions now coincides with the photophile phase of the second day; yet it is clear that ovipara-production is diminished and not enhanced.

These observations appear to be equally irreconcilable with Pittendrigh's model, which requires that the substrate rhythm should entrain to a similated photoperiod defined by the beginning of the main light period and the end of the subsidiary light break. In the experiments just described, this varies from 9·5 h (Fig. 2*C*) to 34 h (Fig. 2*B*). There are no higher organisms in which the limits of entrainment are known to be as wide as this[11].

(2) Cycles longer than 24 h and incorporating a long or very long dark period which is scanned with light breaks have frequently been used for detecting possible circadian periodicities in plants. The work of K. C. Hamner, in particular, has shown that in some short day plants (such as Biloxi soybean), light interruptions may cause an apparently rhythmic flowering response, in conformity with Bünning's hypothesis[12]. In one series of experiments by Hamner, soybean plants were exposed to cycle lengths ranging up to 72 h, and including a constant "main" light period of 8 h. Light perturbations introduced into the dark period tended to inhibit flowering if they occurred about 16, 40 or 64 h after the beginning of the main light phase, that is, during Bünning's first, second and third scotophile phases.

A number of experiments of similar design have been carried out with the aphid *Megoura*. One example of the results, all of which were alike, is given in Fig. 3. It will be seen that the 1-h light breaks, which were spaced in 4-h steps in a 64-h dark period, failed, with one exception, to reduce the inductive effect of the long nights. In this unique cycle, which evoked a complete reversal of response,

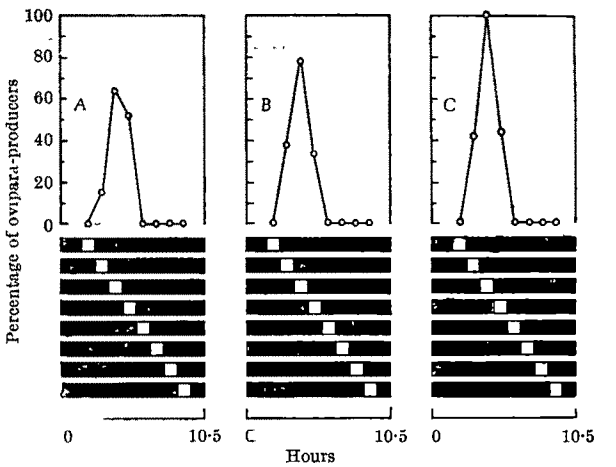


Fig. 2. Photoperiodic reactions in *Megoura*. The three groups of cycles shown in *A*, *B* and *C* all contain a 10·5-h dark phase scanned by 1-h light interruptions. In each group the scanned dark period was preceded by a constant "main" photoperiod (not shown). The duration of this light phase was 13·5 h in *A*, 25·5 h in *B* and 8 h in *C*. In the controls (uninterrupted dark period of 10·5 h) 100 per cent of the aphids produced only oviparous progeny

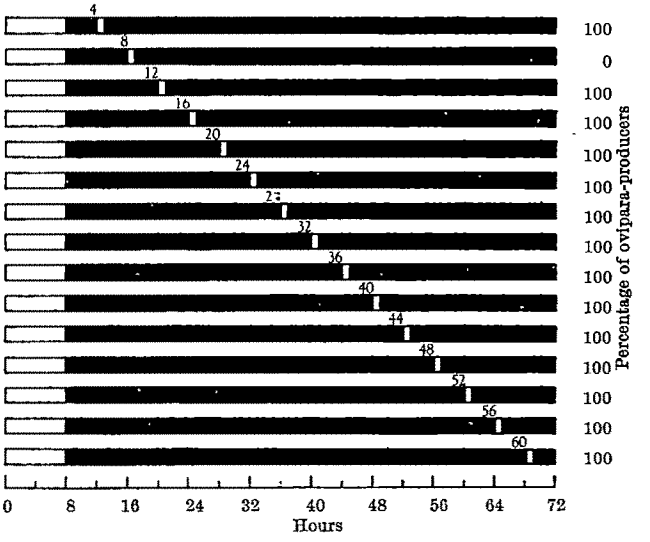


Fig. 3. The effect of introducing 1-h light interruptions into a very long dark period. In the controls (uninterrupted dark period of 64 h) 100 per cent of the aphids produced only oviparous progeny

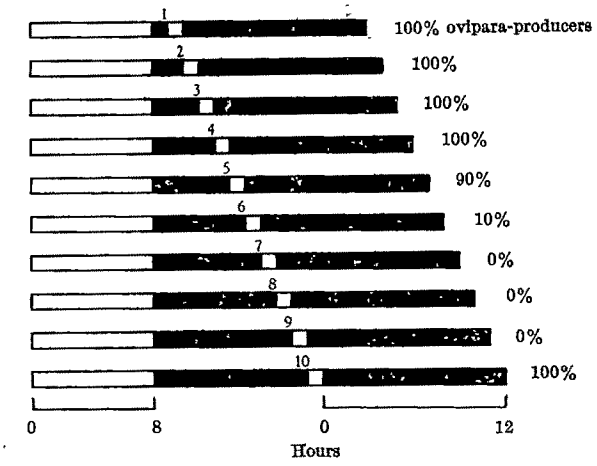


Fig. 4. Photoperiodic reactions in *Megoura*. The effect of introducing 1-h light breaks at different points in the dark period on the response to a subsequent uninterrupted dark period of more than critical length (12 h)

the light break occurs 8 h after the beginning of darkness. Nevertheless, although the long dark phase is obviously highly photosensitive at this point, no trace of a second or third response maximum can be observed with light breaks placed 24 or 48 h further into the dark phase.

While this result provides no indication that the response is rhythmic, it is not, at first sight, easily reconciled with the interval timer hypothesis since, no matter where the light interruptions are placed, each cycle still contains a residual uninterrupted dark period that exceeds the critical length. The explanation of this finding was provided by the observations summarized in Fig. 4. As in the previous experiment, all cycles started with a main light period of 8 h and the following dark period was again scanned by 1-h light breaks; but this series differed in that each interruption was followed by a constant long night of 12 h. When the duration of the dark period, prior to the interruption, did not exceed 4 or 5 h, the long night had the effect expected and was fully inductive. Further, it could also be shown that in such cycles the length of darkness required for complete induction was precisely the critical length, namely, 9·75 h. This must mean that after an early light break the timing process is begun anew.

The response is entirely different if the light breaks follow dark periods of 6–9 h duration (Fig. 4). Induction is then totally inhibited until the dark period before the interruption again exceeds the critical length. This inhibitory effect, which is strikingly reminiscent of the phenomena described by Schwabe[13] in certain short day plants, is also influential outside the cycle in which it occurs. It is well displayed when long days of normal length (such as 16 h light, 8 h dark) are interpolated in a sequence of short days (such as 12 h light, 12 h dark). Table 1 shows that one long day (*L*) is sufficient to cancel out the inductive effect of 2–3 intervening short days (*S*).

Table 1

Regime	Percentage of ovipara-producers
SLSLSLSLS.....	0
SSLSSLSSL.....	0
SSSLSSSLS.....	30

Clearly, this type of regime should be contrasted with those in which adjacent "short days" are separated by long periods of continuous illumination (as in Fig. 1). This result is of interest since it shows that a long day effect is not achieved merely by the absence of inductive long nights, but is at least partially caused by the fact that the short night moiety of the long day cycle is terminated by light after 6–9 h. It should be pointed out, however, that the inhibition is not obtained if the light period preceding the curtailed dark phase is of insufficient length. This is why the cycle 8*L*, 56*D*, 1*L*, 7*D* (Fig. 3) is fully inductive, even though it includes a light-interrupted dark period of 7 h.

The similar effects caused by the introduction of light breaks into dark periods of more than critical length account also for the dual low threshold regions in Fig. 2. But it is now clear that the mode of action of light is entirely different, depending on whether the breaks come early or late. The two photoreactions can be further characterized by means of their action spectra. The response to 1-h interruptions of monochromatic light, placed 1·5 h after the beginning of a 10·5-h dark period, is plotted in Fig. 5. Interference filters with an average bandwidth at half-peak transmission of 7 mμ were used with a tungsten source and light energies measured with a calibrated vacuum photocell. It is sufficient to expose young larvae to eight consecutive interrupted cycles. The curve shows that minimum energies are required at 450–470 mμ in the blue region of the spectrum. The response falls off rapidly both in the near ultra-violet and in the green above 500 mμ. No minor inflexions have yet been established with certainty.

The pronounced blue sensitivity observed in this insect appears to be typical of the photoperiodic responses of arthropods, particularly when these have been tested by extending the main photoperiod with filtered light (the probable equivalent of an early dark period interruption[1,14]). In its general form the aphid action spectrum resembles the spectral sensitivity curve for diapause inhibition in the red spider mite, *Panonychus ulmi*[15].

Since the light energy which drives the photoperiodic response is so strongly dependent on wave-length, it may be assumed that absorption is similarly selective. The photoreceptor should then be a pigment, presumably orange in colour. Unfortunately, the exact site of the receptor is still in doubt. It has been shown that the compound eyes do not form the light pathway and that light acts directly on the brain[16], as it also does on the pupa of the silkmoth, *Antheraea pernyi*[17]. Yet visual

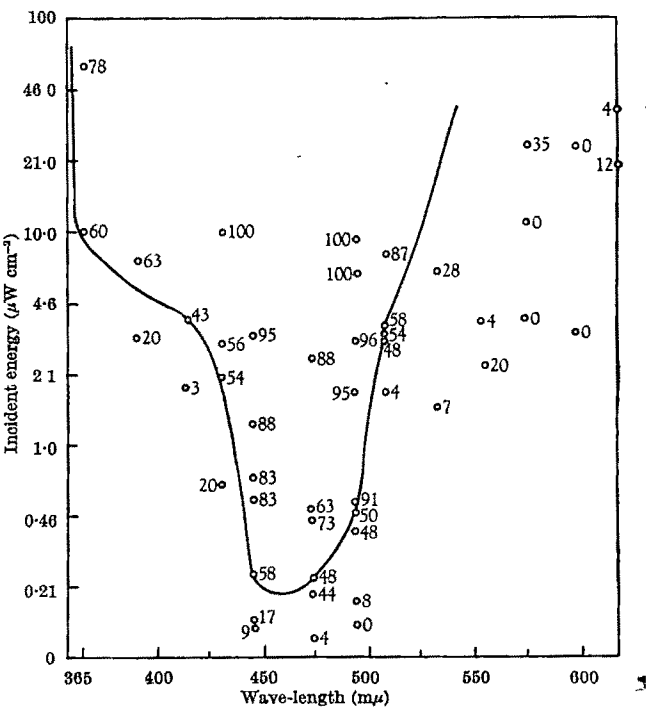


Fig. 5. Action spectrum for the photoperiodic control of progeny type in *Megoura*. Semilogarithmic plot showing the response to 1-h interruptions of monochromatic light introduced into a 10·5 h dark period 1·5 h after its inception. The "main" photoperiod of 13·5 h was of white light (100 ft.-candles). The intensity of the response is given by the percentage of aphids that produced viviparous progeny. These values are also shown by their respective points and the curve is drawn at approximately the 50 per cent response level

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Volume 2, in preparation

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October 1966, about 450 pp., approx. 120s.

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PERGAMON PUBLICATIONS

1965 Transactions of the Third International Vacuum Congress, Stuttgart, Germany

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Edited by **H. Adam**

The main subjects of this meeting gave an interesting and, in the widest sense, an important insight into the significance of high vacuum for our present science and technology.

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162 pages/June 1966 hardcover 75s./$12.00

Vol. 2—Session Papers, to be published in 3 parts—Autumn 1966

Reproductive Biology and Taxonomy of Vascular Plants

Edited by **J. G. Hawkes,** *University of Birmingham*

This volume contains the reports of papers presented at a conference held by the Botanical Society of the British Isles at the University of Birmingham. The papers presented deal with the breeding systems and other aspects of the reproductive biology of flowering plants.

Contents: K. Mather—Breeding systems and response to selection; D. Lewis—The genetic integration of breeding systems; J. L. Harper—The reproductive biology of the British Poppies; J. L. Fyfe—The reproductive biology of some leguminous crops; F. L. Breese—Reproduction in Ryegrasses; L. van der Pijl—Pollination mechanisms in Orchids; J. B. Free—The foraging behaviour of bees and its effect on the isolation and speciation of plants; D. A. Webb—Dispersal and establishment: what do we really know?; P. F. Wareing—Ecological aspects of seed dormancy and germination; J. L. Crosby—Reproductive capacity in the study of evolutionary processes; A. Nygren—Apomixis in the Angiosperms, with special reference to *Calamagrostis* and *Poa;* G. Haskell—The history, taxonomy, and breeding system of apomictic British *Rubi;* T. G. Walker—Apomixis and vegetative reproduction in ferns; S. M. Walters—The taxonomic implications of apomixis; D. H. Valentine—Opening remarks; General discussion.

174 pages/June 1966 hardcover 30s./$5.00

Progress in Aeronautical Sciences

Volume VII

Edited by **D. Kuchemann,** *Royal Aircraft Establishment, Farnborough*

This book is devoted to vortex motions, a specific aspect of fluid mechanics, itself only one particular aeronautical science. The papers were either presented at a recent symposium on the subject or are a survey of particular aspects of the discussion.

Contents: M. Roy—On the rolling-up of the conical vortex sheet above a delta wing; R. Legendre—Vortex sheets rolling-up along leading-edges of delta wings; J. H. B. Smith—Theoretical work on the formation of vortex sheets; M. G. Hall—The structure of concentrated vortex cores; N. Rott and W. S. Lewellen—Boundary layers and their interactions in rotating flows; B. R. Morton—Geophysical vortices; R. Wille—On unsteady flows and transient motions.

228 pages/1966 hardcover 90s./$13.50

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R. T. Holman, *Hormel Institute, University of Minnesota*

This latest edition in an already well-established series deals with the chromatography of lipids, covering such topics as column chromatography, paper chromatography and recent developments in thin layer chromatography.

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132 pages/1966 flexi-cover 40s./$6.50

Infrared Physics

This journal has been established for the publication of scientific papers concerning infrared physics and its application. It is largely concerned with infrared theory, experiment, and instrumentation as applied to infrared detection and transmission, and to problems of atmospheric, meteorological, geophysical, astrophysical and space research. Published quarterly. Specimen copy and subscription rates available on request.

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examination shows the brain tissue of the aphid to be colourless—a circumstance which suggests that the pigment is in low concentration, and in this respect resembles the plant pigment, phytochrome[18].

The dilute nature of the pigment may possibly be advantageous if the action spectrum is to be used for deducing its absorption characteristics. Nevertheless, a close match cannot perhaps be expected until the filtering effect of the cuticle overlying the brain is taken into account. And the possible influence of wave-length on quantum yield and on light scattering[19] is quite unknown. Despite these uncertainties, however, the position of the absorption maximum and the apparent absence of a near ultra-violet peak support the view[20,21] that the chromophore in insects and mites is a carotenoid hydrocarbon.

With later interruptions of the dark period (for example, 7·5 h after its inception) threshold dose rates are effective over a shorter time course, and there are also significant changes in the action spectrum. With 30-min interruptions, a broad band is still found in the blue, but light sensitivity now extends to somewhat longer wave-lengths. The upward arm of the response curve is displaced 30–50 mμ towards the yellow. Red sensitivity is increased, but is still very low.

The fact that the action spectra for early and late interruptions are different, but by no means entirely dissimilar, militates against the suggestion of Bünning and Joerrens (for *Pieris brassicae*[4]) that there are separate blue and red receptors. It seems more likely that time measurement is carried out by means of a linked sequence of dark reactions, probably involving some form of pigment conversion. There are at least four steps, each of which can be defined by the response to light: (1) During the first few hours of darkness, the dark reaction is completely reversible by blue light of low intensity. (2) After 3–4 h of darkness, sensitivity to all wave-lengths rapidly diminishes to zero. Presumably the absorbing pigment is still present, but some other essential component is missing from the system. (3) From about 5 h to the end of the critical dark period, photosensitivity is regained, the dark reaction becoming increasingly responsive to blue-green light. But complete reversibility is no longer possible. Perhaps the irradiated pigment is changed to an unavailable form; at all events, incident light has the distinctive effect of inhibiting earlier (and possibly later) cycles containing dark periods longer than the critical duration. (4) By the end of the critical period the dark reaction is complete, and is then immune to light.

Since it is well known that carotenoids readily form photolabile complexes with proteins[22], it seems possible that this type of association may also be involved in the functioning of the arthropod interval timer.

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NEWS AND VIEWS

Pharmacology in the University of London: Prof. G. A. H. Buttle

PROF. G. A. H. BUTTLE will retire from the Wellcome chair of pharmacology in the School of Pharmacy, University of London, on September 30. Gladwin Buttle's career may be roughly divided into three phases. He went to St. John's College, Cambridge, and afterwards to University College Hospital, where he obtained his medical qualification in 1924. Then in 1926 he joined the Wellcome Physiological Research Laboratories at Beckenham, Kent, where he worked until 1939, first on the actions of some toxins and later on bacterial chemotherapy. Buttle and his colleagues in 1936 were the first to show that sulphanilamide protected mice against experimental meningococcal infections and thus paved the way for the introduction of this drug for the treatment of meningococcal meningitis in man. He was also concerned with the introduction into chemotherapy of 4 : 4′-diaminodiphenyl sulphone which, under the name 'Dapsone', is now the mainstay in the treatment of leprosy by drugs. During the Second World War, Buttle became officer-in-charge of No. 1 Base Transfusion Unit in the Middle East, where he performed wonders in the collection and distribution of blood. In 1942 he was awarded the O.B.E. Later he served in north-west Europe. The third phase of Buttle's career began in 1945, when he was appointed to his present post, in which he has been brilliantly successful as a teacher, administrator, and supervisor of the researches of his pupils and colleagues. He has himself done important work on the transmission of tumours in animals.

Buttle has won the admiration, affection and respect of pharmacologists the world over. We wish him many happy years of retirement.

Prof. E. W. Horton

DR. E. W. HORTON has been appointed to the Wellcome chair of pharmacology in the University of London, tenable at the School of Pharmacy, in succession to Prof. G. A. H. Buttle who is retiring. Dr. Horton, who is thirty-six years old, will take up his appointment on October 1. He was educated at the University of Edinburgh, where he took first-class honours in the B.Sc. degree and graduated M.B., Ch.B. in 1955. The following year he held a full-time research appointment in the Department of Pharmacology under the then Prof. J. H. Gaddum. He then became a member of the scientific staff of the Medical Research Council, Division of Physiology and Pharmacology, National Institute for Medical Research, and later director of therapeutic research and head of the Department of Pharmacology at the Miles-Ames Research Laboratories. In 1963 he was appointed senior lecturer in physiology at the Medical College of St. Bartholomew's Hospital. Dr. Horton's research work has been mainly concerned with the biological properties of certain naturally occurring substances. In collaboration with Dr. D. F. Elliott and Dr. G. S. Lewis, he isolated bradykinin and determined its structure. More recently, he

has been concerned with the identification, distribution and pharmacological actions of the prostaglandins.

Research at East Malling

THE report for 1965 of the East Malling Research Station gives an impressive account of the volume and scope of research at present in progress. It contains twenty-eight research papers, and nearly one hundred additional publications by members of the staff are also listed. The research programme of the Station as a whole is a balance between fundamental and applied studies, all of which contribute to a greater understanding of the factors which influence and control the growth and cropping of fruit plants. The titles illustrate the breadth of the research, and range from "A Polythene Fence to Keep Beetles from Strawberries" to "Some Effects of Gibberellin on the Anatomy of Apple Leaf Discs".

The studies centred around the root observation laboratory are particularly interesting. In this underground laboratory, which has been in use now for about 5 years, observations can be made on undisturbed tree roots growing in soil. Diurnal fluctuations in the growth of cherry roots and nutational movements of apple root tips have been demonstrated using time-lapse cinematography. Nematode infection of roots is being investigated. The construction of a second root observation laboratory (now in use) will make it possible to widen the scope of these fundamental observations on the hitherto much-neglected subject of tree root growth.

One line of research in the pomology section is concerned with attempts to prolong the effects of a single deblossoming of the annual cropping of biennial varieties of apples such as Miller's Seedling. The beneficial effects of laborious deblossoming of half the branches in a bearing year is short lived because the fruitlets produce a hormone which travels to the non-fruiting branches to suppress the development of fruit buds. Ringing of the bases of deblossomed branches has resulted in a more regular supply of good-sized fruit without reduction of crop.

The sterile culture of isolated stem tips is among the research projects of the plant physiology section. If successful, this technique would make growing material available for experimental work during the winter. The possibility that cultured stem tips may become a source of plants free from virus is also being investigated. The importance of growth-promoting and growth-inhibiting substances in the control of shoot growth and flower initiation is being examined. Attempts to extract gibberellins from fruit trees have not yet been successful, but the identity of a growth-inhibitory substance has been established.

In the fruit nutrition section, the responses of trees to irrigation and tolerance to drought are being investigated in relation to form and development of the root system. The influence of soil moisture on the availability of mineral nutrients is also being examined. Intensive experiments have been undertaken to provide information about the rate at which various nutrients are taken up by trees during the year, and to examine the pattern of their distribution within the plant.

Abstracts for Geography

IN the past 6 years, beginning in 1960, geomorphologists have increasingly appreciated the value of *Geomorphological Abstracts*, edited and published as a private venture by Dr. Keith M. Clayton, reader in geography at the London School of Economics. Twenty-seven parts had appeared up to December 1965 and circulation is now world-wide. Encouraged by this success, it has now been decided to cover, probably as six parts a year in each of the four series, the whole field of geography. *Geographical Abstracts A—Geomorphology* will be a continuation of *Geomorphological Abstracts*, but will be numbered with the year and part (1966/1); *Geographical Abstracts B* will cover biogeography, climatology and cartography, the series being edited by Dr. E. M. Yates; *Abstracts C* cover economic geography and are edited by Dr. F. E. Ian Hamilton; *Abstracts D* cover social geography, edited by Dr. Christopher Board. The editors are all members of the Joint School of Geography of King's College and the London School of Economics, and Dr. Clayton continues as publisher under the guise of Geo Abstracts, London School of Economics, W.C.2. Subscription to the whole series has been fixed at £9 or 25 dollars, *A* and *B* being £1 16*s*. each (5.00 dollars), *C* and *D* £2 14*s*. (7.50 dollars) each. Recognizing the great value of this self-sacrificing labour of love, the Royal Society has supported the expansion of the venture, and it certainly deserves to succeed. Some of the abstracts used, especially of works in East European languages, are the English summaries now commonly given; in other cases, authors have provided their own summaries; in all the remainder the abstracts are signed, and one notices how many, especially of the younger university lecturers, have given their collaboration, but a heavy burden has clearly fallen on the editors. All abstracts are in English. Workers in each field will find this an indispensable tool and no serious library covering geography can afford to be without the whole series.

Information for Geologists

AMERICAN geologists are the latest to establish a computer-based information service. The Geological Society of America and the American Geological Institute have agreed to spend 1·5 million dollars of the National Science Foundation's money in the next three years on a project to document geological literature referring to parts of the world other than North America. (The U.S. Geological Survey already runs an abstracting service for its own parish.) The decision to use computers has been forced on the Geological Society of America by the increasing difficulty of keeping its annual *Bibliography and Index of Geology Exclusive of North America* up to date. The current volume, which contains 5,700 abstracts, is three years late. It is hoped that under the new system abstracts will be published monthly at a rate of 20,000 a year.

National Academy of Sciences

THE following were elected new members of the National Academy of Sciences during the 103rd annual meeting of the Academy: PROF. P. BERG, professor of biochemistry, Stanford University School of Medicine; J. BIGELEISEN, senior chemist, Brookhaven National Laboratory; PROF. R. BRESLOW, professor of chemistry, Columbia University; B. B. BRODIE, chief, Laboratory of Chemical Pharmacology and Laboratory of Physiology and Pharmacology of the Autonomic Nervous System, National Heart Institute; T. L. CAIRNS, director of basic sciences, E. I. du Pont de Nemours and Company, Inc.; PROF. E. J. COREY, professor of chemistry, Harvard University; PROF. H. R. CRANE, professor of physics, University of Michigan; PROF. K. DAVIS, professor of sociology, University of California, Berkeley; PROF. H. W. EMMONS, Gordon McKay professor of mechanical engineering, Harvard University; PROF. V. L. FITCH, professor of physics, Princeton University; PROF. R. L. GARWIN, senior staff member, Watson Scientific Computing Laboratory, IBM Corporation, and adjunct professor of physics, Columbia University; PROF. N. H. GILES, Eugene Higgins professor of genetics, Yale University; PROF. E. L. GINZTON, professor of applied physics and electrical engineering, Stanford University; PROF. A. M. GLEASON, professor of mathematics, Harvard University; PROF. C. GROBSTEIN, professor of biology, University of California, San Diego; J. G. HARRAR, president, Rockefeller Foundation; PROF. G. K. HIRST, director, Public Health Research Institute of the City of New York,

adjunct professor of microbiology, New York University College of Medicine; PROF. E. A. KABAT, professor of microbiology, Columbia University College of Physicians and Surgeons; PROF. A. R. KANTROWITZ, director, AVCO-Everett Research Laboratory, and professor, Massachusetts Institute of Technology; PROF. I. KAPLANSKY, professor of mathematics, University of Chicago; PROF. H. G. KHORANA, co-director, Institute for Enzyme Research, and Conrad A. Elvehjem professor in the life sciences, University of Wisconsin; E. F. KNIPLING, director, Entomology Research Division, Agricultural Research Service, U.S. Department of Agriculture; PROF. D. E. KOSHLAND, JUN., professor of biochemistry, University of California, Berkeley; PROF. W. M. KROGMAN, professor of physical anthropology, Graduate School of Medicine and the Evans Institute of Dentistry, University of Pennsylvania; PROF. R. B. LEIGHTON, professor of physics, California Institute of Technology; P. LEVINE, director, Immunohematology Division, Ortho Research Foundation; PROF. V. B. MOUNTCASTLE, professor and director, Department of Physiology, Johns Hopkins School of Medicine; PROF. N. M. NEWMARK, professor of civil engineering and head of department, University of Illinois; PROF. D. E. OSTERBROCK, professor of astronomy, University of Wisconsin; PROF. R. D. OWEN, professor and chairman, Division of Biological Sciences, California Institute of Technology; PROF. F. J. PETTIJOHN, professor of geology, Johns Hopkins University; PROF. G. C. PIMENTEL, professor of chemistry, University of California, Berkeley; E. RACKER, chief, Division of Nutrition and Physiology, Public Health Research Institute of the City of New York; PROF. F. RATLIFF, associate professor (psychology and biophysics), Rockefeller University; PROF. H. A. SCHERAGA, Todd professor and chairman of the Department of Chemistry, Cornell University; PROF. J. STEINBERGER, professor of physics, Columbia University; PROF. H. E. SUESS, professor of geochemistry, University of California, San Diego; PROF. E. W. SUTHERLAND, JUN., professor of physiology, Vanderbilt University; S. M. ULAM, research adviser, director's office, Los Alamos Scientific Laboratory; PROF. O. H. WANGENSTEEN, distinguished service professor of surgery, University Hospitals, and director of the Department of Surgery, University of Minnesota; PROF. S. I. WEISSMAN, professor of chemistry, Washington University; PROF. C. YANOFSKY, professor of biology, Stanford University.

University News:

Adelaide

DR. R. W. R. RUTLAND has been appointed to the chair of geology in succession to Prof. A. R. Alderman.

Canterbury, Christchurch

DR. B. G. WYBOURNE, formerly an associate physicist at Argonne National Laboratory, has been appointed to the newly created second chair of physics, University of Canterbury, Christchurch.

Cardiff

DR. F. JACOBY has been appointed to a personal chair in the Department of Anatomy, University College of South Wales and Monmouthshire.

London

PROF. G. D. DAWSON, professor of experimental neurology in the Institute of Psychiatry, has been appointed to the second chair of physiology at University College.

Sheffield

R. KILPATRICK, at present senior lecturer in pharmacology and therapeutics, has been appointed to the chair of pharmacology.

Southampton

DR. S. H. CROWDY, a research botanist at Imperial Chemical Industries, Ltd., has been appointed to the chair of botany.

Appointments

DR. A. H. KEIL has been appointed professor and head of the Department of Naval Architecture and Marine Engineering at the Massachusetts Institute of Technology, in succession to Prof. C. R. Soderberg, Institute professor (emeritus), who has been serving as acting head of the Department since February 1965, when Dr. H. G. Stever left to become president of the Carnegie Institute of Technology.

Announcements

AN international conference on "Crystal Growth" will be held in Boston during June 20–24. Further information can be obtained from the Conference Secretary, 40 Acorn Park, Cambridge, Massachusetts.

THE 461st meeting of the Biochemical Society will be held in the Heriot–Watt College, Edinburgh, on June 17. Further information can be obtained from the Administrative Officer, the Biochemical Society, 20 Park Crescent, London, W.1.

THE fifth congress of the Fédération Internationale de la Précontrainte will be held in Paris during June 11–18. Further information can be obtained from P. Gooding, Prestressed Concrete Development Group, Terminal House, Grosvenor Gardens, London, S.W.1.

A SYMPOSIUM on "Liquid Helium Technology" will be held by the International Institute of Refrigeration at Boulder, Colorado, during June 16–18. Further information can be obtained from Cryogenic Division, National Bureau of Standards, Boulder, Colorado.

THE fourth congress of the European Federation of Chemical Engineering and the International Chemical and Petroleum Engineering Exhibition will be held simultaneously in London during June 15–24. Further information can be obtained from the Institution of Chemical Engineers, 16 Belgrave Square, London, S.W.1.

THE Australian and New Zealand Association for the Advancement of Science has announced that its thirty-ninth Congress will be held in Melbourne during January 16–20, 1967. Further information and enrolment forms can be obtained from Mr. L. Mann, organizing secretary, 39th ANZAAS Congress, University of Melbourne, Parkville, N.2, Victoria.

A SYMPOSIUM on "Magnetohydrodynamic Electrical Power Generation", arranged by the International Atomic Energy Agency and the European Nuclear Energy Agency, will be held in Salzburg during July 4–8. Further information can be obtained from the Division of Public Information, International Atomic Energy Agency, Karntnerring 11, 1010 Vienna I.

FROM August 1, 1966, Chelsea College of Science and Technology will become a grant-receiving School of the University of London. After the Government, on the recommendation of the Robbins Report, decided that all colleges of advanced technology should be granted university status, it was agreed that in this case it would be best for Chelsea to join the University of London. This has now been approved by the Senate and Court of the University and will be implemented from the beginning of the next session.

THE Psycholinguistics Research Unit of the Medical Research Council, which has been housed in the Institute of Experimental Psychology, University of Oxford, since its establishment in October 1963, will be transferred to the University of Edinburgh from October next. Thereafter the title of the Unit will be changed to Speech and Communication Research Unit. Prof. R. C. Oldfield, who has been directing the Unit in an honorary capacity while holding the chair of psychology at Oxford, is joining the scientific staff of the Council in October and will become the Unit's full-time director.

RESTRICTION OF DEOXYRIBONUCLEIC ACID TEMPLATE ACTIVITY IN CHROMATIN IS ORGAN-SPECIFIC

By PROF. JOHN PAUL and R. STEWART GILMOUR
Department of Biochemistry, University of Glasgow

IT is of crucial importance to the understanding of the molecular basis of cytodifferentiation to determine whether there is a specific restriction of the template activity of deoxyribonucleic acid (DNA) in chromatin. This communication reports experiments which show that there is a specific restriction of this kind.

When chromatin is used as a primer for the synthesis of polynucleotides by bacterial ribonucleic acid (RNA) polymerases, the rate of RNA synthesis is much lower than when DNA is the primer[1,2]. Some specimens of chromatin are less efficient primers than others, and it has been claimed that "repressed" chromatin can be separated from "non-repressed" chromatin; the priming activity of the former is less than that of the latter[3]. Moreover, histones inhibit the synthesis of both RNA by RNA polymerase and DNA by DNA polymerase *in vitro*[1,4,5]. For these reasons it has been suggested that only a restricted range of DNA sequences is available for transcription in chromatin, the majority being masked by basic proteins; this is in analogy with the appearance of RNA-synthesizing "puffs" in the giant chromosomes of diptera[6]. However, this assumption is specious because the rate of transcription may be influenced by many factors other than the specific masking of DNA. For example, chromatin and synthetic nucleohistones are rather insoluble in dilute salt, and Sonnenberg and Zubay[7] have shown that shearing chromatin by sonication increases its solubility and makes it a more efficient primer for RNA synthesis. To test the hypothesis it is necessary to determine (for example by molecular hybridization) whether RNA formed on a chromatin template is transcribed from every kind of DNA sequence in the primer or from a restricted range of DNA sequences.

We have already reported that RNA is transcribed *in vitro* from only 5–10 per cent of the DNA templates present in calf thymus chromatin[8]. Two further questions immediately arise from this observation. (1) Is the RNA formed *in vitro* homologous with RNA formed in the same organ *in vivo*? We shall show in this article that it is. (2) Is there a difference in the range of DNA molecules available as templates in chromatin from different organs? We shall show that there is such a difference. In particular, RNA is transcribed from some common DNA sequences in rabbit bone-marrow and thymus chromatin but, in addition, some unique sequences are available in thymus chromatin which are not available in bone-marrow chromatin; similarly, unique sequences are available in bone-marrow chromatin which are not available in thymus chromatin.

To test the first question the following experiments were performed. Nuclei were first prepared from calf thymus tissue[8]. A fraction of these was used to prepare chromatin; another fraction was used to prepare RNA ("normal" RNA). The chromatin was immediately added to an incubation mixture containing *Micrococcus lysodeikticus* RNA polymerase[9] and tritium-labelled ATP, UTP, GTP and CTP[8]. The polynucleotide material formed after 4–5 h incubation was isolated and purified. To permit the prediction of saturation values, varying amounts of this labelled RNA were then hybridized with constant amounts of calf thymus DNA by the method of Gillespie and Spiegelman[10]. Later, annealing mixtures were made up in which constant amounts of DNA and constant amounts of labelled RNA (at a concentration approaching that required to saturate the DNA) were heated at 65° C for 17 h in the presence of varying amounts of "normal" thymus RNA. The amounts of labelled RNA which remained hybridized to DNA were then determined. Fig. 1 shows that, as the concentration of "normal" RNA is raised, increasing amounts of labelled RNA are prevented from annealing with DNA. By constructing double reciprocal plots it can be shown that, at an infinite concentration of "normal" RNA, all the labelled RNA would be excluded; this provides evidence for the identity of the two kinds of RNA. To exclude artefacts, RNA from *Escherichia coli* was also used to compete out the labelled RNA. It did compete with some of the labelled RNA, but analysis of linear regressions derived from the data showed that only a small part of the RNA was affected.

A similar experimental design was used to test whether RNAs prepared *in vitro* with rabbit thymus and bone-marrow chromatin templates were homologous, first with "normal" RNAs from the homologous tissues, and second with "normal" RNAs from the heterologous tissues. Nuclei were prepared from each organ; chromatin and "normal" RNA were prepared from them. The chromatin was used as a primer to make labelled RNA as before. Fig. 2 shows that when labelled RNA made with a thymus chromatin primer was hybridized with DNA in the presence of increasing amounts of "normal" thymus RNA, marked competition occurred; the line extrapolated to approximately zero, indicating a high degree of homology between the two RNAs and confirming the previous experiment. On the other hand, when the same labelled thymus RNA was hybridized with DNA in the presence of "normal" bone-marrow RNA, the competition curve was less steep. Fig. 3 presents the results obtained when the experimental system was reversed. In this case it could be demonstrated that "normal" bone-marrow RNA and labelled RNA made in the presence

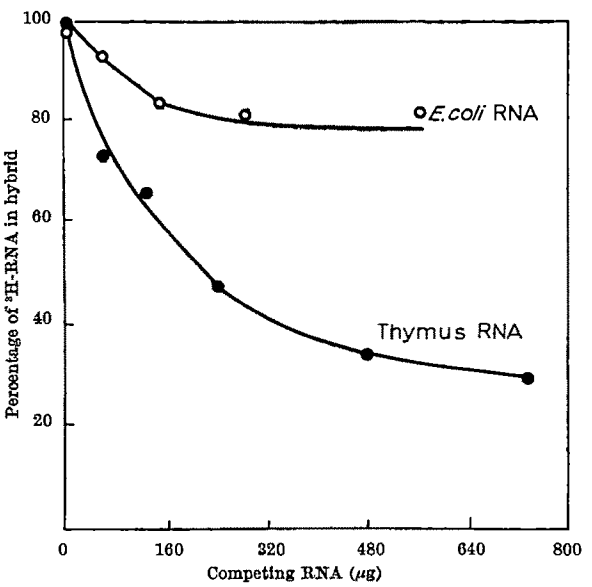


Fig. 1. Test for homology between calf thymus RNA and tritiated RNA made *in vitro* with a primer of calf thymus chromatin. Annealing mixtures contained 1 μg calf thymus DNA, a near-saturating amount of synthetic chromatin-primed ³H-RNA and varying amounts of "normal" thymus RNA. As a control (upper curve) similar annealing mixtures were prepared in which *E. coli* RNA replaced "normal" thymus RNA. The lower curve can be shown to extrapolate to zero at an infinite concentration of "normal" thymus RNA

of a bone-marrow chromatin primer were essentially homologous. On the other hand, "normal" thymus RNA apparently competed less effectively with the labelled RNA for sites on DNA. The conclusion from these experiments is that in rabbit thymus and bone-marrow chromatin some common sequences of DNA are readily available for transcription by *M. lysodeikticus* polymerase; in addition, unique sequences are available in each type of chromatin. In both cases less than 10 per cent of the DNA is available for transcription—figures of 5 per cent for thymus and 7·5 per cent for bone marrow being calculated.

The data in Figs. 2 and 3 were not sufficiently precise to permit accurate calculation of the relative amounts of common and unique sequences of DNA transcribed in the two types of chromatin although theoretically this should be possible.

These experiments would seem to provide evidence that, as a consequence of cytodifferentiation in mammals, specific sections of the genomes are rendered unavailable for immediate transcription. The nature of the "masking" material is such that chromatin can be readily isolated with the specific pattern of masking undisturbed. These experiments do not provide any information about the nature of the mechanism except that, because the removal of proteins and RNA during preparation of DNA from chromatin makes all the DNA available for priming, the "masking" mechanism is probably effected by some of these.

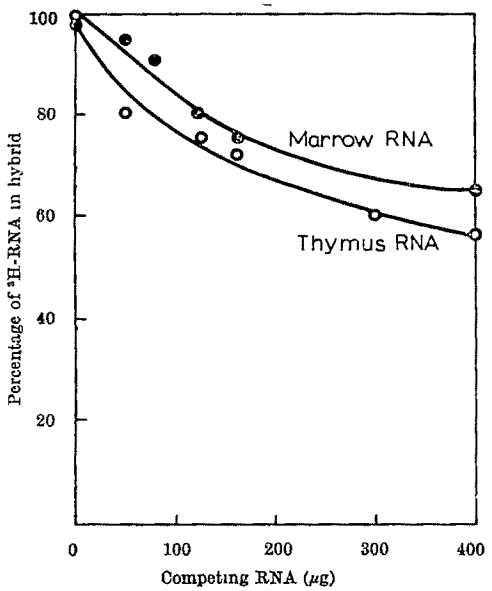


Fig. 2. Test for homology between rabbit thymus or bone-marrow RNA and RNA made *in vitro* with a rabbit thymus chromatin primer Annealing mixtures contained 1·4 μg rabbit DNA and a near-saturating amount of synthetic thymus chromatin-primed ³H-RNA. To each annealing mixture was added varying amounts of competing RNA, either "normal" thymus RNA or "normal" bone-marrow RNA

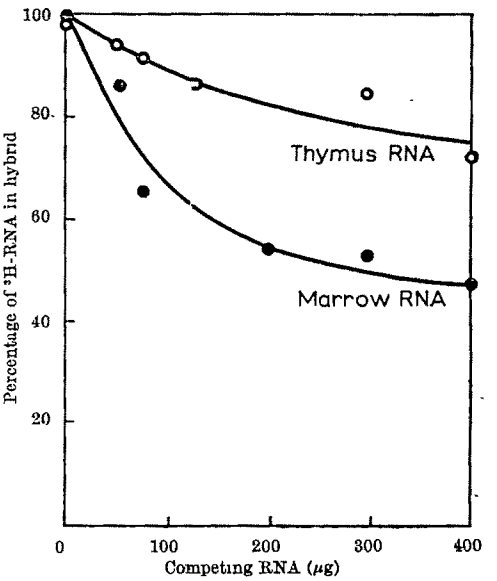


Fig. 3. Test for homology between rabbit bone-marrow or thymus RNA and tritiated RNA made *in vitro* with a rabbit bone-marrow chromatin primer. This is the complementary experiment to that shown in Fig. 2. The conditions were identical except that the synthetic ³H-RNA was made with bone-marrow chromatin instead of thymus chromatin

These experiments, in conjunction with observations on plant tissue[1] and diptera[2], suggest that in differentiated cells only a part of the genetic complement may be available for transcription. It would be convenient to have a word to describe this, and we propose "nome" (from the Greek *nomos*, meaning "the law"). The nome would be defined as that part of the DNA complement of a cell which is directly available for transcription by DNA-dependent RNA polymerase (*E.C.*2.7.7.6.).

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SYNTHESIS OF NUCLEIC ACIDS AND NUCLEAR PROTEIN IN REPLICATING ANIMAL CELLS

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RECENT investigations[1–5] have shown that in replicating animal cell cultures freshly derived from normal tissues, the process of net deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and nuclear protein syntheses is closely associated during interphase. Similar relations between the accumulation of DNA and bound arginine have also been found in nuclei of replicating plant cells[6,7] and were suggested some time ago for DNA and histone[8].

These results were obtained by making physical measurements[9–12] on single cells. Some of my own experiments[1,2] were carried out in conjunction with time-lapse photography, a technique[1,2,13] that made possible the sorting out of the cell population with respect to age within the synthetic cycle, and also demonstrated that some 95 per cent of the cells in the cultures used[1] were in a cycle leading to division, a situation not readily obtained in other living systems. The value of this combination[1–4] of physical methods has been shown by the differences I have demonstrated, in the nuclear RNA and protein synthetic cycles, between replicating cells freshly derived

from normal tissues and those derived from tumours[1-5]: in the latter case, in contrast with freshly derived normal cells, a large part of the accumulation of nuclear protein and RNA is dissociated from the replication of genetic material DNA during interphase. I have suggested[1-4] that this dissociation reflects a fundamental loss of control in tumour strain cells.

Because of the importance of these results it would be of interest to separate and characterize the growth of various nuclear protein fractions by bulk biochemical methods, but it is difficult to attempt this except by using a population of cells synchronized with respect to mitosis. Such procedures are open to the objection that the method of production of synchrony may introduce into the cell cycle some feature not normally present (see, for example, the remarks of Schaechter *et al.*[14], Smith *et al.*[15] and Seed[4]): however, it is possible that certain mechanical methods[31] may be free from these objections.

A system of cells *in vivo* has been adopted by other authors[16-18] who have attempted to use regenerating liver as a synchronized dividing cell population for the investigation of the relations between DNA, RNA and nuclear protein synthesis in replicating cells. The deductions of these authors[16-18] are at variance with results obtained by single-cell methods, and it is the purpose of this article to point out why the conclusions from the regenerating liver system are unreliable so far as replicating cells are concerned.

It is well known that regeneration following partial hepatectomy results in a peak of DNA synthesis at around 20–26 h, with a subsequent wave of cell division at around 26–30 h: this process may again be followed by another less-well-synchronized cycle with mitosis some hours later (see, for example, the recent review by Bucher[19]). It is also established that after two-thirds hepatectomy, only some 20–30 per cent of the remaining cell population in the liver is in DNA synthesis at the peak of regeneration[20,21], while one-third hepatectomy may result in an even smaller response[19]. It seems extremely probable, therefore, that removal of a large part of the liver will also act as a stimulus to RNA and protein synthesis in the differentiated, non-replicating cells forming the majority of the remaining population; it will also probably result in increased demands on these cells[19].

My principal objection to the interpretation[16-18] of the protein and RNA experiments on regenerating liver is that no account has been taken of the effect of these non-replicating cells forming the bulk of the remaining cell population: this effect is very important.

Thus if we study the curves (Figs. 1 and 2) of Evans *et al.*[17], from the time of hepatectomy to the first mitosis, there is a similar timing of increase between the amount per cell of DNA and a histone (II) fraction, but a considerable decrease per cell in other protein fractions over much of the period. For example, in their[17] Fig. 1, following hepatectomy, one of the fractions (protein III) decreases by almost 20 per cent, another (protein II) by more than 20 per cent, another (protein I) by about 50 per cent and another (histone I) by almost 70 per cent, each averaged over the whole cell population: the last two figures demonstrate conclusively that effects from non-replicating cells are being observed because only about 20 per cent of the cell population in their experiment is actually taking part in a cycle of replication at the DNA peak (see the height of the DNA peak in their[17] Fig. 2, which is consistent with the work of Grisham[20] and Looney[21] previously cited on the percentage of synthesizing cells).

Once one has demonstrated that the regenerating liver system is showing major protein fluctuations derived from the non-replicating cells, then it is clear that results from that system, considered as a whole, cannot be taken to represent the behaviour of replicating cells so far as protein synthesis is concerned. Similar objections apply to the work of Umana *et al.*[18], who observe a drop of some 60 per cent in the histone/DNA ratio for the whole cell population in the first 10 h after hepatectomy, before DNA synthesis has begun: again the magnitude of this drop in histone content cannot be caused solely by the replicating cells which comprise only 20–30 per cent of the cell population at the DNA peak[20,21]. However, without taking into account the effect of the non-dividing cells forming the bulk of the population, these authors again go on to interpret their results in terms of the behaviour of replicating cells[18].

Furthermore, returning to Fig. 1 of Evans *et al.*[17], the beginning of the second cycle of synthesis following the first mitosis appears to have a form very different from that of the first cycle, following hepatectomy: the histone (I) fraction that rises most steeply here is the one that fell most rapidly after hepatectomy. Another marked inconsistency between the first and second cycles exists in the specific activity of nuclear RNA (see Fig. 2 of Holbrook *et al.*[16]): a maximum at about the middle of the first cycle is matched by a minimum at the middle of the second cycle.

A second objection is therefore the lack of a steady state in the system, due presumably to the different kinetics of the effect of hepatectomy on the replicating cells and the reaction of the non-replicating cells which form the bulk of the population.

Finally, the difficulty of relating observations of amino-acid uptake[16,18,22] into protein with net protein synthesis in regenerating liver has been emphasized by the experiments of Eagle *et al.*[23-25] with animal cells. These authors[23-25] grew animal cell cultures in medium deficient in one or more amino-acids essential to cell growth. Although under these conditions there was no net increase of bulk protein or total mass in the cultures, it was found that the cells continued to incorporate labelled amino-acids into their proteins at a high level for periods up to 72 h. In addition, it was found that replicating cultures incorporated labelled amino-acids into cell protein at an appreciably faster rate than could be accounted for by the amount of net synthesis or growth, and that they possessed a turnover rate similar to the non-growing cells. Eagle *et al.*[23-25] contrasted these results on animal cells with those obtained for bacteria[26-28] and yeast[29], where turnover in growing cells is much less than in non-growing cells. Conclusions similar to these with animal cell cultures have recently been reported by Bidwell *et al.*[30] for replicating plant cell cultures.

In addition to the observations I have made here, it would seem, therefore, that in order to obtain meaningful results on the accumulation of cellular protein for a new animal or plant cell at the next mitosis, methods measuring net amount of substance must be used[1-5].

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PROPOSED MECHANISM FOR THE TURNOVER OF MYOSIN AND ITS RELATION TO MUSCULAR DYSTROPHY

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MANY cells and cell components have been shown through the use of isotopic atoms to be in a state of 'dynamic equilibrium' in the living animal.

The work of Kruh, Dreyfuss, Schapira and Gey shows that this is true of the muscle protein myosin. Using ^{14}C-labelled glycine, these authors measured a half-life for myosin of 30 days in the rat[1] and of 20 days for the mouse[2]. Myosin turnover in other vertebrate species is indicated by good amino-acid incorporation into myosin in adult rabbit muscle[3].

A half-life of 30 days shows that myosin is normally broken down and normally synthesized within the mature living muscle cell since the lifetime of muscle fibres is almost that of the animal. Most of the myosin of muscle is found in the structure of the *A* region and, more specifically, in the *A* filaments[4]. From this, it is clear that the structure of the *A* region turns over, either as a whole or by replacement of individual molecules. In some experiments with mice and rats there is a period following the incorporation of ^{14}C-glycine in which no isotope appears to be lost from myosin. This period is almost equal to the half-life of myosin, and Dreyfuss, Kruh, Schapira and Gey have suggested that the isotope is incorporated into a structure equivalent to the *A* filaments and is not lost until this structure breaks down[1,2].

This article indicates a possible mechanism leading to the breakdown of the *A* filaments and the turnover of myosin.

Muscle is sensitive to heat in a manner which reflects the stability of its 'molecular architecture'. The temperature at which obvious changes in the non-enzymatic structure of the *A* region have been detected is about 50° C for short periods of heating[5,6].

In vivo experiments on the thermal stability of muscle are complicated because either the relaxing or the stimulating system may be more sensitive to heat than the myofibrillar material and because the internal milieu cannot be defined accurately or changed easily. Glycerinated muscle is more amenable to experimental analysis under a variety of conditions and there is evidence to show that it is functionally intact[7] and has an appearance under the electron microscope comparable to that of non-glycerinated muscle[8]. Whether it is equivalent in other respects remains an open question.

Birefringence, a distinguishing property of the *A* region in vertebrate muscle, was used as a measure of the sensitivity of the *A* filament to heat, since a large decrease in birefringence occurs on heating[5] and the amount of the decrease can readily be measured. The data to be considered are based on the determination of a transition temperature as a measure of the stability of the *A* filaments. This value is defined in these experiments as the temperature of heating which causes a 20 per cent decrease in the birefringence of a glycerinated muscle fibre. (Muscle was glycerinated in 50 per cent glycerol, 0·02 M KCl, 0·002 M EDTA, 0·005 M phosphate at a final *p*H of 7·0 (23° C). The initial treatment was for twenty-four hours at 0° C followed by storage in fresh glycerol solution at about −10° C. Mouse carcasses were glycerinated without dissection but were tied in a standard position. Rabbit psoas muscle was separated into small fibre bundles and tied to sticks with slight stretching.) The usual experimental procedure consists in heating for 2 min in 0·1 M potassium chloride and 0·005 M phosphate at *p*H 7·0. Details of this transformation will not be presented here, but the pertinent properties are that the birefringence decreases to less than half its initial value over a range of about 3° C and this loss of birefringence is correlated with a loss of *A* filament structure seen under the electron microscope.

Heating muscle fibres for prolonged periods decreases the transition temperature (Fig. 1) and the question arises whether appreciable thermal denaturation occurs at body temperature and, if so, whether this could be the factor regulating muscle turnover. Referring to Fig. 1, it seems unlikely, although not impossible, that a half-life of 30 days could be explained on a basis of *in vivo* thermal denaturation at the normal rabbit body temperature of 39·4° C. (ref. 9). A half-life of years seems a more reasonable estimate.

With the method available, it is difficult to assess the physiological validity of this conclusion since (1) glycerinated muscle was used (2) 0·1 M potassium chloride at *p*H 7 is a very crude approximation to the physiological environment, (3) an extrapolation of log $1/t_{0.8}$ against $1/T$ may not be applicable to this type of transition, and (4) the transition temperature defined as a 20 per cent decrease in birefringence cannot be interpreted exactly nor can it be measured with high precision. For these reasons, the data were not elaborated on.

The measured value of the transition temperature in Fig. 1 clearly occurs at lower temperatures with longer heating times and, as an estimate, we can consider that heating for 22 h gives a value 5° C below that obtained by heating for 2 min. This point is pertinent to the measure-

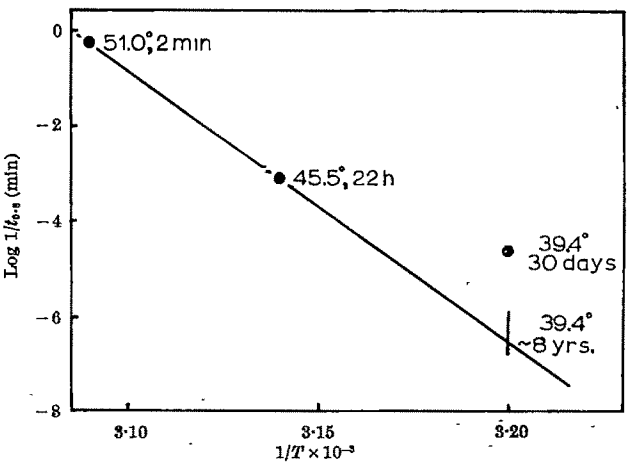


Fig. 1. A plot of the transition temperature ($t_{0.8}$) against $1/T$. T is the absolute temperature and $t_{0.8}$ is the time of heating which caused a drop in retardation to 0·8 of the initial value. Glycerinated rabbit psoas in 0·1 M potassium chloride, *p*H 7·0

ments which follow, since they were all obtained with 2 min heating times.

A good *in vivo* experiment which will establish whether thermal denaturation of the *A* region is of physiological importance in muscle turnover seems difficult to carry out. Biochemical investigations of turnover in poikilothermic animals may be one means of approaching this problem. A consideration of certain pathological conditions, the muscular dystrophies, may also provide pertinent *in vivo* information. The two types of dystrophy considered here are (1) those dystrophies induced by vitamin E deficiency in a wide variety of vertebrates (ref. 10, p. 636) and (2) the genetically determined dystrophy found in 129/*Re* strain mice which are homozygous for the recessive autosomal gene *dy* (ref. 11).

This latter dystrophy was studied because it was thought that muscles from these mice might be more sensitive to heat and provide an *in vivo* correlation between decreased thermal stability and the increased rate of myosin turnover which has been observed[2]. Such genetically controlled changes in the thermal stability of specific proteins have been demonstrated in micro-organisms[12]. Table 1 shows that there is no indication of a significant difference in the transition temperatures of muscle from dystrophic mice and muscle from normal controls and certainly none of a magnitude to affect appreciably the rate of denaturation at body temperature.

Some effects of vitamin E deficiency have been investigated in detail and there is evidence that a major property of vitamin E is its protective action as an intracellular antioxidant[13], either free or built into structure. Deficient animals have higher levels of lipid peroxide[14] in addition to the characteristic muscular dystrophy. Wills[15] has studied the effect of succinyl peroxide (a model lipid peroxide) on enzymes, and has shown that those enzymes which are sensitive to sulphydryl inhibitors are particularly sensitive to low levels of succinyl peroxide and to somewhat higher levels of hydrogen peroxide. Amino-acids containing sulphydryl groups as well as histidine protected these enzymes against inactivation much as they protect against free radicals and peroxides arising from X-irradiation. In parallel with this, nutrition experiments have shown that sulphur-containing amino-acids counteract in part the dystrophy arising from vitamin E deficiency[16].

The increased lipid peroxide levels characteristic of vitamin E deficient animals have not been found in 129/*Re* strain mice with the genetically determined dystrophy[17]. These mice do, however, have a higher level of dialysable sulphur-containing compounds than is found in their non-dystrophic littermates and a much higher percentage of this sulphur is in the oxidized form[18]. This latter observation suggests some similarity between the two dystrophies and prompts the idea that muscular dystrophy may result from the action of metabolic factors which decrease the average lifetime of muscle proteins through oxidation rather than reflecting a property of the myofibrillar structure *per se*. This view is emphasized because of the similarity of interpretation of two types of chemical treatment which decrease the transition temperature of glycerinated muscle fibres.

Salicyl-(γ-hydroxymercuri-β-methoxypropyl) amide-*o*-acetate (mersalyl, Winthrop Laboratories), a monofunctional organic mercurial with binding specificity for sulphydryl groups, reduced the transition temperatures at *p*H 7 by about 10° C for rabbit, frog and mouse skeletal muscle (Table 2). This observation suggests that *in vivo* factors which affect the sulphydryl groups of myosin may decrease the stability of the *A* filaments. The magnitude of this decrease in stability is large enough for thermal denaturation to occur at body temperature.

Table 1

Muscle	*N*	*Dy/Dy*	*N*	*Dy/dy*	*N*	*dy/dy*
Thigh	5	52·3° C	2	53° C	4	52·7° C
Back	3	52·3° C			3	53° C
Lower leg	4	51·1° C			4	51·2° C
Muscle average		51·9° C control				52° C dystrophic

Transition temperatures of glycerinated mouse muscle in 0·1 M potassium chloride at *p*H 7. *dy/dy* denotes the homozygous dystrophic mouse which gives a pathological picture of progressive muscular dystrophy. *Dy/Dy* and *Dy/dy* mice appear normal. The *dy/dy* animals were glycerinated in two groups, one when about 45 days old and the other when about 75 days old. *N* is the number of individuals measured in each category

Treatment of glycerinated rabbit and mouse skeletal muscle with 1 per cent hydrogen peroxide for 1 h gave a decrease in the transition temperature of about 10° C as is shown in Table 3. Likely sites of chemical change are the sulphydryl group of cysteine and the imidazole group of histidine. With longer treatment the transition temperature continued to decrease.

Table 2

Animal	*p*H	Mersalyl pretreatment (−)	Mersalyl pretreatment (+)	Difference (° C)
Rabbit	7·0	50·5° C	42·7° C	7·8
Frog	7·0	43·5° C	32·1° C	11·4
Mouse	7·0	51° C	39° C	12

Transition temperature of glycerinated muscle in 0·1 M potassium chloride at *p*H 7 after pretreatment with 0·5 mM mersalyl in 0·1 M potassium chloride at *p*H 7. The transition temperature was determined after washing the pretreated fibres for at least 1 h to remove excess mersalyl.

Table 3

Animal	Hydrogen peroxide pretreatment (−)	Hydrogen peroxide pretreatment (+)	Difference (° C)
Rabbit	51° C	42° C	9
Mouse	51° C	41° C	10

Transition temperature of glycerinated muscle pretreated with 1 per cent hydrogen peroxide in 0·07 M potassium chloride. The transition temperature was determined in 0·1 M potassium chloride at *p*H 7.

On the basis of these considerations it is proposed that intracellular oxidation affecting imidazole and sulphydryl groups decreases the ability of myosin to form a stable aggregate. This chemically modified myosin is eliminated from the *A* filament at body temperature in a process which may involve the breakdown of the whole *A* filament[1,2]. It is presumed that the myosin is denatured when released from the aggregate and that the muscle cell has some means of recognizing and catabolizing 'denatured' myosin.

There are several possible sources of low levels of peroxides and of free radicals in the normal animal which could, if the *A* filaments accumulate damage, account for the rate of myosin turnover. Parenthetically, it is suggested that anserine, carnosine and other low-molecular-weight histidine-containing compounds found in vertebrate muscle are there in a protective role

The structure of the *A* filament appears to be sensitive to the same types of effects which affect normal function both in binding to actin and in splitting adenosine triphosphate[19,20], so there should be a good correlation between the loss of myosin function and the release of ineffective myosin from the *A* region. The advantage of maintaining a normally functioning muscle fibril through elimination of damaged molecules is clear.

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OXIDIZING AGENTS AS POTENTIATORS OF THE RETINOTOXIC ACTION OF SODIUM FLUORIDE, SODIUM IODATE AND SODIUM IODOACETATE

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ELSEWHERE we have shown[1] that in the rabbit a single intravenous injection of 50 mg sodium fluoride/kg body-weight produces, usually within 24–48 h, a characteristic retinal lesion—generally a sharply defined broad band of oedema one disk distance below the disk, followed in the succeeding days by pigmentation. This effect was, however, seen in only twelve out of sixty-three treated animals. It was also shown that if rabbits were fasted for 24 h prior to the injection the incidence of retinal lesions rose to about 70 per cent (nineteen out of twenty-seven). Other potentiating factors—raising the incidence of lesions to about 50 per cent—were dinitrophenol, triiodothyronine and synthalin *B*. Experimentally induced 'Alloxan' diabetes had no potentiating effect.

In a search for yet other potentiating agents, *N*-ethylmaleimide, an inhibitor of —SH enzymes, was used. A series of such inhibitors had already been found ineffective by themselves as retinotoxic agents[2]. After trials of maleimide in the maximum dose tolerated (5 mg/kg) also proved ineffective, it was used as an adjuvant to fluoride. In a series of preliminary experiments, twenty-one rabbits were given an intravenous injection carrying 50 mg sodium fluoride/kg body-weight and 5 mg *N*-ethylmaleimide/kg body-weight in 4–6 ml. of solution; six animals died within 24 h, and of the remaining fifteen all but one showed a typical fluoride lesion, generally within 48 h.

At this stage the *N*-ethylmaleimide in stock had all been used and with a fresh supply disconcerting results were obtained. Four rabbits treated with 40 mg/kg and six with 50 mg/kg with the maleimide as an adjuvant were all negative. The possibility that the previous maleimide had become oxidized in storage was suggested by Dr. H. W. Reading, and chemical tests of such residue as was available supported this. Dr. W. H. Inman, Dr. Frank Rose and Dr. E. H. P. Young of Imperial Chemical Industries, Ltd., kindly arranged for two oxidized derivatives of maleimide to be prepared for our use—*N*-ethylmaleamic acid and epoxysuccinic acid. Both these agents proved remarkably non-toxic: intravenous injections of doses of up to 125 mg/kg were well tolerated by the rabbit and were not retinotoxic. As adjuvants to sodium fluoride the two agents differed markedly from each other. Fluoride together with doses of 5 mg, 10 mg, and 125 mg/kg epoxysuccinic acid gave no lesion in the three rabbits tried. In contrast, sodium fluoride 50 mg/kg with 10 mg/kg of ethylmaleamic acid gave typical fluoride lesions of the retina in the five survivors of seven animals tried.

These observations were followed up by a more systematic attempt with various oxidizing agents as adjuvants to sodium fluoride. As with —SH enzyme inhibitors, a series of oxidizing agents had been tried earlier as potential retinotoxic substances and except for sodium periodate and iodine pentoxide—both related to sodium iodate, an established retinotoxic agent—all had been found ineffective[3].

As in previous investigations, young adult Dutch rabbits were used, and the procedures were those already recorded. Ten oxidizing agents were tested as adjuvants in rabbits treated with sodium fluoride 50 mg/kg, but only one—potassium permanganate—was used in investigations of other retinotoxic agents.

Table 1 sets out the various oxidizing agents used as adjuvants to sodium fluoride 50 mg/kg intravenously. In all cases the oxidizing agent was injected first, followed immediately by the fluoride. It will be seen that *p*-benzoquinone, sodium sulphate and borax gave no result, while the remaining agents, three sodium salts—persulphate, perchlorate and chromate—and four potassium salts—dichromate, permanganate, bromate and nitrate—gave a high incidence of lesions: fourteen out of sixteen survivors were affected with typical fluoride lesions.

These observations were taken to establish the potentiating effect of oxidizing agents on the retinotoxic action of sodium fluoride, and also suggested that potassium permanganate in a dose of 10 mg/kg was probably as well tolerated as any of the oxidizing agents and only this substance was used in subsequent trials.

Table 2 sets out the results obtained with sodium iodate. The retinotoxic dose of iodate is 25 mg/kg and this gives lesions in all animals so treated. To test the potentiating action of potassium permanganate on iodate, smaller doses of iodate that give only occasional results were used: thus a dose of 17·5 mg/kg gave lesions in 0–20 per cent of animals treated, and 15 mg/kg gave only one affected in a series of nine (ref. 4). It will be seen from Table 2 that all the rabbits treated with sodium iodate 17·5 mg/kg and with potassium permanganate 10 mg/kg showed retinal lesions, and that with different doses of iodate between 15 and 20 mg/kg and different doses of permanganate between 5 and 15 mg/kg, eight out of nine survivors were affected.

Table 3 shows the results obtained with potassium permanganate 10 mg/kg as adjuvant to subliminal doses

Table 1. OXIDIZING AGENTS USED WITH SODIUM FLUORIDE (50 MG/KG BODY-WEIGHT) INJECTED INTRAVENOUSLY IN IMMEDIATE SEQUENCE IN THE RABBIT

Agent	Dose (mg/kg)	No of animals used	Effect on the retina: Typical fluoride lesion	None	No. died
Sodium persulphate	100	4	2	—	2
Sodium persulphate	75	1	1	—	—
Potassium dichromate	10	2	2	—	—
Potassium permanganate	5	2	2	—	—
Potassium permanganate	10	1	1	—	—
Potassium bromate	75	1	1	—	—
Sodium chromate	15	2	2	—	—
Sodium perchlorate	50	4	2	1	1
Potassium nitrate	30	2	1	1	—
p-Benzoquinone	25	2	—	1	1
Borax	1,000	1	—	1	—
Sodium sulphate	750	1	—	1	—

Table 2. EFFECT OF ADJUVANT DOSES OF POTASSIUM PERMANGANATE (5–15 MG/KG) ON SUBLIMINAL DOSES OF SODIUM IODATE (12·5–20 MG/KG)

Dose of sodium iodate (mg/kg)	Dose of potassium permanganate (mg/kg)	No. of animals used	No. of retinal lesions: present	absent	No. died
12·5	5	1	—	1	—
15	15	3	1	—	2
17·5	10	5	5	—	—
18·5	5	1	1	—	—
20	5	2	1	1	—

Table 3. EFFECT OF POTASSIUM PERMANGANATE 10 MG/KG AS ADJUVANT TO SUBLIMINAL DOSES OF RETINOTOXIC AGENTS

Retinotoxic agent	Effective dose (mg/kg)	Incidence of retinal lesions with full doses (per cent)	with smaller doses	Doses (mg/kg) used with potassium permanganate as adjuvant	No. of animals used	Retinal lesion present	absent	No died
Sodium iodate*	25	100	17·5 mg/kg: c. 20 per cent	17·5	5	5	—	—
Sodium iodoacetate	30	c. 75 in survivors	20 mg/kg: 0–20 per cent	20	8	8	—	—
				25	2	—	1	1
				20	2	—	2	—
Diphenylthiocarbazone	30	c. 100 in survivors	20 mg/kg: 0–20 per cent	17 5	6	—	6	—
				15	4	1	1†	2
Di(*p*-aminophenoxy)pentane	5	100	1 mg/kg. 0–20 per cent	1	8	—	8	—
				2	2	—	2	—
				3	3‡	1	2	—
				4	2	2	—	—

* Extracted from Table 2.
† Also negative in one rabbit with 5 mg/kg and another with 15 mg/kg or permanganate as adjuvant.
‡ Of three controls (without permanganate) two were positive and one negative.

of three other retinotoxic agents. It is clear that sodium iodoacetate repeats the positive results seen with iodate: in both cases a dose that would give only some 20 per cent of results was almost entirely effective when permanganate was given as an adjuvant. No such results were seen, however, with diphenylthiocarbazone and di(*p*-aminophenoxy)pentane.

(1) *The potentiating effect of oxidizing agents on sodium fluoride.* Borax, *p*-benzoquinone and sodium sulphate have proved ineffective, but the results are conclusive in showing that inorganic oxidizing agents, with the possible exception of sodium sulphate, powerfully potentiate the retinotoxic action of fluoride. The significance of the two failures recorded with sodium perchlorate and potassium nitrate (in addition to positive results) needs to be explored further. In the light of these findings, the potentiating effect of dinitrophenol and triiodothyronine on fluoride previously reported becomes more explicable, for both substances are fairly powerful oxidizing agents.

(2) *The limitations of the potentiating action.* Sorsby and Harding[4] have shown that fasting for 24 h, which had proved the most effective potentiating factor with fluoride, was equally effective with sodium iodate, for in the fasted rabbit doses of 17·5 mg/kg and 15 mg/kg gave retinal lesions in twenty-seven out of thirty-one animals treated (against three out of twenty unfasted rabbits). Fasting was, however, without effect on the action of sodium iodoacetate, diphenylthiocarbazone and di(*p*-aminophenoxy)pentane. The potentiating effect of fasting was therefore limited to only two of the five established retinotoxic agents. In the present investigation, oxidizing agents have been seen to be effective rather more widely—in three out of the five agents: fluoride, iodate and iodoacetate.

(3) *The mode of action of retinotoxic agents.* Whether the five known retinotoxic agents, each of which rapidly produces a lesion on single intravenous injection when effective, have a common mode of action is still open to question. The differences in ophthalmoscopic appearances and in the histological picture are not such as to warrant the assumption of a different mechanism, but the available evidence from the investigation of potentiating factors does suggest that diphenylthiocarbazone and di(*p*-aminophenoxy)pentane probably act in a different manner from iodate, iodoacetate and fluoride. For the time being no clear answer is possible, for the evidence from investigations of protection against the retinotoxic effect is still more puzzling. It is known that cysteine and other thiol donors are protective against the action of iodate[5,6], but no such protection is seen with any of the other retinotoxic agents. In contrast, there is no evidence that the use of inhibitors of —SH enzyme systems leads to retinal lesions—an assumption made by Noell[7] for the action of iodoacetate, but not supported by work on other thiol inhibitors[2].

We thank Dr. H. W. Reading of the Wernher Research Unit on Ophthalmological Genetics (Medical Research Council), Royal College of Surgeons, and Dr. W. H. Inman, Dr. F. Rose and Dr. E. H. P. Young of Imperial Chemical Industries, Ltd., for their help with the problems presented by the oxidation of *N*-ethylmaleimide, and Imperial Chemical Industries, Ltd., for preparing two oxides of maleimide.

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EFFECT OF ADRENALINE AND ADRENERGIC BLOCKING AGENTS ON THE BASAL OXYGEN CONSUMPTION OF THE PERFUSED RAT HEART

ADRENALINE has several well-known effects on the physical and chemical activities of cardiac muscle. The metabolic effects include increased glycogenolytic and lipolytic activities and increased oxygen consumption (Qo_2). The latter effect, first described by Barcroft and Dixon[1] in 1907, has generally been considered secondary to the increase in the stroke-work of the heart. However, some investigators have suggested that at least part of the increased oxygen consumption reflects a direct effect on cardiac metabolism[2]. Recently, this has been confirmed by studying the oxygen consumption after the addition of adrenaline to arrested perfused rat hearts[3] and to dog hearts under controlled haemodynamic conditions[4]. Challoner and Steinberg[3] suggested that the effect of adrenaline on oxygen consumption of the arrested rat heart (basal Qo_2) was secondary to the lipolytic effect, as the addition of free fatty acids to the perfusate may increase the oxygen uptake in a perfused heart.

We have studied the effects of adrenaline on basal Qo_2 in rat hearts perfused with a modified Krebs Ringer bicarbonate buffer gassed with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide. 10 mmoles glucose and 1 per cent albumin (bovine, fraction V, Sigma) were always added to the buffer. The hearts were perfused in a Morgan perfusator as described previously[5]. By this method the perfusate can be supplied to the heart through the aorta as well as through the left atrium, thus permitting the heart to perform a physiological type of work *in vitro*. The aortic pressure was continuously recorded with a Statham '*P23Gb*' transducer. After transfer to the perfusator the hearts were allowed to work against a diastolic pressure of 75 cm of water for 10–15 min. The minute volumes under these conditions were 25–30 ml. The hearts were arrested by exchanging a portion of the perfusate with a similar medium in which potassium had been substituted for sodium, giving a final concentration

of potassium of 35 m.equiv./l. in the perfusate. When the pumping activity had ceased, the flow direction of the perfusate was reverted to retrograde perfusion through the aorta. Qo_2 was calculated from the arterio-venous pO_2 differences measured with a Clark oxygen microelectrode and a Beckman 'Physiological Gas Analyser', and from the oxygen solubility constant. Flow-rates were measured with a Palmer phototransistor drop counter. Perfusions were carried out at a temperature of 32° C. The total volume of perfusate was 40 ml. in all experiments.

In one series of experiments the perfusate was enriched with palmitic acid (40 μmoles/g albumin), in another series with 5 mmoles each of sodium pyruvate, sodium lactate and β-hydroxy-butyric acid. The effect of adrenaline on basal Qo_2 could be demonstrated regardless of the type of perfusate used and even when no glucose was added. This indicates that at least part of this effect in arrested hearts is mediated through mechanisms other than that of increasing the availability of substrates. We therefore suggest that the effect is not secondary to increased lipolysis or glycolysis, but reflects a more direct metabolic action of adrenaline.

In the experiments presented here we have studied the effects of adrenergic blocking agents on the adrenaline-induced increase in basal Qo_2. Fig. 1 shows the oxygen consumption of one heart in the working and arrested states in a typical experiment. Following potassium-induced arrest the Qo_2 curve fell steeply for about 4 min. A further slight decline in Qo_2 was usually seen during the subsequent control period. The addition of 0·025 μg/ml. of adrenaline to the potassium-arrested hearts did not always affect the Qo_2 as measured by our method, in spite of the fact that this dose was sufficient to affect rate and force of contraction in the working heart. However, 10 μg adrenaline injected into the aortal tubing in the arrested retrogradely perfused heart, giving a final concentration of 0·25 μg/ml. in the perfusate, caused an increase in basal Qo_2 in all experiments. At lower potassium concentrations this amount of adrenaline would induce contractions in the arrested hearts. In the present experiments no physical activity could be detected by pressure recording or by direct inspection.

After the addition of 10 μg of adrenaline, as described earlier, the increase in the basal Qo_2 was about 50 per cent (Table 1) and occurred within 5 min. The adrenergic β-blockers DCI (1 - 3′,4′ - dichlorophenyl) - 2 - isopropyl-aminoethanol hydrochloride, Aldrich Chemical Co. Inc.), 2 μg/ml., and propanolol (1-isopropylamine-3-(1-naphthyl-oxy)-propan-2-ol, 'Inderal', I.C.I. Ltd), 0·2 μg/ml., added

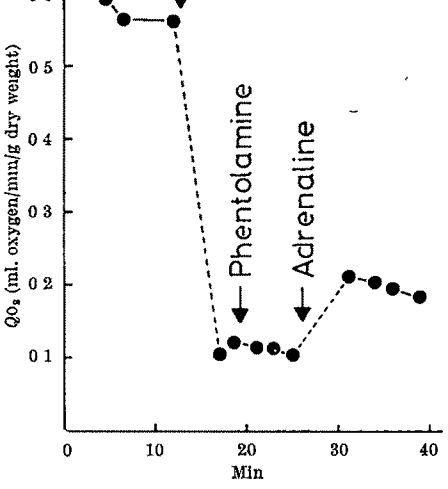


Fig. 1. Qo_2 during one typical experiment from the phentolamine-group

Table 1. EFFECTS OF ADRENALINE ON OXYGEN CONSUMPTION IN ARRESTED PERFUSED RAT HEARTS AFTER THE ADDITION OF α AND β ADRENERGIC BLOCKING AGENTS

Group	Qo_2* before the addition of adrenaline	Change in Qo_2 after the addition of adrenaline (ΔQo_2)
Control (ref. 7)	0·15 ± 0·02†	± 0·08 ± 0·01†
Phentolamine added (ref 4)	0·14 ± 0·02†	+ 0·07 ± 0·02†
DCI added (ref. 3)	0·11 ± 0·02†	− 0·002
Protranol added (ref 3)	0·14 ± 0·03†	− 0·001

* ml oxygen/min/g dry wt.
† Standard error of the mean.

to the perfusate 5 min prior to the adrenaline completely abolished the adrenergic effect on basal Qo_2 (Table 1, Fig. 1). The adrenergic α-blocker phentolamine ('Regitin', Ciba) 2 μg/ml., on the other hand, did not block this effect of adrenaline. Like the glycolytic and lipolytic effects of adrenaline, the effect on basal Qo_2 thus seems to be mediated through the β-type of adrenergic receptors.

Adrenaline causes a rapid increase in the amount of cyclic 3′5′-adenosine monophosphate (AMP) in the rat heart[6–8]. This nucleotide accelerates reactions involving utilization of ATP (ref. 9). The increased rate of such energy-requiring reactions might contribute to the increase in basal Qo_2.

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PRELIMINARY work which I carried out (unpublished) while at the Laboratory of Metabolism of the National Heart Institute does not confirm the lack of stimulation by adrenaline after β-adrenergic blockade as reported by Hauge and Øye. In a similar preparation, control Qo_2 after β-blockade was 0·17 as against 0·20 ml. oxygen/min/g dry weight determined after the addition of adrenaline ($\bar{d} = +0·03 \pm 0·003$ (S.E.), $p = 0·02$, $n = 6$). It must be pointed out that these were preliminary experiments and that a different β-blocker was used, 2 isopropyl-1-[2 naphthyl]ethanol hydrochloride, at different doses of both blocker (0·02 mg/ml.) and adrenaline (0·05 μg/ml.) than those used by Hauge and Øye. An α-blocker was not studied.

The mechanism of production of the 'metabolic' effect of adrenaline is not clear. The authors' conclusion that persistence of the effect in the presence of exogenous substrates would implicate a mechanism unrelated to substrate availability is not of necessity true. This will not be settled until the effect of both exogenous substrate and hormone on the substrate flow through the immediate endogenous oxidative precursor pool can be defined. The working hypothesis developed by Dr. Daniel Steinberg and myself is based on studies demonstrating the adrenaline-increased oxygen consumption in arrested rat heart to be accompanied by glycerol release and increased intracellular free fatty acid (FFA) concentration[1,2]. Separate studies have shown that exogenous FFA are able to increase intracellular FFA concentration[3] and that such exogenous FFA levels can stimulate oxygen uptake

through non-phosphorylation-dependent (oligomycin insensitive) pathways of mitochondrial oxidative electron flow[2-5]. We therefore suggest that the metabolic effect of epinephrine may be secondary to stimulation of endogenous substrate flow from lipolysis through such non-phosphorylating pathways.

Experimental evidence to date does not provide a definitive choice between Hauge and Øye's suggestion of a primary action of adrenaline on ATP utilization with secondary increase in oxygen consumption and our hypothesis of adrenaline-increased lipolysis with increased FFA flow through non-phosphorylating oxidative pathways.

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MODIFICATION OF THE ELECTROKINETIC RESPONSE OF BLOOD PLATELETS TO AGGREGATING AGENTS

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DESPITE widespread interest in the ability of blood platelets to adhere to each other, the mechanism by which they do so remains obscure. It is well known that a variety of substances, including adenosine diphosphate (ADP), adenosine triphosphate (ATP), noradrenaline, thrombin and collagen[1-3] will cause platelet aggregation in a stirred system. It has been suggested[4,5] that ADP provides a final common pathway for all these agents. The surface of platelets can be studied by measurement of their electrophoretic mobility, and we have shown[6] that low concentrations of the aggregating agents cause platelet surface charge to increase, while higher concentrations, of the order which cause aggregation if the mixture is stirred, reduce the surface charge. We have shown some differences between the behaviour of ADP and of noradrenaline in their effect on surface charge, and we considered that a study of the effect of various methods of inhibiting the changes in surface charge might shed light on the nature of these changes.

Blood was obtained by clean venepuncture from healthy volunteers and from patients with a variety of medical and surgical conditions, excluding those with recent myocardial infarction, pulmonary embolus, and inflammatory states, or patients who had recently undergone surgery. Platelet-rich citrated plasma was prepared as described by Emmons and Mitchell[7] and was diluted with nine volumes of platelet-poor plasma prepared by centrifugation at 750*g* for 15 min. The mixture was then brought into contact with unsiliconized glass and the post-contact electrophoretic mobility[8] was measured in the horizontal capillary apparatus designed by Bangham *et al.*[9] using a potential gradient of 2·66 V/cm and a temperature of 25° C. The mobility of the platelets is expressed as μ/sec/V/cm.

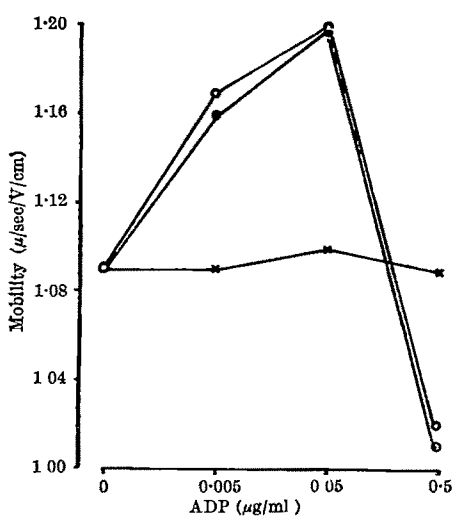


Fig. 1. The effect of 10^{-4} M NEM (×), and 10^{-4} M NEM plus equimolar l-cysteine (●), on the changes in platelet electrophoretic mobility induced by ADP ($n=3$). O, Control

The effect of a variety of agents was investigated: *n*-ethylmaleimide and *p*-hydroxymercuribenzoate (both from Sigma), which block sulphydryl groups; potassium cyanide, an inhibitor of cytochrome oxidase; sodium fluoride, an inhibitor of the synthesis of phosphopyruvate, and sodium monofluoroacetate (MFA), an inhibitor of aconitase and so of the tricarboxylic acid cycle. MFA is thought to exert its effect after conversion to monofluorocitrate (MFC)[10] and purified MFC was also studied. We assessed the effect of these substances on the changes in platelet mobility induced by ADP, ATP, noradrenaline (all from Sigma), thrombin ('Fibrindex', Ortho), and an extract of collagen[3]. At least two concentrations of each of these aggregating agents were used: a low concentration which we have shown to cause a maximal increase in mobility, and a higher concentration which causes a significant decrease in mobility[6].

The inhibitors were dissolved in 0·85 per cent sodium chloride solution and were added to the platelet-rich citrated plasma/platelet-poor plasma (PRCP/PPP) mixture. Parallel samples of the PRCP/PPP mixture to which saline alone had been added were set up as controls. None of the inhibitory agents was found to affect the post-contact electrophoretic mobility, and after the addition of the inhibitors, the mixtures were incubated at 25° C for 5 min. The agents known to change platelet mobility were then added, and 10 min later mobility was measured in the test and control mixtures.

A, Sulphydryl Blockers

(1) *n-Ethylmaleimide.* 10^{-4} M *n*-Ethylmaleimide (NEM) abolished both the increase in platelet mobility induced by 0·05 μg/ml. of ADP and the decrease caused by 0·5 μg/ml. This inhibition could be prevented if a source of thiol groups was supplied, in the form of equimolar l-cysteine (Sigma) as is shown in Fig. 1. Reduction of the concentration of NEM progressively reduced its effect, but its inhibitory action was still detectable at 10^{-7} M (Table 1).

Table 1. MEAN PERCENTAGE INHIBITION BY *n*-ETHYLMALEIMIDE (NEM) OF THE INCREASE IN PLATELET ELECTROPHORETIC MOBILITY CAUSED BY 0·05 μG/ML. OF NORADRENALINE AND ADP

Concentration of NEM (M)	Noradrenaline $n=3$	ADP $n=2$
10^{-3}	100	—
10^{-3}	74	—
10^{-4}	49	100
10^{-5}	37	63
10^{-6}	0	32
10^{-7}	—	6

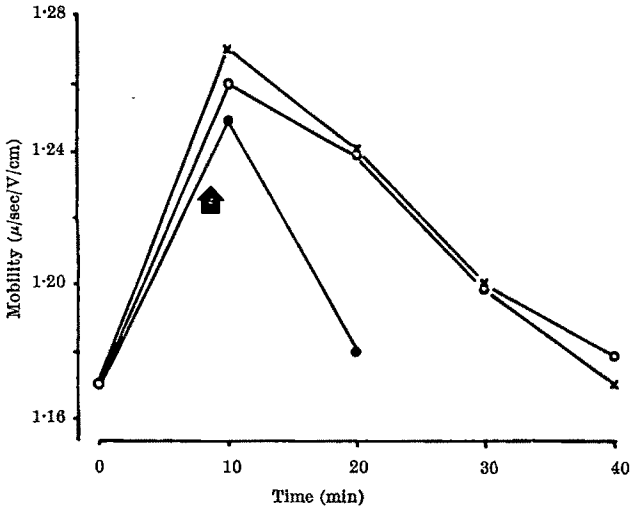


Fig. 2. The effect of 10^{-3} M NEM (●) and 1 mg MFA/ml. (×) on the time course of the platelet electrokinetic response to 0·05 μg ADP/ml. O, Saline

The changes in mobility induced by noradrenaline were also abolished by NEM, but higher concentrations of the latter were necessary (Table 1). Equimolar cysteine opposed the inhibitory action of NEM on the changes induced by noradrenaline in exactly the same way as it had done with ADP.

When NEM was added 9 min after the addition of 0·05 μg/ml. ADP or noradrenaline and the platelet mobility was measured 1 min later, the increase in mobility usually produced by these aggregating agents was found to be unaffected but the subsequent time course of this increase was markedly altered. In the control system, platelet mobility took 30 min to return to its initial level whereas, in the presence of NEM, mobility returned to its starting value in 10 min (Fig. 2). NEM is thus able to reverse the effects of ADP and noradrenaline as well as to prevent them.

10^{-3} M NEM was also found completely to abolish the increase and decrease in mobility induced by 0·5 and 5·0 μg/ml. of ATP, 0·01 and 1·0 u./ml. of thrombin and by dilute and concentrated extracts of collagen.

(2) *p-Hydroxymercuribenzoate.* The effects of *p*-hydroxymercuribenzoate (HMB) were similar to those of NEM. In a final concentration of 10^{-3} M it abolished both the increase and the decrease in mobility induced by ADP, ATP, noradrenaline, thrombin and collagen; this inhibition was prevented by l-cysteine in equimolar concentration. Like NEM, it rapidly reversed the effects of noradrenaline when added to a platelet mixture 9 min after the addition of the noradrenaline.

B, Monofluoroacetate and Monofluorocitrate

Monofluoroacetate (MFA) and monofluorocitrate (MFC) were found to have precisely similar effects; they did not alter the biphasic change in mobility induced by ADP and ATP. With noradrenaline, thrombin and collagen, MFA and MFC did not significantly affect the decrease in mobility induced by the higher concentrations, but they completely abolished the increase in mobility induced by the lower concentrations of these agents. The effect of MFA on noradrenaline is shown in Fig. 3. Abolition of the increase in mobility was found with MFA and MFC at 1·0 mg/ml.; at 0·1 mg/ml. they reduced the mobility change, and at 0·001 mg/ml. they had no effect. Though MFA and MFC could prevent the increase in mobility produced by noradrenaline, they were not able, as the sulphydryl blockers had been, to reverse the mobility change once it had occurred. Fig. 2 shows the effect of adding MFA 1·0 mg/ml. 9 min after noradrenaline had been added to a PRCP/PPP mixture.

C, Cyanide and Fluoride

$1·5 \times 10^{-3}$ M potassium cyanide and $2·5 \times 10^{-4}$ M sodium fluoride had no effect on the biphasic change in mobility induced by ADP, ATP, noradrenaline, thrombin and collagen.

D, Pyruvate Kinase

ADP can be removed from a system by the enzyme pyruvate kinase (PK) by way of the reaction:

Phosphoenolpyruvate (PEP) + ADP = Pyruvate + ATP

where the equilibrium of the reaction favours the production of ATP (ref. 4).

Saline solutions of PK and PEP (Sigma) were added to PRCP/PPP mixtures and the platelet mobility compared with control mixtures to which saline alone had been added. PEP in a final concentration of 0·005 μg/ml., PK in a concentration of 0·1 mg/ml., and a mixture of PK 0·1 mg/ml. and PEP 0·005 μg/ml. had no effect on the post-contact mobility of platelets.

PRCP/PPP mixtures were incubated with PK 0·1 mg/ml. and PEP 0·005 μg/ml. for 5 min at 25° C. ADP and the other aggregating agents were then added and the platelet mobility was measured in the usual way 10 min later. The increase and decrease in mobility induced by the two concentrations of ADP (Fig. 4) and of ATP was abolished by the enzyme system, and this effect was found to occur

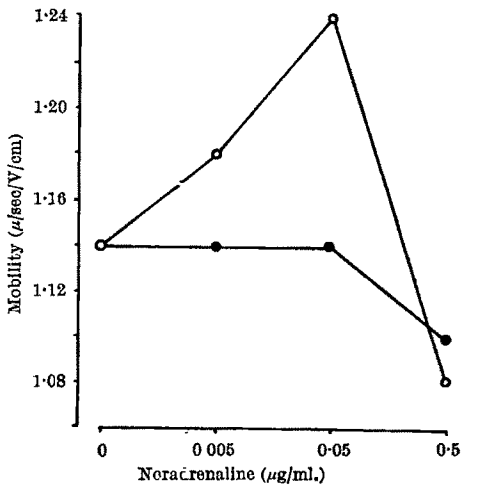


Fig. 3. The effect of 1 mg MFA/ml on the changes in platelet electrophoretic mobility induced by noradrenaline ($n = 4$). O, Without MFA; ●, with MFA

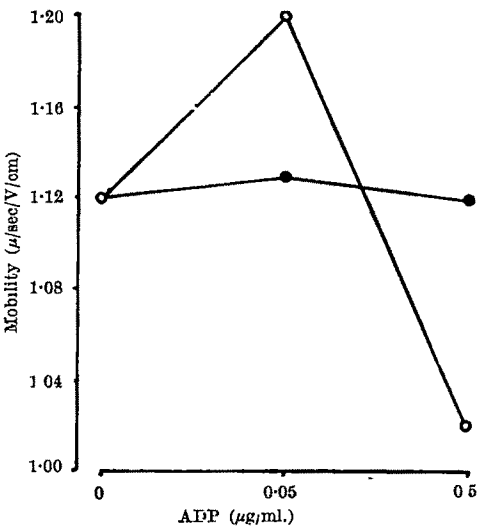


Fig. 4. The effect of PK and PEP (●) on the changes in platelet electrophoretic mobility induced by ADP ($n = 3$). O, Control

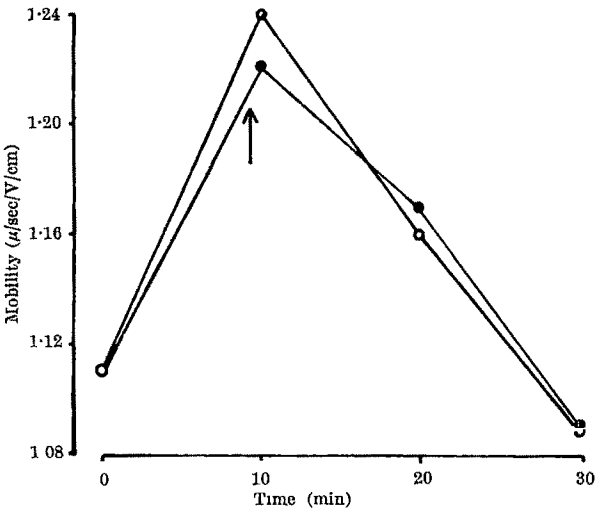


Fig. 5. The effect of PK and PEP (●) on the time course of the platelet electrophoretic response to 0·05 μg ADP/ml. ○, Saline

with concentrations as low as PK 0·001 mg/ml. and PEP 0·0005 μg/ml. When the enzyme system was added 9 min after the addition of ADP or ATP, and the platelet mobility was measured 1 min later, the usual change in mobility was still seen, and the subsequent time course of the change in mobility was unaffected (Fig. 5). Thus the enzyme system, like MFA and MFC, is capable of preventing the mobility changes but not of reversing them once they have occurred, differing in this respect from the sulphydryl blockers.

The enzyme system did not affect the changes in mobility induced by noradrenaline, thrombin and collagen.

E, Dipyridamole

A pyrimido-pyrimidine compound, dipyridamole (*RA8*, 'Persantin', Boehringer Ingelheim), has been shown to inhibit ADP-induced platelet aggregation *in vitro* in a concentration of 10^{-4} M, but to be without effect on noradrenaline-induced aggregation[11]. Its effect on the electrokinetic properties of platelets was studied by adding dipyridamole to citrated whole blood *in vitro* to give a final concentration of 10^{-4} M and preparing a PRCP/PPP mixture from the blood in the usual way. The agent had no effect on the post-contact platelet mobility or on the increase and decrease produced by low and high concentrations of ADP. The increase in mobility produced by noradrenaline was, however, abolished.

We have shown that ADP, ATP, noradrenaline, thrombin and collagen extract cause a biphasic change in the electrophoretic mobility of platelets, low concentrations producing an increase in mobility, while higher concentrations decrease mobility[6]. ADP, like the platelet, is negatively charged, and the increase in mobility caused by low concentrations is probably the result of simple adsorption of this 'exogenous' ADP on to the platelet surface. Since noradrenaline, which is positively charged, produces the same increase in mobility as ADP, we have suggested that this agent, and thrombin and collagen, act by releasing ADP from within the platelet on to its surface ('endogenous' ADP). The decrease in mobility produced by higher concentrations of all the agents is probably the result of adsorption of calcium which occurs when an appropriate charge density and spatial arrangement of ADP molecules has developed.

The effect of the various substances described in this article on the electrophoretic response of platelets to aggregating agents provides some parallels with, and some differences from, the results obtained by the aggregation technique of investigating platelet behaviour. Fantl[12] showed that thiol groups are present on the platelet surface, and *n*-ethylmaleimide and *p*-hydroxymercuribenzoate, which abolish the electrokinetic response of the platelets, have been found[13–15] to prevent aggregation by any of the agents at present recognized, and to cause disruption of pre-formed aggregates. Cyanide and fluoride have been shown to be without effect on either aggregation or electrophoretic response.

Monofluoroacetate has no effect on aggregation as measured by the optical density technique[15]. Its property of inhibiting the increase in electrophoretic mobility caused by noradrenaline, thrombin, and collagen has therefore no parallel when platelet function is assessed by measuring aggregation. Pyruvate kinase also behaves differently in the two systems: it inhibits aggregation by ADP, ATP, noradrenaline, thrombin and collagen[4,16], and yet does not affect the changes in mobility induced by the latter three substances.

We have already shown that there are significant differences between the electrokinetic actions of ADP and noradrenaline[6]; the former induces changes in platelet surface charge in the presence of inorganic ions, glucose and fibrinogen, while noradrenaline, thrombin and collagen require some other cofactor. The experiments described here provide further evidence that aggregating agents are of two distinct types. First, exogenous ADP, the effect of which is abolished by pyruvate kinase, but not by monofluoroacetate. Second, noradrenaline, thrombin and collagen, which are unaffected by pyruvate kinase, but cannot increase platelet mobility in the presence of monofluoroacetate. A further difference between the effect of ADP and noradrenaline on platelet surface charge is that dipyridamole abolishes the increase in mobility caused by noradrenaline, but has no effect on the increase produced by ADP.

It appears that when exogenous ADP is added to a platelet suspension, it is adsorbed on to the platelet surface; for this, both calcium[6] and thiol groups are necessary. Noradrenaline, thrombin and collagen, on the other hand, cause the release on to the platelet surface of endogenous ADP or some similar substance. Monofluoroacetate can prevent this release, but once the endogenous ADP is on the platelet surface it is unable to remove it. Thiol groups are, however, necessary if both exogenous and endogenous ADP are to remain on the platelet surface, the sulphydryl blockers being able to reverse the effect of noradrenaline and of ADP. It would appear that pyruvate kinase acts by removing exogenous ADP from the system before it can become adsorbed on to the platelet surface, but that the enzyme cannot remove adsorbed ADP or endogenous ADP.

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NUMBER AND LOCATION OF ACETYLCHOLINESTERASE MOLECULES AT MOTOR ENDPLATES OF THE MOUSE

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THE mammalian motor endplate is a highly specialized structure, at which the terminal axonal twigs of the motor nerve come to lie in a series of shallow gutters in the surface of the striated muscle fibre. Numerous light and electron microscope studies have elaborated its detailed structure. This endplate is cholinergic, and the enzyme acetylcholinesterase (AChase) occurs there at relatively high concentrations. The occurrence of AChase at these endplates has been demonstrated by several classical histochemical studies with the light microscope and, more recently, by histochemical techniques modified for electron microscopy[1]. Up to the present time, however, no technique has been evolved to measure the amount of AChase in single endplates, or to describe its detailed distribution in quantitative terms.

It is possible to block the action of AChase with a number of organophosphorus compounds, such as diisopropylfluorophosphate (DFP), which form a covalent bond with the serine hydroxyl group at the active centre of the enzyme. This reaction has been proposed by Barnard and Ostrowski as a possible basis for the cytochemical demonstration of the enzyme[2]. Radioactive DFP would introduce labelled diisopropylphosphoryl (DP) groups at the active site of the enzyme, and the localization of the latter should be demonstrable autoradiographically, both with the light and electron microscope. Measurements of the radioactivity present could be used to indicate the number of enzyme molecules per motor endplate. DFP is not specific for AChase, but reacts with several other enzymes. Furthermore, since DFP is lipophilic, it is difficult to remove loosely bound DFP from treated tissues. Any measurement of AChase that uses labelled DFP as a reagent must, therefore, distinguish clearly between these two sources of unwanted radioactivity on the one hand, and the phosphorylation of AChase by DFP on the other.

Measurement of the Total DFP-reactive Sites per Endplate. The measurement of the total number of molecules of DFP bound per endplate was made in the present experiments with the technique of beta-track autoradiography. Levinthal and Thomas[3] have demonstrated the precision of this technique in quantitative studies, and Levi and Rogers[4] have investigated its technical requirements in detail. Small fragments of the sternomastoid muscle and diaphragm of male Swiss mice were dissected out, and the endplates lightly stained by brief (5–10 min) reaction in the Koelle acetylthiocholine method (ref. 5, p. 890). After fixation in cold formalin (*p*H 7·4), DFP, labelled with phosphorus-32 (Radiochemical Centre, Amersham, and New England Nuclear Corp., specific activity 40–80 mc./mmole), was applied at 10^{-4} M for 20 min at room temperature, in 0·02 M phosphate buffer (*p*H 7·4). Extensive washing followed, both in phosphate buffer and in unlabelled DFP (10^{-3} M). Individual lengths of muscle fibre were then obtained under the dissecting microscope, some bearing stained endplates, some without. These were pipetted on to a gelatinized slide, and coated with a 60-μ layer of Ilford '*G5*' nuclear research emulsion. After exposure periods of 15–16 h, development and fixation took place, following the procedures outlined by Levi and Rogers[4].

At the exposure times used, beta-tracks, numbering up to ten on each endplate, could be clearly seen entering the emulsion from the stained endplates (Fig. 1). In each experiment, the track density per unit surface area of the muscle fibres without endplates was determined, and the observed track count from each endplate corrected for this muscle background. Since the specific activity of the ^{32}P-DFP was known, a conversion of the observed track counts to the number of molecules of DFP bound to each endplate was possible after appropriate corrections for geometrical factors and for radioactive decay.

The value for the number of sites of phosphorylation per endplate was reasonably consistent from one experiment to another. It was not altered by incubation in ^{32}P-DFP for 30 min instead of 20 min, or in 10^{-3} M instead of 10^{-4} M ^{32}P-DFP, indicating that the reaction was complete in the conditions used routinely. Nor was this value significantly influenced by the formalin fixation, since material that was not fixed until after the labelling procedure was completed gave the same number of reactive sites per endplate. Prior treatment of the muscle with unlabelled DFP (10^{-4} M) for 20 min completely prevented the uptake of radioactivity at endplates.

With phosphorus-32, it is reasonable to ignore self-absorption within the tissue. Similarly, the failure of very low energy beta particles to give rise to recognizable tracks[4] is quite insignificant with this isotope. Latent image fading was excluded, on the basis of an investigation of the grain spacing in the beta-tracks. A chemographic artefact was consistently found, however, in material exposed for more than 24 h, resulting in the desensitization of the emulsion immediately adjacent to the tissue. Only

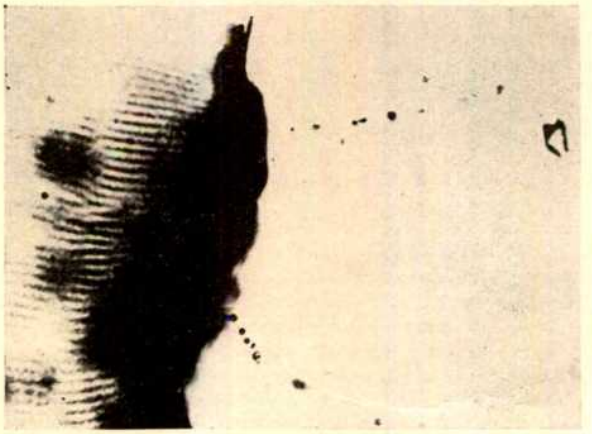


Fig. 1. Beta-track autoradiograph of a sternomastoid endplate treated with ^{32}P-DFP. The tracks of 2 beta particles can be seen entering the emulsion from the stained endplate. (× *c.* 556)

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the data from autoradiographs exposed for less than 24 h were, therefore, used in the calculation of absolute values.

For the sternomastoid muscle, the observed number of phosphorylated sites per endplate decreased with increasing time of incubation in the acetylthiocholine-containing medium used to stain the endplates (Fig. 2). It has not proved possible to identify endplates satisfactorily on unstained material, nor to stain the endplates after reaction with DFP. From the data shown in Fig. 2, it seems reasonable to extrapolate to zero incubation time, and assume that the uptake of labelled DFP in unstained endplates would be $7{\cdot}0–10{\cdot}0 \times 10^7$ molecules/endplate in the sternomastoid, and (from similar data) $2{\cdot}5–3{\cdot}5 \times 10^7$ for the diaphragm.

The latter figure is in close agreement with the estimate, given in a recent note by Waser and Keller[6], of $2{\cdot}4 \times 10^7$ sites per diaphragm endplate, obtained by an independent autoradiographic technique. However, their claim that all these sites are AChase cannot be accepted in the light of the evidence on specificity presented in the following section.

After labelling muscle material with ^{3}H-DFP, sectioning, and preparation of grain density autoradiographs, a low, but significant, level of labelling was found over endplates. It is difficult to calculate the total number of DFP-reactive sites per complete endplate from such material, without accurate knowledge of the grain yield of the autoradiographs and the geometry of the endplates. However, the ratio of labelling between endplate and muscle fibre was compatible with that found in track autoradiographs, and the grain counts over endplates were within the range expected from the grain yield figures available in the literature[7].

Barnard, Ostrowski, *et al.*[8] previously applied ^{3}H-DFP to mouse diaphragm, and found heavily labelled areas which they deduced to be AChase at motor endplates. It will be shown in a subsequent publication that those results failed to distinguish endplates from mast cells; it is a matter of much interest, which will be explored elsewhere, that mast cells have a far higher concentration of DFP-reactive sites. In the present work, with ^{3}H-DFP at higher specific activities, endplates could always be clearly identified as the source of the radioactivity measured.

The Specificity of Phosphorylation with DFP. The proportion of this total number of DFP-reactive sites that is contributed by the active centres of AChase was found by two independent methods. In the first of these, pyridine-2-aldoxime methiodide (2-PAM) was used. This compound is a specific reactivator for the DP-AChase complex, removing the DP group and restoring activity to the enzyme. It has been shown to reactivate organophosphate-

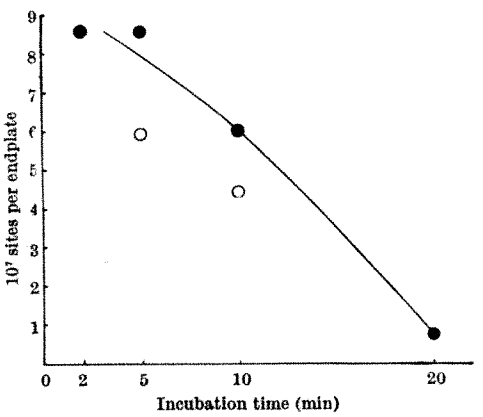


Fig. 2. Effect of the period of incubation in the Koelle acetylthiocholine medium on the number of sites subsequently phosphorylated by ^{32}P-DFP, in sternomastoid endplates. In each individual experiment, the mean value was derived using 12–48 endplates. The closed circles (and the curve) show a series of four experiments all using the muscle of one animal, reacted together. The open circles are the means of two (5 min) or five (10 min) experiments on other specimens

Table 1. CONTRIBUTION OF ACHASE TO THE TOTAL SITES PHOSPHORYLATED BY DFP IN ENDPLATES OF DIAPHRAGM AND STERNOMASTOID

Muscle	Specific agent	Autoradiographic technique	AChase as percentage of total DFP-reactive sites
Diaphragm	2-PAM	Beta-track	37
		Grain density	31
			36
			36
			46
	284C51	Beta-track	31
		Grain density	34
	Eserine	Grain density	34
Sternomastoid	2-PAM	Beta-track	33
			37
	284C51	Beta-track	51

Each value is the mean of a separate experiment involving groups of 12–48 endplates in beta-track autoradiographs and counts from fifty or one hundred sections of endplates in grain density measurements.

inhibited pseudocholinesterase (Chase) also, but at about 0·01 per cent of the rate for AChase[9]. After 20 min treatment with 2-PAM at 10^{-3} M (Table 1), 30–40 per cent of the radioactivity was removed from endplates previously labelled with DFP. There was no further loss of radioactivity after 30 or 60 min exposure to 2-PAM. The same decrease in this range was obtained from endplates of either sternomastoid or diaphragm muscles, whether labelled with ^{32}P-DFP and examined by beta-track autoradiography, or with ^{3}H-DFP and examined by grain density autoradiography. Using a complementary procedure, endplates were treated with unlabelled DFP, followed by 2-PAM, blocking all the reactive sites except those of AChase. In these circumstances, only one third of the total number of reactive sites could then be labelled with ^{3}H-DFP.

'Ageing' of the DP-AChase complex involves the loss of one isopropyl group: it has a half-time of about 12 h at room temperature, and is very much slower at 4° C (ref. 10). The 'aged' complex cannot be reactivated by 2-PAM. In the present material the time between DFP application and 2-PAM reactivation was 35 min at room temperature, and 2–3 h at 4° C. The effect of 'ageing' on these measurements is thus negligible, and they can be interpreted as showing that the active centres of AChase represent about one third of the total DFP-reactive sites.

In an alternative approach, the AChase was protected from radioactive DFP by the presence of tightly bound specific inhibitors, and the reduction in labelling was measured. The inhibitor '284C51' (Burroughs Wellcome) is highly specific for AChase when used at 3×10^{-5} M (ref. 11). This compound, when applied to the muscle fragments before, during, and after labelling with DFP, reduced DFP uptake by (on average) about one third (Table 1). Eserine, at 10^{-5} M, should protect both AChase and Chase. In a similar experiment using eserine, the reduction in labelling was again about one third, suggesting that little, if any, Chase is present at the endplate. Ethopropazine (Warner Chilcott Laboratories) at 3×10^{-5} M protects Chase alone[12]. In two experiments, values of 5 per cent and 15 per cent protection were obtained. Taken in conjunction with the eserine results, it is probably safe to deduce that less than 10 per cent of the DFP is reacting with Chase.

Direct Estimation of AChase Molecules per Endplate. Accepting that 30–40 per cent of the radioactivity at mouse sternomastoid and diaphragm endplates represents phosphorylation of AChase, the number of molecules of this enzyme at one endplate becomes $2{\cdot}1–4{\cdot}0 \times 10^7$ (sternomastoid) and $0{\cdot}8–1{\cdot}4 \times 10^7$ (diaphragm). The sternomastoid value has been checked directly by liquid scintillation counting, making use of exhaustive extraction by a 2-PAM solution.

First, the reduction in labelling with ^{3}H-DFP following staining by the Koelle technique was measured: after 10 min staining, the radioactivity eluted by 2-PAM (10^{-3} M) solution from small fragments of muscle rich in endplates was 55 per cent of that from similar but unstained material. This is in good agreement with the value obtained by extrapolation to zero staining time in Fig. 2.

Small unstained fragments of sternomastoid were labelled with ^{3}H-DFP and, after very extensive washing, the radioactivity that was eluted with 2-PAM was measured. The fragments were then treated by Koelle's technique to stain the endplates, which were counted. Similar treatment of fragments without endplates provided an estimate of the muscle background. In a further experimental design, stained, labelled endplates were obtained by microdissection, and the radioactivity eluted from these by 2-PAM was measured: the results were corrected for muscle background, and for the reduction in labelling known to result from the staining procedure.

Six experiments by the above techniques have given estimates ranging from $1{\cdot}2$ to $4{\cdot}4 \times 10^7$ molecules AChase per endplate, with a mean value of $2{\cdot}4 \times 10^7$, confirming the values obtained by beta-track autoradiography (Table 2).

Table 2. Number of Molecules of AChase per Endplate of Mouse Sternomastoid and Diaphragm, determined by Three Independent Techniques following Treatment with Radioactive DFP

Muscle	Technique	No. of molecules of AChase per endplate
Sternomastoid	Beta-track	$2{\cdot}1$–$4{\cdot}0 \times 10^7$
	Liquid scintillation counting	$1{\cdot}2$–$4{\cdot}4 \times 10^7$
	Electron microscope autoradiography	1–2×10^7
Diaphragm	Beta-track	$0{\cdot}8$–$1{\cdot}4 \times 10^7$

The figures are the ranges of all determinations.

The Detailed Distribution of AChase at Sternomastoid Endplates. For an evaluation of the distribution of AChase within the endplate with higher resolution than that available with the light microscope, the technique of electron microscope autoradiography was used following the methods described by Salpeter and Bachmann[13]. Fragments of sternomastoid rich in endplates were fixed in 1 per cent buffered glutaraldehyde (*p*H 7·4) at 4° C. AChase only was labelled in this material, using the sequence: unlabelled DFP, 2-PAM reactivation, labelling with ^{3}H-DFP, and the usual washing. Post-fixation in osmium tetroxide and embedding in 'Epon' followed.

The necessary conditions for the quantitative evaluation of electron microscope autoradiography, such as the control of the uniformity and thickness of the emulsion, have been described by Bachmann and Salpeter[14]. The level of radioactivity in the present material was so low that relatively thick sections (1000 Å) were used. For ease of scanning and grain counting, monolayers of Ilford '*L*4' emulsion (giving a purple interference colour) were developed with 'Microdol *X*'. The resolution thus obtained was about 1800 Å.

Control experiments with light microscope autoradiography indicated that glutaraldehyde fixation decreased the uptake of labelled DFP by about 10 per cent only.

Developed grains were counted from large areas of muscle and expressed as grains per unit volume of underlying tissue. This procedure showed a significant localization of radioactivity at the endplates, where the grain density was 25 times higher than over the muscle. The endplates were subdivided into three major compartments (Fig. 3): (i), the terminal axon, which forms 25–35 per cent of the total volume; (ii), the post-junctional zone, which is 40–50 per cent of the total volume (this includes the sarcolemmal folds and the primary cleft that separates them from the terminal axon); (iii), the teloglial cap, or overlying connective tissue with tongues of Schwann cell cytoplasm, which is 20–30 per cent of the total volume. Of more than 60 developed grains seen over endplates, 85 per cent lay over the junctional folds (Figs. 3 and 4), giving a grain density approximately 45 times that of the muscle background. 10 per cent of the grains occurred over the teloglial cap, a density ten times the muscle background. Only 5 per cent lay over the terminal axon.

In the conditions of autoradiography used, each developed grain represents approximately ten disintegrations within the section[15]. On this basis, the junctional fold region contains about 19,000 molecules AChase per μ^3: the connective tissue cap has about 4,000 per μ^3. Averaged over the whole endplate, the concentration of AChase is about 10,000 molecules per μ^3.

We have attempted to relate these figures to those obtained by beta-track autoradiography and liquid scintillation counting, by converting them to the number of molecules of AChase per complete endplate. To do this, we have estimated the mean volume of a sternomastoid endplate from combined light and electron microscope measurements. The estimates obtained in this way have a considerable element of uncertainty: the values range from 1,000 to 2,000 μ^3, depending on the precise assumptions made regarding the proportion of the stained endplate formed by synaptic gutters. On this basis, we get figures of 1–2×10^7 molecules AChase per sternomastoid endplate. In spite of the uncertainties of this conversion, this figure is in good agreement with those given by the other techniques (Table 2).

Autoradiographic cytochemistry, with labelled enzyme inhibitors as reagents, can clearly give precise information on the number and location of enzyme sites, in suitable circumstances. The quantitative agreement between beta-track counts, electron microscope autoradiography, and liquid scintillation counting is most encouraging.

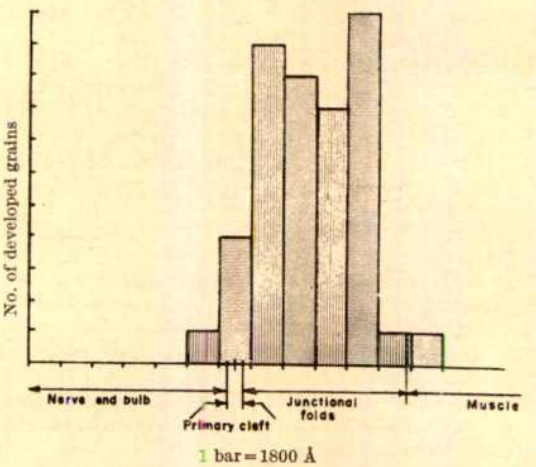


Fig. 3. Histogram based on the distribution of developed grains seen over fifty sections through sternomastoid endplates, labelled with ^{3}H-DFP and studied by electron microscope autoradiography

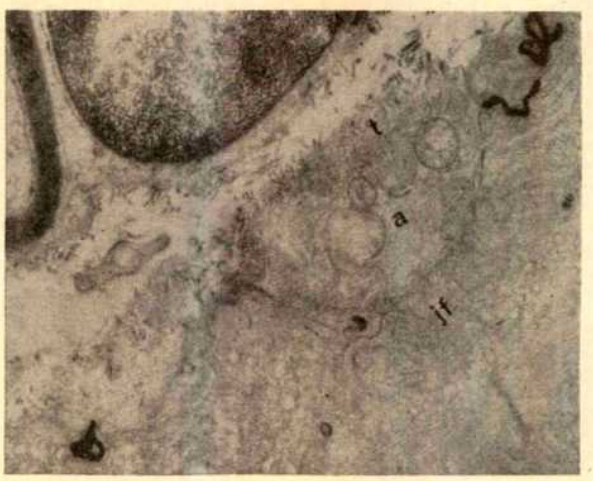


Fig. 4. Electron microscope autoradiograph of a motor endplate in the sternomastoid muscle of the mouse, after labelling the AChase (only) with ^{3}H-DFP (using a 2-PAM routine). Three developed grains lie over the post-synaptic junctional folds. *a*, Terminal axon; *jf*, junctional folds (× 12,000)

The results indicate that there are 7·0–10·0 × 10^7 and 2·5–3·5 × 10^7 sites that react with DFP in endplates of mouse sternomastoid and diaphragm respectively. About 35 per cent of these are active centres of AChase, and probably less than 10 per cent are Chase. Of the AChase molecules, approximately 85 per cent lie in the junctional fold region of the endplate, approximately 10 per cent in the overlying connective tissue and perhaps about 5 per cent in the terminal axon.

The nature of the remaining reactive sites, which form 50–60 per cent of the total, is under investigation. The consistent labelling of these sites, persisting through exchange with unlabelled DFP at high concentrations, and blocked by prior treatment with unlabelled DFP, suggests that these sites are enzymes phosphorylated by DFP. The presence of esterases other than AChase and Chase has been demonstrated histochemically at the endplate[5,15] (and confirmed similarly by us on the muscles investigated here), but the methods used do not permit any deductions to be made as to their relative amount. It may be possible to determine the nature and distribution of these sites, using extensions of the methods described here.

We thank Miss A. O'Connor, of Cornell University, for assistance with the electron microscope autoradiography, and Dr. K. Ostrowski of the Medical Academy, Warsaw, Poland for interest and advice. This work was supported by grants from the National Institutes of Health, U.S. Public Health Service.

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REGIONAL DIFFERENCES IN THE RATE OF TURNOVER OF NOREPINEPHRINE IN THE RAT BRAIN

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NOREPINEPHRINE is present in different concentrations in specific areas of the mammalian brain[1,2] and recent histochemical findings have clearly demonstrated its precise localization in a complex system of neurones[3,4]. Norepinephrine, which acts as a neurotransmitter substance in the peripheral sympathetic nervous system[5], may also have a similar function in the central nervous system.

Although there have been many reports of changes in the level of norepinephrine in the brain in response to centrally acting drugs[1,6], little is known about the dynamic aspects of the metabolism of this catecholamine in the central nervous system. Measurements of the rate of turnover of brain norepinephrine have been hampered by the existence of a blood–brain barrier to catecholamines[7], which prevents the labelling of central stores by the systemic administration of radioactive norepinephrine. An alternative approach has been to use radioactive precursors of norepinephrine, such as tyrosine or 3-4-dihydroxyphenylalanine (DOPA), which more readily enter the brain[8,9].

After the introduction of labelled norepinephrine or its precursors into the brain, the rate of turnover of the endogenous amine can be measured by following the rate of decline of its specific activity.

The injection of very small amounts of ^{3}H-norepinephrine into the lateral ventricle of the rat brain by a stereotaxic technique[10,11] offers a convenient method for the labelling of brain norepinephrine stores.

Radioactive norepinephrine appears to be selectively taken up and retained in catecholamine-containing neurones, where it mixes with the endogenous stores of norepinephrine[12]. ^{3}H-norepinephrine disappears from the brain in a multiphasic fashion; the initial phase during the first 12 h after injection accounts for the disappearance of approximately 93 per cent of the accumulated amine[12]. The rate of disappearance of ^{3}H-norepinephrine from the whole brain during this major initial phase indicates a half-life of approximately 4 h, which is in good agreement with previous estimates of the rate of norepinephrine turnover in the brain[8,9]. ^{3}H-norepinephrine, however, distributes in more than one pool of endogenous norepinephrine and this has led to difficulties in the use of this technique for studies of norepinephrine turnover in peripheral tissues[13]. In order to test the validity of using the rate of disappearance of ^{3}H-norepinephrine as a measure of turnover in the brain, this method has been compared with two alternative experimental approaches.

α-Methyl-*p*-tyrosine has recently been shown to act as an effective inhibitor of norepinephrine synthesis *in vivo*[14]; this drug is thought to act by inhibiting the rate-limiting step in the biosynthetic pathway, the conversion of tyrosine to DOPA (ref. 15). The rate of disappearance of the endogenous norepinephrine stores from the brain after inhibition of synthesis with this drug can be used as an alternative measure of the rate of utilization of the catecholamine. Another approach has been to measure the rate of disappearance of ^{3}H-norepinephrine endogenously formed in the brain from ^{3}H-dopamine. In addition we have investigated the possibility that differences in the rate of norepinephrine turnover may exist between various anatomical regions of the rat brain.

Rat brains were rapidly dissected at 4° C into several regions: cerebellum, hypothalamus, medulla oblongata, striatum, mid brain, hippocampus and cerebral cortex, using a procedure which will be described in detail elsewhere[16]. The rate of turnover of norepinephrine in the various regions was measured by the following methods.

In method 1 a large dose of the synthesis inhibitor L-α-methyl-*p*-tyrosine (200 mg/kg) was administered intra-

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† Fellowship: Foundations' Fund for Research in Psychiatry.

peritoneally and the endogenous norepinephrine content of the various brain regions was measured at different times after this treatment. Norepinephrine was isolated from brain extracts by alumina adsorption[17] and was assayed fluorometrically[18].

In method 2 a small amount (1·5 μg) of ^{3}H-dopamine (1·5 c./mole, generally labelled, New England Nuclear Corp., Boston) was injected into the lateral ventricle of the rat brain, and the disappearance of the radioactive norepinephrine formed from the labelled precursor was measured. ^{3}H-norepinephrine was extracted from brain tissues and separated from labelled dopamine and other radioactive metabolites by a combination of alumina adsorption and ion-exchange chromatography[19].

For method 3 the disappearance of ^{3}H-norepinephrine from various brain regions was measured after the intraventricular injection of a small amount (0·15 μg) of DL-7-^{3}H-norepinephrine (5·0 c./mmole, New England Nuclear Corp.). ^{3}H-norepinephrine was assayed after isolation on alumina[20].

Table 1. RATE OF NOREPINEPHRINE (NE) TURNOVER IN RAT HYPOTHALAMUS
Results obtained by three experimental methods

Time (h)	Method 1 NE content (mμg/g)	Method 2 ^{3}H-NE content (mμg/g)	Method 3 ^{3}H-NE content (mμg/g)
0	1,790 ± 102 (8)	—	—
1	1,500 ± 232 (5)	—	—
2	1,020 ± 47 (3)	34·7 ± 2·6	42·9 ± 2·5
3	1,000 ± 93 (5)	—	—
4	710 ± 50 (3)	—	31·5 ± 2·7
6	520 ± 30 (8)	20·6 ± 0·8	23·5 ± 1·7
8	—	—	14·0 ± 1·1

Sample of experimental data obtained by three different methods for measuring rate of norepinephrine (NE) turnover in rat hypothalamus. In method 1 animals received a single intraperitoneal injection of L-α-methyl-p-tyrosine (200 mg/kg) and the norepinephrine content of hypothalamus was assayed at various times thereafter. Results are mean values ± standard errors of the mean; number of animals in each group is indicated in brackets. In method 2 the content of ^{3}H-norepinephrine was measured at various times after the intraventricular injection of ^{3}H-dopamine (1·5 μg). Values are means for five determinations; each sample was obtained by pooling tissue from two brains. For method 3 the ^{3}H-norepinephrine content was assayed at various times after the intraventricular injection of ^{3}H-NE (0·15 μg). Values are the means for four determinations ± standard errors of the mean.

An example of results obtained by the three methods is given in Table 1 for the hypothalamus. The data obtained by method 3 for this region, which has the highest norepinephrine concentration in the brain, are compared with results obtained by this method for the cerebellum, a region containing only small amounts of endogenous norepinephrine (Fig. 1). Results obtained by the three different methods for the hypothalamus and cerebellum are illustrated and compared in Fig. 2. The rates of disappearance of endogenous norepinephrine (method 1) and of ^{3}H-norepinephrine (methods 2 and 3) are expressed by slopes (Figs. 1 and 2) calculated by the method of least squares analysis. For each region the slopes obtained were compared by an analysis of variance[21]. It was concluded that for any given region of the brain the slopes or disappearance rates obtained by the three methods did not differ significantly.

Table 2. NOREPINEPHRINE (NE) TURNOVER RATES IN HYPOTHALAMUS AND CEREBELLUM OF RAT ESTIMATED BY THREE EXPERIMENTAL METHODS

	Method 1	Method 2	Method 3
Cerebellum	2·25	2·33	2·03
Hypothalamus	3·31	5·28	3·82

Summary of estimates of half-life of norepinephrine (NE) in rat cerebellum and hypothalamus by the three methods described in the text. Half-life was determined from the calculated slopes (Figs. 1 and 2) by the expression: half-life $= \frac{\log_{10}2}{\text{slope}}$.

Differences in slopes between regions were, however, apparent (Figs. 1 and 2). For example, the estimated half-life for norepinephrine in the cerebellum and hypothalamus by all three methods indicated a more rapid turnover in the cerebellum (Table 2). Estimates for the half-life of norepinephrine in other brain areas were intermediate between those for the hypothalamus and

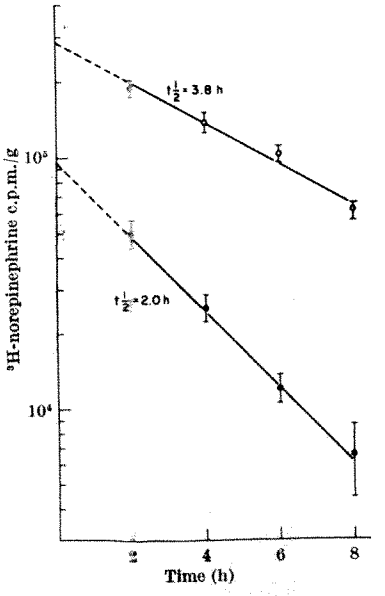


Fig. 1. ^{3}H-norepinephrine content of rat cerebellum (●) and hypothalamus (○) at various times after the intraventricular injection of 0·15 μg D,L-^{3}H-norepinephrine. Each point is the mean of four determinations ± standard error of the mean; slopes were calculated by least squares analysis

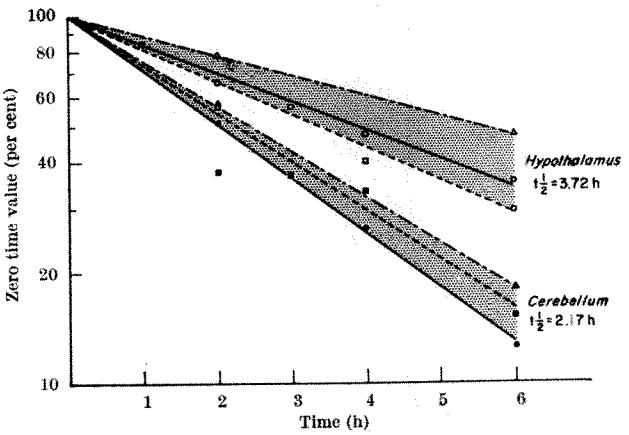


Fig. 2. A comparison of the results obtained for rat hypothalamus and cerebellum by the three experimental methods described in the text. Zero time values for each method for the two regions were adjusted to 100 per cent for purposes of illustration. Each point is the mean value for three to eight determinations (Table 1). Weighted mean values for half-life of norepinephrine in each region are shown in the figure. ——, Method 1; — · —, method 2; - - -, method 3

cerebellum. The estimated half-life for norepinephrine in the cerebellum by method 3 was significantly lower than that obtained for any other brain region. The rate of norepinephrine formation in any region can be estimated from the turnover rate and the size of the endogenous norepinephrine pool. Since the hypothalamus has a high endogenous norepinephrine content (1·79 μg/g) and the cerebellum a low content (0·17 μg/g), the rate of norepinephrine formation per gram of tissue is actually higher in the hypothalamus than in the cerebellum, although the rate of turnover is faster in the latter region. The estimated rates of norepinephrine formation in hypothalamus and cerebellum are 234 mμg/g/h and 42 mμg/g/h, respectively.

The close agreement among the estimates of norepinephrine turnover by the three different experimental procedures used in the present investigations supports the conclusion that the disposition of ^{3}H-norepinephrine after intraventricular injection is representative of the metabolism of the endogenous stores of norepinephrine in the brain. This simple and reliable procedure can therefore

be used to obtain valid estimates of the rate of norepinephrine turnover in the brain under various experimental conditions. With this technique it is possible to introduce considerable amounts of radioactivity into the brain without appreciably altering the size of the endogenous catecholamine pools (Table 1).

The present results demonstrate for the first time significant differences in norepinephrine turnover rates in specific areas of the brain. The cerebellum, containing only low concentrations of norepinephrine, has a rate of norepinephrine turnover almost twice that of the whole brain or hypothalamus. These results emphasize the importance of considering the dynamic aspects of catecholamine metabolism in the brain. Measurements of the changes in the levels of norepinephrine in the brain under various experimental conditions can only reflect gross changes in the complex disposition of this amine in the central nervous system and cannot yield precise information on the rates of formation and utilization of the catecholamine. Norepinephrine-containing neurones in various regions of the rat brain have different anatomical characteristics[4]. Our results also show variations in the rate of metabolism of norepinephrine in these neurones in different areas of the brain, which may reflect regional differences in their physiological activity.

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INHIBITION OF THE AGGREGATION OF DISSOCIATED EMBRYO-CHICK FIBROBLAST CELLS BY ADENOSINE TRIPHOSPHATE

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IT has been shown that there exists in many types of cells a nucleotide triphosphatase (ATPase) which is capable of hydrolysing exogenous adenosine triphosphate (ATP)[1]. According to evidence provided by electron micrographs it also appears as if the plasma membrane is a site of ATPase activity[2].

The possibility of a contractile protein with ATPase activity existing at the cell surface was first raised in 1952 (ref. 3). The contractile protein was envisaged as being intimately linked with osmotic work. In view of the fact that contractile proteins are involved in the movement of cells, the suggestion that these proteins may be coupled with processes entailing the movement of cell surface elements was not unreasonable. However, only in 1951 was it first suggested that the mechanisms responsible for the motility of cells may resemble the contractile system of striated muscle[4]. In 1954 it was demonstrated that glycerol-extracted fibroblast cells exhibited active contraction in the presence of exogenous ATP[5]. In 1956 an actomyosin-like protein with ATPase activity was isolated from tumour cells[6], and recently a protein with the typical properties of an actomyosin-type was extracted from human platelets[7]. It has also been shown that the ATP disappearing from the platelets during viscous metamorphosis is mainly converted into ADP, which, after being secreted, induces platelet clumping[8]. Apparently the ATP which is being split is at the surface, which suggests that the actomyosin-like molecule with ATPase activity is likely to be located at the platelet surface. This possibility is supported by the report that an actomyosin-like protein has been obtained from the outer membrane of liver cells[9].

It seemed therefore suggestive to assume that a contractile protein with ATPase activity existing at the cell surface might be capable of utilizing exogenous ATP in the same way as an actomyosin-like system. Moreover, since actomyosin-like protein threads extracted from non-muscle cells are capable of contracting to 20 per cent of their original dimensions by the addition of ATP[5], there remained the possibility that contraction of an actomyosin-like protein situated at or near to the cell surface might affect its adhesive properties. On this assumption experiments were devised to test the effects of nucleotide phosphates on the adhesiveness of chick embryo fibroblast cells.

The cells were obtained from the muscle tissue of 10-day-old chick embryos. The tissues were washed in warm Hanks's solution and then treated with a 0·2 per cent trypsin (Difco 1 : 250) solution for 3–4 min at 37° C. The cells were afterwards washed and resuspended in Hanks's solution, *p*H 7·2.

The technique of reaggregation was first applied to embryonic cells in 1944 (ref. 10). When cells of a young chick or mouse embryo are chemically separated and subsequently rotated in suspension to make them collide, they will readily aggregate. In the present investigation reaggregation was quantitatively estimated by a turbidimetric method, which has previously been applied to platelets[11] and tissue cells[12]. The cell preparations were placed in siliconized 2-ml. test tubes. These were inserted in absorptiometers (*EEL* 'Quantitrator' with 'Unigalvo 20' attached), the suspensions being kept at 37° C, and stirred by a magnetically rotated rod covered with siliconized glass. An increase in aggregation within the suspension brings about a decrease in optical density. The changes in optical density were measured at 600 mμ. The density of single cells in the test preparations was adjusted to about 8 million cells/ml. by using a haemocytometer.

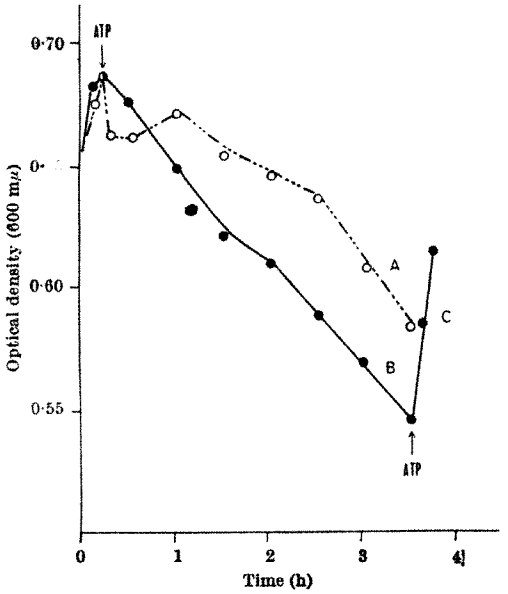


Fig. 1. Effect of exogenous ATP on the optical density of a suspension of chick-embryo fibroblast cells at 16×10^{-6} cells/ml. rotated in Hanks's solution at 37° C. The curves are related to the introduction of ATP into the medium. (*A*) 1×10^{-3} M ATP introduced after 15 min; (*B*) nil (control); (*C*) precipitous rise in control curve after the introduction of ATP at a final concentration of 1×10^{-3} M

In the control preparations the process of reaggregation was completed at the end of about 4 h. That the process is a cumulative one with the aggregates gradually increasing in size as single cells are added to them is reflected in the downward inflexion of the optical density curve of the untreated preparation in Fig. 1. It is not easy to explain the slight but significant initial increase in optical density in the first 30 min. But it may well be due to the cells recovering from the trypsin treatment and washings, and relaxing centripetally, thus reflecting more light. The fall in optical density which followed this initial rise ended when the process of aggregation came to a halt. This occurred after about 4 h under the conditions of the experiments.

When ATP was introduced into the suspension of cells in Hanks's solution by adding 0·02 ml. of ATP in Hanks's solution at 1×10^{-1} M to give a final concentration of 1×10^{-3} M, the process of reaggregation was inhibited. Fig. 1 shows that immediately after the ATP was introduced there was a slight fall and compensatory rise in optical density. The decrease in optical density which followed was significantly less than it was in the case of the control preparations. This is shown by the difference in steepness of the slopes of the optical density curves in Fig. 1. Examining and comparing samples taken from untreated and treated preparations also showed conclusively that at any given time in the first 2 h aggregation in the former was conspicuously less advanced (Fig. 2). After more than 2 h, the rate of decrease of optical density in the treated preparations compared favourably with that recorded for the controls (Fig. 1).

When ATP was added to a suspension of control cells containing newly formed aggregates, there was a release of cells from the aggregates, and a breaking up of the aggregates into smaller ones as well as a corresponding sharp rise in the optical density of the preparation (Fig. 1). This further indicates that the state of ATP-sensitive elements at or near the cell surface may be an important factor in adhesion.

Attention was next turned to plotting the extinction of exogenous ATP in the treated cell preparations against time. In the method adopted for estimating ATP, a sample of 0·25 ml. of the treated cell suspension was removed and cold perchloric acid at 0·6 M strength was added to prevent further hydrolysis. The sample was then centrifuged at 3,000 r.p.m. for 5 min and 0·2 ml. of the supernatant was added to a mixture of D-3-phosphoglyceric acid at 6×10^{-3} M and DPNH at $1{\cdot}2 \times 10^{-2}$ M in the presence of 0·1 M triethanolamine buffer.

$$3\text{-PG} + \text{ATP} \rightleftharpoons 1,3\text{-PG-P} + \text{ADP} \quad (1)$$

$$1,3\text{-PG-P} + \text{DPNH} + \text{H}^+ \rightleftharpoons \text{GAP} + \text{P} + \text{DPN}^+ \quad (2)$$

Phosphoglycerate kinase is added to mediate the first reaction, and glyceraldehyde phosphate dehydrogenase the second. DPNH is estimated by photometry at 340 mμ

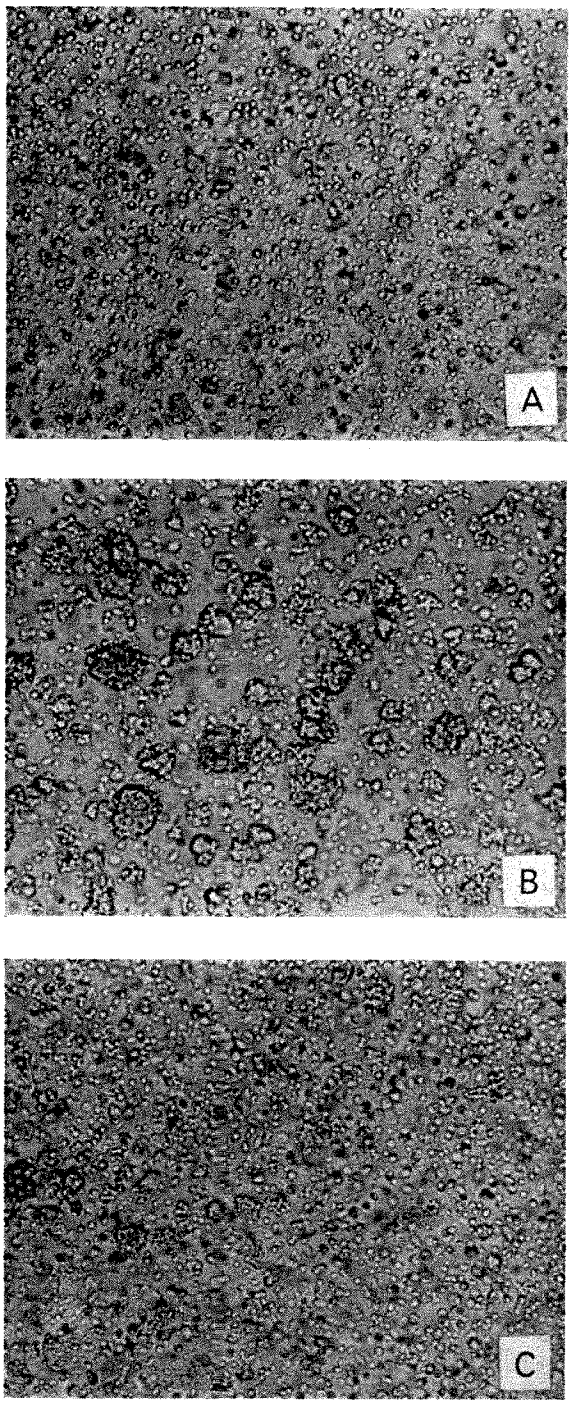


Fig. 2. Effect of exogenous ATP on the aggregation of chick-embryo fibroblast cells rotated in Hanks's solution. *A*, Cells in isolation at the beginning of the period of their rotation; *B*, aggregates of cells in Hanks's solution after being rotated for 2 h; *C*, state of aggregation of cells after 2 h in Hanks's solution which contained ATP at 1×10^{-3} M at the beginning of the 2-h period

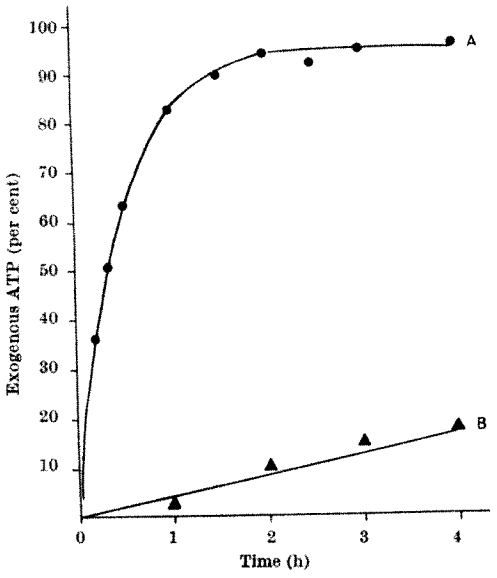


Fig. 3. Extinction of exogenous ATP at a concentration of 1×10^{-3} M in a rotated suspension of chick-embryo fibroblast cells at 16×10^{6} cells/ml. in Hanks's solution (curve *A*) and in cell-free Hanks's solution (curve B)

before and after the addition of the enzymes. The reactions proceed stoichiometrically and the difference in optical densities relative to the decrease in DPNH is proportional to the number of μmoles of ATP. Details of the method are in the ATP Test Combination obtainable from Boehringer and Sohn, Mannheim, Germany.

When ATP was introduced into Hanks's solution to give a final concentration of 1×10^{-3} M there was an estimated 15 per cent loss of ATP at the end of 3 h (Fig. 3). The extinction curve for ATP in the presence of cells at a density of 8 million cells/ml. shows that 85 per cent of the exogenous ATP had disappeared after 1 h, and 95 per cent after 2 h before reaching what appears to be a lower limiting level (Fig. 2). This relatively rapid break-down of ATP over the first 2 h coincided with the period when cell aggregation was inhibited (Figs. 1 and 2). These results strongly suggest that it was the presence of the exogenous ATP which inhibited aggregation, for by the time the ATP had reached its lower limiting level, aggregation entered a phase during which the rate was comparable with that observed in the control preparations.

Inosine triphosphate (ITP) was also shown to be capable of inhibiting aggregation, but it was slightly less effective than ATP. Adenosine diphosphate (ADP) neither enhanced nor inhibited aggregation.

It can be concluded that ATP inhibited aggregation, but it is less easy to explain the precise mechanism by which this compound produced the effect. One main reason for this is that our knowledge of the chemical organization of the cell surface is still very limited because of technical difficulties which hamper the characterization of the different components of the cell surface. An effect resulting from an interaction between a chemical substance and cells is usually explained in terms of the substance acting on the cell surface to alter its permeability allowing an influx of extracellular material which causes a disturbance of the intracellular ionic balance. Notwithstanding this kind of broad explanation, it is difficult to discard the one-sided possibility that the inhibitory effect on cell aggregation by exogenous ATP is due to ATP-splitting by an ATPase situated at the cell surface. Moreover, since ATP-splitting activity is a typical property of contractile proteins of the actomyosin-type[13] and since ATP induces contraction of this protein[5,13], the possibility of a mechanism of this kind lying directly under or at the cell surface influencing adhesion cannot obviously be dismissed.

However, the likelihood of the inhibitory effect of the exogenous ATP being due to the ATP chelating the calcium and magnesium ions has not been overlooked. But the amount of ATP introduced was certainly insufficient to chelate all the calcium and magnesium ions present. Furthermore, calcium-ATP is regarded as being ineffective as an energy source[14], which implies that in this state it is non-hydrolysable. Yet the results of the present work show that the exogenous ATP was broken down relatively rapidly in the presence of cells until it reached a lower limiting level (Fig. 3). Thereafter, the cells regained their ability to aggregate at a rate comparable with that of the 'control' cells (Fig. 1). This recovery would not have occurred if the ATP was acting as a chelating agent depriving the cells indefinitely of the calcium and magnesium ions they require for aggregation.

One of us (V. A. K.) during the course of the present work has succeeded in extracting an actomyosin-like protein with ATPase activity from embryo-chick fibroblast cells without disrupting them, and from the evidence so far it seems as if the protein in question is being removed from the peripheral region of the cell. This was achieved by immersing the cells for 3 min at 0–4° C in a 'Weber-Edsall' solution (buffered 0·6 M KCl). This is the conventional method used for extracting actomyosin-type protein[13]. The cells recovered from this treatment and remained viable. The amount of protein of the actomyosin-type removed in this way was relatively small, but it is of interest that the treated cells failed to aggregate under the conditions of the experiments described above. This suggests that it may not be too far-fetched to assume that there is a link between the actomyosin-like protein and adhesion. However, this aspect of the work is still in progress, and the results will be published in due course.

The several excellent reviews on the subject of cell contact and adhesion[15] make it abundantly clear that there is a multiplicity of factors capable of influencing both the adhesion and de-adhesion of cells. Yet, curiously enough, the search for basic cellular mechanisms capable of initiating changes in the adhesive properties of the cell surface has not so far alighted on contracting and relaxing systems involving contractile actomyosin-like proteins operating at or subjacent to the surface. It would seem, however, that the link between these proteins and cell motility[5], and their presence in many types of cells[16,17] particularly at the cell surface[9], together with the evidence from the present investigation, converge in support of our suggestion that the activity of proteins of the actomyosin-type accumulated at the cell surface may well have an important role in adhesion.

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EFFECT OF VITAMIN A DEFICIENCY ON THE RETINA OF THE EXPERIMENTAL RABBIT

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TANSLEY[1,2] reported that severe vitamin A deficiency in the rat and in the dog produced characteristic histological damage in the rod and pigment layers of the retina. Confirmatory findings for the rat came from Johnson[3] and for the horse from Anderson and Hart[4]. No such damage was, however, observed by Mann, Pirie, Tansley and Wood[5] in a series of fifty vitamin A-deficient rabbits, although three animals showed localized changes which were interpreted as evidence of spontaneously occurring hereditary retinal degeneration such as is frequently seen in inbred rat colonies—a reading supported by the fact that two of three affected rabbits were littermates and that the same lesion was seen in another littermate which served as one of the ten controls in this experiment.

Johnson[6] and Wald[7] suggested that the structural retinal derangement described in the vitamin A-deficient rat might be responsible for the lasting or permanent effect of nightblindness which persists after the deficiency has been relieved. More recently Dowling and Wald[8,9], and in a series of communications summarized and listed in Dowling[10], have reported that, while vitamin A acid can replace vitamin A in maintaining rats in good health, such replacement is inadequate for the retina (as was originally suggested as a possibility by Moore[11]): they observed extensive degenerative changes in the rods and the pigment epithelium in vitamin A-deficient rats kept generally fit on supplements of vitamin A acid. The changes in the retina recall those seen in retinitis pigmentosa in man and in spontaneously occurring hereditary retinal degeneration in the rat. These findings are therefore of particular interest in that they may help to elucidate not only the role of vitamin A in the metabolism of the retina, but also the mechanism of retinitis pigmentosa in man—probably the commonest of the hereditary blinding affections.

These are fundamental issues. The present investigation of the significance of vitamin A aldehyde in the metabolism of the retina in the intact animal was undertaken in the rabbit partly because spontaneous retinal degeneration has not been definitely recorded in this animal and mainly because in the rabbit it is possible to watch any developing lesion by means of an ophthalmoscope.

Dutch rabbits were used throughout. Two separate experiments were carried out:

(1) In the first experiment of six young litter mates, two were used as controls—one being kept on a vitamin A-deficient diet without any supplement and the other on the deficient diet supplemented by vitamin A palmitate. The remaining four were kept on the same diet deficient in vitamin A but supplemented by vitamin A acid. Three of the four animals so treated were kept for 30 months and one for 22·5 months under the conditions to be described; the first of the two controls was destroyed at 12·5 months, and the second accidentally at 14·5 months.

(2) The second experiment began 5·5 months later; six baby rabbits from three litters were used. All the six animals, the weights of which ranged from 560 g to 640 g, were kept on a vitamin A-deficient diet until they ceased to gain weight, when their diet was supplemented by vitamin A acid. One was destroyed at 14·5 months and a second at 18·5 months, both for histological studies. The remaining four were kept for 24 months.

In both series, all animals were submitted to ocular examination, including ophthalmoscopy, at intervals, generally of 2 months. Electroretinography was carried out in six animals 4 months before they were destroyed for histological examination: three of the first batch at 26 months and three of the second batch at 20 months, with a repeat examination of one animal in each series at 30 months and 24 months, respectively.

Dietetic procedures

(*a*) *The vitamin A-deficient diet.* The composition of the vitamin A-deficient diet used is given in Table 1.

Table 1

Groundnut meal	17·5 g
Sugar-beet pulp	10 g
Oatmeal	27 g
Fine wheatfeed	13 g
Molasses	3 g
Linseed oil meal	23 g
Common salt	0·5 g
Dried brewer's yeast	4 g
Salt mixture†	2 g
D-α-tocopheryl acetate*	40 mg/rabbit/week
Vitamin D_3*	1,000 I.U./rabbit/week

* In oil by mouth until 13 months when these vitamins were incorporated in the diet (vitamin D in protected form).

† The salt mixture supplied consisted of (g/kg diet): sodium chloride, 1·1; $CaH_4(PO_4)_2{\cdot}2H_2O$, 8·7; ferric citrate, 0·8; sodium dihydrogen phosphate, 2·3; $MgSO_4{\cdot}7H_2O$, 1·7; potassium chloride, 5·4; potassium iodide, 0·02; sodium fluoride, 0·0009; $MnSO_4{\cdot}4H_2O$, 0·0044.

This diet is similar to that used by Lamming, Woollam and Millen[12]. Chemical analysis showed it to contain less than 0·07 I.U. true vitamin A/g, together with β-carotene equivalent to 0·08 I.U. provitamin A/g and a xanthophyll fraction estimated as 1·1 μg/g. From the seventeenth month of the first experiment (12·5 months of the second experiment) each batch of basal diet was tested for absence of vitamin A before being used.

(*b*) *Supplements used.* When vitamin A acid was given the dose was 15 mg/kg of diet. Vitamin A palmitate (for control rabbit *B*) was given in a dose of 2·5 mg/kg of the vitamin A-deficient diet. After 7 months, vitamin A palmitate was omitted from the diet and given to the rabbit by mouth, at the rate of 40 mg/week for 2 months followed by 0·8 mg/week until the end of the observations at 14·5 months.

(*c*) *Nutritional history*

The first series: The six litter mates—whose mother was put on a vitamin A-deficient diet 12 days after their birth—were kept on a vitamin A-deficient diet from the age of 12–25 days, and on this diet with the acid supplement for the next 10 days. Thereafter, rabbits Nos. 1–4 were continued on this supplemented diet, while control rabbit *B* had vitamin A palmitate as the supplement instead of vitamin A acid, and control rabbit *A* had the vitamin A-deficient diet without any supplement.

All the rabbits grew well except control rabbit *A* which showed a decline in weight and necessitated giving it

vitamin A acid supplement at the age of 4·5 months. It finally developed a flaccid paralysis 8 months later and was destroyed.

The second series: The six rabbits were all maintained on a vitamin A-deficient diet for a preliminary depletion period of up to 5·5 months of age before giving them the vitamin A acid supplement. They had all reached a weight plateau at this time and they showed weight gain on receiving the supplement.

Change of diet after the onset of the retinal lesion. Five and a half months before the end of the experiment in both series, when the retinal lesion was fully established, rabbits Nos. 1, 4, 7, 8 and 9 were given modified diets to determine whether the lesion could be reversed. Rabbit No. 1 was given a normal diet (diet *S.G.*1 (Rank)) and the remaining rabbits (Nos. 4, 7, 8 and 9) were given the vitamin A-deficient diet and an oral dose of 6 mg vitamin A palmitate/rabbit/week. These modifications were continued until the end of the experiment (at 30 months in the first series and at 24 months in the second).

Accidental administration of vitamin A palmitate. On one occasion, at 13 months and 7·5 months in the two series respectively, all survivors receiving vitamin A acid supplement were accidentally given 0·6 mg vitamin A (instead of an intended dose of vitamin D). These animals were later put back on a vitamin A-deficient diet without any supplement for 5 weeks and then returned to their diet containing vitamin A acid supplement.

(*d*) *Estimation of vitamin A and vitamin A aldehyde.* At the end of the experiment estimation of the concentration of vitamin A and vitamin A aldehyde was carried out for several tissues. The procedures used were:

Vitamin A. This was estimated in liver and brain by the method of Ames, Risley and Harris[13]. Very concentrated extracts of tissues believed to be deficient in vitamin A were prepared and tested, giving a lower limit of sensitivity of about 2–3 I.U./g tissue (that is, about 1 μg/g).

Vitamin A aldehyde. This was measured by the thiobarbituric acid method of Futterman and Saslaw[14]. The eyes were preserved at −10° overnight, allowed to thaw, and then dark-adapted. Each eye was dissected under a dim red light, and the cornea, lens and vitreous were removed. The remainder of the eye was extracted with alcohol in an M.S.E. top-drive steel-bladed homogenizer, using the 10-ml.-sized goblet. The amounts of retinene in a single rabbit retina proved too small to be detected by this procedure. (At the time these estimations were carried out, it was not realized that storage of the eyes *post mortem* destroyed the vitamin A aldehyde content and the capacity for its regeneration. Subsequent estimations have shown that it is necessary to carry out the estimations as soon as possible after enucleation.)

RESULTS

The salient ocular findings and the conditions under which they were observed have been set out in Table 2. Rabbit *A*, kept on a vitamin A-deficient diet without any supplement, was in a poor state by 4·5 months and had to be put on vitamin A acid supplement for the rest of the experiment. It had developed typical xerosis of the conjunctiva and cornea at 7·5 months and at 9·5 months it showed white dots at the fundus similar to the lesions observed at the same time in rabbits Nos. 2 and 4 which were thriving on the same deficient diet supplemented by vitamin A acid from the beginning. The same fundus lesion was observed 6 weeks later in the two remaining treated animals of the first experiment (Nos. 1 and 3), but at no time during the 14·5 months' observation was a fundus lesion seen in control rabbit *B*, which received the vitamin A-deficient diet supplemented by vitamin A palmitate. The five rabbits on a vitamin A-deficient diet supplemented by vitamin A acid therefore all showed a fundus lesion at about 10 months.

It will also be seen from Table 2 that all six rabbits in the second experiment—all kept on a vitamin A-deficient diet supplemented by vitamin A acid—developed fundus lesions, first clearly seen at 13·5 months.

The fundus lesions were not the only ocular changes. As can be seen from Table 2, xerosis of the cornea was present in one of the four treated animals in the first series (in addition to the expected corneal lesions seen in the control animal *A*) and in four of the six animals in the second series. It is possible that this complication (which interfered with opthalmoscopic examination) was partly

Table 2. (i) OBSERVATIONS ON SIX LITTER MATES BORN ON OCTOBER 19, 1961

No. of animal	Diet	Period of observation (months)	Xerosis of cornea at (months)	Fundus lesion first observed at (months)	Severity of lesion towards the end of the second year	Modification of diet for the last 5·5 months (after lesion fully established)	Effect on lesion
A	Vitamin A deficient	12·5	7·5	9·5	(General condition very poor at 4·5 months; given vitamin A acid from then on; destroyed at 12·5 months because of flaccid paralysis)		
B	Vitamin A deficient + vitamin A palmitate	14·5	—	—			
1	Vitamin A deficient + vitamin A acid	30	—	11	+ +	Normal diet	No regression
2	Vitamin A deficient + vitamin A acid	22	+ 7·5	9·5	+ +		
3	Vitamin A deficient + vitamin A acid	30	—	11	+ + +	None	
4	Vitamin A deficient + vitamin A acid	30	—	9·5	+ + +	Intensive vitamin A palmitate	No regression

(ii) OBSERVATIONS ON ANOTHER SIX LITTER MATES BORN ON APRIL 4, 1962

No. of animal	Diet	Period of observation (months)	Xerosis of cornea at (months)	Fundus lesion first observed at (months)	Severity of lesion towards the end of the second year	Modification of diet for the last 5·5 months (after lesion fully established)	Effect on lesion
5	Vitamin A deficient + vitamin A acid	18·5	—	13·5	+ + +		
6	Vitamin A deficient + vitamin A acid	24	± 5	13·5	+ + +	None	
7	Vitamin A deficient + vitamin A acid	24	± 5	13·5	+	Intensive vitamin A palmitate	No regression
8	Vitamin A deficient + vitamin A acid	24	+ 5	13·5	+ +	Intensive vitamin A palmitate	No regression
9	Vitamin A deficient + vitamin A acid	24	—	13·5	+ +	Intensive vitamin A palmitate	No regression
10	Vitamin A deficient + vitamin A acid	13·5	+ 5	13·5	+ + when destroyed at 13·5 months		

In this experiment the vitamin A acid supplement was only given after 5·5 months when the animals ceased to gain weight on the vitamin A-deficient diet.

responsible for the delay in establishing the presence of fundus lesions in the second series.

The Fundus Lesion

(i) *Ophthalmoscopic appearances.* The fundus lesion in all these animals was highly characteristic. The first changes were inconclusive and consisted of an indefinite breaking up of the reddish background, suggestive of a geographical map. Within some 6–8 weeks these changes had become more defined by the appearance of white dots in the lower half of the fundus, beginning at about 1 disk distance below the disk, and extending downwards. These white dots first appeared at the periphery, the central area below the disk becoming affected last. That the lesion was definitely pathological, and not a physiological variant of the rather wide range of the normal appearances of the rabbit fundus, was not beyond question until about 9 months from the beginning of the observations.

Once the white dots appeared in the fundus they progressed steadily and sometimes rapidly, so that the indicated in Table 2, two of the four treated animals in severity of the lesions varied in the different animals. As the first series were regarded as severe (Nos. 3 and 4), the remaining two being moderately affected. Likewise, only two of the second series (Nos. 5 and 6) were severe; the rest, except for No. 7 which showed a mild lesion, were moderately affected. The range of ophthalmoscopic appearances is shown by Figs. 1*a*, *b* and *c*.

The fundus lesion proved irreversible. Three of the rabbits in the first series had survived at 24 months, and four of the second series at 18 months. Four of these seven animals were given intensive vitamin A therapy (6 mg vitamin A palmitate/week) and one was kept on a normal rabbit diet; none of these five animals showed any regression of their lesion during the 6 months of observation.

(ii) *Electroretinography.* Rabbits Nos. 1, 3 and 4 had electroretinographic examination at 26 months, and rabbit No. 1 a repeated test at 30 months. Rabbits Nos. 6, 8 and 9 had such an examination at 20 months with a repeat test for rabbit No. 6 at 24 months. These tests showed a consistent pattern. In all animals the *b* wave was most affected, being small or absent; the amplitude was not influenced by dark adaptation. Lesser and indefinite anomalies were seen in the *c* wave. The photoptic components of the electroretinogram were normal. In contrast to the normal rabbit, which shows a predominantly rod type of electroretinogram, these rabbits showed a cone type of tracing.

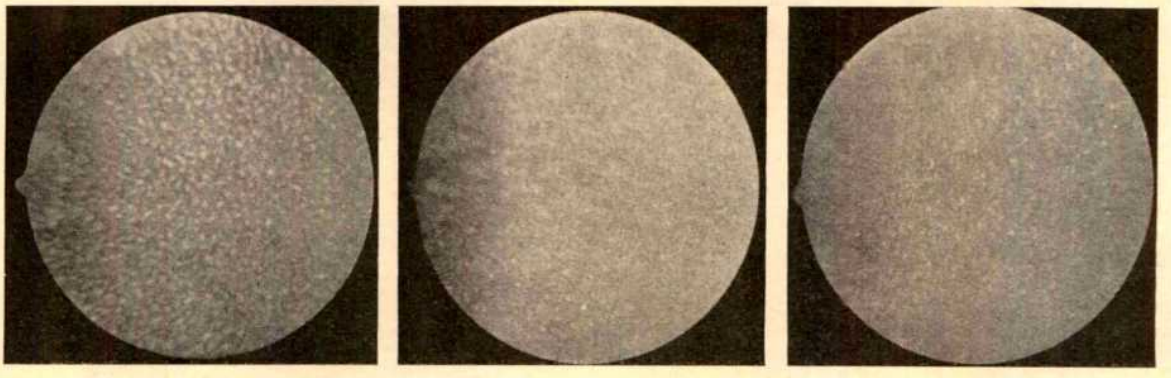
(iii) *Histological appearances.* All animals were destroyed by the injection of air into the vein of the ear except control rabbit *B* and rabbit No. 5, both of which died from an overdose of anaesthesia, the first while having an abscess of the upper jaw treated, and the second while undergoing an electroretinographic test. In all animals the eyes were excised immediately after death and those destined for histological study were fixed in acid Zenker solution. Paraffin sections were stained with haemalum and eosin. Both eyes were used for histology in rabbits Nos. 2, 5 and 10 and in the control rabbits; in the animals destroyed at the end of the experiment (Nos. 1, 3, 4, 6, 7, 8 and 9) one eye was kept for biochemical tests. Of the eleven animals kept on a vitamin A-deficient diet supplemented by vitamin A acid (ten from the beginning and one after 4·5 months) sections for histological study were obtained in only nine.

Pathological changes were recognized in all the sections. They were most prominent in the area centralis, but extended into the mid-peripheral area as well. Debris appeared between the outer rod segments and the pigmented epithelium. It consisted partly of foamy, lightly eosinophilic material coating the pigmented epithelium and of local aggregations of circular masses staining much more deeply with eosin (Fig. 2*a*). In addition, a few large macrophage-like cells were observed (Fig. 2*b*). Occasionally, these contained pigment granules or small masses of material resembling the eosinophilic debris. The pigmented epithelium itself in the area centralis had a ragged appearance and there was patchy loss of pigment. In places the macrophage-like cells were seen adhering to normal-looking pigmented epithelial cells. Elsewhere the latter appeared in process of rounding up as though they were transforming into macrophage-like cells (Fig. 2*c*).

Changes in the retina proper were slight. Local thinning of the outer nuclear layer was seen, often in association with aggregates of debris. The visual cells which remained appeared largely intact, though some of the deeply staining eosinophilic debris may have been derived from degenerated rod outer segments.

Biochemical Control

The following observations were made as to the vitamin A concentration in the liver: (1) Control rabbit *A* on a vitamin A-deficient diet, destroyed at 12·5 months. The liver contained no vitamin A. (2) Control rabbit *B*, on the same diet supplemented by vitamin A palmitate, accidentally killed at 14·5 months. The liver showed 70 mg of vitamin A. (3) Treated rabbits on vitamin A-deficient diet and vitamin A acid supplement. No. 10 destroyed at 14·5 months, No. 6 destroyed at 24 months, and No. 3 destroyed at 30 months. The liver contained no vitamin A. (4) Treated rabbits on vitamin A-deficient diet supplemented by vitamin A acid, and given a normal diet (No. 1) or vitamin A palmitate supplement subsequent to the development of the retinal lesion (rabbits Nos. 4, 7, 8 and 9). Vitamin A was present in the liver in the



a *b* *c*

Fig. 1. Fundus appearances in rabbits maintained on vitamin A-deficient diet supplemented by vitamin A acid. *a*, A severe lesion in rabbit No. 6, maintained on the vitamin A-deficient diet for 24 months. *b* and *c*, A moderate lesion in rabbit No. 9 and a mild lesion in rabbit No. 7, respectively; both animals had intensive vitamin A supplement during the last 5·5 months of the 2-year period of observation without effect on their lesions

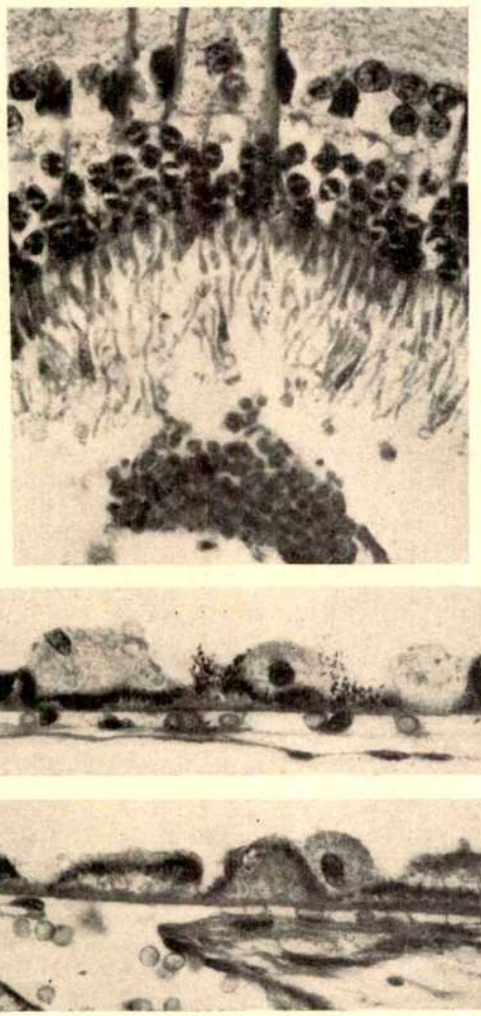


Fig. 2. Histological appearances. *a*, Retina. Ventral mid-periphery showing an aggregation of deeply staining eosinophilic debris. There is also local thinning of the outer nuclear layer. (Haemalum and eosin × 600.) *b*, Pigmented epithelium. Ventral mid-periphery showing macrophage-like cells adhering to it (the retina has become detached during preparation). (Haemalum and eosin × 600.) *c*, Pigmented epithelium. Ventral mid-periphery showing one macrophage-like cell and a pigmented epithelial cell rounding up. The coating of foamy debris can also be seen. (Haemalum and eosin × 600)

following quantities: 5 mg, 25 mg, 27 mg, 32 mg and 41 mg, respectively.

In addition, the brains of rabbits Nos. 3, 4, 6, 7, 8 and 9 were examined for vitamin A with negative results. The retinae of these animals were tested for vitamin A aldehyde also with negative result by the method used.

DISCUSSION

(1) *Vitamin A aldehyde in the visual purple cycle.* In the mammal, visual pigment consists of a molecular compound between retinene (vitamin A aldehyde) and the protein opsin. Vitamin A alcohol, the precursor of retinene, is oxidized in the eye to its aldehyde (retinene) under the action of an enzyme, alcohol dehydrogenase—the same enzyme system being responsible for the reduction of retinene to vitamin A following the bleaching action of light. A further degree of biological specificity is found in the visual cycle in that a particular geometrical configuration of retinene is obligatory for the synthesis of visual pigment. Vitamin A and retinene exist in a variety of *cis-trans* isomeric forms, and, in the visual pigment, retinene is in the 11-*cis* configuration. Isomerization of vitamin A and/or retinene occurs in the eye under the action of a specific enzyme, retinene isomerase[15]. The primary action of light on the visual pigment is to convert the 11-*cis* retinene into its all-*trans* isomer, and then to break apart the molecular bond between retinene and the protein opsin. A cyclic series of reactions involving synthesis and bleaching of visual pigment is therefore continuously occurring in the retina under the influence of light stimulation and dark adaptation.

In this mechanism vitamin A acid has no place. It is itself the oxidation product of retinene and so far, within the body, no system has been demonstrated which is capable of reversing the process.

(2) *The histological findings in the present investigation.* The following observations are relevant: (i) It is highly probable that the white dots seen with the ophthalmoscope are aggregates of eosinophilic debris. (ii) The macrophage-like cells might be modified pigmented epithelial cells. They also resemble the 'interstitial cells' described in normal mouse retina[15a]. (iii) The aggregates of eosinophilic debris recall the bundles of extracellular lamellae described by Dowling and Sidman[16] in rats with inherited retinal dystrophy. (iv) The pathological changes differ from those seen in known retinopathies induced by various chemical agents. (v) These changes also differ from those described by Dowling and Wald[17] in vitamin A deficiency in the rat in which changes appear first in the rod outer segment, there is fairly rapid diminution in the number of visual cells, and the pigmented epithelium remains normal.

(3) *The fundus lesion observed in the present investigation as the counterpart of retinitis albi punctatus.* The outstanding feature of the observations in the present investigation on vitamin A aldehyde deficiency in the rabbit is the lack of any substantial effect on the rods. The striking fundus lesion seen ophthalmoscopically has a surprisingly slight histological basis: only the pigment epithelium shows any substantial changes, and even these are relatively mild. The electroretinographic tracings are consistent with disturbed dark adaptation and, though they do not exclude damage to the rod system, they do not support such an assumption either.

The lesion in the rabbit is in fact reminiscent of the features seen in vitamin A deficiency in man and in retinitis albi punctatus. In both these affections, night-blindness is an outstanding feature, and in both white dots on the fundus are the characteristic ophthalmoscopic appearances.

The white dots which can be seen using the ophthalmoscope in vitamin A deficiency are not a feature widely known. It is not seen by ophthalmologists practising in affluent countries, but in the Far East, with its massive nutritional problems, it seems to be well recognized since it was first described by Uyemura in 1928 (ref. 18) (Fig. 3). (The literature is reviewed by McLaren[19].) It is likely that in man the associated—and possibly earlier—corneal

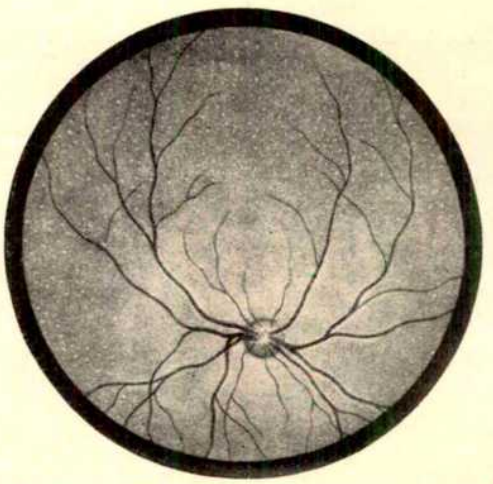


Fig. 3. Fundus appearances in vitamin A deficiency. (After Uyemura (ref. 18))

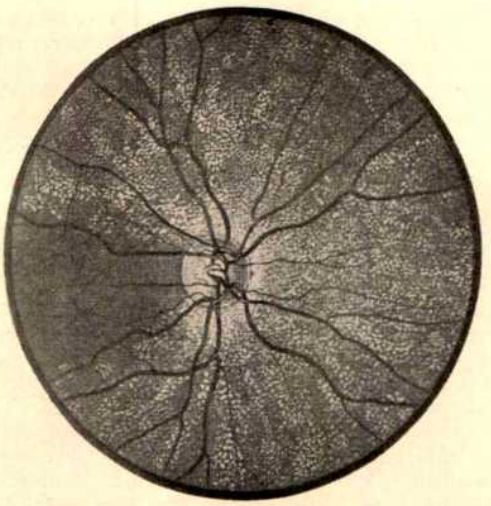


Fig. 4. Fundus albi punctatus. (After François (ref. 21))

xerosis makes the recognition of this fundus lesion difficult in many cases, and it is perhaps significant that, in such of the rabbits as developed corneal xerosis in the present experiment, the corneal lesion preceded a recognizable fundus lesion.

Retinitis albi punctatus has the same ophthalmoscopically visible white dots (Fig. 4) and nightblindness. It is a well-established, though rare, genetic affection, described by Lauber in 1910 (ref. 20), and shows a recessive mode of inheritance. Its clinical importance lies in the fact that, unlike retinitis pigmentosa, it is a stationary affection. It is apparently congenital; it does not lead to pigmentary disturbances, shrinking fields of vision and ultimately total or sub-total blindness. Its mild course excludes any possible involvement of the rods, and the nightblindness and the white dots are presumably the result of an anomaly in the pigment layer—as they appear to be in the rabbit. Though histological evidence is lacking in retinitis albi punctatus, and inconclusive in vitamin A deficiency in man, it is not unreasonable to assume that the experimental lesion in the rabbit is a closely allied disturbance—possibly their counterpart.

These findings in the rabbit fully substantiate those of Wald and Dowling in the rat as to the adequacy of vitamin A acid for the body as a whole, and the dependence of the pigment epithelium (and the visual pigment) on vitamin A aldehyde. They differ, however, in limiting the dependence to this tissue and this cycle: the integrity of the rods is apparently not dependent on vitamin A aldehyde. In the rabbit, in any event, the deprivation of vitamin A aldehyde does not give a picture similar to retinitis pigmentosa, but a considerably less serious disturbance, and it is possible that the pigment epithelium is the major and perhaps the exclusive site of conversion of vitamin A into the specific chromophore for the visual pigment.

Further investigations on both the rat and the rabbit are needed to assess this discrepancy in the findings and its bearing on the fundamental problem whether vitamin A deficiency is an aspect of retinitis pigmentosa with its affection of the rods.

We thank the directors of Vitamins, Ltd., for providing facilities for the animal experiments at their Walton Oaks Experimental Station, and Messrs. Roche Products, Ltd., and their medical adviser, Dr. A. L. Morrison, for generous supplies of vitamin A acid. Dr. Hisako Ikeda of the Institute of Ophthalmology and Mr. A. I. Friedmann of the Royal College of Surgeons and the Royal Eye Hospital carried out and interpreted the electroretinographic tests, while Dr. David Lucas of the Radiobiological Research Unit, Harwell, was responsible for part of the histological study of the retinae. We also thank Miss E. G. Nairne, Miss E. J. Castle and Mr. R. Harding for technical help.

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CELL WALL SURFACE OF THE HIGHER PLANTS

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RECENT work with the electron microscope considerably clarifies earlier observations on the surface of the cell wall in shoot and in root. The present article deals with the surface of the cell wall in the growing regions of the higher plant: (1) external surface of the outer epidermal wall; (2) lining of intercellular space system; (3) intracellular lining.

The External Surface

Shoot-cutin. The outer wall of epidermal cells in the shoot is covered with a cuticle which appears at an early stage of development in the seed. In certain species an additional coating of wax is more or less conspicuous, while, in others, mineral substances such as silica or calcium carbonate may encrust or partially impregnate the cell wall.

The precursors of cutin and other surface substances elaborated within the living protoplast pass to the surface along plasmodesmata through pits in the outer epidermal wall. A pit is defined here, as in previous publications, as a collection of pores in the structural cellulose wall. In the epidermal wall of the rind of the fruit of *Citrus*, and also in the *Citrus* leaf, pits are visible under the light microscope[1,2]. Ultrasonic fragmentation is an excellent method for tearing or peeling the cuticle of any tissue. In the leaf of *Bergenia* the larger pits are demonstrable under the light microscope when stained with ruthenium red and Sudan 3. In the same material and in the onion under the electron microscope minute pits and innumerable pores are clearly defined[3]. They resemble in structure the pits ubiquitous in the internal cell walls[4]. Plasmodesmata are still present in the pits and in the pores unless

they have been removed by ultrasonic treatment. In texture the isolated cuticle resembles a very fine artificial rubber sponge.

The plasmodesmata appear to be anchored in the cuticle of the outer wall of all epidermal cells. The sensitive contractility of slender protoplasmic strands is familiar to all observers of living cells. The disturbance inevitable during the preparation of material for examination may cause the retraction of the plasmodesmata into the cellulose layer of the wall. At the same time the elastic cuticle, stretched by the turgor of the cell, shrinks instantly and no visible plasmodesmatal cuticular pores remain. However, the thinner areas which are obvious in cuticular electron micrographs may mark the former location of the plasmodesmatal endings.

Shoot-wax. The characteristic 'bloom' on leaves such as *Eucalyptus*, onion and cabbage, and on fruits such as grapes, is due to the presence of wax. About 100 years ago De Bary described and illustrated various types of wax rodlets and postulated the presence of wax canals[5]. Under the light microscope, wax canals are visible in the epidermis of the orange rind, in the onion leaf and in the seed of *Cercidium*[6]. The close distribution of rodlets in the onion leaf is evident under the electron microscope[3]. In the leaf of beet, rodlets are present in the youngest leaves and eventually fuse into rugose plaques[7].

Root. The epidermis of the root, like that of the shoot, is covered with a cuticle which is defined at an early stage of embryonic development. In fresh material of soil-grown seedlings, and in the feeder roots of older plants, a cuticle covers the entire surface of the growing primary region of the root including the root hairs. All roots are also coated with a more or less conspicuous layer of mucilage[8].

In the roots of certain azaleas, mucilage occurs as a conspicuous sheath[9]. In the smoke tree, a desert xerophyte, the mucilage of the extremely long tap root of the one-year seedlings dries out during the rainless season, except occasionally for a sheath near the tip when the surrounding soil still retains some trace of hygroscopic moisture[10].

Pits occur in the outer epidermal wall of all soil-grown roots examined. The larger pits are visible under the light microscope and the smaller may be faintly indicated in the root hairs of certain gymnosperms. Under the electron-microscope the smaller pits and pores are clearly defined[11,12].

Lining of the Intercellular Space

Apical meristems. The ideal shape of isodiametric cells is tetrakaidekahedral[13], but this ideal changes constantly during cell division and growth The polyhedral cells are closely packed and interconnected by plasmodesmata through pits which are ubiquitous on all cell faces. As the cells expand and divide, the cell corners round off and minute intercellular spaces arise within about 20μ, or two cells distant from the apical surface of stem or root[14]. The breaking of the plasmodesmata is virtually a wounding of the living protoplast. The intercellular system, the respiratory system of Haberlandt[15], develops in general as an air-filled continuum as seen in fresh material and also clearly defined in the meticulous slides of Schuepp[16]. The wall surface of the intercellular continuum is lined with a non-cellulosic lipid layer, tenuous in younger tissue but clearly defined during later growth. In microchemical reactions it resembles cutin and suberin, but, as its chemical composition is not yet known, it is termed a 'lipid' layer.

Leaf. The cells of the leaf primordia, like those of the apical meristems, are polyhedral and closely packed. In the mature leaf the volume of intercellular space and the internal surface vary with the species[17]. In the spongy mesophyll the cells are lobed or branched. The larger cells in the middle of the leaf areole as a rule remain in contact with eight surrounding cells, while the smaller, nearer to the veinlets, are in plasmodesmatal contact with six others. The ellipsoidal palisade cells also vary in size, in diameter and length. In general, the larger are in contact laterally with about eight cells, the smaller with six. The entire surface of the intercellular space is lipid lined. In the region of abscission 'internal suberization' is conspicuous and the walls of the abscizing leaves are impregnated with suberin, and resistant to the action of 80 per cent sulphuric acid[18].

Endodermis and stele. Intercellular space occurs in cortical and also in stelar parenchyma as seen clearly in *Ricinus* and other species. Suberin deposition on the Casparian strip of the endodermis begins at the outer and inner edges of the radial wall which are nearest to the cortical and stelar intercellular spaces. Pits occur on all faces of the endodermal cells. As seen under the electron microscope, some become occluded by microfibrils during later growth[12].

In *Ricinus* in fresh material, clearly defined intercellular spaces around the spiral vessels are visible under the light microscope. These spaces, like all others, are lined with lipid. Structural details are visible under the electron microscope[19].

Flower. The presence of intercellular space in all parts of the flower including the petals is well known. In flowers which last for a few hours, or at most a day, such as the primulas, the intercellular lining is tenuous as in the meristematic region. In contrast, in long-lasting flowers, such as orchids and camellias, the lipid lining resembles in thickness that of a xerophytic leaf[20].

Fruit. The lining of intercellular space is notable during the development of the outer integument of the seed of *Cercidium*[6]. In the same seed the inner integument differs from other tissues examined. At no time during development is air observed in the extensive intercellular space which surrounds the long-armed parenchymatous cells. This space is filled continuously with amorphous mucilage. Nevertheless, in this mucilaginous mass, a lipid layer appears about 1–2μ from the cellulosic parenchymatous walls. This layer is visible under light and electron microscopes and is similar in microchemical reactions to cutin and suberin. It is suggested that the differentiation of this layer may indicate the existence of an oxygen gradient in the mucilaginous mass. The mucilage elaborated within the protoplast is extruded through pits which are present in the arms and body of the integumentary cells.

Intracellular Lining of Xylem Vessels

The protoplasts of the spiral vessels of *Ricinus* are interconnected through pits by plasmodesmata with contiguous vessels and with surrounding xylem parenchyma cells. The pits are visible under light and electron microscopes[19]. In the differentiating vessels a lipid-like lining is seen in living cells under the light microscope. After the death of the protoplast the empty vessels are lined with a suberin-like layer, which, as seen under the electron microscope, resembles in texture the isolated external cuticle and the intercellular lipid lining.

Discussion

Fatty substances, lipids, essential components of protoplasm, furnish the precursors of cell wall surface materials. In all living cells examined the chloroplasts are surrounded by coronae of lipid droplets visible under light and phase microscopes and which can be immediately stained with sudan black. The same is true of leucoplasts and elaioplasts. It is probable that these droplets are concerned in the formation of surface material precursors[21].

In a recent review, Napp-Zinn[22] pointed out that pits in the outer epidermal wall of higher plants were described in *Elymus arenaria* and in *Cycas revoluta* by Hugo Von Mohl in 1842. In the following hundred years they were

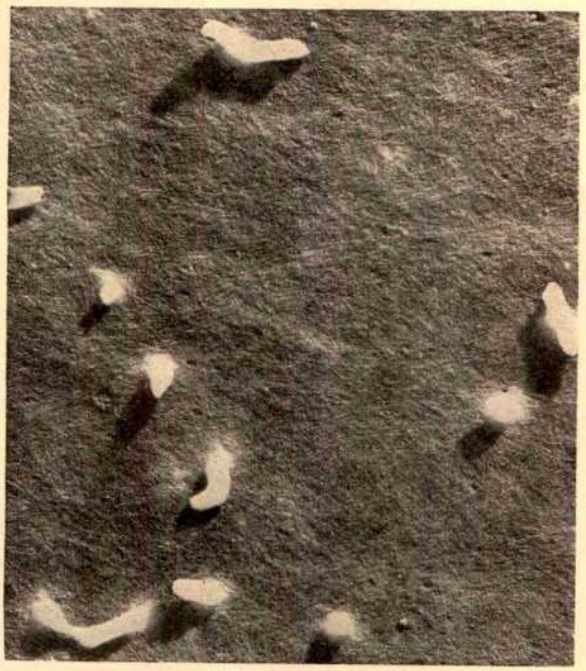


Fig. 1. Rhizoplane of mustard (*Brassica nigra*) root from garden soil; electron micrograph (*c.* × 19,200). Part of outer epidermal wall of non-piliferous cell showing microfibrillar network with clearly defined pits and bacteria *in situ*. The thin coating of mucilage has been removed during ultrasonic treatment. Bacteria may be attached in or near pits (ref. 12)

reported by many other observers, but were apparently ignored in text-books. To-day, as seen under the electron microscope, epidermal pits are similar in structure to those of internal cell walls.

The nature of the strands in epidermal pits, plasmodesmata or ectodesmata, is at present a topic of lively discussion[23]. Whatever the result of the present-day controversy, the strands presumably furnish the principal path for the transport of the precursors of surface materials, cutin and wax in the shoot and cutin and mucilage in the growing root.

Haberlandt noted that the wounding of living tissues in air stimulates the formation of traumatic acid and the deposition of a film of suberin on the wounded surface[15]. Priestley demonstrated experimentally that suberin deposition depends on the presence of oxygen[24]. The breaking of plasmodesmatal strands during the formation of intercellular space is virtually a wounding of the protoplast. The deposition of the lipid lining of intercellular space already evident in the meristem may, therefore, be considered a wound reaction.

The deposition of a lipid layer in the inner integument of the seed of *Cercidium* has been noted. It is suggested that this deposition may depend on the presence of an oxygen gradient in the mucilaginous mass. An investigation of other mucilaginous tissues in which at no time during development is air observed in intercellular space should prove interesting.

Thus, in conclusion, in all plants which have been examined, pits are present in the epidermis of all shoots and roots, including the root hairs. Retraction of plasmodesmata into the cellulosic layer of the cell wall probably results from the shock inevitable during the preparation of material for examination. In the isolated shrunken elastic cuticle, cuticular pores do not remain visible but their previous location may be indicated by the ubiquitous thinner areas evident in electron micrographs. Formation of intercellular space entails the breaking of the plasmodesmata and is therefore a wounding of the protoplast. Lipid deposition on the surface of intercellular space is the first visible reaction to wounding. Deposition of lipid in the mucilaginous mass in the inner integument of *Cercidium* may depend on the presence of an oxygen gradient.

The occurrence of pits in the outer wall of the growing root may indicate the preferred points of entrance for bacterial plant pathogens as seen in the electron micrograph (Fig. 1).

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INSULIN-LIKE EFFECTS OF ADENOSINE-3′,5′-MONOPHOSPHATE ON RAT STRIATED MUSCLE

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RECENT investigations[1–5] suggest that adenosine-3′,5′-monophosphate (cyclic AMP) acts as a cellular mediator involved in the tissue response to a number of hormones, including catecholamines, glucagon, adrenocorticotrophic hormone and vasopressin. It is clear that cyclic AMP may produce alterations in the activity of intracellular enzyme systems[1,6,7], but the steps correlating intracellular accumulation of the nucleotide with the cellular response to the hormone are not known.

This article describes two insulin-like effects of cyclic AMP on striated muscle: increased glucose uptake and decreased extracellular space. These effects are discussed in terms of ultrastructural alterations recently observed to occur under various physiological conditions.

Glucose-uptake experiments. Diaphragms from 230–250-g female Sprague–Dawley rats were rapidly removed and dissected in halves. One member of the pair of hemidiaphragms served as control for the other. The hemi-

diaphragms were placed in 20-ml. conical flasks containing 1 ml. of freshly gassed (oxygen–carbon dioxide, 95 per cent : 5 per cent) bicarbonate-buffered media[8] containing glucose 2·5 mg/ml., 1 unit insulin/ml., 10^{-3} M cyclic AMP (final concentration), 10^{-3} M 5′ AMP, or an equal volume of buffered media (volume of addition, < 10 per cent of initial volume); the flasks gassed again, stoppered, and shaken in a water bath (T = 37° C, 144 c/m). After 1 h the tissues were removed, blotted and weighed. Glucose determinations on the incubation bath before and after incubation were obtained by the glucose oxidase method[9].

Extracellular space determinations. Rat femoral musculature (intact limbs with skin and surface connective tissue removed; approximate weight, 10 g) was placed in 14 ml. of gassed incubation media (*vide supra*) containing 1 μc./ml. carboxyl-^{14}C-inulin (New England Nuclear Corporation: specific activity = 3·57 μc./mg). The limbs were gassed and shaken continuously for 1 h at 37° C in media containing 1·5 mg/ml. of glucose but otherwise identical with that used in the glucose-uptake experiments. Cyclic AMP was added to the bath containing one of the limbs to a final concentration of either 10^{-3} M or 2×10^{-3} M. The bath containing the paired limb received a volume of buffered media equal to that of the cyclic AMP solution but was otherwise untreated and therefore served as a control. Following incubation, the tissues were dipped three times in three 14-ml. solutions of incubating media free of labelled inulin, dissected free of fat, connective tissue and bone, and then the muscle was thoroughly minced. The mince was weighed and made to a 10 per cent (w/v) mixture with 0·25 M sucrose and homogenized in glass tubes with loose- and then tight-fitting pestles. Portions (0·01–0·05 ml.) of bath or tissue homogenate were placed in glass scintillation vials containing 14 ml. of scintillation fluid and counted (efficiency approximately 80 per cent) in a liquid scintillation counter. (The scintillation fluid consisted of: 8·25 g 2,5-diphenyloxazole, 0·103 g 1,4-*bis*-2-(5-phenyloxazolyl)-benzene, 113 g naphthalene, 320 ml. toluene, 40 g 'Cab-O-Sil', all made to one litre with 'spectroquality' *p*-dioxane. The chemicals listed were purchased from the Packard Instrument Co., La Grange, Illinois, with the exception of the *p*-dioxane which was purchased from the Matheson, Coleman and Bell Co., Cincinnati, Ohio.) The extracellular volume was calculated as the ratio of the inulin concentration in the homogenate to the final concentration of inulin in the bath.

Rat hemidiaphragms exposed to 10^{-3} M or 2×10^{-3} M cyclic AMP *in vitro* take up significantly ($P < 0{\cdot}001$) more glucose from a glucose-containing medium (initial concentration 250 mg per cent) than do paired untreated (control) hemidiaphragms. On the other hand, adenosine-5′-monophosphate (5′-AMP) does not appear to increase significantly ($P = 0{\cdot}3$) the uptake of glucose by the rat hemidiaphragm. Similar experiments conducted with insulin (medium concentration 1 U./ml.) show that the glucose uptake of the insulin-treated hemidiaphragms is significantly ($P = 0{\cdot}05$) greater than the glucose uptake of the cyclic AMP-treated hemidiaphragms (Table 1, Figs. 1 and 2). Although further increase in the concentration of cyclic AMP in the experimental medium failed to increase glucose uptake, the possibility remains that the quantitative difference between the effect of insulin and that of cyclic AMP may be due in part to the relatively low permeability of tissues to cyclic AMP (refs. 1 and 10).

Table 1. EFFECT OF INSULIN AND CYCLIC AMP ON GLUCOSE UPTAKE IN RAT DIAPHRAGM: GLUCOSE UPTAKE IN MG/G/H ± STANDARD ERROR

Control	Insulin	C-AMP	5′-AMP
3·40 ± 0·1 (22)*	5·20 ± 0·33 (9) Insulin versus control $P < 0{\cdot}001$	4·51 ± 0·15 (9) C-AMP versus control $P < 0{\cdot}001$	3·82 ± 0·22 (4) 5′-AMP versus control $P = 0{\cdot}3$
		Insulin versus C-AMP $P = 0{\cdot}05$	

* Numbers in parentheses represent the number of hemidiaphragms tested.

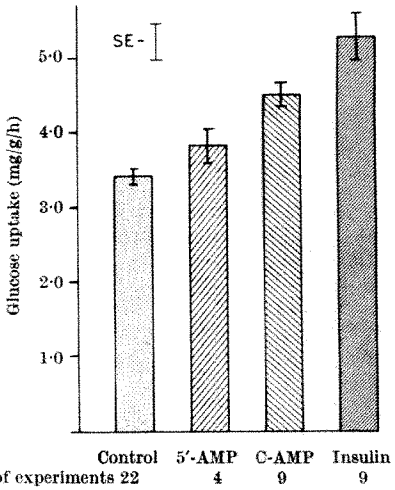


Fig. 1

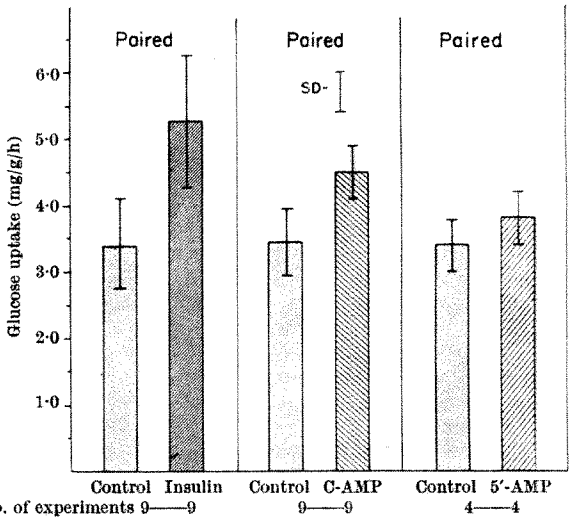


Fig. 2

The inulin space of rat femoral muscles treated with cyclic AMP was less than that of paired control femoral muscles (Table 2). The wide variation in the diminution of the extracellular space observed with cyclic AMP may be due in part to differences in the ratio of surface to volume of muscle mass which would affect the extent of penetration of cyclic AMP into the muscle. Creese and Northover[11], Fritz and Knobil[12], and Randle and Smith[13] have shown previously that insulin decreases the inulin space in the rat diaphragm and that this effect may be correlated with other parameters of insulin action.

Table 2. INULIN SPACE (VDIN) IN FEMORAL MUSCULATURE TREATED WITH 2 mM CYCLIC AMP COMPARED WITH INULIN SPACE IN PAIRED CONTROL FEMORAL MUSCULATURE

Experiment	$\frac{\text{VDin of treated tissue}}{\text{VDin of control tissue}}$
1	0·7
2	0·5
3	0·5
4	0·4
5	0·8

Cellular ultrastructural components thought to be involved in extra-to-intracellular translocation of substances have recently been under intensive study. Electron microscopic investigations of striated muscle reveal the transverse tubule (central element of the triad) as an invagination of the sarcolemma[14,15]; the lumen of this tubule, therefore, is continuous with the extracellular space[16,17]. There is evidence that the transverse tubule of the triad functions as a direct channel for the internal passage of electrolytes, metabolites and depolarizing currents[18–20]. This transverse tubular system, which appears to be similar to the 'special region' of Hodgkin

and Horowitz[22] and the 'intermediary space' of Adrian and Freygang[23], possesses the faculty of altering its capacity by dilation[24–26].

It has been shown that the transverse tubules swell when the muscle cell membrane is hyperpolarized by chloride ion withdrawal procedures or by electrical stimulation[24–26]. If swelling of the transverse tubules is thus generally associated with hyperpolarization, the demonstration by Zierler[27,28] that insulin hyperpolarizes the muscle cell membrane raises the possibility that the action of insulin on cell membrane transport involves not only the sarcolemma but also its extensions into the triadic system (transverse tubules and terminal cysternae of the sarcoplasmic reticulum). This speculation is an extension of that raised by Krahl[29], who suggested that insulin may act on the cytoskeleton of Peters[30], and by Hechter[31], who suggested that both insulin and ACTH may act on the endoplasmic reticulum of their target cells.

The finding that both cyclic AMP and insulin diminish the inulin space of muscle without changing the total water content of the tissue would be expected if the triadic system and the muscle cell were swelling at the expense of the extracellular space. The evidence for almost exclusive localization of labelled insulin at the sarcolemmae and in the soluble fraction of the muscle cell[32,33] is in accord with the idea that the hormone may be acting on the transverse tubular invaginations of the sarcolemmae.

In considering the possibility that the insulin-induced glucose transport in muscle involves the mediation of cyclic AMP it must be emphasized that, before cyclic AMP can be established as a mediator of insulin action, effects on other known parameters of insulin action must be tested, the relationship of epinephrine and cyclic AMP in muscle must be clarified, and direct tissue analyses for cyclic AMP must be carried out.

In summary, cyclic AMP has been found to increase the glucose uptake of the rat diaphragm and decrease the extracellular space of striated muscle, but its effects on other parameters of insulin action have not yet been investigated. The possibility has been raised that insulin may attach and act on the transverse tubular extensions of the sarcolemmal membrane, swelling the transverse tubular system and cell and diminishing the interstitial space—effects which would stretch the sarcolemma and expand pathways by which water, glucose and amino-acids could enter the cell. A hormonally sensitive, possibly contractile, system of this type involving the membrane systems of the cell as a whole could also account at least in part for the known effects of insulin on protein synthesis by facilitating translocations of substrates within the cell.

Crystalline insulin, Lot 795372, was a generous gift of Dr. O. K. Behrens of the Eli Lilly Co., Indianapolis, Indiana. 3′,5′-Cyclic adenosine monophosphate (cyclic AMP) and 5′-adenosine monophosphate (5-AMP) used as the sodium salts were obtained from the Sigma Chemical Co., St. Louis, Missouri.

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AN APPROACH TO THE ORGANIZATION AND CLASSIFICATION OF VERTEBRATE VIRUSES

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RECENTLY, Lwoff *et al.*[1] attempted to arrange viruses into an orderly system using as a basis the definition that a virus is a biological entity which exhibits in its life-cycle an infectious particle containing only one type of nucleic acid. In order to classify all viruses which replicate in vertebrates, insects, plants and bacteria, two characteristic features of virions were used: (1) the chemical constitution of the genetic material DNA or RNA; (2) the architecture of the viral capsids. These authors also suggested that other properties of the viral nucleic acids and proteins could be used for further subdivisions.

In contrast to the system of Lwoff *et al.*[1], a more recent attempt at virus classification by the Provisional Committee for Nomenclature of Viruses[2] makes use of the Linnaean system. However, the basic approach to virus characterization is similar to that of Lwoff *et al.*, in so far as the viruses are divided into two sub-phyla according to whether they contain DNA or RNA and then further sub-divided into classes and orders according to the nature and characteristics of the viral coats.

The importance of the viral genetic material as the carrier of the genetic information has already been stressed by Crick and Watson[3]. It is the nucleic acid which controls, during the replicative cycle of the virus in the host cell, the structure of the proteins of the viral coat, which provides a protective shell for the viral nucleic acid. In addition, conditions have been developed for the use of naked viral nucleic acid molecules as infectious material[4]. The existence of a virus as a naked nucleic acid[5] defective in regard to its ability to produce coat protein emphasizes the inadequacy of the use of

virus morphology for the purpose of classification. Moreover, the viral coat contains only a few of those proteins coded by the viral genomes, and in several virus groups components specific to the host cell are incorporated into the viral coats and determine their morphology. In addition, the coating of the viral nucleic acid molecules, which is the last step in virus maturation, is not always an efficient process[6].

In contrast to the use of coat architecture, the physical properties of the viral nucleic acids may provide a better tool for classification. The two types of nucleic acids, DNA or RNA, which appear in viruses could be further characterized according to the nature of their polynucleotide chain, either double stranded or single stranded which can attain a linear or circular form. The viral genome contains all the information necessary for its replication and determines the fate of the host cell. With the aid of cellular mechanisms, the viral genomes transcribe their genetic information. The viral nucleic acids are generally stable, except for mutational changes, and retain their full complement of genetic material through many cycles of replication. The amount of information encoded in the virus genome is related to the molecular size of the viral nucleic acid[7].

The present approach to the organization and classification of vertebrate viruses uses the viral nucleic acids as the most characteristic component of the virus which controls all other phenotypic viral properties. As shown in Table 1, the viruses are divided into two major groups, as already suggested[1]: (1) DNA viruses; (2) RNA viruses. The next step in the organization is the division of each of the major groups into two sub-groups according to the physical properties of the viral nucleic acids: (*a*) double-stranded molecules; (*b*) single-stranded molecules. Each is further sub-divided into: (i) linear; (ii) circular forms. The further division is according to the molecular weight of the specific nucleic acid.

It should be stated that the use of the molecular shape of the nucleic acids (linearity or circularity of the double-stranded DNA or RNA molecules) is based on analytical and morphological properties of molecules which are sensitive to shearing after extraction from the virions. The development of more accurate techniques for the preservation of the extracted molecules will make possible a reconsideration of the physical characteristics of viral nucleic acids.

As indicated in Table 2, further characterization and definition of the different vertebrate virus groups are based on: (1) The phenotypic expressions of the viral genomes, (*a*) the structure and the shape of the viral coats, and (*b*) the general morphology of the virions as viewed in the electron microscope. (2) It is also suggested that the cellular site of viral nucleic acid replication be included as a criterion of the different virus groups. The site of virus replication might indicate the nature of the cellular mechanisms utilized by the viruses for replication and maturation. The nomenclature of the different virus groups is the same as that used by Andrewes[8] and as recently reviewed[7,9–11].

A further division of each group into sub-groups and species can be effected on the basis of: (1) the differences in the molecular weights of the nucleic acids of different virus strains; (2) the guanosine + cytosine content in the viral nucleic acids; and (3) differences in the chemical compositions o viral protein coats and envelopes.

The advantages of the proposed scheme are: (1) the group of vertebrate viruses at present known can be distinguished from each other by the nature of their nucleic acids; (2) it will be possible to classify viruses which lack distinct coats; (3) the characteristics will be indicated of viruses not yet known to replicate in vertebrates but known to exist in other cellular systems, for example,

Table 1. PROPOSED SCHEME FOR THE ORGANIZATION OF VIRUSES BASED ON THE PROPERTIES OF THE VIRAL NUCLEIC ACID

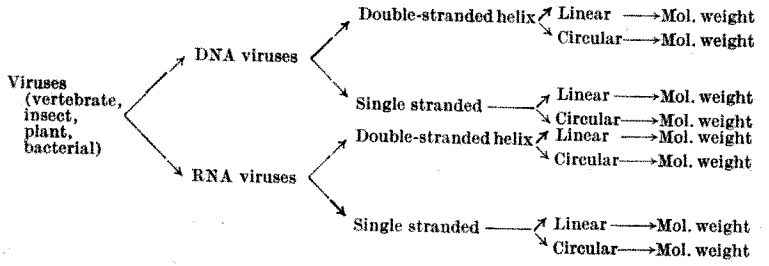


Table 2. PROPOSED SCHEME FOR THE CLASSIFICATION OF VERTEBRATE VIRUSES*

Nucleic acid	Physical properties		Mol. wt. (daltons)†	Virus coat: Protein	Virus coat: Lipid	Virus morphology: Symmetry	Virus morphology: Size (mμ)	Replication site: Synthesis	Replication site: Maturation	Virus group	
DNA	Double-stranded helix	Linear	160×10^6 (12, 13)	'Core'	Lipoprotein coat (viral)	Helical	250 × 300	Cytoplasm	Cytoplasm	Poxvirus	
			70×10^6 (14)	162 cylindrical capsomeres	Membrane (nuclear origin)	Cubical	75	Nucleus	Nucleus and cytoplasm	Herpes virus	
			23×10^6 (9, 15)	252 capsomeres	None	Cubical	70–90	Nucleus	Nucleus	Adenovirus	
		Circular	4×10^6 (16)	42 or 92 capsomeres	None	Cubical	45 (mean)	Nucleus	Nucleus	Papovavirus	
	Single stranded	Linear	Not yet described								
		Circular									
RNA	Double-stranded helix	Linear	8×10^6 (17)	92 cylindrical capsomeres	None	Cubical	60–90	Cytoplasm (mitotic apparatus)	Cytoplasm	Reovirus	
		Circular	Not yet described								
	Single stranded	Linear	10×10^6 (18)	Nucleoprotein	Membrane (cytoplasmic origin)	Spheroid	72 (Nucleoid 30)	Cytoplasm	Cytoplasm	Avian leucosis, sarcoma	
			10×10^6 (19)	Nucleoprotein	Membrane (cytoplasmic origin)		80–110	Cytoplasm	Cytoplasm	Mouse leukaemia	
			$2–3 \times 10^6$ (20, 21)	Nucleoprotein	Membrane (cytoplasmic origin)	Helical	60–200	Nucleus and cytoplasm	Cytoplasm (cell membrane)	Myxovirus	1
								Cytoplasm only			2
			2×10^6 (22)	Nucleoprotein	Membrane (cytoplasmic origin)	Helical (24)	20–100	Cytoplasm	Cytoplasm (intracytoplasmic membrane)	Arbovirus	
		Circular	2×10^6 (23)	42 capsomeres	None	Cubical	27	Cytoplasm	Cytoplasm	Picornavirus	
			Not yet described								

* The nomenclature and properties of the different virus groups are according to Andrewes (ref. 8) and recent review articles (refs. 7, 9–11).
† Numbers in parentheses refer to references.

single-stranded DNA bacteriophages[25]; (4) every new virus isolate will be classified on the basis of the nature of its nucleic acid. The proposed scheme (Table 1) could also be used for classifying viruses which replicate in insects, plants and bacteria, for example:

(1) Viruses containing double-stranded, linear DNA molecules: (*a*) Insect viruses: molecular weight 156 × 10^6 daltons (ref. 13)—tipula iridescent; 76 × 10^6 daltons (ref. 13)—nuclear virus from *Bombyx mori*. (*b*) Bacteriophages: 130 × 10^6 daltons (ref. 25)—T_2 phage.

(2) Viruses containing single-stranded, circular DNA molecules: Bacteriophages: 2 × 10^6 daltons (ref. 26)—φ × 174.

(3) Viruses containing double-stranded, linear RNA molecules: Plant viruses: 10 × 10^6 daltons (ref. 17)—wound tumour virus of clover.

(4) Viruses containing single-stranded, linear RNA molecules: (*a*) Plant viruses: 2 × 10^6 daltons (ref. 27)—tobacco mosaic; turnip yellow mosaic. (*b*) Bacteriophages: 1·3 × 10^6 daltons (ref. 28)—f_2.

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EFFECTS OF 6-DAY MATERNAL DEPRIVATION ON RHESUS MONKEY INFANTS

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BOWLBY[1] concluded that if a young child is deprived of maternal care for a prolonged period, there may be far-reaching effects on his future development. His views, based in part on earlier work[2–4], have given rise to much research and some controversy. While his basic thesis is well supported[5], it opens up many problems, such as measuring the extent of the effects produced by maternal deprivation, assessing their irreversibility, and understanding the mechanisms involved.

Research on the effects of temporary maternal deprivation has depended largely on retrospective studies of individuals who have had a separation experience in the past[6], and on studies of the immediate consequences of maternal deprivation[7–9]. For ethical reasons, experimental studies of maternal deprivation in human subjects are out of the question, although a few workers have taken advantage of an already existing condition to provide experimental alleviation[10,11]. Control of the deprivation circumstances, or of the post-deprivation conditions, has thus nearly always been impossible, and retrospective investigations are sometimes open to the criticism of possible bias (genetic or otherwise) in sample selection.

Can the symptoms of maternal deprivation seen in man be studied experimentally in animals? Monkeys are an obvious possibility, since adult behaviour is known to be markedly affected by the social conditions of rearing[12], and immediately following brief periods of separation the pattern of mother–infant interaction is disturbed[13,14]. Although gross species comparisons can be misleading, whether or not maternal deprivation produces similar consequences in monkeys and men is an empirical question.

Accordingly an attempt has been made to study the effects of a period of maternal deprivation on the subsequent behaviour and mother–infant interaction of rhesus macaques. In the present experiment the age at separation was 30–32 weeks, and the period of separation 6 days. Four infants were used, special care being taken to assess their individual pre-separation characteristics, and to study their behaviour not only immediately the mother was returned, but for several months afterwards.

The animals concerned lived in groups each consisting of a male, two or three females and their young. Each group was housed in an outdoor cage 18 ft. × 8 ft. × 8 ft. communicating with an inside room 7·5 ft. × 6 ft. × 4·5 ft. (ref. 15). Each infant was watched for 6 h a week (birth to 8 weeks) or a fortnight (weeks 9/10–30) to compare mother–infant relations with those of eight control infants studied previously which had no separation experience. On the three pre-separation days each mother–infant pair was watched from 1000 h G.M.T. until the infant had been off the mother for 2 h, or until 1430 h, whichever was the earlier. Just before 1000 h on day 4 the mother was caught and placed out of sight of the infant in a room about 60 yd. away. Further watches were made immediately the mother was removed (day 4, or first day of separation) and on days 5, 7 and 9, when the mother was returned on day 10, and on days 11, 12, 14, 16, 23, 30 and 37. Routine watches were also made for 6 h a month for the rest of the first year.

The method of observation was similar to that already described[16]. In addition, during the pre-separation, separation and post-separation periods (that is, days 1–37) a second observer recorded the amount of locomotor activity (that is, the number of sectors of the cage entered in each half-minute period), and the type of activity shown, while the infant was off its mother. Only certain parameters of the infants' behaviour which indicate the more obvious consequences of the deprivation experience will be considered here: a more detailed analysis is in preparation.

During the pre-experimental period, the behaviour of these four infants was generally similar to that of the control subjects living under similar conditions previously studied: if anything, they spent on average rather

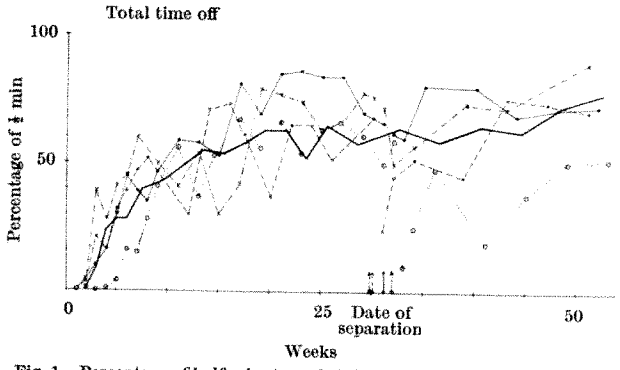


Fig. 1. Percentage of half-minute periods in which infants were recorded off their mothers. Thick line—median for eight control animals which had no separation experience. Linda, —·—×; Doris, ——●; Hilary, ——○; Tim, ····○

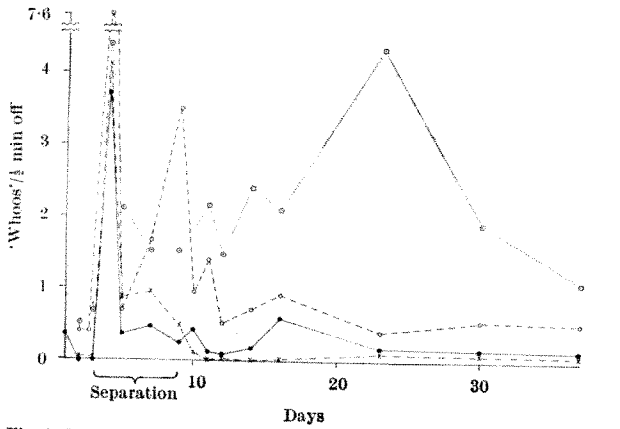


Fig. 2. Frequency of 'whoo' calls while off mother during pre-separation, separation and post-separation periods. Conventions as in Fig. 1

more time off their mothers (Fig. 1), and a higher proportion of that time at a distance from their mothers, than the median for the controls. During the pre-separation watches the inter-individual differences between the four subjects can be summarized as follows. Ranking the individuals with respect to one another, the amount of time spent off the mother decreased in order from Linda, Doris, Hilary to Tim: this was due to the behaviour of the infants rather than to restrictiveness by the mothers. Linda was an active infant who spent frequent short periods away from her mother, during which she did not spend long at a distance from her. The others spent less time at a distance from their mothers in the order Doris, Hilary, Tim: all had higher scores in this respect than Linda, but showed (in the same order) increasing scores of social and manipulative play and locomotor activity.

During the separation period, the infants directed some of the behaviour which they had previously directed towards their mothers towards other adults in the group, but they received much less care from these than they had previously from their mothers. For example, before separation they were recorded on their mothers in 41–53 per cent of half-minute observation periods, and on other adults for less than 1 per cent, but during the separation they were on other adults for only 0–24 per cent (mean 17 per cent).

When the mother was removed there was an immediate increase in 'whoo' calls—a call characteristically given by an infant separated from its mother[17]. The rate of calling fell off somewhat during the rest of the separation period, but in all four subjects was still higher than the pre-separation mean 4 weeks after the mothers' return (Fig. 2).

During the mother's absence, the behaviour of the infants can be described as 'depressed'. They usually sat in a characteristic hunched posture, and crept about on their hind legs. Almost immediately after the mothers' removal they became less active than they had been previously when off their mothers (Fig. 3). During the course of the separation period all four infants became active for more of the time (that is, they sat still less), but they became progressively less active when not sitting still. Three of the four infants returned to the pre-separation level of activity within a week of the return of their mothers, but Tim showed less activity on every watch up to 4 weeks afterwards. The amount of social and manipulative play shown changed in a manner generally similar to that of general locomotor behaviour.

On the first day of the return of their mothers, all four infants spent a high proportion of their time on her (Fig. 4). The more they had been off the mother before separation, the less clinging were they on her return, and the more quickly did they return to behaviour similar to that seen before separation. Thus Linda spent only a little more time on her mother than before separation, but Tim scarcely left his mother on the first day of her return. Similarly, while Linda and Doris were leaving their mothers almost as much as before separation within a week of the return of their mothers, in Hilary and Tim the effects persisted for a month or longer. When Tim was a year old he was still off his mother less than he had been before separation, although the median for the control animals was increasing during this time (Fig. 1).

In all four infants the amount of time spent off the mother recovered in the first few days after the mothers' return, but then regressed for a while, the permanent recovery occurring more slowly (Fig. 4). The reason for this is not apparent: although the proportion of attempts by the infant to gain the nipple which were rejected by

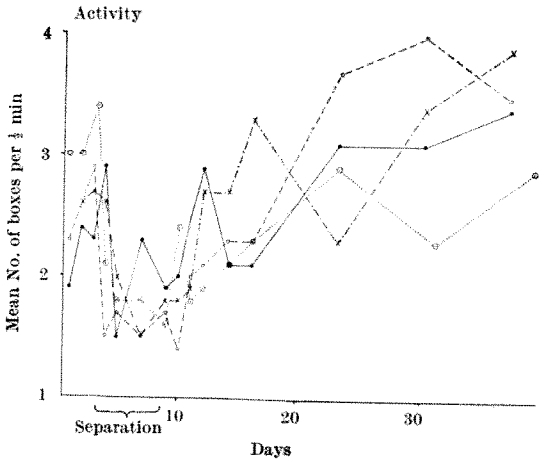


Fig. 3. Activity changes during the pre-separation, separation and post-separation periods. The cage was divided into sixteen 'boxes', and activity measured by the number of boxes entered per half minute while off the mother. Conventions as in Fig. 1

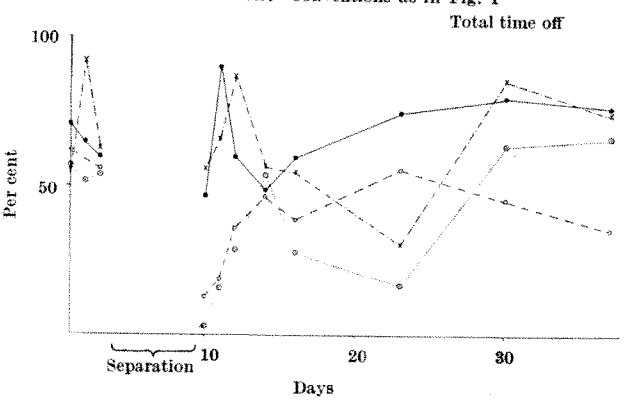


Fig. 4. Percentage of half-minute periods in which infants were recorded off mothers during pre-separation, separation and post-separation periods. Conventions as in Fig. 1

the mother varied directly with the amount of time spent off the mother both during the pre-separation period and later in the post-separation period, it did not do so during the period in which this initial recovery and subsequent temporary regression occurred.

Not only did the infants leave their mothers less often during the post-separation period, but they went less far from her when they were off her. On this measure, all four infants recovered to their pre-separation levels about a fortnight after the return of their mothers.

These data show that some rhesus infants of this age are adversely affected by a 6-day removal of their mothers, that the severity of the effects varies with the nature of the pre-separation mother–infant relationship and that they may persist for at least some weeks after the return of the mother. The consistency between individuals is adequate for conclusions of some generality to be drawn, but the individual differences are also revealing. The symptoms shown by the infants parallel in many ways those of human infants undergoing a similar experience, with the exception that none of the monkeys showed a phase of detachment from the mother on her return, similar to that described in children[8,18].

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ESTIMATION OF THE TOTAL HYDROGEN CONTENT OF THE HUMAN BODY

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METHODS are now available for the estimation of several important body constituents by non-destructive testing which can be applied to the living subject. Total-body water can be determined by the method of isotopic dilution using deuterium oxide or tritium oxide as the tracer. Estimates of the 'exchangeable' fractions of total sodium and potassium can also be obtained by this method using radioactive tracers, and determination of the total potassium content by measurement of the γ-radiation from the naturally radioactive isotope potassium-40 is a well-established technique, the applications of which are increasing. Recently the application was described of body radioactivity measurement to the estimation of total-body sodium, chlorine and even calcium following neutron activation *in vivo*[1]. This article describes a method of estimation of the total hydrogen content of the body. The method is based on the emission of 2·23 MeV γ-rays when hydrogen atoms capture thermal neutrons; the γ-rays are measured by scintillation spectrometry with thallium-activated sodium iodide. We have investigated the possibility of using cosmic radiation as a source of fast neutrons which are first moderated by, and then captured in, the body. The use of lead for the low-background shield for the measurement of the capture γ-rays increases the number of neutrons available, as evaporation neutrons are produced in the lead.

The γ-ray spectra in Fig. 1 illustrate the method. Curve 1 is the background spectrum (0·04–3·34 MeV) of the spectrometer, which uses four crystals of thallium-activated sodium iodide, each 6 in. in diameter by 3·5 in. thick. Curve 2 is the background spectrum when a 66-kg phantom was placed on the stretcher to simulate a human. The phantom[2] consisted of right circular and elliptical cylinders of polythene filled with 54 kg of distilled water, and it contained 7·8 kg of hydrogen. The two curves are very similar between channel numbers 17 and 130 (corresponding to approximate γ-ray energies of 210 keV and 1·7 MeV), but below channel 17 there is a marked increase in the counting-rate due to γ-rays which have been scattered and degraded by the phantom. Between channels 130 and 190 there is another increase with a well-defined peak at channel 170 (2·23 MeV). We have

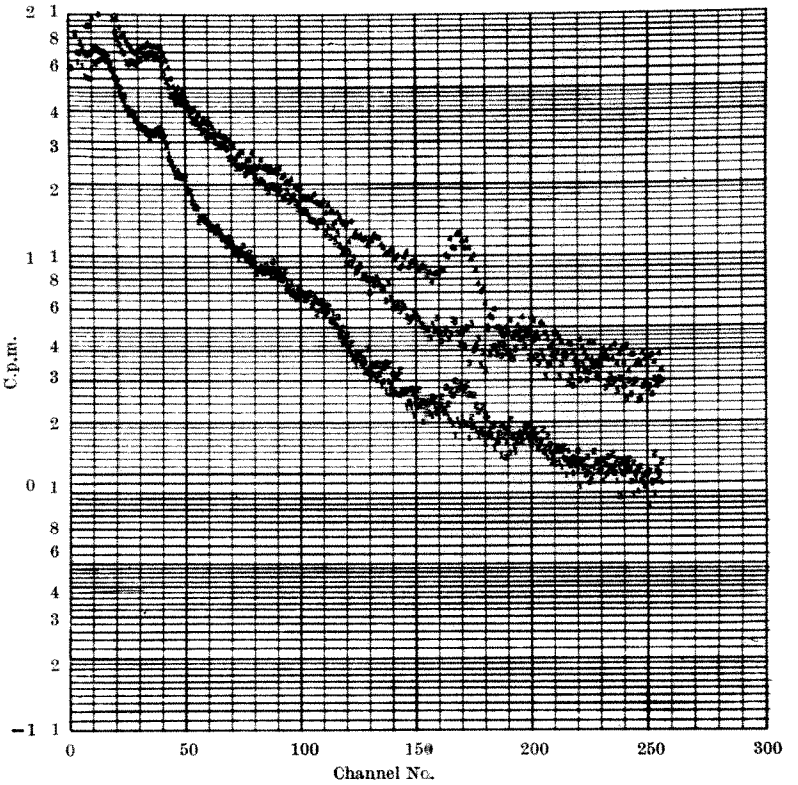


Fig. 1. Computer-produced plot of γ-ray spectra showing the effects on the spectrometer background (curve 1) of placing the polythene and water phantom on the stretcher (curve 2) and of a heavily-shielded 2-mc. radium-beryllium neutron source 6 ft. from the mid-point of the stretcher, without (curve 3) and with (curve 4) the phantom. The numbers −1, 0, 1 and 2 to the left of the vertical scale identify the powers of ten at the respective scale points

proved that this is due to neutrons (*a*) by investigating variations in the counting-rate in the peak at different barometric pressures and (*b*) by measuring the background spectrum with a 'phantom' composed of 63 kg polytetrafluoroethylene (PTFE) which contains no hydrogen. The results of (*a*) showed that the counting-rate in the 2·23-MeV peak varied from about 14 c.p.m. at a pressure of 716 mm mercury to 8·5 c.p.m. at 765 mm mercury. The coefficient was approximately −10 per cent per cm of mercury, in agreement with the coefficient for the neutron and neutron-producing components of cosmic rays[3]. The results of experiment (*b*) showed that while the PTFE phantom produced an increase in counting-rate at low energies (below channel 17) there was no increase at higher energies and no trace of a peak at 2·23 MeV. The results of both experiments showed that the peak was due to cosmic-ray neutrons reacting with the hydrogen in the polythene and water phantom, and not to some unidentified long-lived radioactive contaminant in the phantom.

Gamma-ray spectra taken with humans on the stretcher of the equipment also showed the peak at 2·23 MeV, and we have attempted to estimate the total-body hydrogen content. Because the intensity of the neutrons varied markedly with barometric pressure, two measurements of background were made each day, one with and one without the polythene and water phantom. The second of these was used as the counter background for the measurements of the areas of the peaks in the spectra from the subjects and from the phantom. In effect the latter was used as a measurement of the 'source strength'.

Calibration. The equipment was calibrated roughly to take account of the effects of variations in body size, which affect both the moderation of the fast neutrons and the number of capture γ-rays produced, as well as their self-absorption in the body and the geometrical efficiency for their detection by the scintillation counters. In order to increase the counting-rate for the calibration measurements a 2 mc. radium-beryllium fast neutron source was placed about 6 ft. from the centre of the stretcher, outside the 4-in. lead shield and with an additional 6 in. of lead shielding around it. The effect of this source on the background γ-ray spectrum (an increase by a factor of 2–2·5) is shown by curve 3 of Fig. 1, while the increased counting-rate when the phantom was placed on the stretcher is shown in curve 4. The area under the 2·23-MeV peak was increased ten-fold by the additional neutron source. Measurements were made of the peak area (c.p.m.) for a number of phantom sizes and shapes, with various configurations of the component parts of two phantoms (total weights 66 kg and 84 kg). The counting-rates per kg of hydrogen were plotted as a function of phantom weight, with the counting-rate from the 66 kg phantom normalized to that due to cosmic-rays alone at an average barometric pressure of 755 mm mercury. The calibration factor (c.p.m. per kg of hydrogen) varied from 1·53 to 1·07 for phantom weights in the range 40 to 84 kg.

Calculation of body hydrogen. After subtraction of the counter background the counting-rate in the energy range 2·08–2·35 MeV (channels 161–181) from each subject's γ-ray spectrum was corrected to correspond to a barometric pressure of 755 mm mercury. The hydrogen content was then calculated from the appropriate calibration factor for the subject's weight. Table 1 shows typical counting-rates with their statistical standard errors for counting times of 2^{11} sec (about 34 min) for the subject and 2^{13} sec (~ 136 min) for the background.

From the last two entries in Table 1, the hydrogen content is 6·7 ± 1·1 kg (9·5 ± 1·6 per cent of the body-weight). The hydrogen contents of twenty-seven adult males and of ten adult females were calculated in this way and the average concentrations were 9·47 per cent of body-weight for the men and 8·65 per cent of body-weight for the women. These values are similar to, although a little lower than, the value of 10 per cent quoted by Hawk *et al.*[4] and adopted by the International Commission on Radiological Protection[5]. However, there is evidence that there may be a systematic error on our estimates of body hydrogen. Thus, the 63 kg PTFE phantom reduced the background in the 2·23-MeV peak region by about 1 c.p.m., while above the peak there was a decrease of 4 c.p.m. in the energy range 2·51–3·34 MeV. These effects were presumably due to absorption of cosmic radiation or γ-rays produced by cosmic rays. On the other hand, the polythene and water phantom increased the background (79·6 c.p.m.) above the 2·23-MeV peak by 2·4 c.p.m. The difference between these two effects on the counting-rate may be explained, at least in part, by the fact that the neutrons produce an increase in the background when the polythene and water phantom is present. This effect is well demonstrated by curves 3 and 4 of Fig. 1; with the neutron source present the counting-rate in the energy range 2·51–3·34 MeV increased from 210 c.p.m. to 261 c.p.m. when the phantom was placed on the stretcher, presumably due to γ-rays produced as a result of inelastic scattering of the neutrons by oxygen atoms, as well as some produced by capture. Thus the true background in the region of the 2·23-MeV peak may be indeterminate because the neutron-produced increase (other than that due to hydrogen capture γ-rays) is masked by the decrease due to absorption of electromagnetic radiation.

From a careful investigation of our data, we conclude that there might be a systematic error, probably not exceeding 1 c.p.m. in the background counting-rate at 2·23 MeV, which would correspond to about 1 kg of hydrogen.

At present, knowledge of the hydrogen content of the human body appears to have no major application, although it is of interest that it can be estimated by body 'radioactivity' measurement without administration of radioactivity or exposure of the subject to extra radiation. Should it be considered important to determine body hydrogen with some precision, this could be done by increasing the neutron flux inside the shielded room as we did for our calibration measurements. This increased the counting-rate in the 2·23-MeV peak by a factor of about ten, while only increasing the background by a factor of 2·3. A dose-rate of 19 μrem/h was measured with a rem counter[6] on the stretcher, with the 2 mc. radium-beryllium source outside the shield. Thus it should be possible to measure the hydrogen content of a subject with a statistical error of less than 5 per cent, with a radiation dose of about 10 μrem of fast neutrons which should be quite acceptable. At the same time the potassium content of the subject could be measured with similar or better precision; from the lean body mass (which may be calculated from the equation[7]:

$$L.B.M. = 0{\cdot}0119\,K + 6{\cdot}04\,SA$$

where K is the potassium content in milliequivalents and SA is the surface area in m^2) the associated hydrogen content can be calculated. The difference from the total-body hydrogen might be useful in some applications, provided it has not too large an uncertainty.

Table 1

	C.p.m. in 2·23 MeV peak
Background	37·0 ± 0·5
Subject plus background (764 mm mercury)	43·7 ± 1·1
Response due to subject	6·7 ± 1·25
Correction for pressure	+0·9
Corrected response	7·6 ± 1·3
Calibration factor (70 kg)	1·14

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OBSERVATIONS ON CAESIUM ENRICHMENT AND THE POTASSIUM/RUBIDIUM/CAESIUM RELATIONSHIP IN ECLOGITES FROM THE ROBERTS VICTOR MINE, SOUTH AFRICA

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THE possibility of either eclogite or peridotite as a constituent of the upper mantle has often been suggested. Quantitative data, particularly on trace elements, are, however, either lacking or often unsatisfactory. We are accordingly undertaking a systematic study of the composition of eclogites and peridotites, with particular reference to their trace-element content. Most samples are from the kimberlite pipes of Southern Africa. Whereas peridotites are a widespread occurrence, the majority of eclogites being studied come from the pipe of the Roberts Victor Mine, Orange Free State.

Considerations of heat flow and the chondritic earth model both indicated that the alkalis would be an interesting group of elements for study. Preliminary investigations made it immediately apparent that the eclogites from the Roberts Victor Mine were enriched in caesium relative to potassium and rubidium, as compared with common igneous rocks. The caesium content of eclogites and peridotites from other localities did not show the same anomalous behaviour. In the light of this discovery the potassium, rubidium and caesium contents of a suite of seventeen eclogites from the Roberts Victor Mine have been determined. Two eclogites from the Jagersfontein Mine, Cape Province, South Africa, and two from the Dodoma Mine, Tanganyika, have been included for purposes of comparison. Five kimberlites from Roberts Victor have also been studied.

Previous accurate work on the alkali content of eclogites from the Roberts Victor Mine is limited to Heier[1], who reports the potassium content of seven specimens. Three of these, together with one sample from Jagersfontein and one sample from Tanganyika, form part of the present collection. Faure and Hurley[2] have reported a single rubidium value of 6·90 p.p.m. for a Roberts Victor eclogite.

Williams[3] suggested that the eclogites had undergone considerable secondary alteration; this conclusion was also reached by Heier[1]. These points are to be borne in mind when discussing the results reported here.

These results have been obtained as follows:

Rock type and locality	Potassium (%)	Rubidium (p.p.m.)	Caesium (p.p.m.)
Roberts Victor eclogite (ref. 17)	Flame	Emission	Emission spectrograph
Eclogite from other localities (ref. 4)	Photometer	Spectrograph	—
Roberts Victor kimberlite (ref. 6)		X-ray fluorescence	Emission spectrograph

Emission Spectrography

The comparatively wide range in concentration of rubidium, caesium and the internal standard potassium, coupled with differences in sensitivity of the analysis lines (potassium 6939 Å; rubidium 7947 Å and 7800 Å; caesium 8521 Å) and the problem of self-absorption, meant that it was not possible to obtain results for all the samples under identical conditions. However, only two sets of conditions were necessary. Synthetic standards were used. *W*–1 was treated as an unknown sample and five of the eclogites were used to correlate the two procedures. All standards and *W*–1 were arced in triplicate, all samples in duplicate. The average values obtained for *W*–1 were 23 p.p.m. for rubidium and 0·79 p.p.m. for caesium, both of which are close to previously reported values. The correlation between the results obtained by the two different sets of conditions was good. The relative deviations obtained using the formula:

$$C = \sqrt{\frac{\Sigma d^2 \text{ as } \%}{2N}}$$

(where C is the relative deviation, d^2 as % is the $\frac{\text{difference between two duplicates} \times 100}{\text{average}}$ and N is the number of samples) were:

Line Å		Run No. 1 (%)	Run No. 2 (%)
Rubidium	7947		
	7800	5·6	5·3
Caesium	8521	8·2	5·3

These figures refer to the eclogites only.

Full details of the spectrographic technique will be given at a later date.

The results of the present work given in Table 1 can be summarized as follows:

(1) *Potassium and rubidium abundance.* (*a*) *Eclogite:* The potassium and rubidium concentrations in the Roberts Victor samples would seem to be greater than the level of concentration in the Jagersfontein and Tanganyikan eclogites. (*b*) *Kimberlite from Roberts Victor:* The potassium and rubidium concentration here is of the order of ten times the level found in the eclogites from the same locality.

(2) *Caesium abundance.* (*a*) *Eclogites:* The caesium concentration of the Roberts Victor eclogites is extremely high (the average is 5·9 p.p.m.). It may be significant that the average caesium content of the kimberlite from the same area is also ~6 p.p.m. The caesium content of the Jagersfontein and Tanganyikan eclogites has not specifically been sought. Indications are, however, that the concentration is less than 0·3 p.p.m., and that the potassium/caesium ratios are greater than 5,000. One further point of interest is the wide range reported in the Roberts Victor eclogites from 0·59 p.p.m. in a sample with a potassium/caesium ratio of 7,320, to 16·4 p.p.m. in a sample with a potassium/caesium ratio of 158. (*b*) *Kimberlite from Roberts Victor:* Owing to the erratic behaviour of kimberlites in the d.c. arc and the possibility of a matrix effect, the caesium values quoted in this article must be taken as indications only. (It should be noted that the quoted relative deviation does not refer to the results obtained for kimberlites.)

(3) *Potassium and rubidium correlation.* A strong correlation is apparent between potassium and rubidium in all the samples analysed (Fig. 1). However, with the data now available, it is not possible to establish whether the relationship is linear (that is, whether a uniform potassium/rubidium ratio holds over the whole range of potassium and rubidium content, or whether there is a slight tendency for rubidium to concentrate relative to potassium. In the latter case, the potassium/rubidium ratio may approach that of the kimberlite, as indicated by the dashed line (Fig. 1). The average potassium/rubidium ratio of the Roberts Victor eclogites (367) is distinctly greater than the average for the kimberlites (246) which is close to the estimated crustal average of 230 (ref. 4).

(4) *Potassium and caesium correlation.* (*a*) *Roberts Victor eclogites:* No correlation is apparent between

Table 1

Rock type	Locality	No.	Potassium (%)	Rubidium (p.p.m.)	Caesium (p.p.m.)	K/Rb	K/Cs
Eclogite	Roberts Victor, South Africa	*RV* 372	0·156	2·9	10·7	534	146
Eclogite	Roberts Victor, South Africa	*RV* 374	0·660	21·7	1·0	304	6,620
Eclogite	Roberts Victor, South Africa	*RV* 377	0·456	11·8	0·63	385	7,270
Eclogite	Roberts Victor, South Africa	*RV* 378	0·259	7·9	16 4	326	158
Eclogite	Roberts Victor, South Africa	*RV* 379	0·395	11·8	8·5	334	464
Eclogite	Roberts Victor, South Africa	*RV* 380	0·338	8·7	6·2	389	549
Eclogite	Roberts Victor, South Africa	*RV* 381	0·164	3·4	0·71	489	2,310
Eclogite	Roberts Victor, South Africa	*RV* 390/4	0·424	8·7	7·2	488	589
Eclogite	Roberts Victor, South Africa	*KRV* 1	0·200	5·5	2·6	364	768
Eclogite	Roberts Victor, South Africa	*KRV* 2	0·310	8·8	3·3	351	960
Eclogite	Roberts Victor, South Africa	*KRV* 3	0·430	16·7	0·59	257	7,320
Eclogite	Roberts Victor, South Africa	*KRV* 4	0·220	7·3	2·4	300	906
Eclogite	Roberts Victor, South Africa	*KRV* 5	0·123	3·5	5·8	354	211
Eclogite	Roberts Victor, South Africa	*KRV* 6	0·750	23·7	4·3	316	1,730
Eclogite	Roberts Victor, South Africa	*KRV* 7	0·220	5·9	8·1	370	272
Eclogite	Roberts Victor, South Africa	*KRV* 8	0·290	7 6	12·8	382	226
		AA 3	0·330	11·1	8·5	297	389
		Average	0·337	9·8	5·9	367	1,817
Eclogite	Jagersfontein, Orange Free State	*JAG* 41	0 138	3·3	—	414	—
Eclogite	South Africa	*JAG* 351	0·094	2·5	—	375	—
Eclogite	Dodoma, Tanganyika	*TAN* 501	0·121	3·8	—	317	—
Eclogite	Dodoma, Tanganyika	*TAN* 1	0·110	2·1	—	513	—
			0·116	2 9	—	405	—
Kimberlite	Roberts Victor	*KRV* 11	3·21	130	8·0	247	4,000
Kimberlite	Roberts Victor	*KRV* 12	2·95	113	4·5	*261*	*6,600*
Kimberlite	Roberts Victor	*KRV* 13	1·67	73	—	229	
Kimberlite	Orange Free State	*KRV* 14	3·26	13·5	6·5	241	4,900
Kimberlite	South Africa	*KRV* 15	2·95	121	4 2	244	7,100
Kimberlite	South Africa	*KRV* 16	3·37	121	6 8	253	5,000
		Average	2·92	116	6 0	246	5,400

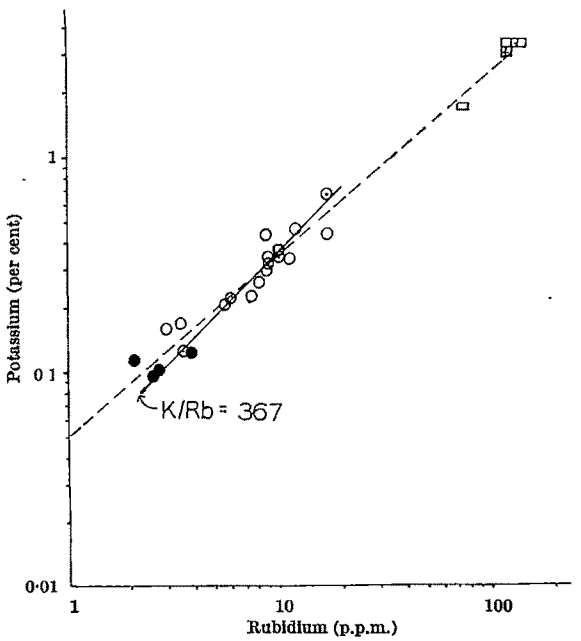


Fig. 1. Plot of potassium (per cent) versus rubidium (p.p.m.) in: ●, Jagersfontein and Dodoma eclogite; ○, Roberts Victor eclogite; □, Roberts Victor kimberlite

potassium and caesium, nor between rubidium and caesium (Fig. 2). Indeed, *RV* 374, *RV* 377 and *KRV* 3 with a caesium content of 1 p.p.m. or less have among the highest potassium and rubidium contents of all the Roberts Victor eclogites. Ahrens *et al.*[5] and Ahrens and Edge[6] have reported average potassium/caesium values of 7,500 for gabbroic and basaltic rocks. On this evidence, and also on the reported potassium/caesium ratios for many different rock types the average potassium/caesium ratio of 1,800 for Roberts Victor eclogites can only be regarded as extraordinarily low. It is, in fact, lower than that found in shales[4] where an enrichment of caesium is to be expected. Taken individually, the ratios are even more unusual, with five potassium/caesium ratios less than 500, and thirteen of the seventeen results less than the average value. (*b*) *Roberts Victor kimberlite:* Bearing in mind the limitations previously mentioned, the only conclusion which can be drawn from the five reported potassium/caesium ratios is that they are the same magnitude as the estimated crustal average[4]. They show that, relative to potassium, caesium is enriched in eclogites compared with the kimberlite in which these inclusions occur.

Caesium in Roberts Victor Eclogite

Finally, it must be emphasized that the caesium concentrations reported here are high even on an absolute scale, the average caesium content being greater than the recommended maximum crustal average of Heier and Adams (4·2 p.p.m.)[4]. Coupled with the ultrabasic nature of the samples and their relatively low potassium and rubidium content a concentration of caesium of this order is impossible to explain, except by invoking some second-

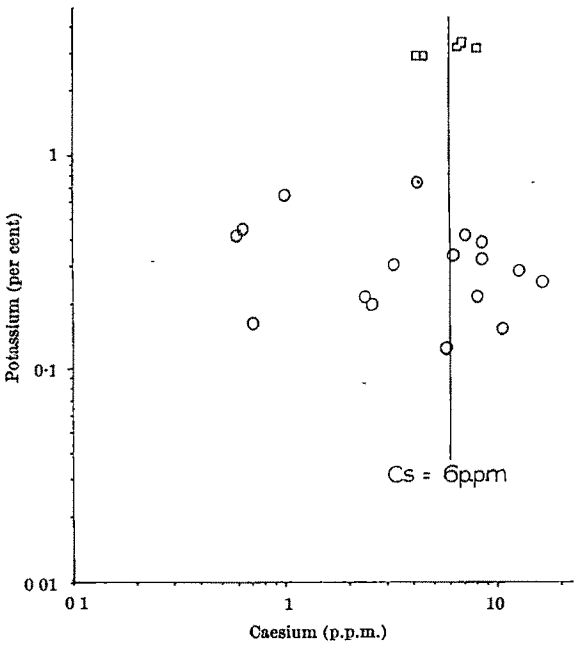


Fig. 2. Plot of potassium (per cent) versus caesium (p.p.m) in eclogite (○) and kimberlite (□) from Roberts Victor Mine

ary process or processes which differentiate between potassium and rubidium on the one hand and caesium on the other.

For one eclogite, *RV* 372, the secondary mineral containing the caesium has been separated and found to have an analcite-type structure. The crystalochemical properties of analcite appear to explain the unusual trends in alkali ratios reported here. Further details of this aspect will be given later.

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CONTINENTAL DRIFT AND OCEANIC HEAT-FLOW

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THE fact that oceanic heat-flow tends to be approximately equal to the heat-flow observed on the continents has been used as an argument against the possibility of continental drift[1]. This argument, in its most simple form, runs as follows. A comparison of the radioactive heat-production of a typical continental and a typical oceanic section shows that the continental heat-flow should exceed the oceanic heat-flow by a factor of 2–3 (ref. 2), provided that the mantle under the oceans and the mantle under the continents have the same content of radioactive elements. As in fact the average oceanic and continental heat-flow are nearly equal (1·43 μcal/cm² sec for continents, 1·37 μcal/cm² sec for oceans, if values above 4 are omitted[3]), some authors have concluded that in a continental section the radioactive elements, together with the light elements, have segregated and accumulated into a continent, whereas under the oceans this segregation has not occurred. Here the radioactive elements would still be distributed through the mantle. This explanation has become a basis for many geochemical calculations[4]. It seems impossible to reconcile the idea of chemical differences characteristic of oceanic or continental areas, extending to depths of several hundred kilometres, with continental drift, which would conceivably bring continents on top of undifferentiated 'sub-oceanic' mantle, and create oceans on top of depleted 'sub-continental' mantle. The general effect would be a significantly higher heat-flow from the continents than under the oceans.

The idea of large-scale chemical differentiation occurring in some sectors of the mantle, and not at all in other sectors, seems a rather unattractive proposition, even without moving the continents. One would expect to see a whole series of transitional areas with some crust developed. As such transitions are virtually absent, and as continental drift is supported by a large amount of evidence, it was decided to see whether an unsteady state of affairs caused by the relatively young age of the oceanic basins would cause the rough equality of oceanic and continental heat-flow. A series of cooling curves were therefore computed for a model in which at time $t = t_0$ a continent was suddenly removed, and the newly formed oceanic section of the mantle was allowed to come to its new thermal equilibrium. As a second boundary condition we chose that the temperature T at the plane of separation was 800° C and that the thermal gradient in the upper mantle was 2°/km (probably a minimum, see ref. 5). This choice does not greatly influence the outcome. The temperature at the new surface will be held at 0° C. The computer programme was run twice, for values of thermal diffusivity $\varkappa = 0{\cdot}01$ cm²/sec (decidedly too low) and 0·03 (probably a maximum). The formula for the model situation outlined above is given by Carslaw and Jaeger[6] as follows:

$$v = V \operatorname{erf} \frac{x}{2\sqrt{\varkappa t}} + kx$$

in which v is the temperature at time t; V, the temperature at time t_0; t the time; $\varkappa$ the thermal diffusivity; x the depth and k the thermal gradient at time $t = 0$, chosen as 2×10^{-5} °C/cm. The cooling curves thus obtained (see Fig. 1) give us direct information on the heat-flow at the surface after different time intervals. As can be seen from these computed heat-flows (Fig. 2), the contribution of the unsteady state cooling (labelled 'excess heat-flow' in Fig. 2) is still of the same order of magnitude as the average continental heat-flow in a steady-state situation even after several hundred million years.

The assumption of 'sudden removal' of a continent, though mathematically attractive, causes very high heat-flows during the first 50 million years or so. In

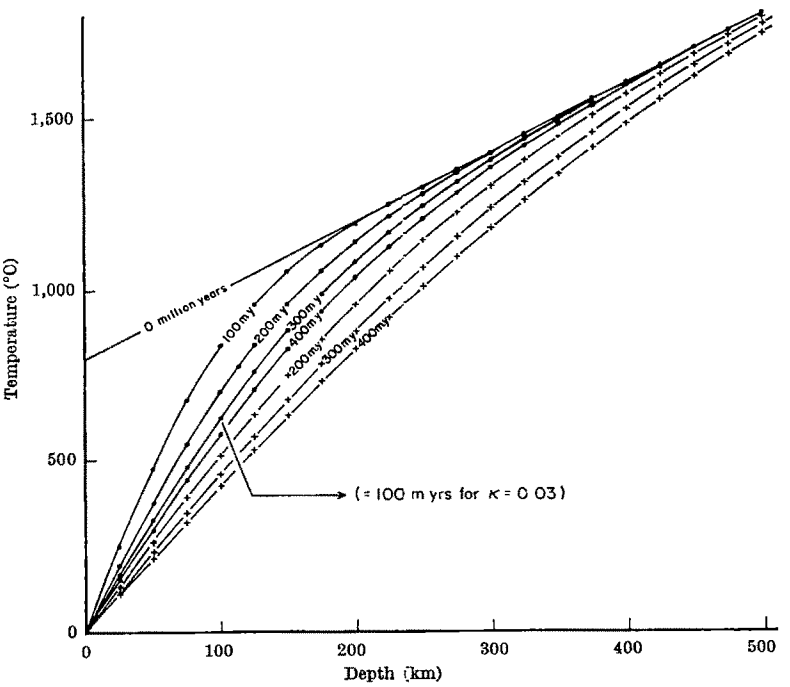


Fig. 1. Cooling curves for unsteady state model. +, $\varkappa = 0{\cdot}03$; ●, $\varkappa = 0{\cdot}01$

order to eliminate this effect, which is solely due to the introduction of a temperature discontinuity by the assumption of 'sudden removal', we recalculated the heat-flow for the case that the temperature at the plane of separation decreases exponentially with time, with a half-life of 20 million years. The resulting heat-flow curves are indicated with dotted lines in Fig. 2. This corresponds roughly to a situation in which a wedge-shaped continental edge is gradually removed.

One could try to incorporate more details in the model, but I feel that it would be unrealistic to try to be more realistic. Therefore heat-contributions from radioactivity in the mantle, or from exothermic reactions which would proceed on cooling, have not been taken into account. Heat contributions from a horizontally spreading layer of hot material, brought up by a convection current, have also not been taken into account. It can easily be seen that their effect would be to counteract cooling, and so maintain a higher heat-flow than expected from the model used. The effect of an increase of the thermal diffusivity with depth, as radiative heat-transport becomes more effective with increasing temperature[7], is more difficult to assess. All these possible refinements of the calculation do not change my basic conclusion, that continental drift itself may be the explanation of the approximate equality of oceanic and continental heat-flow. Once a continent which acted as an 'electric blanket' is displaced by convection currents, the new ocean floor is able to maintain an essentially normal heat-flow for geologically long periods of time.

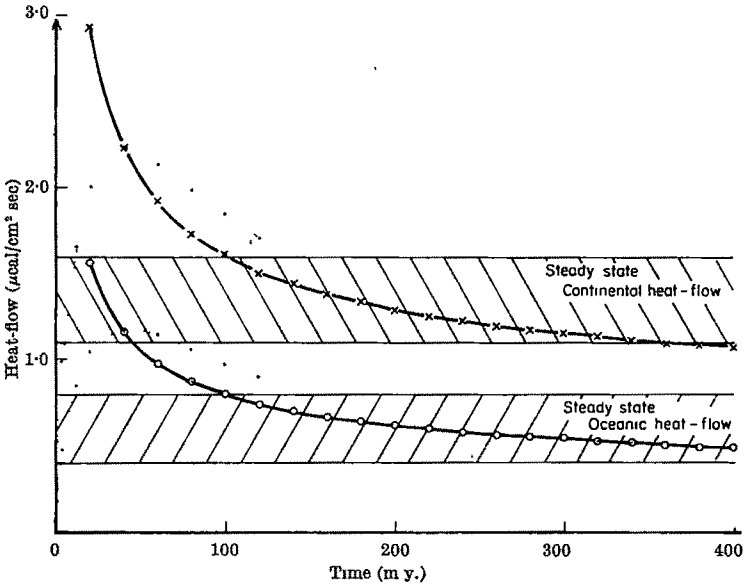


Fig. 2. Excess heat-flow curves from unsteady state model, compared with the heat-flow to be expected from radioactivities of typical continental and typical oceanic sections in a steady state situation. Any heat contribution from other sources than unsteady state cooling must be added to these excess heat-flow curves in order to obtain the true heat-flow. Dotted lines indicate heat-flow for a model in which gradual, rather than sudden, removal of a continent is assumed. ×, Excess heat-flow for $\varkappa = 0{\cdot}03$; ○, Excess heat-flow for $\varkappa = 0{\cdot}01$

I conclude that the approximate equality of oceanic and continental heat-flows cannot be used as an argument against continental drift having occurred in the past few hundred million years, and that there is no valid reason to suppose that continents sit on top of differentiated mantle and oceans on top of undifferentiated mantle.

I would like to point out some of the repercussions of the model outlined here. If there exist very old, undisturbed parts of oceans, these will be characterized by a lower than average heat-flow. It may be expected that near the trailing edge of a drifting continent the heat-flow values are slightly above normal. If it is accepted that the mid-oceanic ridges are the loci of upwelling convection currents, accompanied by volcanism and serpentinization[8] and thus characterized by high heat-flow, an ideal heat-flow profile should show a shallow minimum between the oceanic ridges and the continental slope. As a further consequence of the unsteady-state model it is possible that a layer of low viscosity in which horizontal movement is presumably concentrated is not to be found everywhere at the same depth.

The theory outlined here offers a general explanation of the unexpectedly high average level of oceanic heat-flows, and it removes one of the strongest objections against continental drift. In areas more restricted geographically, especially near the ridges, heat contributions by volcanism, serpentinization and upwelling convection currents will be more important than unsteady state cooling.

I thank Dr. W. S. Morgan for setting up the computer programme, and Prof. H. H. Hess, Prof. W. M. Elsasser, Prof. H. J. Greenwood and Dr. Morgan for their stimulating discussions.

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RADIOCHEMICAL DATA CORRELATIONS FOR SEA-WATER AND CORAL-SURFACE BURSTS

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A SYSTEMATIC investigation and correlation of all available radiochemical data from field test operations is an essential step toward an understanding of the phenomenon of fractionation in fall-out formation. The first step in this direction was an examination by Freiling[1] of the data from high-yield coral-surface and sea-water bursts of Operation Redwing. A subsequent publication[2] by the same author presented the results of correlations of some air-burst data.

This report is based on radiochemical data from three high-yield bursts from Operation Hardtack—one on a coral surface, one on the surface of deep water, and one on the surface of shallow water over a coral bottom. The conditions are similar to those of some of the Redwing events dealt with in ref. 1. Since a slightly different method of making correlations has been used in the present investigation, the Redwing data have been recalculated in accordance with it, so that direct comparisons can be made.

It has been shown[1] that certain relationships between the fractionation behaviour of fission-product radionuclides can be established by a method of logarithmic

plotting. The application of the method is most straightforward if the analytical data on the radionuclides are first expressed as "equivalent fissions of the device" at the time of detonation (rather than disintegrations per minute, or number of atoms present at time of detonation, or the more complicated ratios of nuclides found in the sample normalized to the ratios observed in unfractionated uranium-235 thermal-neutron fission products).

The term 'equivalent fissions' means the number of atoms of material in the device which must have undergone fission in order to produce the amount of a particular radionuclide observed in the sample. In order to express radiochemical results in terms of equivalent fissions of the device, the yields of the nuclides for the device must be known. Then, if the sample contains a_n atoms of nuclide n (at the time of detonation) and if the fractional yield of nuclide n for the device was y_n, the number of equivalent fissions, f_n, is:

$$f_n = \frac{a_n}{y_n}$$

For the purpose of investigating fractionation, it is convenient to form ratios of equivalent fissions of two nuclides, i and j:

$$r_{i,j} = \frac{f_i}{f_j}$$

If nuclides i and j have not fractionated from each other in the sample under consideration, the value of $r_{i,j}$ will be unity (or reasonably near it). Pairs of nuclides of the group zirconium-95, molybdenum-99, cerium-144 and neodymium-147 usually behave this way in land-surface bursts. However, if i fractionates from j, the value of $r_{i,j}$ may be either much less or much greater than unity. For example, strontium-89 generally fractionates from zirconium-95 in land-surface bursts, and both high and low values of the ratio $r_{89,95}$ are commonly observed.

It should be clear that such terms as 'fractionate', 'fractionating', 'fractionation', etc., are meaningful strictly only in so far as they express or imply 'fractionates from some reference substance', etc. A looser usage has become commonplace, however, which categorizes mass chains with volatile members as fractionating and mass chains with all refractory members as non-fractionating. An alternative terminology refers to the two kinds of chains as 'volatile' and 'refractory', respectively. In fact, these latter terms are also commonly applied (through abbreviation of speech) to the nuclides themselves. Thus, strontium-89 is referred to as volatile, although the volatility is characteristic of the bromine, krypton and rubidium in the mass-89 chain and not of the strontium.

Since the mass-89 chain and the mass-95 chain are subject to severe fractionation from each other, the ratio $r_{89,95}$ (equivalent fissions of strontium-89 to equivalent fissions of zirconium-95) is a useful indicator of the degree of fractionation of a sample. The fractionation of any other nuclide i from zirconium-95 can then be related to $r_{89,95}$ in a simple way by making log–log plots. Values of $r_{89,95}$ for a set of samples are used as abscissae and corresponding values of $r_{i,95}$ are used as ordinates. The points can usually be fitted with a fair degree of confidence to the line:

$$\log r_{i,95} = a_i + b_i \log r_{89,95} \qquad (1)$$

Values of a_i and b_i can be determined by linear regression, together with coefficients of correlation and limits of confidence.

In Freiling's study of fractionation data[1], a slightly different method of correlation was presented, in which $r_{95,89}$ was used as abscissa and $r_{i,89}$ as ordinate. This earlier method regards the mass-95 chain and mass-i chain as fractionating from mass 89. The present method regards mass 89 and mass i as fractionating from mass 95. This latter point of view seems preferable as the mass-95 chain (having no volatile members) is usually regarded as non-fractionating.

If data are fitted to equation 1, the resulting slope, b_i, will indicate the degree to which the nuclide i fractionates from zirconium-95 relative to the fractionation of strontium-89 from zirconium-95. If i does not fractionate at all, the slope will be near zero; if i fractionates like strontium-89, the slope will be near one; intermediate fractionation yields slopes between zero and one, while degrees of fractionation greater than that of strontium-89 will give slopes greater than one. The quantity b_i is thus a measure of the volatility of the behaviour of the mass-i chain.

The significance of the intercepts, a_i, is less clear cut. If all the correlation lines pass through the point ($r_{89,95} = 1$, $r_{i,95} = 1$), then some part of the debris may exist that has the same relative proportions of radionuclides as the unfractionated fission-product mixture. Interpretation of the significance of the intercept values is complicated by the fact that they may be strongly affected by calibration errors in the determinations, as well as by errors in the nuclide yield estimates for the device.

Experience gained so far in making correlations of this kind indicates that strontium-89 and zirconium-95 are most satisfactory choices for reference fractionating and non-fractionating nuclides. However, in the case of the Hardtack data, zirconium-95 was not determined, and it was necessary to choose cerium-144 instead. The results seem satisfactory. For silicate-surface bursts it seems that the choice of molybdenum-99 would serve equally well. If necessary, one might choose caesium-137 instead of strontium-89, since these two nuclides show similar fractionation behaviour.

The data used in this article pertain to samples collected from three events of Operation Hardtack in 1958. The three events were all high-yield detonations. The first was a land-surface burst on a coral island; the second was a water-surface burst fired on a barge in deep water; and the third was a water–land-surface burst fired on a "Landing Craft Utility" (LCU) in 15 ft. of water over a coral reef.

The data for all particulate samples from these events were utilized in this investigation. All samples were collected by aircraft, except for one rocket-collected sample from the water-surface event. Some of the samples are from low altitude (1,000 ft.), the others from high altitudes (39,000–60,000 ft.). Some of the latter were separated into 'coarse' and 'fine' fractions.

Figs. 1 and 2 are examples of the log–log plots of the data. Fig. 1, the plot for strontium-90, illustrates the case of a nuclide that fractionates from cerium-144 almost as strongly as strontium-89, while Fig. 2, for molybdenum-99, illustrates a case of little or no fractionation from cerium-144. Data from all three events are included on each of the figures, and the correlation behaviour seems to be slightly different from one event to the other. In Fig. 1, for example, most of the data points for the land-

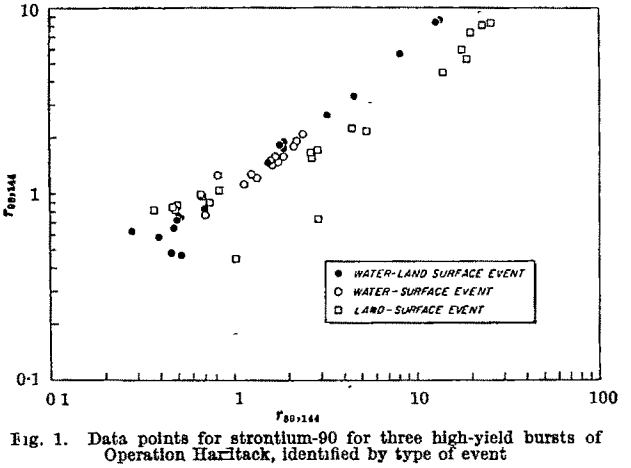


Fig. 1. Data points for strontium-90 for three high-yield bursts of Operation Hardtack, identified by type of event

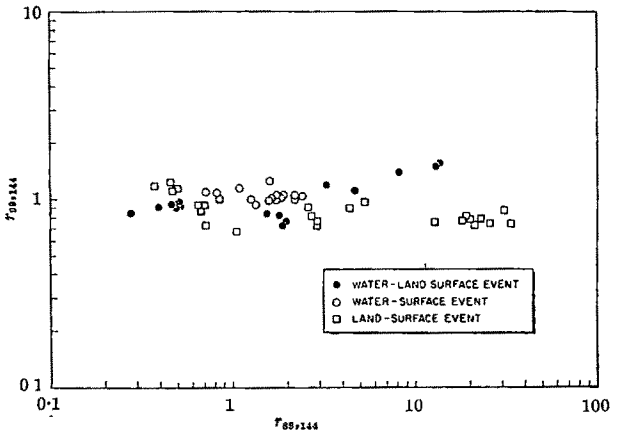


Fig. 2. Data points for molybdenum-99 for three high-yield bursts of Operation Hardtack, identified by type of event

surface event seem to lie on a line somewhat displaced from the line on which lie the data points for the other two events. In Fig. 2, the points for the water–land surface event suggest a slight positive correlation, while those for the land-surface event suggest a slight negative correlation. In view of these apparent differences, the data for the three events were correlated separately.

The points in Figs. 1 and 2 illustrate the wide range of fractionation observed in these samples. The values of $r_{89,144}$ vary by a factor greater than 100. However, for the water-surface event data point the range of this value is compressed to a factor of 3·4.

Figs. 3 and 4 show the same data points used in Figs. 1 and 2, but in this case the plotting symbols indicate the type of sample rather than the event. It is noteworthy that the data points for the low-altitude samples are contained in a relatively narrow range, the $r_{89,144}$ values being usually less than one. The samples collected at high altitudes, on the other hand, show nearly the complete range of $r_{89,144}$ values.

It is tempting to speculate that the narrow range of fractionation observed in the water-surface event, as compared with the other two events, was caused by some differences in the burst conditions. However, there is no assurance that the sampling of the cloud was sufficiently extensive to display the complete range of fractionation that actually existed, so that the absence of extremely fractionated samples in the water-surface case may be fortuitous.

The samples which were separated into fine and coarse fractions give some indication of the effect of particle size on the radionuclide fractionation. In all cases the $r_{89,144}$ value was lower for the coarse fraction than for the corresponding fine fraction. However, the r-values for fine fractions or coarse fractions, each considered as a class, do not fall into any well-defined range; on the contrary, they show wide variation.

The parameters for fitting the following line to the data:

$$\log r_{i,144} = c_i + m_i \log r_{89,144} \qquad (2)$$

are presented for the three Hardtack events in Tables 1, 2 and 3: the 95 per cent confidence limits are given as estimates of error. A positive value of the slope (with a coefficient of correlation sufficiently large to indicate that the data follow the equation reasonably well) means that nuclide i fractionated from cerium-144 in the same qualitative way that strontium-89 fractionated from cerium-144. The magnitude of the slope indicates the relative extent of the fractionation of nuclide i and strontium-89. A negative value of the slope, with a reasonably large negative coefficient of correlation, indicates fractionation in a reverse sense, that is, cerium-144 fractionated from i in the same way that strontium-89 fractionated from cerium-144. If nuclide i did not fractionate from cerium-144, both slope and coefficient of correlation should have small absolute values. Intermediate values of the coefficient of correlation (usually associated with relatively large confidence limits) indicate that the fit of the equation to the data is not sufficiently good to justify the drawing of conclusions.

Tables 1, 2 and 3 include values of the parameters for equation (1) as recalculated to fit the data from Operation Redwing. The Redwing burst referred to in Table 1 is described by Freiling[1] as a burst of megaton range at the surface of a coral atoll; the burst identified by *DWB* 1 in Table 2 was a sub-megaton burst at the surface of deep water, while *DWB* 2 was a burst of megaton range at the surface of deep water; and the burst in Table 3 was a burst of megaton range at the surface of shallow water.

Linear correlations of the Hardtack data were also made, by fitting the data to the following equation:

$$r_{i,144} = k_i + j_i r_{89,144} \qquad (3)$$

The data appear to fit equation (2) and equation (3) about equally well. The two kinds of correlation give the same indications as to the fractionation behaviour of the nuclides. For reasons discussed by Freiling[1], the logarithmic correlation is preferred, and the parameters of the linear fit are not presented in this article.

The land-surface burst of Operation Hardtack should compare with the coral-surface burst of Operation Redwing. The correlations for the latter, shown in Table 1, are based on zirconium-95 as reference nuclide, while those of the former are referred to cerium-144. As can be seen from the table, the Redwing data show the possibility of slight fractionation of cerium-144 from zirconium-95. Nevertheless, the slopes for the other nuclides agree for the two events within the 95 per cent

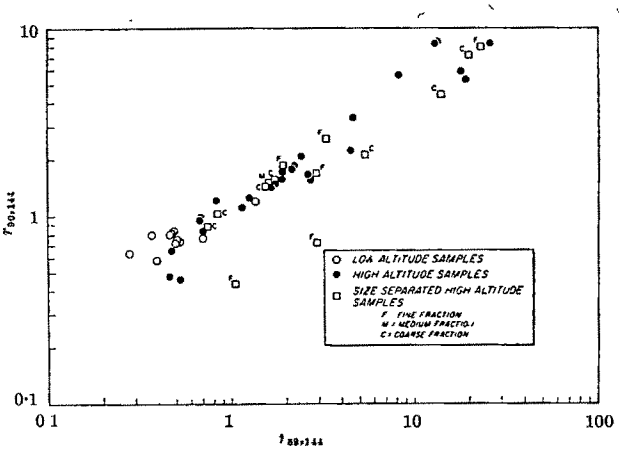


Fig. 3. Data points for strontium-90 for three high-yield bursts of Operation Hardtack, identified by type of sample

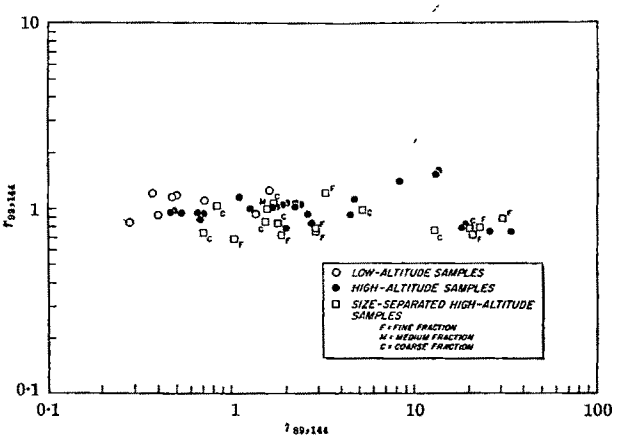


Fig. 4. Data points for molybdenum-99 for three high-yield bursts of Operation Hardtack, identified by type of sample

confidence limits. The fairly clear indication (based on the coefficient of correlation) of slight negative fractionation of uranium from cerium-144 in the Hardtack burst, as compared with the inconclusive case for negative fractionation of uranium from zirconium-95 in the Redwing burst, may also reflect a small degree of positive fractionation of cerium-144 from zirconium-95. The difference in behaviour is small relative to the other uncertainties in the data, and the Hardtack and Redwing correlations may be regarded as essentially in agreement.

Comparison of the parameters for the two water-surface bursts in Table 2 tends to support the observation that cerium-144 is slightly less refractory than zirconium-95. The Redwing burst shows positive fractionation of molybdenum-99 and uranium from zirconium-95 (for the *DWB* 2 event), but there is no conclusive evidence of fractionation of these nuclides from cerium-144 in the Hardtack burst.

Table 1. LEAST-SQUARE PARAMETERS FOR FITTING EQUATION 2 TO LAND-SURFACE EVENT DATA

Nuclide	Slope	Intercept	Coefficient of correlation	No. of data points
Calcium-45	−0·14 ± 0·09	1·0 ± 0·2	−0·60	20
Strontium-90	0·59 ± 0·08	1·1 ± 0·2	0·95	29
Yttrium-91	0·19 ± 0·02	0·84 ± 0·05	0·96	26
Molybdenum-99	−0·08 ± 0·03	0·97 ± 0·06	−0·68	29
Caesium-136	0·05 ± 0·08	0·96 ± 0·14	0·25	29
Caesium-137	1·0 ± 0·05	1·2 ± 0·09	0·99	29
Europium-156	−0·15 ± 0·03	0·89 ± 0·06	−0·90	24
Uranium-237	−0·08 ± 0·03	0·90 ± 0·03	−0·76	24
Calcium	−0·24 ± 0·09	1·1 ± 0·16	−0·78	22
Sodium	0·23 ± 0·23	1·1 ± 0·35	0·96	4
Sodium-24	0·05 ± 0·56	0·92 ± 1·0	0·70	3
The following parameters for the coral-surface burst in Operation Redwing compare with the above:				
Strontium-90	0·76 ± 0·12	1·0 ± 0·25	0·99	7
Molybdenum-99	−0·11 ± 0·11	0·86 ± 0·24	−0·73	7
Caesium-137	1·0 ± 0·38	0·58 ± 0·87	0·97	6
Cerium-144	0·08 ± 0·12	0·85 ± 0·25	0·62	7
Uranium-237	−0·02 ± 0·07	0·80 ± 0·15	−0·33	7
Uranium-239 (Neptunium-239)	−0·02 ± 0·06	0·82 ± 0·13	−0·38	7

Table 2. LEAST-SQUARE PARAMETERS FOR FITTING EQUATION 2 TO WATER-SURFACE EVENT DATA

Nuclide	Slope	Intercept	Coefficient of correlation	No. of data points
Calcium-45	−1·4 ± 9·0	0·19 ± 3·1	−0·44	4
Strontium-90	0·64 ± 0·15	1·1 ± 0·08	0·93	15
Yttrium-91	0·27 ± 0·13	0·95 ± 0·07	0·78	15
Molybdenum-99	−0·05 ± 0·11	1·1 ± 0·06	−0·23	15
Caesium-136	0·24 ± 0·14	0·96 ± 0·07	0·73	15
Caesium-137	1·1 ± 0·13	0·97 ± 0·07	0·98	15
Europium-156	−0·06 ± 0·11	1·1 ± 0·05	−0·39	10
Uranium-237	−0·14 ± 0·17	1·2 ± 0·09	−0·52	12
Sodium	−0·08 ± 0·89	0·74 ± 0·55	−0·08	9
The following parameters for the surface bursts in deep water in Operation Redwing compare with the above. *DWB* 1 is the sub-megaton event and *DWB* 2 is the megaton range event:				
Strontium-90 –*DWB* 1	0·49 ± 0·43	0·92 ± 0·54	0·84	6
DWB 2	0·59 ± 0·22	1·0 ± 0·11	0·95	7
Molybdenum-99–*DWB* 1	0·11 ± 0·08	1·0 ± 0·10	0·84	7
DWB 2	0·40 ± 0·23	0·70 ± 0·11	0·86	8
Caesium-137 –*DWB* 1	1·4 ± 0·52	0·63 ± 0·36	0·99	4
DWB 2	—	±	—	2
Cerium-144 –*DWB* 1	0·06 ± 0·09	0·87 ± 0·10	0·62	7
DWB 2	0·30 ± 0·44	0·78 ± 0·21	0·57	8
Uranium-23 –*DWB* 1	0·09 ± 0·09	1·1 ± 0·11	0·74	7
DWB 2	0·51 ± 0·40	0·60 ± 0·19	0·79	8
Uranium-239 –*DWB* 1 (Neptunium-239)	0·10 ± 0·12	0·98 ± 0·13	0·69	7
DWB 2	0·43 ± 0·38	0·62 ± 0·18	0·74	8

Table 3. LEAST-SQUARE PARAMETERS FOR FITTING EQUATION 2 TO WATER LAND-SURFACE EVENT DATA

Nuclide	Slope	Intercept	Coefficient of correlation	No of data points
Calcium-45	0·91 ± 0·54	0·98 ± 0·45	0·83	9
Strontium-90	0·74 ± 0·07	1·1 ± 0·09	0·99	18
Yttrium-91	0·47 ± 0·05	0·88 ± 0·07	0·98	18
Molybdenum-99	0·13 ± 0·07	0·95 ± 0·08	0·73	18
Caesium-136	0·63 ± 0·12	1·1 ± 0·15	0·95	18
Caesium-137	1·1 ± 0·10	0·90 ± 0·13	0·99	18
Europium-156	−0·27 ± 0·08	0·88 ± 0·08	−0·89	15
Uranium-237	0·05 ± 0·18	0·92 ± 0·18	0·15	15
Calcium	0·24 ± 0·81	0·27 ± 0·77	0·19	14
Sodium	0·47 ± 0·46	0·20 ± 0·58	0·47	18
The following parameters for the shallow-water burst in Operation Redwing compare with the above				
Strontium-90	0·72 ± 0·12	1·0 ± 0·14	0·99	6
Molybdenum-99	−0·32 ± 0·33	0·76 ± 0·42	−0·74	7
Caesium-137	1·1 ± 0·74	0·64 ± 0·85	0·91	6
Cerium-144	−0·05 ± 0·18	0·76 ± 0·23	−0·32	7
Uranium-237	−0·15 ± 0·22	0·75 ± 0·28	−0·60	7
Uranium-239 (Neptunium-239)	−0·16 ± 0·25	0·78 ± 0·31	−0·60	7

The data on the two water–land-surface bursts in Table 3 do not support the foregoing interpretation. The cerium data from the Redwing burst do not show definite evidence of fractionation from zirconium. Furthermore, the definite positive fractionation of molybdenum-99 from cerium in the Hardtack burst and its failure to fractionate positively from zirconium in the Redwing burst suggest that cerium was slightly more refractory than zirconium in these bursts. There is no apparent reason why water–land-surface bursts should show this difference in behaviour, as compared with land-surface or water-surface bursts.

In all three Hardtack events, caesium-137 fractionated to about the same extent as strontium-89. These observations are in good agreement with the Redwing correlations. The Hardtack correlations of the yttrium-91 data show positive fractionation in all three events. There is indication that this fractionation of yttrium-91 was considerably more extensive in the water–land-surface burst than in the land-surface burst. The slope from the yttrium-91 correlations for the water-surface burst is intermediate to the other two. However, the limits of confidence are rather large, and the possibility of agreement with one of the other two bursts cannot be excluded.

The molybdenum-99 and caesium-136 data indicate that fractionation in the water–land-surface burst was not intermediate to that in the land burst and water burst. Molybdenum showed slight negative fractionation in the land event, no fractionation in the water event, and positive fractionation in the water–land-surface event. Similarly, caesium-136 fractionation increased in the order: land surface < water surface < water–land surface.

The calcium data for the land-surface burst show evidence of slight negative fractionation from the reference nuclide. The calcium data for the water-surface burst are few and inconclusive. In the case of the water–land burst, the data are contradictory because the calcium-45 correlation definitely indicates fractionation, while that for macroscopic calcium does not. The unreliability of these data (as well as of the calcium data on the water-surface burst) is suggested by the large confidence limits.

No firm conclusions can be drawn from the sodium data. The high coefficient of correlation, associated with large 95 per cent confidence limits, in the land-surface case may be due to the scarcity of data points. In the water–land surface case the coefficient is inconclusive and the limits still large, although there are a large number of datum points. In the water-surface case the correlation is clearly insignificant, but the very wide 95 per cent confidence limit for the slope suggests that the data are erratic.

The uranium data suggest negative fractionation in the land-surface burst but no significant fractionation in the water–land-surface burst. For both europium and uranium, in the case of the water-surface burst, the data are inconclusive.

It is noteworthy that, in all seven cases reported here, the uranium-237 data agree with the molybdenum-99 data. This may indicate that these nuclides do not fractionate from each other in the situations under discussion, although Freiling and Kay have shown[2] that they fractionate considerably from each other in air-burst debris.

In summary, the correlations presented in this article from the Hardtack data compare well with the correlations of similar data presented by Freiling[1]. Although the Hardtack data are more extensive, they are not sufficient in scope and precision to give much insight into such questions as the relative fractionation behaviour of zirconium-95 and cerium-144.

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LETTERS TO THE EDITOR

GEOPHYSICS

Semi-annual Variation in Upper-atmosphere Density

A SEMI-ANNUAL variation in upper-atmosphere density, with maxima in April and October, and minima in January and July, was discovered in 1960 and has been generally accepted as a feature of the upper atmosphere at heights of 250–600 km. In a recent article, Anderson[1] suggested that the variation is a disguised effect of latitude. Anderson's hypothesis can be tested by analysing the orbit of a satellite of a near-circular polar orbit which samples all latitudes in a nearly equal degree. If the semi-annual variation is recognizable in the orbit of such a satellite, it cannot be due to changes in latitude.

Samos 2 (1961 α 1) is a suitable satellite, having an orbital inclination of 97°, a mean height near 500 km and a mean eccentricity of about 0·005. The motion of *Samos* 2 during 1961 and 1962 has been analysed using orbital data given in *Spacetrack Bulletins* and orbits recently determined at the Royal Aircraft Establishment[2].

Fig. 1 shows the monthly change Δa in the semi-major axis of the *Samos* 2 orbit, expressed as a fraction of the Earth's equatorial radius R. Small corrections have been made to allow for the effects of an oblate atmosphere with day-to-night variation in air density[3,4], the small variation in eccentricity due to the odd harmonics in the Earth's gravitational potential, and the decline in solar activity between 1961 and 1962. The change in semi-major axis is an indication of the average (over all latitudes) of the air density at heights near 500 km. The last four points in 1962 are likely to be the least accurate, because the orbital data are not so complete at these times. Satisfactory values are not available for February in either year.

The mean of the values for 1961 and 1962, shown by the solid line, reveals a semi-annual variation, the maximum values of $10^6\ \Delta a/R$ being 73 in April and 89 in October, and the minimum values being 44 in July and January. Thus at 500 km height in 1961–62, there is a clear semi-annual variation, independent of latitude, with the maximum density exceeding the minimum by a factor of 2·0.

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Ionospheric Storms and Small Pressure Fluctuations at Ground Level

IN the past a number of attempts have been made to establish relationships between events occurring in the troposphere and in the ionosphere[1–3]. However, none of them has as yet been decisive. Recent work[4,5] has shown that it may be possible to link the two regions by the passage of gravity waves from one region to the other. Infrasonic waves, recorded at ground level near polar regions[6], indicate a possible relationship between aurora and these infrasonic waves. Earlier[7], infrasonic waves had been recorded at Washington, D.C., at times of magnetic activity travelling at speeds somewhat higher than the local speed of sound. Maeda and Watanabe[8] and, more recently, Iyengar[9] have discussed the possibility of pressure waves being generated at auroral heights. Also, Maeda and Young[10] have discussed the periods of pressure waves from aurorae. Bowman[11] has suggested that pressure waves generated near polar regions may be responsible for ionospheric storms at the times of magnetic storms. This report gives some results of possible troposphere–ionosphere relations from the analysis of microbarograph records, which have been made at Brisbane since July 1963. The type of microbarograph used is described by Jones and Forbes[12].

At about 0310 h on August 19, 1963, the microbarograph record at Brisbane begins a period of increased intensity, for the short-period oscillations (period less than 1 min),

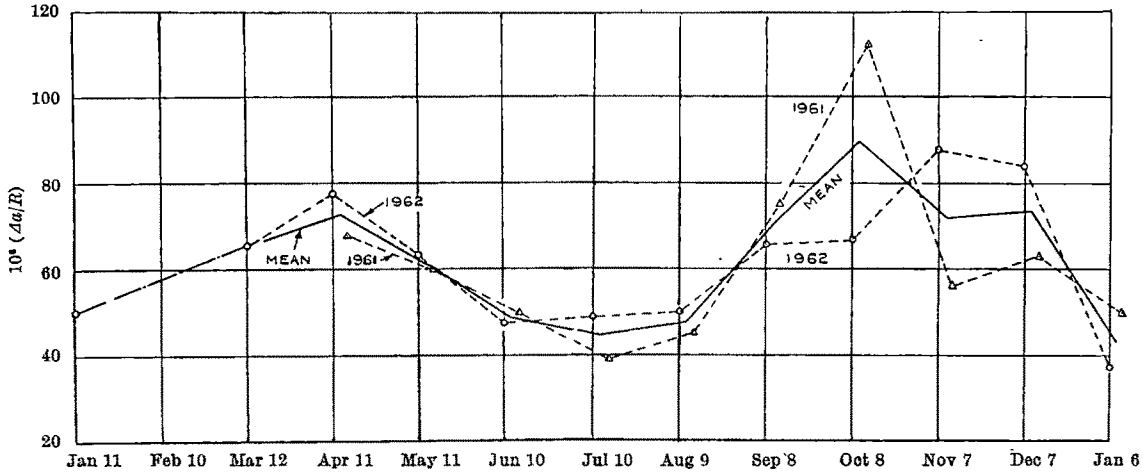


Fig. 1. Monthly change Δa in the semi-major axis of the *Samos* 2 satellite during 1961–1962, after correction for solar activity, etc.

which lasts for about 20 min. For the rest of the night this short-period activity is low. Several hours of the activity during the night can be seen from Fig. 1*A*, where the reported increased activity is also shown. Fig. 2 shows tracings of ionograms taken at Brisbane around this period. At 0310 h there is a dramatic F_2-layer height rise characteristic of ionospheric-storm behaviour, and after about 30 min the layer becomes stable again. Around these times the tracings of ionograms from Townsville (Fig. 2) also show the passage of a well-defined disturbance. Tracings of Canberra ionograms for the period are also shown in Fig. 2. Although the disturbance is not so well defined at Canberra as at the other stations, it is nevertheless well identifiable. There is a sudden height rise in the F_2-layer between 0150 and 0200 h of about 40 km and a burst of intense sporadic-*E* occurrence around these times.

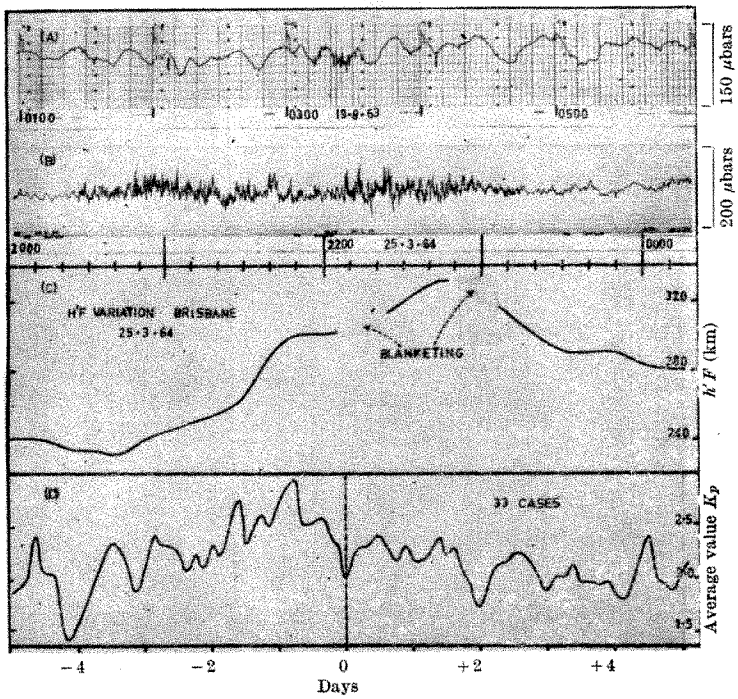


Fig. 1. (*A*) Microbarograph record on 19.8.63; (*B*) microbarograph record on 25.3.64; (*C*) *h'F*, variation at Brisbane on 25.3.64; (*D*) average K_p values for 5 days before and 5 days after selected tropospheric ionospheric events

This type of sporadic-*E* event often accompanies ionospheric disturbances. Inspection of the second- and third-multiple traces shows the height rise more clearly. Using times when it was thought the disturbance could first be detected, 0200 h for Canberra, 0250 h for Brisbane and 0300 h for Townsville, a calculation was made of the velocity of the disturbance, assuming that it advanced with a straight front. All times used are Eastern Australian Standard Time. The velocity of travel so calculated was about 300 m sec^{-1}, in a direction approximately north-east. Allowing for the fact that at Townsville and Canberra the ionograms were taken at 15-min intervals and at Brisbane at 10-min intervals, the calculated velocity is comparable with that found by Bowman[11] for ionospheric storms. Although a sudden commencement does not precede these events, there is an increase in the K_p value from 4− to 6+ about 6 h previous to these events.

During July 1, 1963, to December 31, 1964, some thirty-three cases have been found in which changes on the microbarograph record at the times of ionospheric height rises suggest a possible tropospheric–ionospheric association. Fig 1*B* shows an especially clear-cut example where the period of increased microbarograph activity coincides with the movement of the F_2-layer to greater heights (Fig. 1*C*). For the thirty-three cases the average value of the K_p index has been obtained at 3-h intervals for 5 days on either side of the events, using a superimposed epoch method. The high values of average K_p which occur prior to the zero time, reaching a peak about 18 h before, suggest that the ionospheric height rises are those produced by ionospheric storms. Fig. 1*D* shows the plot of average K_p values.

An analysis of ionograms at Brisbane for the 18-month period (July 1963–December 1964) revealed 123 occasions in which a height rise (F_2 layer) of 50 km or more occurred, or in which there was a drop in critical frequency of the F_2 layer greater than 0·5 Mc/s. On forty-nine occasions there was definitely no corresponding series of microbarograph oscillations, while on forty-one occasions the presence or absence of the microbarograph oscillations could not be decided owing to general confusion ('noise') on the records. For the thirty-three cases mentioned above, seventeen appeared

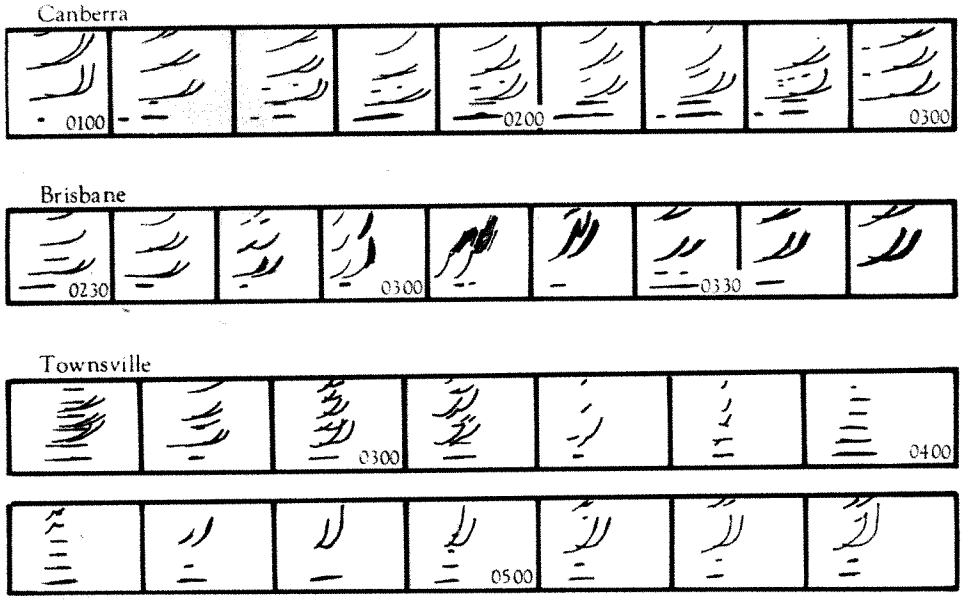


Fig. 2. Ionograms from Canberra, Brisbane and Townsville at selected times on 19.8.63 showing the progression of an ionospheric disturbance

as short-period oscillations (less than 1 min) sometimes resembling noise (Fig. 1*B*). For six of these cases, the period was greater than 1 min but less than 3 min. The remaining ten cases had periods greater than 3 min.

The results presented here appear to support Bowman's suggestion[11] that ionospheric storms are associated with pressure waves travelling from polar regions in the sound channel at an altitude of about 80 km, provided it is permissible to assume that coupling between this channel and the troposphere can occur in some cases (around 40 per cent) but not in others.

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PHYSICS

Isotope Shifts in the Near Infra-red Bands of Diatomic Sulphur

STUDIES of the near infra-red bands of diatomic sulphur (S_2) showed[1,2] that they form two systems of bands involving (*a*) $^3\Pi_g$—$^3\Delta_u$ and (*b*) $^3\Pi_g$—$^3\Sigma_u^+$ transitions with $^3\Pi_g$ being the common initial level for both the systems. Bands of the isotopic molecules, $^{32}S\ ^{34}S$ and $^{34}S_2$ (in addition to $^{32}S_2$), have now been obtained. Vibrational and rotational analyses of these bands confirm the observations of earlier studies[2] and show that the emitter of the near infra-red bands is the diatomic sulphur molecule. Further, the isotope shifts provide a correct vibrational assignment of the bands in both the systems. The results of these investigations are presented in this communication.

The near infra-red bands are excited by a micro-wave discharge of 2,450 mc/sec through trace quantities of sulphur enriched with 37·2 per cent of sulphur-34 and 2–3 mm of helium. The spectra are photographed in the first order of a 6·6 m concave grating spectrograph at a dispersion of 1·1 Å/mm and shown in Fig. 1. The bands in (*a*) are due to $^{32}S\ ^{34}S$, $^{32}S_2$, and $^{34}S_2$, with their intensities decreasing in that order. In juxtaposition are the bands of $^{32}S_2$ in (*b*) obtained in discharges through natural sulphur[2].

The sub-bands at 6983·8 Å and 7069·6 Å belonging to $^3\Pi_g$—$^3\Delta_u$ transitions and those at 7506·8 Å and 7433·6 Å of $^3\Pi_g$—$^3\Sigma_u^+$ transitions show no significant isotope shifts (Fig. 1). This clearly indicates that the bands involve 0–0 transitions. The 0–1, 0–2, 0–3 bands, due to $^{32}S\ ^{34}S$ and $^{34}S_2$, show progressively larger shifts towards the shorter wave-length side of $^{32}S_2$ bands.

Isotope shifts of the bands due to $^{32}S\ ^{34}S$ and $^{34}S_2$ are calculated from the relation:

$$\nu_0 - \nu_0^i = (1-\rho)\{\omega_e'(v'+\tfrac{1}{2}) - \omega_e''(v''+\tfrac{1}{2})\} - (1-\rho^2)\{\omega_e'\chi_e'(v'+\tfrac{1}{2})^2 - \omega_e''\chi_e''(v''+\tfrac{1}{2})^2\}$$

where $\rho = \sqrt{\mu/\mu^i}$, μ and μ^i are the reduced masses of $^{32}S_2$ and $^{32}S\ ^{34}S$ (or $^{34}S_2$).

The lower-state vibrational frequencies and their successive differences are known from the observed bands. However, the vibrational constants of the common upper state $^3\Pi_g$ are not known, since vibrational levels with $v' \geqslant 1$ are predissociated. An anharmonicity equal to that of the lower state is assumed in calculating the isotope shifts. A value of 500 cm^{-1} for ω_e' gives a reasonable fit of the observed isotope shifts for the bands of $^{32}S\ ^{34}S$ and $^{34}S_2$ molecules, as can be seen from Table 1.

Table 1. ISOTOPE SHIFTS OF THE NEAR INFRA-RED BANDS OF DIATOMIC SULPHUR

$v'-v''$	Band origins of $^{32}S_2$ in cm^{-1}		Isotope shifts, $\nu_0-\nu_0^i$, in cm^{-1} ($^{32}S_2$–$^{32}S\ ^{34}S$) Observed	Calculated	($^{32}S_2$–$^{34}S_2$) Observed	Calculated
		(i) $^3\Pi_g$–$^3\Delta_u$ system				
	$^3\Pi_{2g}$–$^3\Delta_{2u}$	$^3\Pi_{1g}$–$^3\Delta_{2u}$				
0–0	14,318·0$_7$			0·1		0·2
		14,144·3$_7$		0·1		0·2
0–1	13,834·8$_8$		−7·2	−7·0	−14·2*	−14·1
		13,661·2$_3$	−7·2	−7·0	−15·1*	−14·1
0–2	13,356·7$_0$		−14·1	−13·9	−28·9*	−28·1
		13,183·1$_1$	−14·2	−13·9	−29·2*	−28·1
0–3	12,883·6$_0$		−21·1	−20·7		
		(ii) $^3\Pi_g$–$^3\Sigma_u^+$ system				
	$^3\Pi_{1g}$–$^3\Sigma_u^+$	$^3\Pi_{2g}$–$^3\Sigma_u^+$				
0–0	13,451·9$_8$			0·1		0·3
		13,317·5*		0·1		0·3
0–1	12,974·3$_3$		−7·1$_8$	−6·9	−14·9*	−13·9
		12,840·6*	−6·9	−6·9	−14·6*	−13·8
0–2	12,501·9$_7$		−14·2*	−13·7		
		12,368·2*	−14·6*	−13·7	−29·3*	−27·7

ν_0 refers to $^{32}S_2$ and ν_0^i to the isotopic molecule $^{32}S\ ^{34}S$ or $^{34}S_2$.
* Values from band head data.

Previous studies of the $^3\Pi_g$—$^3\Delta_u$ bands of $^{32}S_2$ showed that in the $v'=0$ level predissociation occurs at $J'=34$ in the $^3\Pi_{2g}$ state and at $J'=16$ in the $^3\Pi_{1g}$ state. Present studies of the rotational structure of the $^{32}S\ ^{34}S$ bands show that these predissociations occur at $J'=35$ and $J'=17$ levels of the $^3\Pi_2$ and $^3\Pi_1$ states respectively. Further, in the case of the $^{32}S\ ^{34}S$ molecule, the distinction between the symmetric and antisymmetric rotational levels that exists in $^{32}S_2$ or $^{34}S_2$ disappears as a result of which no alternate lines are missing. It is indeed found that the

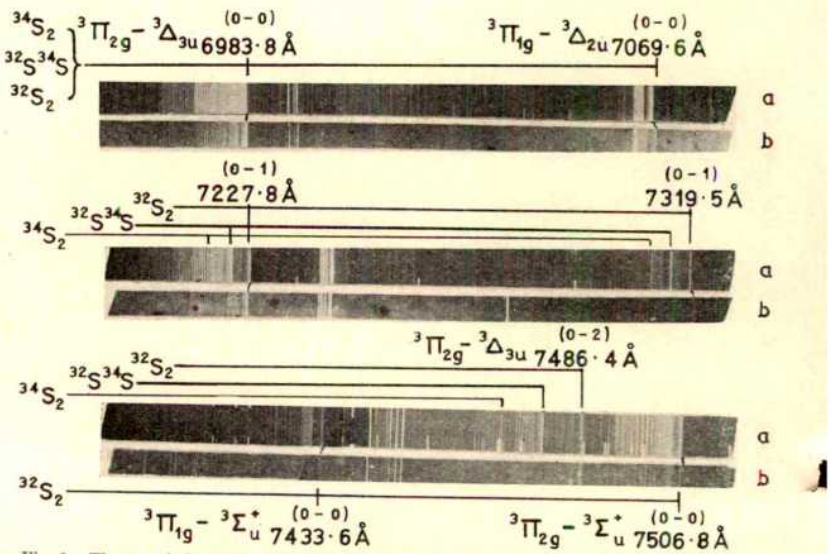


Fig. 1. The near infra-red bands of diatomic sulphur taken at a dispersion of 1·1 Å/mm. Bands in (*a*) are due to $^{32}S\ ^{34}S$, $^{32}S_2$ and $^{34}S_2$ while those in (*b*) are of $^{32}S_2$ obtained in discharges through natural sulphur. Because of a slight difference in the dispersion, the $^{32}S_2$ bands in (*a*) do not coincide exactly with those in (*b*)

even as well as odd rotational lines are present in the $^3\Pi_g$—$^3\Sigma_u^+$ transition.

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Precursor Shock produced by 0·25-g Chemical Explosions

J. M. DEWEY, in a letter[1] written some months ago, described precursor shocks produced by a 500-ton hemispherical charge of 2,4,6-trinitrotoluene (TNT) fired on the ground. He suggested that the precursor shocks were probably formed by ground waves feeding energy into the air ahead of the air shock.

We have fired 0·25-g spherical charges of penta-erythritol tetranitrate (PETN) on a steel plate and photographed the air shock-pattern using the schlieren technique. These photographs have shown a precursor shock and in some firings a portion of the precursor was downward-facing (similar to Dewey's description). To investigate his explanation of precursor formation we positioned a soft plasticene wall close to the charge, the object of this being to separate the direct air shocks from any ground-induced waves on one side of the charge. A photograph of the wave pattern from one of the firings is shown in Fig. 1; before the final exposure was made, 60 μsec after charge detonation, two separate exposures were taken to superimpose (i) a 5-cm grid and (ii) the charge position on the photographic plate. The steel-driven air shocks are visible on the right of the picture, and are similar to those observed by Boys[2]. Waves transmitted through the plasticene wall are also visible. On the left-hand side the precursor is seen leading the main air shock. A series of photographs has shown that the precursor is eventually overtaken by the main air shock which it then lags behind, becoming progressively weaker. A firing on a wooden plate showed that the precursor was less advanced of the main air shock at 60 μsec, and its intersection with the main air shock occurred at a reduced height above the surface.

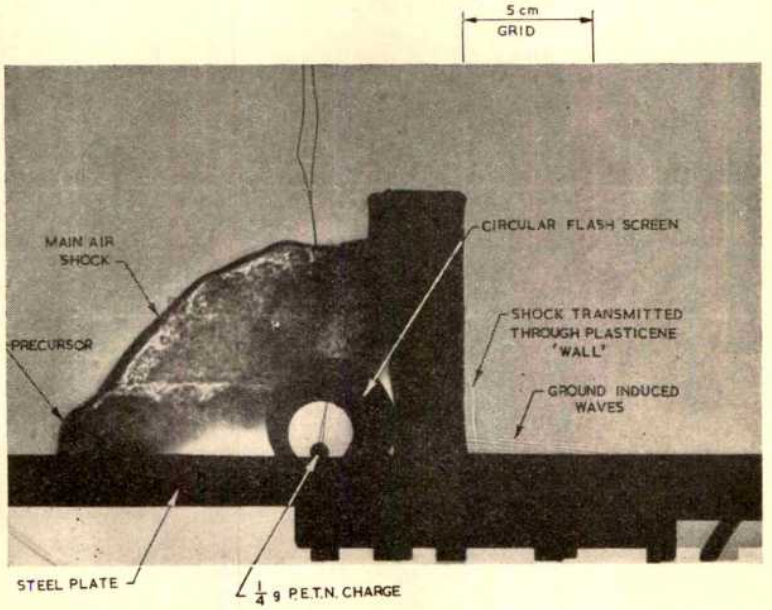


Fig. 1. Photograph of wave pattern from 0·25 g charge fired on a steel plate

These results suggest that, under certain conditions, precursors may be produced by the feedback of energy into the air from strong ground waves interacting with the main air shock.

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Adsorption of Oxygen on Ultra-thin Titanium Films

A COMMON preparatory method used in the investigation of thin films is evaporation of a metal on to a non-metallic substrate. The actual formation of the film on the substrate is an intriguing process, for a collection of independently nucleated islands must grow together to form a continuous, two-dimensional body. This process has been examined with an electron microscope by Bassett *et al.*[1] and they have documented the case of gold on sodium chloride.

During the period in which the metal film is composed of discontinuous islands, electronic conduction through the film is difficult to explain by any mechanism other than tunnelling. Neugebauer and Webb[2,3] have considered this problem in association with the fact that such films have a negative temperature coefficient of resistance. Their conclusions are embodied in a conduction mechanism in which an electron must first be activated before it can tunnel across the gap separating the islands. The basic parameters which must be considered in this mechanism are related by the following equation*:

$$\sigma = \frac{A\,de^2\sqrt{2m\varphi}}{rh^2}\exp\left[-\frac{\varepsilon}{kT}\right]\exp\left[-\frac{4\pi d\sqrt{2m\varphi}}{h}\right] \qquad (1)$$

* Symbols are: σ, film conductivity; A, constant; d, inter-island distance; r, island radius; e, electronic charge; m, electronic mass; ε, activation energy; T, temperature; φ, metal work function; h, Planck's constant; k, Boltzman constant.

The dependence of equation (1) on the island radius, film temperature, and change in inter-island distance caused by thermal expansion of the substrate has been confirmed[2,3], but the tunnelling parameters, that is, metal work function and change in inter-island spacing caused by oxidation of the metal islands, have not been examined experimentally.

In the present investigation, the adsorption of oxygen on ultra-thin titanium films has been used to test the effect of a change in work function and inter-island distance on film conductivity. During the chemisorption of a monolayer of oxygen, the change in film conductivity caused by a change in work function can be determined. If the change in work function is known from other measurements, a value for the inter-island distance can be calculated from equation (1).

The reaction of oxygen with titanium does not stop with the adsorption of a monolayer of gas. Even at room temperature, an oxide layer continues to form. The oxide growth serves to increase the tunnelling distance between the metal islands if the assumption is made that tunnelling takes place from the surface of the metal and not from that of the oxide.

Equation (1) can be modified to include the empirical rate equation for the room-temperature oxidation of titanium[4].

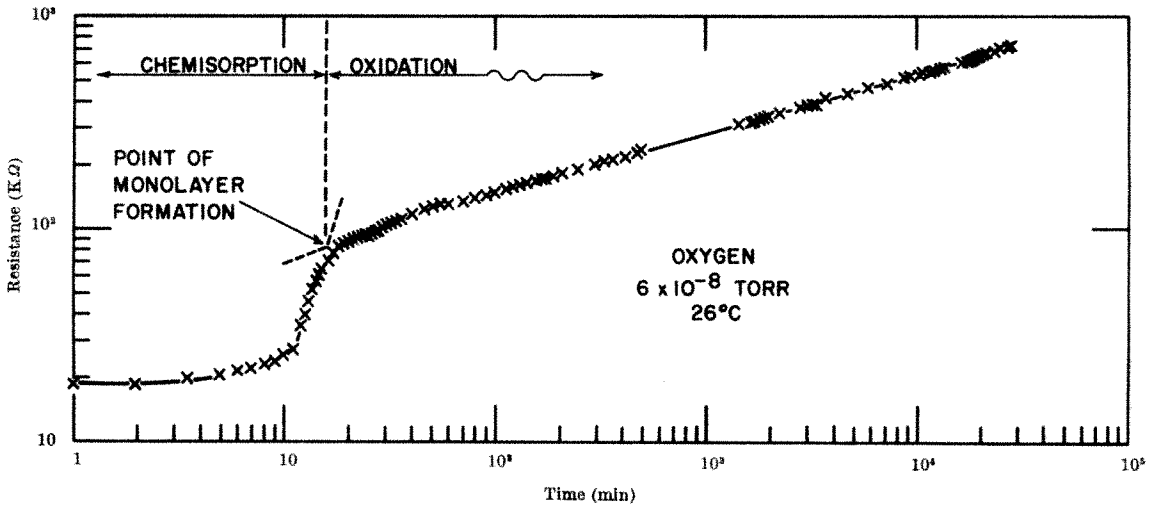


Fig. 1. Change in titanium film resistance

$$s = K \log (at + t_0) \qquad (2)$$

where s is the oxide thickness, t, the time, and K, a, and t_0 are constants. (t_0 may be disregarded when t is large.)

The tunnelling distance, d, is related to the oxide thickness, s, by the fact that d increases twice as fast as s. This increase may be related to a change in film conductivity, σ, by differentiating equation (1) with respect to d. Since s is a function of time (equation 2), and d is related to s, σ may be expressed as a function of time as follows:

$$\frac{\Delta \log \sigma}{\Delta \log t} = \frac{0{\cdot}87K}{d_0}\left[1 - 9{\cdot}26 \times 10^7\ \varphi^{1/2}\ d_0\right] \qquad (3)$$

where d_0 is the inter-island distance before oxidation has begun and K is the oxidation rate constant. Using the value of d_0 calculated from chemisorption data and the slope of the log–log plot of conductivity versus time, a value for the room-temperature oxidation rate constant of titanium can be found.

An ultra-high vacuum system has been used in examining the effect of adsorbed oxygen on the resistance of discontinuous titanium films. A base pressure of 1×10^{-9} torr and a substrate temperature range of $+250°$ to $-196°$ C were available. The gas inlet system was used to maintain a dynamic oxygen pressure between 1×10^{-8} and 1×10^{-6} torr. Titanium was evaporated on to glass slides at a rate of 10 Å/min and at a pressure no greater than 2×10^{-8} torr. The film thickness was controlled by halting the evaporation at a prescribed value of resistance. Immediately after evaporation, the titanium was exposed to oxygen and the film resistance was monitored as a function of time. Oxygen pressure was kept constant throughout the run.

Titanium films with a resistance in the neighbourhood of 20 kΩ (≈5 Å thick) were shown to be discontinuous by the fact that the temperature coefficient of resistance was negative[2]. Alternating current resistance as a function of frequency and electron diffraction also supported this conclusion.

The results of oxygen adsorption at room temperature on a 20-kΩ titanium film are shown in Fig. 1. The induction period was found to increase with a decrease in pressure but proved to be independent of temperature. These properties fit an explanation based on chemisorption. Hence equation (1) could be applied to the data and a value of d_0 calculated. Values of the work function and conductivity for the initial titanium film and the film covered with an oxygen monolayer were substituted into equation (1) expressed in log form. The resulting pair of equations was solved simultaneously and a value of d_0 equal to 5 Å was found for the 20-kΩ titanium film.

The break in the log resistance versus log time curve indicated the formation of a complete monolayer of oxygen. It was at this point that the reaction between titanium and oxygen slowed down, as shown by a rise in the vacuum system pressure. The continued reaction of oxygen with titanium beyond the point of monolayer formation produced the log–log linear plot. This could be attributed to oxidation, for it showed a small dependence on temperature but proved to be independent of pressure.

Using the value of d_0 obtained above and the slope of the linear portion of Fig. 1, a rate constant for the room temperature oxidation of titanium was calculated to be approximately 2×10^{-9} cm. This number agrees with the extrapolated results of Andreeva and Alekseeva[5] ($K = 1{\cdot}6 \times 10^{-9}$ cm).

The results of this study confirm the tunnelling explanation for conduction in discontinuous metal films. The dependencies of σ on φ and d, as stated by the equation of Neugebauer and Webb, allow the calculation of an inter-island distance and an oxidation rate constant which agrees with published data.

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Effect of an Electron-scavenging Solute on the Radiolysis of *Cyclo*hexane

ALTHOUGH a considerable body of evidence exists indicating that solutes of a radical scavenging nature depress the yields of the principal products (hydrogen, *cyclo*hexene, di*cyclo*hexyl) from the gamma radiolysis of *cyclo*hexane[1], the effect of electron scavenging solutes is less well characterized. Scholes and Simic[2] have shown that in the presence of nitrous oxide, a powerful electron but feeble hydrogen atom scavenger, the hydrogen yield is decreased by a factor of two ($G(H_2) = 2{\cdot}78$ at $[N_2O] = 100$ mM), $G(N_2)$ being 3·75 at the same concentration. In the course of similar investigations on carefully de-aerated *cyclo*hexane we have observed a marked increase in the gamma radiolysis yield of di*cyclo*hexyl (see Table 1). The measured yields are independent of dose rate in the range 0·2–2·5 M rads/h and independent of nitrous oxide concentration in the range 15–130 mM. Impoverishment of nitrous oxide in the liquid phase was avoided by the use of low total doses (< 0·5 M rad) and by performing

Table 1. γ-RADIOLYSIS YIELDS IN THE PRESENCE OF NITROUS OXIDE

$[N_2O]$ (mM)	Initial $[I_2]$ (mM)	$G(R_2)$	$\Delta G(R_2)$	$G(-I)$	$\Delta G(-I)$
0	0	1·63	0 85	—	—
15–130	0	2·48		—	—
0	0·59	0·3	0 0	5.1	1·80
45	0·59	0 3		6 9	
0	0 89	0·3	0 0	5·06	1·69
45	0 89	0·3		6·75	
0	6·22	0 3	0·0	5·52	1·75
45	6·22	0·3		7·27	

G(*cyclo*hexanone + *cyclo*hexanol) < ~0·1.

irradiations on samples in which gas–liquid equilibration was facilitated by a high surface to volume ratio of the liquid.

The radiolysis yields obtained in the presence of iodine, a very effective *cyclo*hexyl radical scavenger, are also given in Table 1. It will be seen that there is no change in the residual di*cyclo*hexyl yield ($G(R_2) = 0{\cdot}3$) whether or not nitrous oxide is present, and that iodine disappears to an increased extent in the presence of nitrous oxide, the increase being independent of initial iodine concentration. At initial concentrations less than 0·9 mM, iodine is consumed almost entirely as a result of reaction with *cyclo*hexyl radicals[3]. The enhanced yield of *cyclo*hexyl radicals responsible for the disappearance of solute, $\Delta G(R\cdot)$, may therefore be equated with $\Delta G(-I)$, that is, 1·75. The enhanced di*cyclo*hexyl yield, $\Delta G(R_2) = 0{\cdot}85$, in the presence of nitrous oxide but in the absence of iodine, is equivalent to $\Delta G(R\cdot) = 2\Delta G(R_2) = 1{\cdot}70$ if it is assumed that the additional *cyclo*hexyl radicals react only by dimerization. It is well established, however, that radical disproportionation to *cyclo*hexane and *cyclo*hexene also occurs and we must therefore assume that a part of the additional di*cyclo*hexyl yield is due to processes not involving radicals.

The formation of di*cyclo*hexyl and other radiolysis products has been explained both in terms of reactions involving radicals and ions. *Cyclo*hexyl radicals may be produced either from direct excitation of *cyclo*hexane, or from the excited state produced by ion recombination:

$$RH \rightsquigarrow RH^+ + e \quad (1)$$

$$RH^+ + e \longrightarrow RH^* \quad (2)$$

$$RH^* \longrightarrow R\cdot + H\cdot \quad (3a)$$

$$\longrightarrow C_6H_{12} + H_2 \quad (3b)$$

If β and $(1-\beta)$ designate the probabilities that the excited state formed by ion recombination will decay by reactions (3*a*) and (3*b*) then, for each electron scavenged, there is a loss of not less than β *cyclo*hexyl radicals. Further *cyclo*hexyl radicals may, however, be produced by abstraction:

$$H\cdot + RH \longrightarrow H_2 + R\cdot \quad (4)$$

and the total loss of radicals per scavenged electron therefore lies between β and 2β, the exact value depending on the extent of hydrogen atom abstraction. Because nitrous oxide undergoes dissociative electron capture, we may postulate the following additional steps which result in two *cyclo*hexyl radicals:

$$N_2O + e \longrightarrow N_2O^- \longrightarrow N_2 + O^- \quad (5)$$

$$O^- + RH \longrightarrow R\cdot + OH^- \quad (6)$$

$$RH^+ + OH^- \longrightarrow R\cdot + H_2O \quad (7)$$

It is therefore evident that there will be an overall increase of between $(2-\beta)$ and $2(1-\beta)$ *cyclo*hexyl radicals per scavenged electron. Retention of excitation energy by the water molecule (reaction 7) may also result in the formation of further *cyclo*hexyl radicals.

Although a detailed mechanism cannot be proposed until a complete analysis of product yields has been made, the foregoing sequence is able to explain, at least qualitatively, the main features of our experiments. In addition, the postulated mechanism is able to account satisfactorily for the reported decrease in hydrogen yield in *cyclo*hexane[2] and polyethylene[4], and for the production of water and nitrogen in stoichiometric equivalence to the disappearance of nitrous oxide in polyethylene[4]. While it is possible to account for most of our observations in terms of enhanced *cyclo*hexyl radical production, it is also probable that electron scavenging will enhance ion-molecule reactions of the type discussed by Libby[5].

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CHEMISTRY

Electron Spin Resonance Spectra associated with Nitrogen in Diamonds

MANY diamonds show an electron spin resonance spectrum which has been attributed to the presence of single nitrogen atoms substituted for carbon at a diamond lattice site. Smith *et al.*[1] found four types of nitrogen donors, equally abundant and differing only in their hyperfine axes, these being the four C–N bond directions. They measured the hyperfine coupling constants for $^{14}N(I=1)$, and for $^{13}C(I=\frac{1}{2})$ in the nearest neighbour positions (denoted centre I). Loubser and du Preez[2] found additional lines in the spectrum, which they attributed to interaction of the unpaired electron with carbon-13 at other lattice sites (centres II, III and IV). The hyperfine coupling constants are recorded in Table 1, together with the orbital populations. These populations were obtained using values of $|\psi_{2s}(0)|^2$ and $\langle r^{-3}\rangle_{2p}$ calculated from self-consistent-field atomic wave functions derived by Mayers and by Roothaan and Clementi (see ref. 3). (We have omitted any correction for the δ+ charge on nitrogen and the δ− charge on carbon: this would increase the spin density on carbon at the expense of the nitrogen.)

The calculated spin populations on nitrogen-14 and one of its nearest neighbour carbon-13 atoms are 0·29 and 0·79 respectively, so the electron is almost entirely localized on these atoms, and, contrary to common supposition, predominantly on the carbon. (The total calculated spin density of 1·08 is sufficiently close to unity to give some confidence that the calculations are reasonable.)

Prior to distortion, the bonding between nitrogen and any one carbon atom can be represented by the energy-levels of a pseudo diatomic molecule (Fig. 1), involving overlap of sp^3 hybrid orbitals. The unpaired electron is then accommodated in the σ*-antibonding orbital with a consequent increase in the bond length. As a result of this relaxation, there will be an increase in the *p*-character of the orbitals involved in bonding between nitrogen and carbon, and because the unpaired electron is more closely associated with the carbon atom, a relatively greater increase of *p*-character in the carbon orbital is expected. Thus the energy separation between the nitrogen and carbon *s*–*p* hybrid orbitals will be increased, as depicted in Fig. 1, and this results in an even greater concentration of the antibonding electron on the carbon. In the limit of complete separation, the unpaired electron would be in a pure *p* orbital of the carbon, and the bonding electrons would become a lone-pair on the nitrogen, but the constraints of the lattice prevent such a limit being reached. This accounts for the calculated values of the p/s ratio (λ^2), which are 10·9 for carbon-13 and 3·6 for nitrogen-14.

Loubser and du Preez[2] found three additional groups of lines (centres II, III and IV in Table 1), ascribed to

Table 1

Centre	Hyperfine coupling (gauss) Observed (gauss) $A_\parallel$	$A_\perp$	Isotropic A_{iso}	Anisotropic $2B$	$-B$	$-B$	a_s^2*	a_p^2*	p/s ratio	Relative intensities (approx.)	Hyperfine axis	Ref.
Nitrogen-14	40·8	29·2	33·1	7·70	−3 85	−3·85	0 060	0 23	3·6	180	C_a—N direction	1
Carbon-13 (centre I)	121·4	50·7	74·3	47·2	−23·6	−23·6	0 067	0 73	10·9	1	C_a—N direction	2
Carbon-13 (centre II)	15 1	11·4	12 6	2·5	−1·25	−1·25	0·011	0·038	3·3	3	C_a—N direction (approx.)	2
Carbon-13 (centre III)	9·2		9 2				0 008			3	Isotropic	2
Carbon-13 (centre IV)	4·8		4·8				0 004			3	Isotropic	2

* Calculated using a_s^2 (nitrogen-14) = 550 G a_s^2 (carbon-13) = 1,110 G
a_p^2 (nitrogen-14) = 34 G a_p^2 (carbon-13) = 64·8 G

interaction of the unpaired electron with carbon-13 nuclei other than C_a (centre I). They assigned the centre with the largest hyperfine coupling (centre II) to carbon-13 in one of the three positions basal to the nitrogen (C_b in Fig. 2), on the assumption that the unpaired electron spends more of its time close to the nitrogen. However, even if this assignment is altered to carbon-13 in positions basal to the carbon in the N–C_a bond (C_c in Fig. 2), since our calculations have shown that the greatest spin density is on the C_a carbon, there would seem to be no explanation for the fact that the hyperfine interaction is axially symmetric about the N–C_a bond axis. Therefore, we suggest that the lines of centre II arise from unpaired electron density on the carbon atoms C_d, that

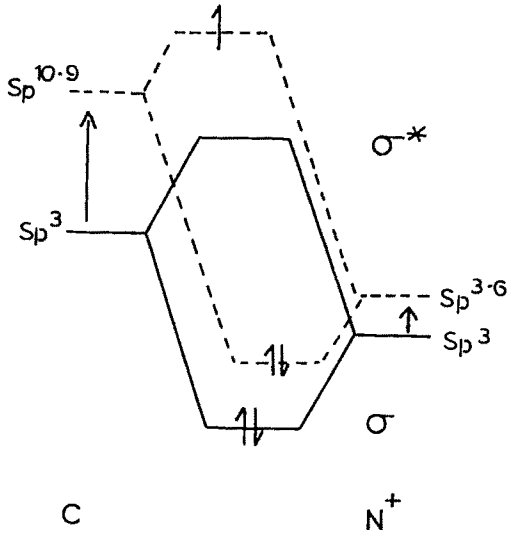


Fig. 1. Energy-level diagrams for the pseudo-molecule CN^+. The full lines represent the undistorted centre, and the dashed lines show the effect of distortion on the energy-levels

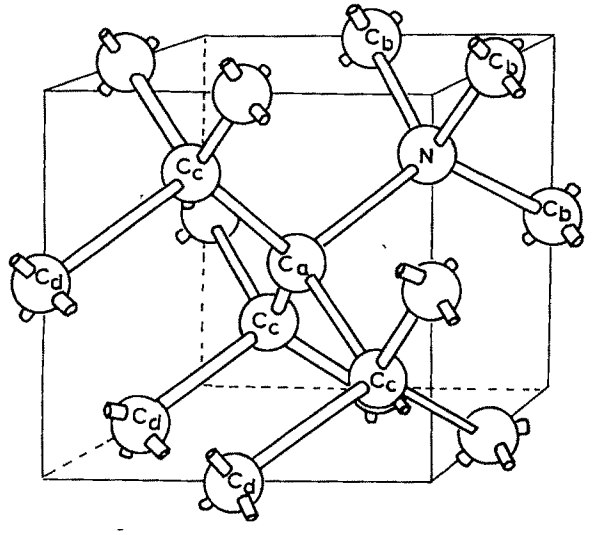


Fig. 2. Part of the diamond lattice showing the nitrogen impurity and the carbon atoms C_a, C_b, C_c and C_d referred to in the text

is, carbon atoms in the β-position with respect to C_a. Hyperfine interaction with the C_d carbons would be expected to have axial symmetry about the C_c–C_d bond, which is parallel to the C_a–N bond direction. Thus the observed dependence of the hyperfine splitting on the direction of the external magnetic field would be explained. It is envisaged that transfer of spin density into the C_c–C_d bond might occur by a mechanism similar to hyperconjugation, the effect being enhanced by the relatively large C_a carbon p-character in the antibonding orbital. Since this effect depends on the fact that the C_c–C_d and C_a–N directions are parallel it is possible to see why interactions with only three of the nine β-carbon atoms are detected. The minor deviations from axial symmetry that were observed can be explained qualitatively in terms of a direct dipolar interaction with spin on C_a. Centres III and IV may be reasonably assigned to carbon atoms C_c and C_b, respectively.

It is interesting to compare the manner in which an extra electron is accommodated in other nominally tetrahedral structures. In phosphorus-doped silicon, electron spin resonance investigations have shown that the phosphorus hyperfine coupling is purely isotropic[4]. The extra electron is no longer mainly localized in any one bond, but is dispersed widely throughout the silicon lattice, with only a very small spin density (0·01) at the phosphorus atom. Thus the electron has avoided the energetically unfavourable antibonding site occupied in diamond by becoming delocalized on to many silicon atoms. This contrast is most readily understood in terms of the presence of relatively low energy 3d levels on the silicon atoms.

In PF_4, however, the unpaired electron causes considerable distortion from the tetrahedral symmetry of the PF_4^+ cation[5], thereby reducing its antibonding character. In this case, the molecule is free to distort in a manner which is virtually impossible for carbon in the diamond lattice. For nitrogen in diamond, the electron in an undistorted frame would be accommodated in an antibonding molecular orbital probably having a_1 symmetry, as in the case of undistorted PF_4 molecule[5]. It becomes localized in a single C–N bond only as a result of the distortion already discussed. It is interesting to note that this distortion does not have the dynamic character often associated with Jahn–Teller distortions, in that the extra electron remains in any one C_a–N σ*-orbital for a time that is long compared with the hyperfine frequencies for carbon-13 and nitrogen-14.

We thank the Science Research Council for a maintenance grant.

Note added in proof. Our attention has been directed to a recent communication[6] in which a molecular orbital treatment is given for this centre and conclusions are drawn which are very similar to our own.

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2537 Å Mercury Photosensitization of Liquid Water

THE solubility of mercury in water is 0·32 μM at 25° C and 1·7 μM at 90° C (ref. 1). The dissolved mercury absorbs the 2537 Å resonance line although not to the same extent as in the vapour phase due to the solvation broadening of its absorption band[2]. It has been shown that mercury-saturated liquid hydrocarbons will undergo photosensitization[3–6] and the results of these investigations have been compared with those from high-energy radiolysis indicating that the process can be of value in the study of the behaviour of molecular fragments and excited states in the liquid. This communication reports some preliminary results in the photosensitization of liquid water and its application to the characterization of the intermediates involved in the radiolysis.

A Hanovia *SC*-2537 low-pressure mercury resonance lamp was used which surrounded the cylindrical cell fabricated from type 7910 'Vycor' (transparent to $\lambda > 2100$ Å). Triply-distilled conductivity water from a tin-lined Barnstead still was shaken with liquid mercury at 80° C for 30 min. Nitrogen or other gases were bubbled through the solution for 20 min before irradiation and, unless otherwise noted, the solution was constantly swept during photolysis. Liquid mercury was always present in order to ensure saturation and the cell and lamp were air thermostated at 80° ± 3° C. The gas phase zone was masked. Only in runs longer than 4 h was evaporation appreciable (> 10 per cent).

Hydrogen peroxide was determined spectrophotometrically[7]. Fig. 1 shows the yield of hydrogen peroxide as a function of exposure time with nitrogen or argon as the bubbling gas. The fall-off of the initial linear rate after 3 h is attributed to photolysis and secondary reactions of the peroxide. The initial quantum yield of hydrogen peroxide formation is about 0·005—based on uranyl oxalate actinometry[8]. No hydrogen peroxide was produced in the absence of mercury.

The formation of hydrogen peroxide can be explained by the combination of OH radicals formed in some primary energy transfer step such as:

$$Hg^* + H_2O \rightarrow H + OH + Hg \quad (1)$$

or:

$$\rightarrow HgH + OH \quad (2)$$

$$\rightarrow Hg + H \quad (2a)$$

where $Hg^* = Hg(6^3P_1)$ and $Hg = Hg(6^1S_0)$. Step 1 is endothermic and can be eliminated. From either step H atoms would be expected to be present; their combination would produce hydrogen. Hydrogen gas is a good scavenger for OH radicals[9], and if not swept from the system would be expected to cause a reduction in the yield of hydrogen peroxide. When no gas was swept through the deaerated solution during exposure, the yield of hydrogen peroxide built up to a steady but low concentration as shown in Fig. 1. Furthermore, when hydrogen was used as the sweeping gas, no hydrogen peroxide was observed for exposures as long as 4 h. In addition, the use of OH scavengers[9,10] such as carbon monoxide, methane, ethane and butane as the sweeping gas caused the complete quenching of the yield of hydrogen peroxide.

This behaviour of the system toward the nature of the sweeping gas strongly suggests that H and OH radicals are present. However, the molecular generation of hydrogen and hydrogen peroxide from an excited state of water according to steps similar to those proposed by Anbar, Guttmann and Stein[11] cannot be immediately discounted:

$$Hg^* + H_2O \rightarrow H_2O^* + Hg \quad (3)$$

$$H_2O^* + H_2O \text{ (or } H_2O^*) \rightarrow H_2O_2 + H + H \text{ (or } H_2) \quad (4)$$

Such an excited state, which could be the precursor of H and OH radicals as well, may be a metastable long-lived

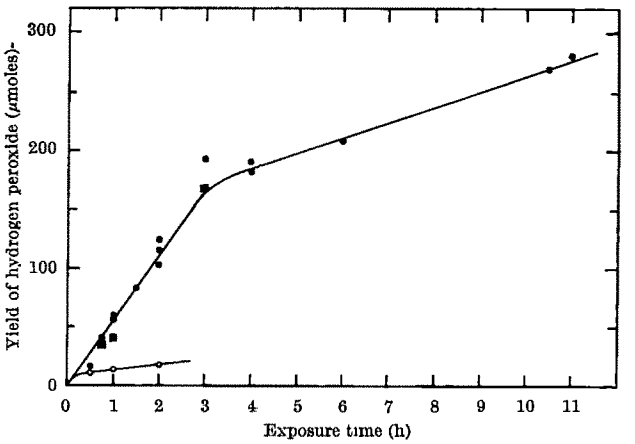


Fig. 1 Production of hydrogen peroxide in the 2537 Å mercury photosensitization of liquid water, ●, nitrogen bubbling; ■, argon bubbling; ○, presaturation with nitrogen but no bubbling during photolysis

triplet state and may be the excited water molecule proposed by several workers[11–13] to explain isotope scrambling and 'residual hydrogen' yields. If H_2O^* is indeed present in irradiated water, its yield, as shown recently[14], is very low.

It is possible to use the technique of liquid phase mercury photosensitization to generate radicals or excited states in liquid water conveniently without the use of ionizing radiation or vacuum ultra-violet techniques.

We thank Prof. G. Stein for his advice in this work, which was partially supported by a grant from the National Institutes of Health.

Note added in proof. M. Chevion, U. Sokolov and G. Stein (unpublished results) have measured the quantum yield of H_2 evolution in the 2537 Å mercury photosensitized decomposition of liquid water and find $\varphi_{H_2} \simeq 0{\cdot}003$, in fair agreement with our value for $\varphi_{H_2O_2}$.

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Polyamide Layer Chromatography of Lactones

POLYAMIDE is a specific chromatographic adsorbent for several kinds of organic compounds, as has been shown by Carelli *et al.*[1] (phenols and fatty acids by powder column), Davidek[2] (antioxidants by powder thin layer), Endres[3] (aromatic nitro compounds and sulphonic acids by powder column) and Wang[4] (indoles by polyamide layer). In the extension of the uses of polyamide layer as a chromatographic media, we found it suitable for the separation of lactones.

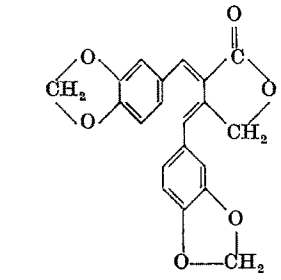
We selected seven lactonic compounds as examples for testing the separation of lactones on polyamide layer.

Table 1 gives the R_F values of lactones in three solvent systems.

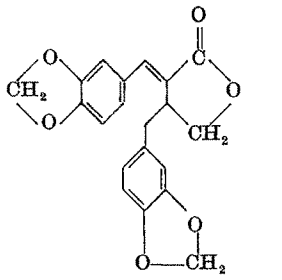
Table 1. R_F VALUES OF LACTONES ON POLYAMIDE* LAYER ACCORDING TO WANG (REF. 7)

Compound	Solvent system† I	II	III
Taiwanin A‡	0·74	0 09	—
Savinin‡	0 74	0 06	—
Santonin§	0·45	0·49	—
Toddalolactone§	0·20	0 58	—
Coumarin§	0 73	0 41	—
Phthalide§	0·65	0·58	—
Ascorbic acid§	0 00	1 00	0·46

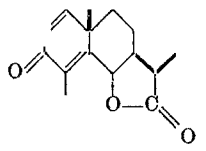
* 'Amilan *CM* 1011' of Toyo Rayon Co., Tokyo, Japan.
† Solvent systems: I, carbon tetrachloride/glacial acetic acid (9/1); II, 90 per cent formic acid/water (1/2); III, *n*-butanol/90 per cent formic acid (9/1).
‡ Visible as fluorescent spots under ultra-violet light.
§ Visible under ultra-violet light after spraying with 0·05 per cent alcoholic fluorescein



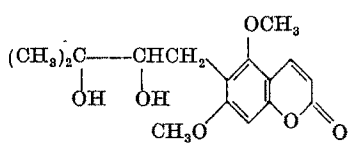
Taiwanin *A*



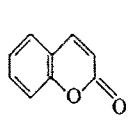
Savinin



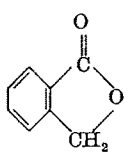
Santonin



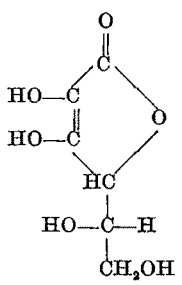
Toddalolactone



Coumarin



Phthalide



Ascorbic acid

Taiwanin *A*[5] and savinin were isolated in our laboratory from *Taiwania cryptomerioides* Hayata. They are typical lignin-type lactones and differ only in the degree of unsaturation. Santonin is a sesquiterpenoid lactone. Its ketonic group probably has some affinity to polyamide. Toddalolactone is a coumarin derivative. The low R_F value of toddalolactone in carbon tetrachloride/glacial acetic acid (9/1) system as compared with coumarin itself reflects on the fact that the two alcoholic hydroxyl groups in the molecule seem to form rather strong hydrogen bonds with polyamide. This is consistent with the behaviour of oestrone, oestradiol and oestriol which, it has been found[6], show a large difference in their R_F values on polyamide layer chromatogram. In the solvent system formic acid/water (1/2), the order is reversed and the hydrophilic hydroxyl groups make toddalolactone move faster with the aqueous mobile phase. The large number of polar groups in ascorbic acid are responsible for it remaining at the origin in the carbon tetrachloride/glacial acetic acid (9/1) system but moving to the solvent front in the formic acid/water (1/2) system. *n*-Butanol/formic acid (9/1) has been found to be the most satisfactory solvent system.

Lactones occur widely as natural products, for example, sesquiterpenoid lactones. It is preferable to separate them on polyamide due to the fact that they are less likely to be decomposed by the adsorbent.

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METALLURGY

Formation of New Intermetallic Phases in Binary Eutectic Systems by Drastic Undercooling of the Melt

IT has earlier been reported[1–3] from our Laboratory that some eutectic binary alloys solidify in an amorphous condition on rapid cooling from the liquid state. The present communication deals with new crystalline phases obtained by subjecting liquid alloys of eutectic systems to severe undercooling during solidification.

A large number of alloys, covering the full composition ranges of four simple eutectic systems[4], namely silver–silicon, silver–germanium, gold–silicon and gold–germanium, were examined in the course of the present work. Small quantities of the homogenized alloys were quenched from the melt on to a copper substrate by the 'gun' technique[5], which has recently been shown[6] to produce cooling rates ranging from 10^6 to 10^8 °C/sec. The quenched thin layers of alloy were subjected to Debye–Scherrer examination at room temperature immediately after quenching as well as after annealing at higher temperatures. Details of alloy preparation, quenching procedure and X-ray examination have been described earlier[7].

Full and partial amorphous solidification was observed in gold–silicon and gold–germanium alloys respectively around the eutectic composition. Slight extension of solid solubility was noted for germanium in both silver and gold. Alloys of the silver–silicon and silver–germanium systems having somewhat higher eutectic temperatures[4] gave no evidence for amorphous solidification. There was no evidence from lattice parameters for any solid solubility of silicon in either silver or gold.

Details of crystal structures and composition of the six new intermetallic phases obtained in the present work are given in Table 1. All these phases, except γ gold–silicon, could be reproduced very well at the indicated composition ranges and proved quite stable at room temperature. Only γ gold–germanium could be obtained strictly homogeneous over an appreciable range of composition. There was strong evidence for X-ray line-broadening characteristic of stacking faults[8] in β silver–silicon, β silver–germanium, γ gold–silicon and β gold–germanium. In case of the three hexagonal close-packed structures, which could be visualized as resulting from face-centred cubic lattices by a change in stacking sequence of the close-packed layers, the stacking faults could be identified as predominantly of the growth type with a random fault for every 5 to 10 layers in the extreme cases. The β gold–silicon, γ gold–silicon and γ gold–germanium structures seem to be complex superlattices, in the light of the relations between their lattice parameters and the unit cell dimension for the adjoining face-centred cubic phase. Among these six phases, β silver–germanium and γ gold–germanium have been shown to be superconducting at 0·85° and 1·63° K respectively[9].

Table 1. Composition Range, Crystal Structure and Relation to Adjoining Face-centred Cubic Phase of the New Intermetallic Phases in Silver–Silicon, Silver–Germanium, Gold–Silicon and Gold–Germanium Systems

Designation of new-phase	Composition range of occurrence	Composition range of homogeneity	Structure	Lattice parameters and relation to $a_{f.c.c.}$	Axial ratio
β Silver–Silicon	5–25 at. per cent Silicon	—	Hexagonal close-packed	$a = 2{\cdot}87$ Å $\simeq a_{f.c.c.}/\sqrt{2}$ $c = 4{\cdot}58$ Å $\simeq 2{\cdot}a_{f.c.c.}/\sqrt{3}$	1·58
β Silver–Germanium	10–30 at. per cent Germanium	20–22 at. per cent Germanium	Hexagonal close-packed	$a = 2{\cdot}90$ Å $\simeq a_{f.c.c.}/\sqrt{2}$ $c = 4{\cdot}72$ Å $\simeq 2\ a_{f.c.c.}/\sqrt{3}$	1·62
α Gold–Silicon	5–25 at. per cent Silicon	—	Face-centred cubic	$a = 7{\cdot}84$ Å $\simeq 2{\cdot}a_{f.c.c.}$	—
γ Gold–Silicon	25–45 at. per cent Silicon	—	Face-centred cubic	$a = 19\ 50$ Å $\simeq 5{\cdot}a_{f.c.c.}$	—
β Gold–Germanium	10–30 at. per cent Germanium	20–22 at. per cent Germanium	Hexagonal close-packed	$a = 2\ 89$ Å $\simeq a_{f.c.c.}/\sqrt{2}$ $c = 4{\cdot}75$ Å $\simeq 2{\cdot}a_{f.c.c.}/\sqrt{3}$	1·64
γ Gold–Germanium	25–65 at. per cent Germanium	40–50 at. per cent Germanium	Body-centred tetragonal	$a = 11\ 63$ Å $\simeq 2{\cdot}\sqrt{2}\ a_{f.c.c.}$ $c = 22{\cdot}49$ Å $\simeq 11/2{\cdot}a_{f.c.c.}$	1·93

There seems to be a close parallel between these new crystalline phases in eutectic systems and the martensitic phases formed in some eutectoid systems (such as iron–carbon and copper–aluminium). In both instances, a new non-equilibrium single phase is produced irreversibly by a non-nucleation-and-growth mechanism from a parent phase in place of a two-phase equilibrium mixture. One may well speculate whether such so-called non-equilibrium phases in eutectic and eutectoid systems are actually the stable phases at room and lower temperatures, but are prevented from formation on slow cooling only by the extremely sluggish nature of the diffusion-controlled peritectoid reactions necessary to produce them. It is significant to note in this connexion that the equilibrium close-packed phases of comparable composition in the copper–silicon and copper–antimony systems[9] are both formed by peritectoid reactions on cooling.

The authors are grateful to Prof. Pol Duwez for suggesting this problem and for valuable comments.

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BIOCHEMISTRY

Collagen-plasticizing Function of Carbohydrate Polymers

The amino-sugar-containing polysaccharides (glycosaminoglycans) of native connective tissues have engaged the attention of a large and diverse group of investigators. However, only a few[1–4] have seriously considered the possible mechanical functions which these macromolecules, either alone or in association with non-collagen proteins, might subserve *in vivo*.

Recent investigations in this laboratory indicate that 'ground-substance' carbohydrates undergo very characteristic alterations (decreased content and, possibly, depolymerization) during natural ageing processes and that these may very largely be responsible for the topological properties of native connective tissue networks[5]. Conversely, it also appears that certain carbohydrate polymers can act *in vitro* much like polymer-siluents, or plasticizers for native and 'purified' collagen matrices[6].

The present investigation was prompted by these observations. It was undertaken specifically to determine whether or not prototype carbohydrate polymers could act as 'good' solvents (and, hence, concurrently, impart both an increase in equilibrium swelling volumes and a decrease in elastic moduli[7]) for specific fibrous protein networks. The matrices selected were goatskin collagens, prepared as outlined previously[8], which were substantially free of all components except collagen. 5 per cent aqueous solutions of 'Dextran-500' and 'Dextran-500 sulphate' (purchased from Pharmacia Fine Chemicals, Inc., New Market, New Jersey) and of dialdehyde starch (100 per cent carbonyl form, kindly donated by Dr. C. L. Mehltretter, U.S. Department of Agriculture, Peoria, Illinois) were used as the 'solvent' phases.

One-inch square segments of the skins were used for the swelling investigations, and dumbbell-shaped specimens (necked-down portion = 1·58 cm) were used with a *TM* model 'Instron' tensile testing apparatus for determination of apparent elastic moduli. (The latter were determined on skin samples cut with their longitudinal axes parallel to that of the animal's vertebral column and were assumed, for purposes of the present comparative investigation only, to behave as if homogeneous and isotropic which they obviously are not. The rate of cross-head travel was 0·5 cm/min in all experiments. The 'modulus' was determined from values of nominal tensile stress-at-break using micrometer measurements of the sample thickness to determine the cross-sectional area of the necked-down portion of the test sample.) The difference in the weight of samples before and after 48 h immersion in each of the solvent phases was corrected for the observed densities of each medium to yield values for the change in sample volume. These were arbitrarily taken as the 'equilibrium' swelling volumes.

Mean values for 10–20 determinations of both equilibrium swelling volume (V_e) and apparent elastic modulus (A.E.M.) in each of the four 'solvent' media (for example, water, dextran sulphate, dextran and dialdehyde starch) are summarized in Table 1, together with data on the observed nominal tensile stress-at-break (σ_u) and change in elongation (ΔL).

Table 1. Physical Properties of Hydrated Goatskin Collagen Matrices in Various 'Solvent' Media

Compound	σ_u	ΔL	A.E.M.	ΔV_e	pH$_i$
Dextran-sulphate	32 9	2·7	1·9	+0·13	7·2
Dextran	91·4	2·4	5 9	+0·08	6·6
Water	99·2	2·3	6·7	—	6·5
Dialdehyde starch	100 9	2·2	7·0	−0·06	7·4

σ_u is the nominal tensile stress-at-break, in kg/cm^2; ΔL is the measured changed in length (cross-head displacement), in cm; A.E.M. is apparent elastic modulus, in dynes/cm^2 × 10^7 after 18 h immersion. ΔV_e is change in volume per g of initial hydrated weight, in ml./g, after 48 h immersion. pH$_i$ is the initial pH of the solvent phase.

The data obviously suggest that unoxidized carbohydrate polymers act, within physiological pH limits and directly on collagen polypeptide chains, as effective plasticizers to 'soften' collagen matrices, more or less permanently. The nominal tensile strengths and apparent elastic moduli of the skin samples were significantly decreased and both the equilibrium swelling volumes and mechanical extensibilities were increased, relative to data obtained for samples in water, in exact accord with the behaviour expected of a 'good' solvent. On the other hand, analogues which have been oxidized to the corresponding dialdehyde form[9] no longer possess this property, and in fact behave, like other aldehydes[8], as potent cross-linking agents for collagen structures.

The possibility thus exists that changes demonstrated at least in the content of the polysaccharide phases of native connective tissues may have very important secondary consequences so far as the mechanical properties of these tissues are concerned. It is also conceivable in the light both of the present data and of an expanding literature on the function of lysosomal enzymes that disruption of the sulphate moiety alone, or more general depolymerization of ground-substance carbohydrates, may, perhaps *per se*, account for the pathogenesis of a number of clinical and experimental abnormalities of the connective tissue system.

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Binding of Aliphatic Aldehydes to Collagen and Elastin Matrices

PREVIOUS publications from this laboratory have demonstrated that specific hydrated aliphatic aldehydes[1,2] react both *in vitro* and *in vivo*[3–5] with the freely rotatable nitrogen atoms[6] of native and 'purified' collagen and elastin chains. The resulting products are highly cross-linked network structures[7] which are characterized by 'locking-in' of dipoles[8] normally present along the respective polypeptide chains. Unfortunately, little is known about the quantitative aspects of the involved reactions. The presently reported investigation was accordingly prompted, in which the binding of two specific C_3 aldehydes to hide powder collagen, extracted aortic elastin, intact (control) bovine thoracic aortae and sonicated bovine thoracic aorta preparations were investigated.

The prototype aldehydes selected were propionaldehyde, a highly charged (specific conductivity, 345 μmho/cm) non-cross-linking agent which exists in solution in the unhydrated or free carbonyl form, and acrolein, a weakly charged (specific conductivity, 9·3 μmho/cm) but potent cross-linking agent which exists in solution in the hydrated aldol (β-hydroxy-carbonyl) form[1].

The hide powder (American Standard) collagen sample, which had been previously limed and neutralized, was purchased from F. R. Marshall, Ridgeway, Pennsylvania, and had a known shelf life of about 3 years. Bovine thoracic aortae, freshly obtained from a local abattoir, were used for the remaining preparations. Elastin was isolated at 45° C for 45 h from defatted aortae according to the formic acid procedure described by Hass[9]. Sonicated aorta preparations were made by sonicating control specimens in 2 per cent sodium hydroxide in sealed 'Pyrex' tubes within the water-filled well of a commercial apparatus ('DiSONtegrator', system 40, Ultrasonic Industries Inc., Engineers Hill, New York) for 24 h. The final temperature was 45° C. All matrices were thoroughly washed in cold, running tap water for 24 h, ground twice in an ordinary household meat grinder and finally dehydrated *in vacuo* over calcium chloride and silica gel at room temperature before use. No attempt was made to achieve uniformity in the particle size of the respective tissue samples.

Microscopic sections, stained by the van Gieson–Verhoeff, periodic acid–Schiff and hematoxylin-and-eosin techniques, revealed the presence of only collagen and elastin fibres, respectively, in the hide powder and aortic elastin samples. The control aorta preparation possessed normal compact architecture with intact cells and fibres. The sonicated preparations were composed of a loosely arranged, entirely acellular matrix with variably disrupted fibres; these stained positively with elastin histochemical stains, and were apparently devoid of any associated ground-substance materials. Electrophoretic patterns of each matrix, obtained after dichloracetic acid solubilization by the method of Milch, Gey and Naughton[10], revealed distinctly different patterns for each of the respective matrices (Fig. 1).

200-mg aliquots of each dried matrix were overlaid with 10 ml. of 0·15-M aqueous aldehyde solutions in stoppered tubes. Three control tubes (aldehyde solution alone) and five experimental tubes (aldehyde + matrix) were used in every run. Between five and seven runs were made with each aldehyde and each matrix. In each, approximately 2 μl. of the supernatant liquid phase were removed from all tubes after 1, 2, 3, 4, 5 and 24 h of interaction and analysed by a gas chromatographic procedure recently described by Clifford and Karmen[11]. (A 'Packard' coiled glass column (10′ × 2 mm internal diameter, catalogue No. 5392910), packed with 14 per cent ethylene glycol adipate, was used with an '*IDS*' model 20 Barber Coleman gas chromatograph. The latter was equipped with a hydrogen flame detector. The inlet temperature was 140° C and the column temperature was 80° C.) The extent of binding was calculated from mean values of the measured differences in supernate aldehyde concentration between the control and experimental tubes at each stipulated time. No corrections were made for volume losses due either to sampling or to evaporation, since both were well within the limits of error of the analytical procedure.

Representative data are summarized in Table 1.

These indicate that essentially identical amounts of both acrolein or propionaldehyde were bound by each matrix over the first 3 h of interaction, in the order: sonicated aorta > hide powder collagen > control aorta > aortic elastin.

Little or no additional binding occurred with propionaldehyde over the ensuing 21 h. With acrolein, however, continued binding over the remaining 21 h of the experimental period was observed with all of the matrices except elastin. Binding capacity was again greatest with the sonicated aorta preparation; lesser amounts were

Table 1. BINDING OF ALDEHYDES TO CONNECTIVE TISSUE MATRICES

Matrix	Propionaldehyde						Acrolein					
	1	2	3	4	5	24	1	2	3	4	5	24
Hide collagen	67 5	64 4	64·0	66 6	70 1	64·4	49·6	63 9	61 0	85·3	95·0	100 9
Elastin	17 0	27·4	25 2	30 5	39·2	41 8	13·1	16 4	25 6	30 7	39 1	40 8
Aorta	19 6	36·2	40 1	36 2	38 8	34·9	16·4	39 5	48 8	49·4	63·9	108·9
Sonicated aorta	56 2	54 9	78 9	80 6	68 8	77 1	51 7	60·9	72 8	102 6	103 0	247 7

Binding is expressed in terms of mg bound/g initial dry weight after stipulated number of hours of interaction.

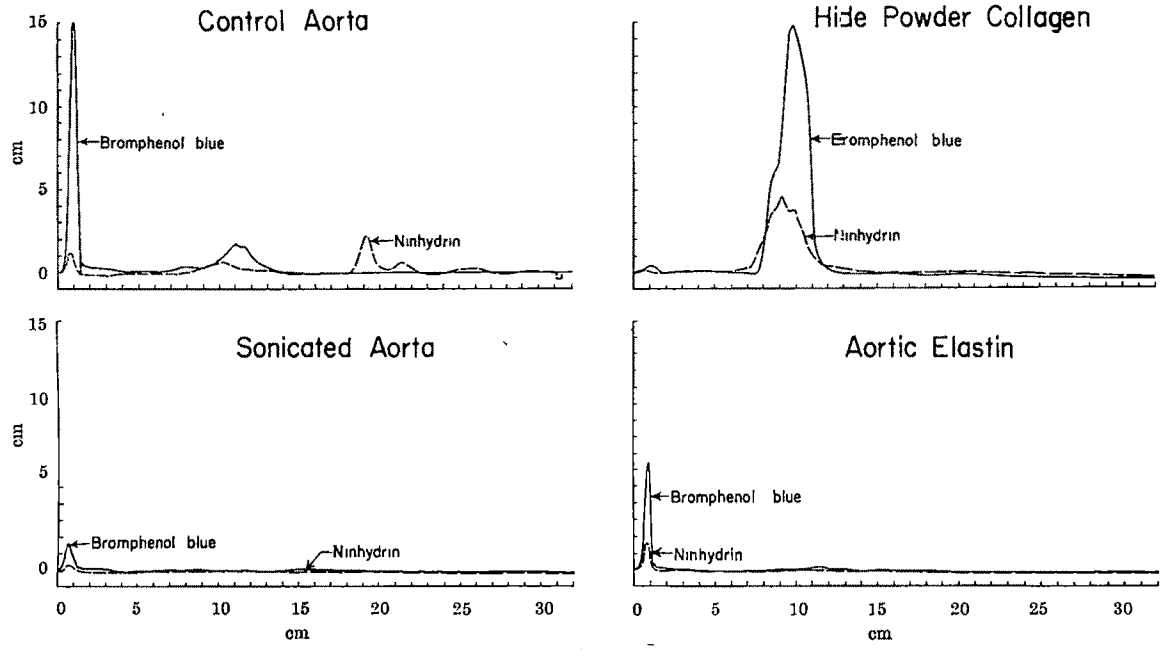


Fig. 1

bound by hide powder collagen and the untreated aorta preparations. Elastin showed the least amount of binding.

Elastin chains prepared as outlined here thus presumably possess but a limited number of sites to which aldehydes can bind, irrespective of their conformation, the time of interaction or the ion-dipole or dipole-dipole nature of the involved reactions. The same appears to be true for all other matrices over the first three hours of interaction. Thereafter, however, additional sites evidently become available for acrolein but not for propionaldehyde molecules. These possibly arise in consequence of chain scission phenomena, which appear to accompany interchain cross-linking reactions at high aldehyde concentrations[8,12], such as used here.

These differences in the binding capacity of each matrix for either of the two aldehydes may be explained, in part, by differences in the gross or 'fabric' structure of the respective matrices. In larger part, however, the differences are probably best accounted for in terms of the network structure of the respective fibres and fibre bundles. Hide collagen fibres with low internal cross-link densities and substantively free of associated carbohydrate components would be expected, in agreement with the experimental observations, to possess relatively more apparent binding sites for aldehydes than do the more highly cross-linked polypeptide chain networks, such as represented by the elastin and the control aorta preparations. Similarly, it might also be predicted that more or less selective extraction of the alkali-labile protein-polysaccharide complexes of native ground-substance materials[13,14] would liberate chemically or sterically hindered protein N—H groups and thus increase relative aldehyde-binding capacities. This also accords with the experimental observations. Finally, 'purified' matrix fibres which possess relatively few reactive amino and/or amide groups per unit length of polypeptide chain, such as elastin[15], would also be expected to possess relatively low aldehyde binding capacities. This, too, is in agreement with what was found.

The data thus support the conclusion that, in fibrous protein networks, the availability of freely rotatable amino nitrogen atoms[6] to reactive aldehydes is governed not only by the number of such groups but also by both the internal (intra- and inter-chain) cross-link density of the respective chains and the presence (and probably aggregation state) of the associated ground-substance protein-polysaccharide copolymers[14]. Furthermore, the quantitative extent of binding appears to be critically dependent on the nature of the binding reactions, becoming generally asymptotic with non-cross-linking aldehydes and increasing progressively (concomitant with and probably due to backbone chain scission) with cross-linking aldehydes.

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Ribonucleic Acid Polymerase Activity in Extended and Contracted Chromosomes

VARIOUS reports have appeared in the literature concerning the absence of RNA synthesis in metaphase cells. An explanation commonly given is that the condensed or compacted state of chromosomes prohibits DNA trans-

scription[1]. This explanation is supported by evidence that regions of condensed chromatin in an interphase nucleus are sites of minimal RNA synthesis[2]. We wish to report some results of recent studies on RNA metabolism during the meiotic cycle which provide additional evidence on the relationship between the physical state of the chromosomes and RNA synthesis. Two features of meiosis are relevant to this report: (1) Cells in early prophase of meiosis (leptotene) synthesize RNA at a much higher rate than those at metaphase[3]. Indeed, it is probable that no RNA is synthesized during metaphase. (2) At leptotene, chromosomes are in their most extended form; at metaphase, they are in their most contracted form. The biochemical and cytological data are thus consistent with the view that the capacities of chromosomes to transcribe DNA into RNA are inversely related to their degree of condensation. We addressed ourselves to the question of whether *in vitro* studies of isolated nuclei and chromosomes could yield information on the respective roles of RNA polymerase and template availability in accounting for established observations. Meiotic cells obtained from anthers of *Tulipa gesneriana* were used in these experiments.

Leptotene nuclei or metaphase chromosomes were isolated from tulip microsporocytes in a 1 : 1 (v/v) glycerol: water mixture containing the following molarities of solutes: sucrose, 0·5; potassium chloride, 0·01; ethylenediamine tetraacetic acid (EDTA), 0·002; sodium–potassium phosphate buffer (*p*H 6·95), 0·01. The cells were disrupted by means of a 'French' pressure cell and the suspension then fractionated by differential centrifugation. Details of the procedure are described elsewhere[3]. The isolated fractions were incubated in 0·2 ml. of medium containing the following components (in μmoles): *tris* buffer (*p*H 7·9), 8·0; manganese chloride, 0·2; magnesium chloride, 0·8 ; 2-mercaptoethanol, 2·5; each of the four nucleotide triphosphates (ATP, GTP, UTP, CTP), 0·08. Assays were carried out at 37° C for 10 min according to the procedure of Chamberlin and Berg[4]. This time interval was chosen because rates were found to be linear for the first 15 min. Assays of RNase and nucleotidases indicated no significant differences in activities between the stages studied. Moreover, the magnitudes of the activities were low enough to be overlooked in determining the comparative rates of RNA synthesis in the different preparations.

Evidence that the RNA synthesized *in vitro* is a product of DNA-dependent RNA polymerase is summarized in Table 1. Omission of any one of the four triphosphates resulted in activities which were below the limits of detection. Treatment with DNase or actinomycin *D* abolished 80–90 per cent of the activity. Higher percentages of inhibition could not be obtained by increasing the concentrations of either DNase or actinomycin *D* (up to 5 μg/ml.). This minor component of RNA synthesis was not further investigated. The efficiency of the isolated fractions in synthesizing RNA may be judged by comparing the results with those of Huang and Bonner[5]. The endogenous activity of their crude chromatin preparations was of the order of 200 μmoles nucleotide incorporated per mg DNA in 10 min at 37° C. The activities of the isolated nuclei or chromosomes are of the order of 2–3 mμmoles. The reason for this ten-fold difference in rate is not apparent since meiotic cells are probably less active in RNA synthesis than embryonic cells.

Table 1. EFFECTS OF VARIOUS FACTORS ON RNA SYNTHESIS BY ISOLATED NUCLEI AND CHROMOSOMES FROM MEIOTIC CELLS OF TULIPS

Preparation	Control	+4 μg DNase	+8 μg DNase	+Actinomycin *D* (0·5 μg/ml)	−GTP	−CTP	−ATP
Nuclei	99	7	7	12	<5	<5	<5
Contracted chromosomes	107	11	11	28	<5	<5	<5

Nuclei were obtained from cells in early prophase. Contracted chromosomes were obtained from cells of which about 70 per cent were in metaphase and the remainder in diakinesis or anaphase. Numbers represent μμmoles of $UT^{32}P$ incorporated per 100 mμmoles of DNA-nucleotide in 10 min at 37° C.

Table 2. RNA POLYMERASE ACTIVITIES IN EXTENDED AND CONTRACTED CHROMOSOMES

Chromosome type	Series 1 ($UT^{32}P$) Intact	Series 1 ($UT^{32}P$) Broken	Series 2 ($GT^{32}P$) Intact	Series 2 ($GT^{32}P$) Broken	Series 3 ($GT^{32}P$) Intact	Series 3 ($GT^{32}P$) Broken +DNA
Extended	68	92	65	150	102	160
Contracted	47	92	48	129	105	175

Conditions of assay and expression of activities are the same as those described for Table 1. Nuclei and chromosomes were broken by rapid dilution of the suspension medium (1·5 M to 0·15 M sucrose). All suspensions were assayed in the presence of 0·5 M sucrose. Each series represents the average of 2–3 sets of measurements made at the same time. Within a series variations were of the order of ±15 per cent. Differences between series are discussed in the text. The labelled substrate used in each series is indicated. 50 mμmoles (nucleotide equivalents) of purified tulip DNA were added in Series 3.

Although considerable variability (one- to two-fold) was found in RNA polymerase activities between different batches of tulips, relative activities with respect to meiotic stage were similar within each set of preparations. One comparison between leptotene nuclei and metaphase chromosomes is shown in Table 1 and three others are shown in Table 2. In general, but not always, polymerase activity of nuclei prepared from leptotene cells is slightly higher than that of metaphase chromosomes. The activity increases if the preparations are partially fragmented but the relative activities of the extended and contracted chromosomes remain the same. Addition of purified DNA also has a stimulating effect, but again, the degree of stimulation is similar for both groups. We infer from these observations that the polymerase enzyme remains associated with the chromosomal complex through the meiotic cycle, and that the absence of RNA synthesis in living cells at metaphase is not due to a loss of enzyme from the chromosomes. The possibility that the enzyme might somehow be inactivated within the living cell during this stage cannot be excluded, but if so, *in vitro* measurements of RNA polymerase activity cannot have much significance in the interpretation of *in vivo* behaviour.

Our failure to observe a differential effect of added DNA on the respective polymerase activities of leptotene and metaphase chromosomes raised the possibility that the enzyme itself might be the limiting factor in the *in vitro* system. In order to test whether extended chromosomes provided better templates for RNA synthesis than contracted ones, purified RNA polymerase prepared from *E. coli* was added to the different fractions. In the case of intact nuclei and chromosomes a two- to three-fold increase in RNA synthesis was observed among the different stages (Table 3). Small differences in activity between different types of chromosomes may be seen in the table, but at best, these would be feeble evidence for a correlation between the microscopic morphology of the chromosomes and the regulation of RNA synthesis in their host cells. Bigger differences were observed when the polymerase was added to fragmented nuclei or chromosomes, an effect which is most easily explained by a more extensive diffusion of the enzyme into the chromosomal network. Even these differences, however, are of insufficient magnitude to account for the observed differences in rates of RNA synthesis within the intact cells. Our data on *in vivo* incorporation of uridine into RNA,

Table 3. EFFECT OF ADDED RNA POLYMERASE ON RNA SYNTHESIS IN EXTENDED AND CONTRACTED CHROMOSOMES

Chromosome stage	Series 1 (intact) Control	Series 1 (intact) +Polymerase	Series 2 (broken) Control	Series 2 (broken) +Polymerase
Leptotene	95	175	65	230
Metaphase	65	135	45	170
Post-meiotic	85	275	45	350

In these tests nuclei from cells which had just completed meiosis are included for comparison. RNA polymerase was prepared from *Escherichia coli* according to the method of Chamberlin and Berg (ref 4). The amount of enzyme used in the tests catalysed the incorporation of 6·4 mμmoles $GT^{32}P$ in 10 min at 37° C in the presence of 100 mμmoles purified tulip DNA. Activities are expressed as in Table 1. Conditions of assay were the same as those described under Table 1 except for a 30-min 'preincubation treatment' which is outlined in the text and under Table 4. The treatment was introduced in order to make these tests comparable with those concerned with the effects of mercaptoethanol

Table 4 EFFECT OF 2-MERCAPTOETHANOL ON RNA SYNTHESIS IN ISOLATED NUCLEI AND CHROMOSOMES SUPPLEMENTED WITH BACTERIAL RNA POLYMERASE

Chromosome stage	Series 1 (intact, 30′)				Series 2 (broken, 30′)			Series 3 (broken, 60′)		
	Control		Polymerase		Control	Polymerase		Control	Polymerase	
	−	+	−	+	+	−	+	+	−	+
Leptotene	100	110	175	1,120	75	230	1,180	95	485	1,600
Metaphase	70	90	140	210	40	160	190	70	165	190
Post-meiotic	85	110	300	435	45	350	550	85	350	625

Conditions of assay were the same as those under Table 3 Nuclei and chromosomes were preincubated for 30 or 60 min at 0° C in the presence of standard medium containing 0 05 M 2-mercaptoethanol. At the termination of the preincubation period, the suspensions were centrifuged and the nuclei or chromosomes resuspended in the standard assay medium. Activities are expressed as in Table 1. The time interval of preincubation and the physical state of the chromosomes or nuclei are shown in parentheses following the series number '+' and '−' indicate the presence or absence of mercaptoethanol during pretreatment.

based on analyses of cell populations similar to the ones used in these studies, indicated a five-fold difference in rate between leptotene and metaphase cells[3].

Striking differences in response were observed when nuclei or chromosomes were pretreated with 2-mercaptoethanol, an agent which causes nuclear swelling[6]. The effect was readily observed under the microscope when leptotene nuclei or metaphase chromosomes were exposed to 0·05 M mercaptoethanol at 0° C for 30–60 min. When various preparations were thus treated and then assayed in a standard incubation medium, two sets of results were obtained. In the absence of added polymerase, no appreciable increases in RNA synthesis were observed (Table 4, Series 1). In the presence of added polymerase leptotene chromosomes showed a fifteen- to seventeen-fold increase in activity; post-meiotic chromosomes showed a seven- to twelve-fold increase; metaphase chromosomes showed a three- to five-fold increase (Table 4, Series 2 and 3). The differences are even more striking if a comparison is made between preparations which were pretreated with mercaptoethanol followed by polymerase addition, and those which were preincubated without mercaptoethanol followed by polymerase addition. Under this regime, the condensed chromosomes showed very little response to the thiol reagent.

We have drawn two conclusions from the results: (1) Mercaptoethanol may have a profound effect on the capacity of chromosomes to act as templates for RNA synthesis *in vitro*. (2) The effect is observed in chromosomes which are already in the extended form as defined by cytological technique, but not in contracted chromosomes. The significance of this observation to the behaviour of chromosomes *in situ* is difficult to assess. The absolute activities of leptotene chromosomes pretreated for 60 min are in themselves impressive. Deproteinized DNA is only four times more active as template material than are these chromosomes (Table 4). Although we have not measured the amount of histone retained by the chromosomes after mercaptoethanol treatment, an appreciable loss of this chromosomal component was not observed when mercaptoethanol was used in the course of DNA preparation[7]. The suggestion has been made, however, that some proteins may be bound to DNA by disulphide bridges[6]. Whatever the effect of this reagent, we doubt whether its effect *in vitro* has any direct bearing on the intracellular regulation of RNA synthesis. More likely, it reflects certain structural changes which occur in the chromosomes during the cycle of contraction and extension.

That a change may be occurring during isolation of chromosomes and nuclei which affects the observed rates of RNA synthesis is evident on comparing the last two series in Table 4. Broken leptotene nuclei are significantly affected by the length of the preincubation period, whether or not mercaptoethanol is added. Metaphase chromosomes, on the other hand, are not affected at all whereas post-meiotic nuclei are slightly affected. The probability therefore exists that, from the very beginning of the isolation procedure, the efficiency of chromosomes to act as templates for RNA polymerase is progressively altered, the degree of alteration depending on the structure of the native chromosome. The use of cell populations which differ with respect to chromosome morphology makes this probability apparent. Differential alterations within a nucleus containing partly extended and partly contracted chromosomes would be much more difficult to detect.

On the whole, these experiments indicate that relatively little information may be gained from the techniques here used for postulating a mechanism to explain the marked changes in RNA synthesis which cells undergo during the cycle of chromosome contraction and elongation. The evidence does invite the conclusion that the enzyme itself is not a determining factor. However, on the much-debated issue of the factors which control template availability in chromosomes, the evidence obtained mainly invites scepticism.

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Hexose Content of Bovine Fibrinogen and Fibrin, and Evidence against its Release by Fibrin Stabilizing Factor

FIBRINOGEN from several species contains a small amount of hexoses as well as some glucosamine and sialic acid[1]. It has been shown that a slight decrease in its hexose content occurs when fibrinogen is clotted with thrombin[2–6], though the fibrinopeptides released by thrombin contain no carbohydrate[5,7]. Recently, evidence has been put forward that this loss of hexose, and of sialic acid, is related to the action of fibrin stabilizing factor (FSF)[1,8–11]. Such evidence is, however, difficult to reconcile with the earlier findings of a loss of hexose in experimental conditions which, because of the absence of Ca^{++} ions, would not have allowed fibrin stabilization to occur[3–6]. Indeed, the fibrin clots described in one report were clearly not stabilized as 1 per cent monochloroacetic acid was used to dissolve them[5]. Furthermore, the failure to demonstrate hexose in clot liquors after clotting fibrin monomer in the presence of FSF and Ca^{++} ions[12] makes it difficult to ascribe the release of hexose, if any, to the action of FSF. The following experiments were made in an attempt to resolve these different findings.

Crude bovine fibrinogen (Armour Pharmaceutical Co., Ltd.) was purified by the method of Laki[13]; the final product contained 94–95 per cent clottable protein and was still contaminated with potentially active FSF. Bovine thrombin (Parke, Davis and Co., Ltd.) was made

up in 0·9 per cent sodium chloride. It was exhaustively dialysed against 0·9 per cent sodium chloride to remove a contaminant found to interfere in the hexose assay. Clotting of fibrinogen was carried out in *tris*-hydrochloric acid buffer (0·05 M, *p*H 7·4) in the presence of cysteine (final concentration 0·01 M) and with or without the addition of calcium chloride (final concentration 0·01 M). In the presence of both of these latter reagents and thrombin, the fibrin formed was proved by the insolubility of control clots in 2 per cent (v/v) acetic acid to be of the fibrin$_I$ type. Conversely, fibrin$_S$ (soluble in 2 per cent acetic acid) was formed when sodium chloride was substituted for calcium chloride. The clotting mixture contained approximately 75 mg of fibrinogen and 20 N.I.H. units of thrombin, in a final volume of 6 ml. Clots were allowed to form at 37° C for 1 h and were then collected on to glass rods, so making it possible to press them out thoroughly and force out the liquors. The clots were washed with distilled water followed by 95 per cent ethanol; they were then air-dried and taken up in 3 ml. of 0·5 N sodium hydroxide for protein and hexose assays. 1 ml. of 20 per cent (w/v) trichloroacetic acid (TCA) was added to the clot liquors, whereon a slight precipitate formed which was centrifuged down and discarded. The supernatants were decanted, made up to 7·5 ml. with water and assayed for hexose. A control consisted of the entire clotting mixture except for thrombin. No clotting occurred and after 1 h at 37° C protein was precipitated by the addition of 1 ml. of 20 per cent TCA; thrombin (20 U.) was then added and the mixture centrifuged. The precipitate was washed with 95 per cent ethanol and then treated as described for the fibrin clots. The TCA supernatant was made up to 7·5 ml. with water and assayed for hexose.

Protein was determined by the method of Lowry *et al.*[14], using bovine serum albumin of known protein content as standard. Hexose was determined by the orcinol–sulphuric acid method[15], using an equimolar galactose and mannose solution as standard. A preliminary hexose assay showed that absorbance at 540 mμ was proportional to the amount of fibrinogen or fibrin used. All assays were done at least in duplicate, with appropriate reagent blanks. The entire experiment was repeated a further three times and the figures obtained were remarkably constant. The average of the results from the four experiments is shown in Table 1.

In no experiment was there found any difference, outside experimental error, in the hexose : protein ratio between fibrinogen and fibrin$_S$ or fibrin$_I$. Neither was there detected any hexose in the liquors derived from either fibrin$_S$ or fibrin$_I$ clots. If the action of FSF is to liberate 16–20 per cent of the total hexose as suggested[1,10,11], then the liquors from the fibrin$_I$ clots in the experiments reported here would be expected to contain 17–20 μg of hexose per 1 ml. Also, a significant drop in the hexose : protein ratio of fibrin$_I$ would be expected, as thrombin liberates only 3 per cent of the total protein from fibrinogen[1]. Fibrin$_S$ and fibrin$_I$ had the same hexose : protein ratio, however, and this together with the fact that no hexose was detected in the fibrin$_I$ clot liquors support the negative findings of Rosenberg and Carman[12] as to the supposed action of FSF. Moreover, these results do not agree with the earlier findings that fibrin (that is, fibrin$_S$) contains up to 20 per cent less hexose than fibrinogen. This is probably because these early hexose determinations were expressed as a percentage of dry weight[3–6], or merely in terms of absorbance at 540 mμ[8], rather than related to protein content. The work of Hormann and Gollwitzer[16,17] has shown that the anthrone and orcinol assays for hexose are subject to interference by tryptophan and that the degree of interference differs in fibrinogen and fibrin. These authors suggest that the reported difference in the percentage of hexose in fibrinogen and fibrin is due to artefacts inherent in the assay, and they found that the absolute amounts of hexose in bovine fibrinogen and fibrin are the same[17]. It should be noted that, in the experiments reported here, it is only when the hexose and protein figures are related that parity between fibrinogen, fibrin$_S$ and fibrin$_I$ is observed.

Table 1. RESULTS OF FOUR EXPERIMENTS TO DETERMINE THE HEXOSE : PROTEIN RATIO OF BOVINE FIBRINOGEN, FIBRIN$_S$ AND FIBRIN$_I$ CLOTS, AND THE HEXOSE CONTENT OF THE CLOT LIQUORS

Material tested	Mg protein per ml. Mean	(Range)	μg Hexose per ml. Mean	(Range)	μg Hexose per mg protein Mean	(Range)
Fibrinogen control	25·6	(24·3–26·0)	269	(261–285)	10·5	(10·1–10·8)
Fibrin$_S$ clot	23·1	(22·0–24·4)	245	(229–273)	10·6	(10·1–11·2)
Fibrin$_I$ clot	21·6	(21·2–21·8)	229	(205–253)	10·6	(9·7–11·6)
Fibrinogen control liquors	—		5·4	(0–8)*	—	
Fibrin$_S$ liquors	—		5·8	(0–11)*	—	
Fibrin$_I$ liquors	—		5·9	(2–11)*	—	

* These figures are below the limits of accuracy for the orcinol method, but they clearly indicate that there is no release of hexose into the fibrin$_S$ or fibrin$_I$ clot liquors.

From these experiments, it is concluded, therefore, that fibrin stabilization by FSF does not cause the release of hexose and that, irrespective of whether or not fibrin is stabilized, its hexose content remains the same as that of fibrinogen.

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Note added in proof. Since this manuscript was prepared, the elegant investigation of Mester *et al.*[18] has come to my notice. These workers were also unable to demonstrate any release of carbohydrate material after clotting fibrinogen.

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Dihydrodiol Formation from Indanones in Rats and Rabbits

DIHYDRODIOLS have been reported as metabolites of cyclic hydrocarbons after the administration of these compounds to animals. *trans*-Dihydrodiols have been isolated from the urine of animals after the separate administration of *cyclo*hexane[1], naphthalene[2], anthracene[3] and phenanthrene[4,5]. Both *cis*- and *trans*-dihydrodiols have been isolated from the urine of animals to which indene[6] and acenaphthylene[7] had been separately administered. Dihydrodiols do not appear to have been previously reported as metabolites of cyclic ketones.

In the present investigation indan-2-one was prepared by the distillation of *o*-phenylene-diacetic acid. The product was purified by steam distillation. Indan-1-one was obtained from Kodak, Ltd., London. (±)*cis*-Indane-1,2-diol (m.p. 98–99° C) and (±)*trans*-indane-1,2-diol (m.p. 158°–159° C) were prepared by the methods described by Brookes and Young[6].

Table 1. DIHYDRODIOLS DETECTED BY PAPER CHROMATOGRAPHY IN THE URINES OF ANIMALS DOSED WITH INDANONES

Substance dosed	Animals	Dihydrodiol detected	R_F values Solvent 1 Biosynthetic	Solvent 1 Synthetic	Solvent 2 Biosynthetic	Solvent 2 Synthetic	Solvent 3 Biosynthetic	Solvent 3 Synthetic
Indan-1-one	Rabbits	*cis*-	0·30	0·30	0·36	0·36	0·60	0·60
		trans-	0·08	0·07	0·08	0·08	0·36	0·36
Indan-1-one	Rats	*cis*-	0·30	0·30	0·35	0·35	0·62	0·61
		trans-	0·07	0·08	0·08	0·08	0·40	0·38
Indan-2-one	Rabbits	*cis*-	0·29	0·31	0·36	0·35	0·60	0·60
		trans-	0·07	0·08	0·10	0·10	0·36	0·36
Indan-2-one	Rats	*cis*-	0·31	0·29	0·35	0·35	0·61	0·60
		trans-	0·10	0·08	0·10	0·09	0·38	0·38

The solvents and detecting reagents have been described by Brookes and Young (ref. 6). Solvent 1, benzene–water–ammonia (sp. gr. 0·88) (5 : 4 : 1 v/v); solvent 2, benzene–water–acetic acid (5 : 4 : 1 v/v); solvent 3, benzene–water–ethyl acetate–acetic acid (4 : 4 : 1 : 1 v/v). The dried chromatograms were sprayed with 2 per cent (w/v) aqueous sodium periodate followed 15 min later by Schiff's reagent. The dihydrodiols were located as yellow spots on a magenta background.

Seven rabbits were each given 0·5 g indan-2-one by stomach tube. In a separate experiment seven rabbits were each given 0·5 g indan-1-one by the same route. The urines were collected daily for 2 days after dosing and stored in a refrigerator.

In experiments with rats, twenty-four rats (body-weight 200–250 g) were each given 100 mg indan-2-one dissolved in 1 ml. of sunflower oil, by subcutaneous injection. The rats were given a second equal dose 24 h later. A similar experiment was also carried out with twenty-four rats. Here, indan-1-one was substituted for indan-2-one as the compound under investigation. The urines were collected daily for 4 days after the initial dosing and stored in a refrigerator. The four urines were examined for dihydrodiols in an identical manner.

The urines were adjusted to *p*H 7 and continuously extracted with ether for 48 h. The ether extracts were reduced to about 20 ml. by evaporation and were then washed with dilute sodium hydroxide solution. Portions of the extracts were then examined by paper chromatography for dihydrodiols. When the presence of dihydrodiols in the urines was detected, attempts were made to isolate the compounds. The ether extracts were dried by the addition of anhydrous calcium sulphate and the filtrate was evaporated to dryness under reduced pressure. *cis*-Indane-1,2-diol was extracted from the residues using boiling *cyclo*hexane[5] and *trans*-indane-1,2-diol with boiling chloroform[5].

As shown in Table 1, both *cis*- and *trans*-indane-1,2-diols were detected in the urines of rats and rabbits dosed separately with indan-1-one and indan-2-one. In isolation experiments, 24 mg of *cis*-indane-1,2-diol, m.p. 98°–99° C (found: carbon, 72·3; hydrogen, 6·7 per cent. Calculated for $C_9H_{10}O_2$: carbon, 72·0; hydrogen, 6·7 per cent), $[\alpha]_D$ + 41° in chloroform, was isolated from the urine of rabbits dosed with indan-2-one. The melting point was not depressed by admixture with synthetic (±)*cis*-indane-1,2-diol. The compound was examined by ultra-violet spectroscopy and paper chromatography and was found to be identical with its synthetic analogue. A small amount (1 mg) of *trans*-indane-1,2-diol was also isolated from the rabbit urine. The amount isolated was insufficient to permit purification by recrystallization. The compound was dissolved in ethanol and identified by paper chromatography. *cis*-Indane-1,2-diol (44 mg), m.p. 96°–98° C, $[\alpha]_D$ + 40° in chloroform, was also isolated from the urine of rats dosed with indan-2-one. The melting point was not depressed by admixture with its synthetic analogue. The ultra-violet light-absorption and the paper chromatographic behaviour of the biosynthetic dihydrodiol was identical with that of the synthetic *cis*-dihydrodiol. In addition, *trans*-indane-1,2-diol (5 mg) was also isolated from the urine. The compound had a melting point at 183°–185° C, $[\alpha]_D$ + 30° in ethanol. The substance was examined by ultra-violet and infra-red spectroscopy and paper chromatography and found to be identical to synthetic *trans*-indane-1,2-diol. Brookes and Young[6] isolated *trans*-indane-1,2-diol, m.p. 184°–186° C, $[\alpha]_D$ + 31° in chloroform, from the urine of rats dosed with indene. They suggested that the compound was probably (+) *trans*-indane-1,2-diol.

Attempts to isolate *cis*- and *trans*-indane-1,2-diols from the urines of animals dosed with indan-1-one were unsuccessful.

In addition to the dihydrodiols, the presence of ketones, other than the compounds dosed, has been detected in the experimental urines by paper chromatography. The possibility that a ketol may act as an intermediate in the formation of the dihydrodiols from the indanones is being investigated.

The infra-red spectroscopy was carried out on the Unicam *SP*.200 spectrometer and the ultra-violet spectroscopy on the Unicam *SP*.700 spectrometer. Elementary analyses were carried out by Drs. Franz and Ellen Pascher, Bonn, Germany.

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Consequences of Inhibition of *Escherichia coli* by Tetracycline Antibiotics

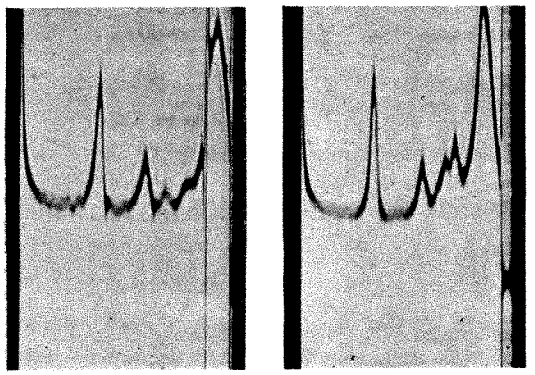
DURING incubation of *Staphylococcus aureus* with tetracycline, chlortetracycline or oxytetracycline, protein synthesis is inhibited to a much greater extent than the synthesis of ribonucleic acid (RNA)[1]. We have found these observations to apply also to inhibition of *Escherichia coli* and have extended them to include two other antibiotics of the tetracycline group. Table 1 shows that during incubation for 2 h with each of five tetracyclines, protein synthesis was almost completely inhibited, whereas increases in cellular RNA of from 56 per cent to 76 per cent were observed.

The RNA made by *E. coli* in the presence of chlortetracycline has been studied in some detail[2]; about 70 per cent is contained in particles that sediment more slowly than ribosomes and that give rise to two boundaries in schlieren diagrams during analytical ultracentrifuging of crude cell-free extracts. Incubation of *E. coli* with either oxytetracycline, tetracycline, demethylchlortetracycline or 6-methylene-5-hydroxytetracycline also led to the accumulation of similar particles which, like those produced by chlortetracycline action, were found to be

Table 1. SYNTHESIS OF RNA AND PROTEIN DURING INHIBITION BY TETRACYCLINE ANTIBIOTICS

Antibiotic	Percentage increase in RNA	Protein
Chlortetracycline	60	4
Oxytetracycline	64	7
Tetracycline	75	3
6-Methylene-5-hydroxytetracycline	76	4
Demethylchlortetracycline	56	2

Cells of *E. coli K*12 were incubated for 2 h at 30° C without aeration in a peptone medium (ref. 2) containing 15 μg of the appropriate antibiotic/ml. Estimations of RNA and protein were made on portions (5 ml.) of the suspension, using orcinol and Folin methods, respectively, and essentially as described by Fraenkel and Neidhardt (ref. 3).



a *b*

Fig. 1. Schlieren diagrams, after centrifuging for 20 min at 187,000*g* and room temperature, for cell-free extracts prepared from *E. coli* K12 in 15 mM potassium dihydrogen phosphate, *p*H 7·0, containing 0·1 mM magnesium acetate. Sedimentation is to the left. The bar angle was 40°. (*a*) Control; cells collected before addition of antibiotic. The extract shows major boundaries caused by 50 *S* and 30 *S* ribosomes, DNA and 'soluble' cell constituents. (*b*) Cells collected after inhibition for 2 h by 15 μg of demethylchlortetracycline/ml. One μg of deoxyribonuclease/ml. was added to the extract to eliminate convection caused by DNA synthesized during inhibition (ref. 2)

readily degraded by ribonuclease and ultrasonic vibrations under conditions which left ribosomes unaffected. Fig. 1 shows typical schlieren diagrams illustrating changes occurring during inhibition by demethylchlortetracycline.

These results suggest that the accumulation of RNA, largely contained in particulate material that sediments more slowly than ribosomes, may be a general consequence of inhibition by antibiotics of the tetracycline group. Particles resembling those accumulated during the action of the tetracyclines are also present after inhibition of *E. coli* by chloramphenicol or puromycin, two other antibiotics that inhibit protein synthesis but permit continued synthesis of RNA; these particles are thought to be precursors of the ribosomes[4,5]. The results of inhibition by these different antibiotics may be similar because chloramphenicol, puromycin and the tetracyclines inhibit protein synthesis at stages after the formation of aminoacyl-transfer RNA complexes[6–8]. According to the hypothesis proposed by Kurland and Maaløe[9], in which synthesis of RNA is suggested as being repressed by species of transfer RNA uncombined with amino-acids, the inhibition of protein synthesis effected by these antibiotics would not then cause an immediate reduction in the rate of synthesis of RNA.

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In vitro Metabolism of *p,p'*DDT in Pigeon Liver

DESPITE the widespread use of DDT*, knowledge of its metabolism in vertebrates is incomplete, and is based almost entirely on experiments carried out on mammals *in vivo*. There have been no unequivocal reports of *in vitro* conversions to support these findings. Woodward, Davidow and Lehman[1] have reported the disappearance of DDT from homogenized liver of rabbits injected intravenously with emulsions of DDT prior to death, but no metabolites were isolated. Judah[2] incubated rat liver, kidney and diaphragm with DDT, but found no DDD or DDE and obtained only a very small conversion to DDA. Peterson and Robison[3] have recently put forward a metabolic pathway for DDT in rats which was based on feeding the pesticide and its postulated metabolites, and analysing residues in the liver and kidney. In the course of these experiments, liver homogenate was incubated for 6 days with DDT, and DDD was isolated with a final ratio of DDT : DDD of 1 : 3, although, as the authors point out, this may have been due to bacterial action. No *in vitro* work has been reported with birds, which are probably the vertebrates most at risk from the agricultural use of DDT. We now wish to report the *in vitro* conversion of DDT to DDD and DDE by incubation of both homogenate and slices of pigeon liver.

All solutions referred to were made up in 0·1 per cent 'Triton *X*-100' (Lennig Chemicals, Ltd.) to facilitate the solution of DDT, which could otherwise be precipitated by centrifugation at 25,000*g*. Pigeons were killed by cervical dislocation and the livers excised and placed on ice as rapidly as possible. Slices were obtained with a Stadie–Riggs microtome[4], and 0·5 g batches were incubated with DDT under gentle agitation for 4 h at 41·5° C in 5 ml. of Krebs–Ringer bicarbonate. The incubation medium was pre-gassed either with oxygen–carbon dioxide (95 : 5) or with nitrogen and carbon dioxide separately. Incubations in the former medium were carried out under oxygen–carbon dioxide, and in the latter under nitrogen. Redistilled propylene glycol, 0·1 ml., containing 20 μg of DDT was added to each flask after the addition of the slices. Reduced glutathione (7·5 mg/flask) was also incorporated into certain flasks. Liver homogenates were obtained with a Potter–Elvehjem[5] apparatus in two volumes of 0·25 M sucrose. Equal portions of homogenate equivalent to 0·5 g of liver were incubated with DDT and 4 ml. of Krebs–Ringer bicarbonate for 4 h or 24 h under exactly the same conditions as already described for slices. Some homogenates used for 24 h incubations were made in sucrose containing 25,000 U./ml. of penicillin *G* to prevent bacterial action, but this addition was shown to have no effect on the results obtained. All incubations were terminated by the addition of 1 ml. of 10 per cent trichloroacetic acid, followed by maceration and extraction by a modification of the method of Taylor, Rea and Kirby[6]. The final hexane extract was subjected to a further clean-up step with concentrated sulphuric acid before examination by gas–liquid chromatography[7,8]. The identity of the metabolites has been confirmed in a number of instances by the use of an additional column packing (*QF*-1) with gas–liquid chromatography or, where sufficient material was available, by the use of thin-layer chromatography. Appropriate blank incubations were run in all cases, and the recovery of DDT, DDD and DDE in the absence of liver was shown to be about 80 per cent.

The preliminary results which are summarized in Table 1 clearly demonstrate that two distinct reactions

Table 1. INCUBATION OF PIGEON LIVER WITH DDT

Tissue preparation	Incubation time (h)	Atmosphere	Special conditions	Molecular recovery (per cent) DDT	DDD	DDE
Homogenate	24	N_2	None	26·5	21·1	0·8
Homogenate	24	O_2/CO_2	None	64·5	0	0·5
Homogenate	24	N_2	Heat treated	32·8	14·5	0·7
Homogenate	24	N_2	Reduced glutathione	16·7	28·7	2·4
Homogenate	24	O_2/CO_2	Reduced glutathione	43·4	0	6·6
Homogenate	4	O_2/CO_2	Reduced glutathione	55·8	0	3·0
Slices	4	N_2	None	15·5	47·6	0·4
Slices	4	O_2/CO_2	Reduced glutathione	63·7	0	1·5

* Abbreviations: DDT, 1,1-di(*p*-chlorophenyl)-2,2,2-trichloroethane; DDE, 1,1-di-(*p*-chlorophenyl)-2,2-dichloroethylene; DDD, 1,1-di(*p*-chlorophenyl)-2,2-dichloroethane; DDA, di(*p*-chlorophenyl)acetic acid.

occur with both slices and homogenates. Under anaerobic conditions there is considerable conversion of DDT to DDD. A very small amount of DDE similar in magnitude to that found under aerobic conditions is also produced. The fact that the conversion to DDD still occurs after a homogenate has been maintained under nitrogen at 75° C for 10 min suggests that this may be a non-enzymatic reaction similar to that of DDT with reduced porphyrins recently described by Miskus, Blair and Casida[9]. Under aerobic conditions the overall reaction of DDT is much slower. A very small amount of DDE is produced unless reduced glutathione is included in the incubation medium, when a four- to six-fold increase in this conversion occurs. This reaction appears to proceed marginally better with homogenates than with slices. No other non-polar metabolite was found in any incubation and, despite a careful search in a separate experiment, neither was DDA. Some incubations using rat liver homogenate under the same conditions suggest that the same two pathways also operate, but that the formation of DDD is even more favoured under anaerobic conditions than in the pigeon, while the amount of DDE formed is slightly less.

Further work on the *in vitro* metabolism of DDT and its metabolites is in progress.

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BIOLOGY

Scanning Electron Microscopy of Biological Material

The scanning electron microscope has been used for biological work[1,2] but in general the results were disappointing because of the poor contrast obtained with secondary or back-scattered electron read-out.

This communication reports some preliminary results obtained with a different contrast mechanism. In these experiments, the fine electron beam is scanned across the specimen and the light, excited by the beam at different points on the specimen, is detected on a photomultiplier and the signal obtained is used to build up the microscope image on the face of a cathode ray tube. This contrast mechanism has been used by other workers[3-4] for studying cathodo-luminescence but, as far as we are aware, it has not been used for biological applications.

Some biological material (such as cotton fibre) is cathodo-luminescent without any treatment; other material can be selectively stained with a cathodo-luminescent dye, such as thioflavin *T*, to render the material visible. Micrographs have been obtained under both conditions; Fig. 1 shows a micrograph of spinach leaf in which the dye, thioflavin *T*, is apparently selectively taken up on the cell walls and gives rise to the high signal from these regions. This conclusion was supported by a similar image obtained by visible light fluorescence microscopy. However, the advantage of the scanning electron microscope is that the ultimate resolution obtainable (about 100 Å)[5] is much higher than is possible with fluorescent light microscopy. At the present time,

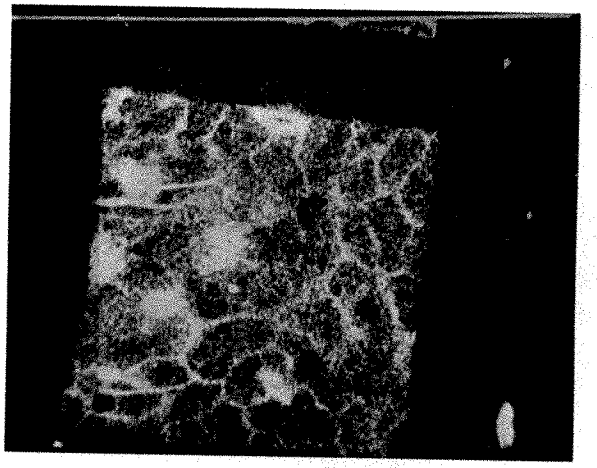


Fig. 1. Scanning electron micrograph of spinach leaf stained with thioflavin *T* using light collection only to build up the image. Beam current, $i = 10^{-8}$ amp; beam voltage, $V = 27$ kV; exposure time, 30 sec. (×240)

the highest useful magnification of scanning micrographs with this contrast mechanism is about 5,000 ×.

A number of problems are associated with this technique. The first is to ensure that the signal is due to light and not to scattered electrons. This has now been solved with a system of light collection that uses metallized mirrors. The second problem is to obtain sufficient signal to build up a useful image (see Fig. 1). This is not simply a question of focusing more electrons into the fine beam since 'poisoning' of the cathodo-luminescent material can occur and has been observed on a number of occasions with beam currents of the order of 10^{-9} amps. Hence, it may be necessary to use longer exposures (up to 30 min) and very stable power supplies for the electron optical column. As an alternative, stains may be found which are more resistant to electron bombardment.

If both these problems can be overcome, it should be possible to realize the potential of this method and to obtain micrographs showing resolution of a few hundred Ångstroms. An obvious extension of this technique is to identify different cathodo-luminescent materials by spectral analysis of the emitted light.

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Lift produced by the Heterocercal Tail of *Acipenser*

The upward forces produced by the heterocercal tails of two species of Selachii, moving transversely through water at various speeds, have recently been measured[1]. It was found that the lift was approximately proportional to the 1·4th power of the transverse speed. The equilibrium of the fish, swimming horizontally, was discussed, and it was estimated that in each case the lift

required for equilibrium would be obtained at swimming speeds around 0·5 body lengths/sec. At higher speeds, more lift would be obtained if the hypocaudal lobe acted passively, but it was thought probable that the lift could be adjusted to the required value by means of the radial muscles of the hypocaudal lobe.

Heterocercal tails were almost universal in the early Osteichthyes, which presumably had negative buoyancy. Among modern Osteichthyes, however, only the Acipenseriformes have a markedly heterocercal tail.

Experiments have now been carried out on the tail of a sturgeon, *Acipenser sturio* L. The fish weighed 9 kg and was about 120 cm long. It was caught on March 15, and the tail arrived at this laboratory, apparently in fresh condition, on March 19. It had been packed in ice for most of the intervening period but had not at any time been frozen. The experiments were performed on March 19 and 20.

The method was that used for Selachii[1]. In some of the experiments the prongs of the apparatus extended 3 cm beyond the leading edge of the hypocaudal lobe, as in the majority of experiments with the rather smaller selachian tails, and in some they extended 5 cm beyond it. There was no appreciable difference between the two sets of results (Fig. 1).

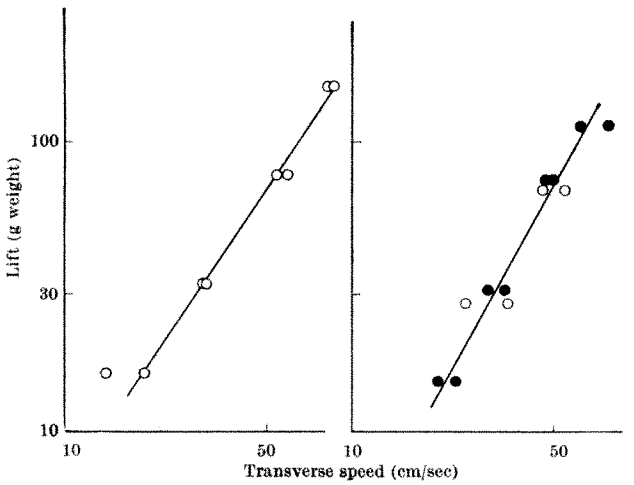


Fig. 1. Graphs of lift against transverse speed for the tails of *Galeichthys* (left, after ref. 1) and *Acipenser*. Measurements made with the prongs extending 3 cm (○) and 5 cm (●) beyond the leading edge of the hypocaudal lobe

The lift was found to be proportional to the 1·7th power of the speed, whereas in the experiments with selachians it was found to be proportional to the 1·4th power.

At any given speed, the lift was close to the lift given at the same speed by the tail of a specimen of the selachian *Galeichthys* (Fig. 1). The specimen of *Acipenser* was, however, much larger than the specimen of *Galeichthys*, which was about 95 cm long and probably weighed about 3·5 kg. If lift is expressed as a percentage of body weight, and speed in body lengths/sec, the lift produced by the *Acipenser* tail at a given speed is only about 50 per cent of that produced by the *Galeichthys* tail.

This difference seems appropriate in view of the difference in specific gravity between the fish. The specific gravity of *Acipenser*, which has a small swimbladder, is 1·05, whereas that of *Galeichthys* is 1·07 (ref. 2). If these values are reliable (they were obtained by the very inaccurate method of direct measurement of weight and volume) *Acipenser* will only require a little more than half as much lift as *Galeichthys* of similar weight when swimming in sea water of specific gravity 1·026. The swimming speeds at which the hypocaudal lobe, acting passively, would give the required lift may therefore be similar. It is not, however, clear whether the proportion of the total lift which must be provided by the tail is the same in both cases. In selachians, the pectoral fins seem to be the main source of lift anterior to the centre of gravity[3], but it seems possible that in *Acipenser* the ventral surface of the snout may be more important. If so, a greater proportion of the lift would have to be provided by the tail to balance the moments.

The hypocaudal lobe of the tail of *Acipenser* has radial muscles which can presumably be used to adjust the lift to the required value.

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Response to Cercal Stimulation recorded in the Cricket Brain

THE responses of the cercal receptors of orthopterous insects have been known for a long time. In cockroaches and crickets nerve fibres from the sense organs synapse in the last abdominal ganglion with giant fibres; in the former insect at least the system is a simple relay initiating escape responses[1,2]. In crickets the cerci possess a variety of mechanoreceptors and have, among others, a tactile function in song and courtship[3,4]. Although the brain is primarily responsible for controlling speed and duration of movement of the animal following cercal stimulation[4,5], neural activity in response to such stimulation has been recorded only as far forward as the suboesophageal ganglion[4].

During a micro-electrode study of responses to visual stimuli in the brain of the house cricket, *Gryllus domesticus*, the results of which will be published elsewhere, we attempted in six preparations to record also units in the brain responding to cercal stimulation. In one of these we were successful, and the data are shown plotted in Fig. 1. A spontaneously firing unit was found which increased its impulse frequency in response to a puff of air directed through a pipette at the ipsilateral cercus. After the initial increase in rate, there was a gradual decline until cessation of the stimulus whereupon there was an inhibitory 'off-effect' followed by a return to base level. The brief decline in frequency immediately following onset of stimulus seems to be real and not artefact; although small, the effect was seen in all eight trials of the unit. The unit did not respond to puffs of air directed at the contralateral cercus or elsewhere on the body, nor did it respond to tactile stimulation of the ipsilateral cercus or of any other area.

Although we attempted to record cercal responses in only six animals, we did survey rather extensive areas of the brain. The fact that we found only one unit which

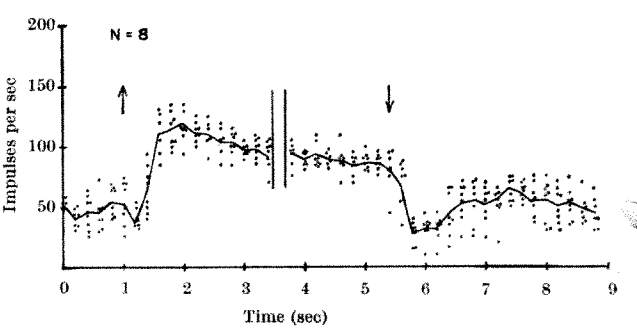


Fig. 1. Response of unit in cricket brain to a puff of air on the ipsilateral cercus. Duration of puff varies from 5–7 sec; all eight responses plotted with respect to beginning and end of stimulus indicated by arrows

exhibited such responses implies that these units are confined to a fairly limited area. No attempt was made to locate the above unit precisely, but its position could be roughly determined from surface contours and measurement of electrode penetration. It was located somewhat lateral and considerably ventral to the mushroom body; the electrode was deep enough to be quite possibly recording from the vicinity of fibres entering the brain from the circumoesophageal connectives. The occurrence of the unit here reported seems to be the first direct evidence of electrical activity within the insect brain as a result of cercal stimulation.

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An *in vivo* Method for studying the Hair Cycle

STUDIES of the hair cycle have been carried out for many years on fixed material[1,2] and, more recently, in tissue culture[3]. In neither case can environmental influences on the initiation of the hair cycle, such as microcirculatory changes and presence or absence of oedema, be determined satisfactorily. In the following *in vivo* method, single follicles may be mapped and observed repeatedly at any time-intervals thereafter.

Young mice, 3–4 weeks old, of *BUB* (albino) strain, or C_3Hp (lightly pigmented) strain, are anaesthetized intraperitoneally with 'Nembutal', 75 mg/kg. The mouse rests horizontally on a holder with a long coverslip covering the dorsal surface of the pinna and adhering to it by means of glycerine. The holder fits on to the stage of a light microscope, the ear being transilluminated from below.

Blood vessels are mapped using sequential magnifications of 10–600 times, and the hair follicles, marked by the prominent sebaceous glands, are mapped in relation to the smaller blood vessels. The diameter of mapped blood vessels can be measured with a calibrated ocular micrometer, and comparative measurements made at different times and under different conditions.

The hair follicles in the apex of the mouse pinna are in the resting stage (telogen) until the animal reaches an age of about 6 months. They can be experimentally activated by various means, including applications of a dekeratinizing agent (commercial hair remover such as 'Neet'), or by plucking the hair. 'Neet' applied for 5 min will remove the external hair, and exert a minimal effect on regrowth. If applied for a longer time, it often causes vasodilatation and oedema, which can be observed under the microscope, and it hastens regrowth. Once the follicles are activated, all stages of the hair cycle except early anagen can be observed and studied.

The activity of the apical follicles of the pinna is much slower than the follicles of body skin, both with regard to spontaneous and induced cycles, thus facilitating time analyses. Figs. 1–3 show the same area of pinna on day 10 after plucking the hair (follicle inactive), on day 24 (follicle in anagen), and on day 32 (follicle in catagen).

The method has been used to study vasodilatation and oedema in relation to the hair cycle. It is at present being used to explore endogenous factors which are involved in the initiation of anagen.

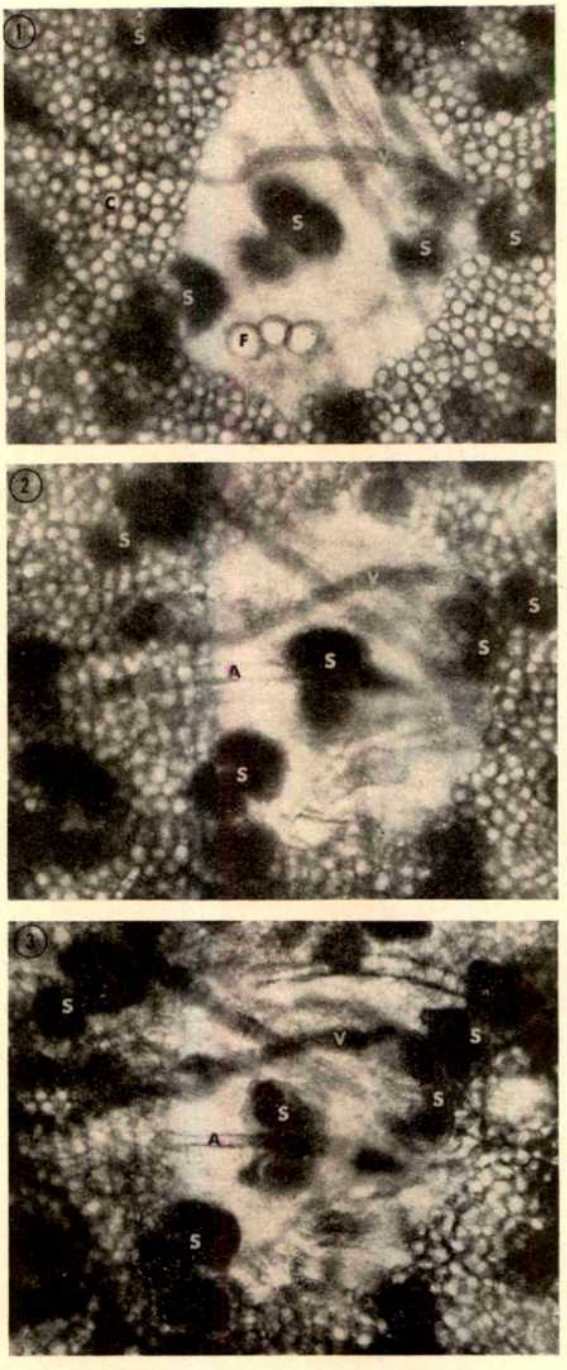


Fig. 1. Area of living mouse pinna, transilluminated, on day 10 after plucking the hairs. Sebaceous glands (*S*) are all that can be seen of the pilosebaceous units. The follicles are inactive. *C*, Cartilage; *F*, fat cell. (×150)
Fig. 2. Same area as Fig. 1, on day 24 after plucking. Follicle *A* (*A*) is in full anagen. *V*, Venule. (×150)
Fig. 3. Same area as Figs. 1 and 2, on day 32 after plucking. Follicle *A* is in late catagen. (×150)

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ENTOMOLOGY

Reversion of the Toxicity of Tricalcium Phosphate to Insects by Trehalose

EARLIER we reported[1] data on the exceptional toxicity of tricalcium phosphate to insects. The symptoms of toxicity on larvae were supernumerary moulting, mottling of cuticle, reduction in growth, malformation and death. Further experiments were carried out to investigate the action of sugars on the toxicity of tricalcium phosphate. The sugars (A.R.) were incorporated into tricalcium phosphate at the 5 per cent level. These premixes were mixed with broken rice at the 0, 0·05, 0·1, 0·5, 1·0 and 2·0 per cent levels. The test insects, second instar larvae of *Corcyra cephalonica* drawn from laboratory cultures, were released on 10 g of the medium. There were ten replicates for each treatment. Observations were made on the growth, mortality and histological changes in the larvae during 15 days' incubation at 25° C and 65 per cent relative humidity. Staining for fat and tissues was carried out by the procedures described by Conn and Darrow[2]. The results are given in Table 1.

The results of the experiments reveal that most of the sugars significantly enhanced the toxicity of tricalcium phosphate to insects. Only trehalose, a disaccharide, exerted any marked effect on the toxicity of tricalcium phosphate—it reversed its action. Even with the highest concentration of tricalcium phosphate (2 per cent and above) in the medium, trehalose produced almost total detoxification and no deaths were recorded among the test insects. This detoxification mechanism required further investigation. Whereas with the incorporation of glucose, sucrose, fructose, lactose, xylose, mannose and rhamnose, the toxicity of tricalcium phosphate was markedly increased, raffinose and galactose reduced it to some extent.

Histopathological investigations of *C. cephalonica* larvae showed that tricalcium phosphate reduced the fat deposition in the larvae, disintegrated the striated border and the midgut epithelium and induced great degenerative changes in the alimentary canal and other surrounding tissues (Fig. 1). In the absence of trehalose, these pathological symptoms could be observed at concentrations of 0·5 per cent and greater of tricalcium phosphate after a period of 6 days. These histopathological symptoms were absent in larvae fed on tricalcium phosphate containing trehalose. It was of interest to note that when the quantity of trehalose in tricalcium phosphate was small, and when the mixture was incorporated into the growth medium at the 2 per cent level (tricalcium phosphate 20 mg/g + trehalose 1 mg/g), the larvae grew

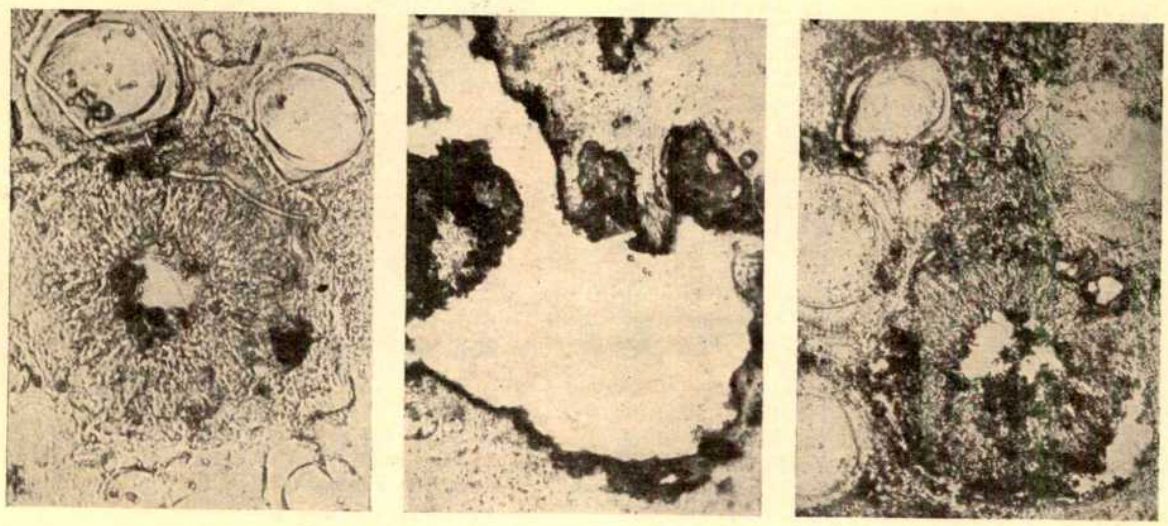
Table 1. EFFECTS OF SUGARS ON THE TOXICITY OF TRICALCIUM PHOSPHATE TO *Corcyra cephalonica* LARVAE
Percentage of mortality (after 2 weeks)

Tricalcium phosphate (mg/g)	Sugar (mg/g)	Glucose	Fructose	Mannose	Maltose	Galactose	Xylose	Rhamnose	Lactose	Sucrose	Trehalose	Raffinose	No sugar*
0	0	0 (832)	0 (832)	0 (832)	0 (832)	0 (832)	0 (832)	0 (832)	0 (832)	0 (832)	0 (832)	0 (832)	0 (832)
0·5	0·025	0 (830)	0 (821)	0 (827)	0 (825)	0 (824)	0 (830)	0 (828)	0 (830)	0 (830)	0 (834)	12† (730)	0 (830)
1	0·05	55† (415)	70† (625)	25† (823)	40† (560)	0 (820)	0† (630)	75† (592)	55† (720)	20† (331)	0 (834)	20 (727)	0 (830)
5	0·25	100 —	80 (122)	40 (622)	90 (125)	12† (620)	80 (155)	80 (102)	80 (110)	90 (115)	0 (824)	22 (722)	55† (125)
10	0·5	— —	100 —	100 —	100 —	20 (616)	100 —	90 (102)	100 —	100 —	0 (789)	64 (423)	100 —
20	1·0	—	—	—	—	55 (422)	—	100	—	—	0 (774)	88 (223)	—

* Only tricalcium phosphate.

† The lowest concentration at which pathological symptoms were observed.

Figures in brackets show weight of 100 larvae in mg.



a *b* *c*

Fig. 1. Transverse section through the midgut of *C. cephalonica* larvae after four days of feeding on broken rice diets. *a*, Control showing ntact midgut and surrounding tissues; *b*, 10 mg tricalcium phosphate/g rice, showing disintegration of midgut and surrounding tissues; *c*, 10 mg tricalcium phosphate + 0·5 mg trehalose/g rice, showing no abnormal change in the midgut and surrounding tissues

as well as with the control medium in which tricalcium phosphate was absent. The reversion of the toxicity of tricalcium phosphate to insects by trehalose, as indicated by the histological and growth investigations, is of considerable interest. On the other hand, glucose, sucrose and inulin exerted a considerable potentiating effect on the toxicity of tricalcium phosphate to larvae of *C. cephalonica*, and induced rapid pathological changes in tissues. Similar interactions of sugars on the toxic effects of tricalcium phosphate were also observed with larval stages of *Tribolium castaneum* and *Ephestia cautella*. These results on the pathological effects of the tricalcium phosphate potentiating effect of some of the sugars and the detoxifying action of trehalose should have far-reaching effects in any future search for a specific insecticidal substance.

Wyatt and Kalf[3] reported that the non-reducing disaccharide α-α-trehalose was the major sugar in insect plasma (compared with mammals). It is believed that it plays a significant part in carbohydrate transport in insects, perhaps conveying glucose units from fat body glycogen to the site of the metabolism in other tissues. Since glucose, fructose and sucrose occur in small amounts or in traces in the larval blood, and trehalose is present to the extent of 90 per cent of the blood sugar level[3], the role of tricalcium phosphate seems to be related to the imbalance created in the energy metabolism of larva. This imbalance is accelerated by sugars such as glucose, sucrose, mannose, fructose, rhamnose and xylose, and corrected by trehalose.

The results obtained on the outstanding toxicity of tricalcium phosphate and the reversion of its toxicity by trehalose and enhancement of toxicity by glucose and other sugars on insects indicate that the comparative biochemistry of insects and mammals is involved in this specific toxicity. Vertebrates are characterized by an endo-skeleton with 99 per cent of the body calcium bound in the hydroxyapatite crystallites of bone and teeth. Their requirement of calcium salts contrasts strongly with that of invertebrates. Other physiological and biochemical differences between the mammals and arthropods[4] also seem to be implicated in the action of tricalcium phosphate on insect as a metabolic poison even at low concentration. These aspects are being investigated.

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Generation-to-generation Passage of the Nuclear Polyhedral Virus of *Spodoptera exempta* (Wlk.)

GENERATION-TO-GENERATION passage of polyhedral viruses has been reported for a number of insects[1–5]. Previous work with the nuclear polyhedral virus of *Spodoptera exempta* (Wlk.) indicated that generation-to-generation passage of the virus occurred in this insect, but the evidence was inadequate to decide whether the virus passed within the egg or by contamination of the egg shell[6]. This communication presents the results of experiments on this question.

A virus-free culture of *S. exempta* was maintained in the insectary from the middle of 1964 and throughout the period of the experiments until the end of March 1965. No deaths due to polyhedrosis occurred in these 9 months and no signs of the disease were observed. The two experiments next described were done simultaneously in the same room as that in which the virus-free stock was maintained. Identical methods were used, and larval food, which alone could not be sterilized, was distributed at random between the virus-free and experimental larvae. In the first experiment, carried out during February 1965, egg batches from three parent moths of the virus-free stock were each divided into three and the following treatments carried out: (*a*) control, untreated; (*b*) eggs dipped 15 min in a virus suspension of known concentration; (*c*) eggs dipped as in (*b*), air-dried and then dipped 90 min in 10 per cent formalin and 0·1 per cent 'Teepol' and finally rinsed in distilled water. The results presented in Table 1 show that dipping in virus suspension led to 100 per cent mortality due to polyhedrosis; additional treatment with 10 per cent formalin and 0·1 per cent 'Teepol' completely prevented death due to polyhedrosis, presumably by destroying the virus contaminating the egg shell.

Table 1. DIPPING EXPERIMENTS WITH EGGS OF VIRUS-FREE STOCK OF *Spodoptera exempta*

Batch No.	Treatment	Total larvae	Percentage dead due to polyhedrosis	Percentage pupated
1	(*a*) Control, untreated	33	0	78·8
	(*b*) Dipped 15 min. in suspension of 5,000 polyhedra/mm³ + 0·1 per cent teepol	44	100	—
	(*c*) As (*b*), followed by dipping 90 min. in 10 per cent formalin + 0·1 per cent teepol	46	0	91·3
2	(*a*)	21	0	61·9
	(*b*)	34	100	—
	(*c*)	30	0	66·7
3	(*a*)	4	0	50·0
	(*b*)	12	100	—
	(*c*)	8	0	0
4	Virus-free stock	82	0	90·2

Table 2. OCCURRENCE OF POLYHEDROSIS IN OFFSPRING OF WILD MOTHS OF *Spodoptera exempta*

Moth and date caught	Treatment	Total larvae	Percentage dead due to polyhedrosis	Percentage pupated
1	*A*	130	0	81·5
(22.1.65)	*B*	48	6·3	89·6
2	*A*	41	100	—
(29.1.65)	*B*	185	0	90·3
3	*A*	172	0	79·1
(31.1.65)	*B*	120	15·0	4·2
4	*A*	163	1·2	71·8
(31.1.65)	*B*	125	10·4	36·8
5	*A*	225	2·7	42·2
(31.1.65)	*B*	185	18·4	43·8
6	*A*	149	20·1	26·8
(2.2.65)	*B*	115	33·9	2·7
7	*A*	99	100	—
(5.2.65)	*B*	80	0	2·5

Treatments: *A*, control, untreated; *B*, eggs dipped 90 min in 10 per cent formalin + 0·1 per cent 'Teepol'.

In the second experiment, wild female moths, which could be expected to carry polyhedrosis virus, were caught alive in a Robinson-type mercury-vapour light trap, and the eggs from each were divided into two. One moiety was left untreated; the eggs in the other were dipped 90 min in 10 per cent formalin and 0·1 per cent 'Teepol' and then rinsed in distilled water. The eggs were allowed to hatch and the resulting larvae from each batch were reared as an aggregate. All larvae that died were examined for polyhedra, as Giemsa-stained smears, using an oil-immersion objective. The results of this experiment are presented in Table 2, and show that larvae from eggs laid by all seven moths became infected with polyhedrosis. It is known from individual rearing that only a proportion of larvae from a particular egg batch will show infection[6]. This probably accounts for the absence of polyhedrosis in the untreated moieties from moths 1 and 3. Since polyhedrosis appeared in larvae from five of the seven batches of eggs that were surface-sterilized, passage of the virus in the interior of the egg would appear to have occurred.

It is unlikely, though not impossible, that the last-mentioned infections resulted from contamination of larval food. The larvae in the experiments and the virus-free culture were fed from a common food source throughout. Under these circumstances the chances of giving

contaminated food only to larvae in the second experiment were very small ($P = 0.006$).

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PHYSIOLOGY

Tonic Activity of Lung Receptors in Normal and Pathological States

ADRIAN[1] was the first to investigate the activity of single afferent vagal fibres. He showed that, in normal breathing, the vagal effect on respiration was due mainly to the impulses from the stretch endings in the lungs. These impulses have an inhibitory effect on the respiratory centre and thus contribute to the self-regulation of breathing.

Respiratory acceleration is one of the most significant symptoms of lung diseases. On the basis of Adrian's investigation it was supposed that dyspnoea depended on the increased sensitivity of stretch endings in the lungs[2]. Investigation of the activity in single afferent vagal fibres during experimental congestion[3–5], oedema[6,7], embolism[8] and pneumonia[9] did not, however, confirm this assumption.

The single-fibre technique allows easy detection of the activity of the group *A* vagal fibres, that is, the pulmonary stretch receptors. Using this method, it is, however, very difficult to detect the activity of the non-medullated *C* fibres. It has been shown histologically that most of the vagal fibres, including their bronchial branches, belong to the *C* group[10].

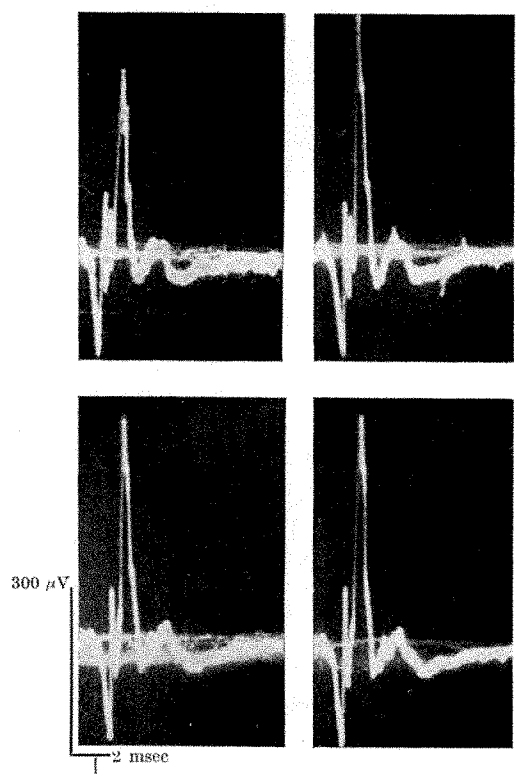


Fig. 1. Records of the rise of the action potential of the *A* fibres of cat's cervical vagus. Top, before oedema of the lungs. The amplitude of the *A* elevation during inflation (left) is smaller than that of the *A* elevation during deflation (right). Bottom, during oedema of the lungs. The amplitude of the *A* elevation during inflation (left) increases and becomes just equal to that of the *A* elevation during deflation (right). In this and subsequent records the distance between the stimulating and recording electrodes was 58 mm. The nerve was stimulated once per sec during inflation or deflation. Each record was made by superposing five sweeps

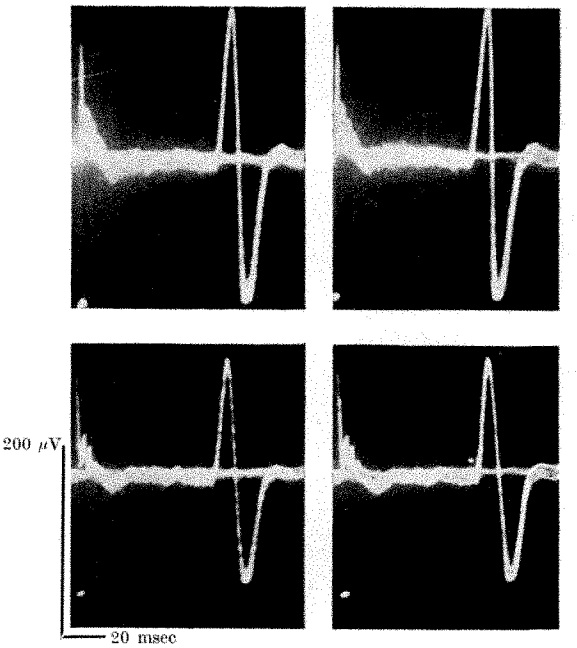


Fig. 2. Records of the rise of the action potential of *C* fibres of cat's cervical vagus. Top, before oedema of the lungs. The amplitude of the *C* elevation during inflation (left) is equal to that of *C* elevation during deflation (right). Bottom, during oedema of the lungs the amplitude of *C* elevation decreases equally during inflation (left) and deflation (right)

To investigate the whole activity of pulmonary receptors during oedema and pneumonia we used the method of Douglas and Ritchie[11], which allowed us to detect the activity of both the *A* and the *C* fibres.

The experiments were carried out on cats anaesthetized with urethane and chloralose. The chest was widely opened and artificial respiration was carried out. The right cervical vagus was cut just below the nodose ganglion. A pair of stimulating platinum electrodes was placed close to the peripheral cut end of the nerve, and another pair of recording electrodes was placed about 50–90 mm more distally. For stimulation of the nerve, rectangular pulses of 0·1–0·5 msec duration were used, the stimuli used being supramaximal for both *A* and *C* components. To exclude the impulses from other organs, the left and right vagi were cut over the diaphragm and the majority of cardiac branches of the right vagus were also cut. Oedema and pneumonia were evoked by the injection of 0·5 c.c. of 40 per cent glucose solution and 2·0 c.c. of hot water, respectively, in each lobe of the right lung.

Before the injection, there was a regular variation in the size of the *A* wave of the evoked antidromic vagal action: the potential was at a maximum during deflation, when the natural orthodromic activity was least, and at a minimum during inflation, when the natural orthodromic activity was at a maximum (top records of Fig. 1). The size of the evoked action potential varied with the amount of spontaneous activity in the vagus. The greater the degree of inflation, the smaller was the evoked action potential.

On the other hand, the size of the *C* wave of the evoked action potential did not change with changes in the respiratory volume (Fig. 2, top). This is in agreement with Adrian's observation that only the stretch receptors show periodic activity in normal breathing.

Both the oedema and the pneumonia caused characteristic changes in the size of the evoked action potential.

The size of the *A* wave increased during inflation and sometimes became equal to that of the *A* wave during deflation (Fig. 1, bottom). This means that there is no increase in sensitivity of the stretch endings following injury to the lung. On the contrary, there is some decrease in sensitivity of the stretch endings.

The *C* wave also underwent modification. During the oedema and pneumonia the size of the *C* wave of the antidromic action potential was decreased both during inflation and deflation (Fig. 2, bottom). This shows that pathological processes increase the sensitivity of the non-medullated *C* fibres.

It is known that oedema and pneumonia are accompanied by an increase in the concentration of potassium ions. In this connexion it seems to be interesting that the size of the *C* wave also increases after intravenous injection of potassium ions[12].

These experiments give a new explanation of the mechanism of the respiratory acceleration during pathological processes in the lungs. It has recently been shown that cathodic polarization evoked facilitation of respiratory neurones[13]. It is possible that the constant increase of impulses from *C* fibres also evokes facilitation and this could be the reason for the observed respiratory acceleration.

The tonic activity of the lung receptors can have great significance not only in the pathological breathing but also in the normal breathing. It has been shown that the lung receptors take part in the regulation of the activity of the gamma motoneurones of the respiratory muscles[14–20]. The activity of the gamma motoneurones is mainly tonic. It is therefore possible that it can be supported by the tonic activity of the aforementioned lung receptors.

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Potentials evoked *in vitro* in Preparations from the Mammalian Brain

MANY parts of the mammalian brain, isolated and examined in simple media *in vitro*, afford metabolic responses to electrical stimulation. It has not hitherto been established whether this implies neuronal responses detectable electrically. Thus in spite of much effort, no responses were recorded from the surface of slices of mammalian cerebral cortex[1], though metabolic sequelae to stimulation were observed.

In the present experiments, slices 0·35 mm thick were prepared from the anterior portion of the piriform lobe

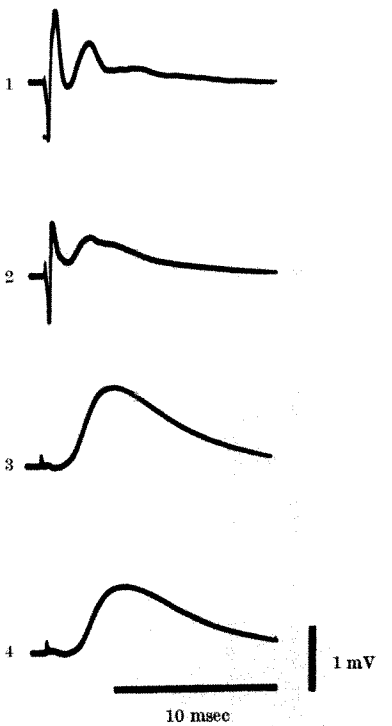


Fig. 1. Distribution of the evoked potential recorded from the surface of the slice in response to a single shock to the lateral olfactory tract. A recording electrode was put either on the tract (record 1) or at different distances along a line perpendicular to the tract and 5 mm from the stimulating electrodes. This gave records at distances of 0·5 mm (record 2), 1·5 mm (record 3) and 2·5 mm (record 4) from the tract. The action potential of the tract decreased in amplitude from record 1 to 4, while the *N*-wave attained its maximum height at a distance of 1·5 mm. Upward deflexions, negative. Calibration, 1 mV and 10 m.sec

(prepiriform area) of the guinea-pig cerebral cortex. The samples were incubated in a glucose-saline medium saturated by 95 per cent oxygen and 5 per cent carbon dioxide at 37° C in apparatus previously described[2]. The stimulating electrodes were a pair of ball-tipped silver wires connected to a Grass stimulus-isolation unit. The recording electrode was also a ball-tipped silver wire. The stimulus was applied to the frontal end of the lateral olfactory tract, which sends axon terminals extensively to the nerve cells in the prepiriform area[3,4].

Fig. 1 illustrates potentials generated on the surface of the slice in response to a single shock to the lateral olfactory tract. The potential recorded on the tract (record 1) was composed of an initial biphasic sharp deflexion, and a following slow negative wave—the latter being called the '*N*-wave' in this report. The initial deflexion was the action potential of the nerve fibres in the lateral olfactory tract. Thus, when the electrode was placed on the prepiriform area and the distance between the tract and the recording point was successively increased, the initial sharp deflexion decreased in amplitude in record 2 and disappeared in records 3 and 4. On the other hand, the *N*-wave increased in amplitude at these latter points. The irregular descending phase of the *N*-wave in record 1 became smoother in records 3 and 4.

By further investigating the properties of the *N*-wave, it was revealed that the *N*-wave was enhanced when it resulted from a stimulus which was preceded by a conditioning shock at short interval; it was obliterated by high-frequency stimulation (85 c/s) and was potentiated after tetanic stimulation (post-tetanic potentiation). The *N*-wave was sensitive to sodium phenobarbitone, to γ-aminobutyric acid and to chlorpromazine.

These findings exclude the possibility that the *N*-wave is the action potential of the slowly conducting nerve fibres in the lateral olfactory tract but indicate that it is a postsynaptic potential generated at cortical neurones.

It is therefore concluded that sections from the mammalian brain can maintain *in vitro* the synaptic organization which elicits the postsynaptic potential[5,6].

It is not quite clear whether or not these neurones retain the intact spike-generating mechanism. However, irregularities on the *N*-wave in records 1 and 2 might indicate that the neurones near the tract produce spike potentials. If so, the smaller amplitude of the *N*-wave in these records can be explained by thinking that the postsynaptic potential was abolished by a spike potential or that it was diminished by polysynaptic inhibitory mechanisms.

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An Effect of Stimulus Colour on Average Steady-state Potentials evoked in Man

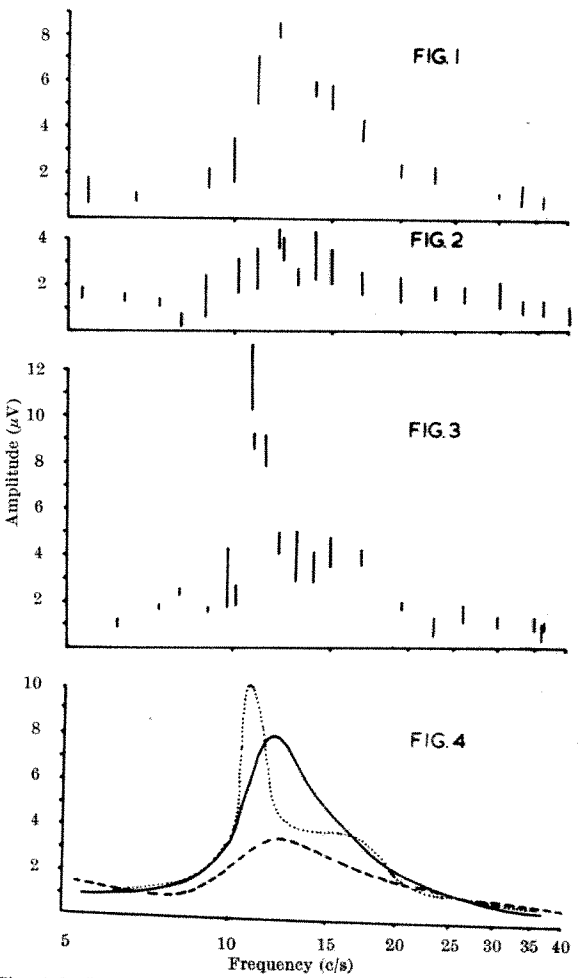
SCALP potentials evoked by sinusoidally modulated light have been measured for various monochromatic stimuli of equal retinal illuminations. The correlation apparatus made use of the orthogonality of sinusoids to extract from the electroencephalogram the sinusoidal evoked component at stimulus frequency (synchronous component), and to measure its amplitude and phase[1,2]. It was found that if the light stimulus were viewed with good voluntary fixation, and if its frequency and peak intensity were accurately maintained constant, an average steady-state régime occurred from about 12–20 sec after stimulus onset until at least 70 sec, during which the running average of the amplitude and phase of the synchronous component remained constant[1]. Data for one subject are described.

The stimuli were all of 9,000 trolands retinal illumination, of 14° subtense with a central fixation cross and with dark surround. An artificial pupil was used with Maxwellian view. The first electrode was placed 8 cm above the inion, the second 5 cm to the right of this and the earth placed 1 cm above the inion. Monochromation was by interference filters of about 10 nm half-width, and peak transmissions at 435, 589 and 634 nm. The colour vision of the subject was examined by the Ishihara test and found to be normal.

Figs. 1, 2 and 3 show the relationship between the peak-to-peak amplitude of the synchronous component of the evoked potential, and the modulation frequency for 100 per cent sinusoidal modulation. In Fig. 4, curves drawn through each of Figs. 1, 2 and 3 have been superposed to show the differences between the synchronous components of the potentials evoked by the red, yellow and blue stimuli. It can be seen that for both blue and red the low-frequency flank of the amplitude peak is very steep, but that the high-frequency flank is less steep for blue than for red. Red has the most selective amplitude peak, and yellow the least. It should be mentioned here that with the dark surround used, scattered light was found to make an important contribution to the evoked potential. Any dependence of this contribution on colour, however, might be expected to result in bodily shifts of the data of Figs. 1–3 along the ordinate, without affecting the shapes of the graphs.

The graphs of the phase of the synchronous component versus stimulus frequency all show a rapid phase shift near the amplitude peak followed at higher frequencies by a linear region of reduced slope. The similarity shown in Fig. 5 between the graphs of phase versus stimulus frequency in the vicinity of the amplitude peaks suggests that neither variations in the selectivity nor the contribution of a resonant-like filter is a major factor underlying the different amplitude-frequency characteristics of different colours shown in Figs. 1–4. Van der Tweel[3,4] has found that the amount of harmonic distortion may be very different at quite closely separated stimulus frequencies, and has suggested that this distortion may occur at an early stage in the visual pathways, while the more central region acts as a filter which responds selectively in the region of 10 c/s.

If the amount of harmonic distortion generated at a particular stimulus frequency depended on the colour of the stimulus, a peaking of the amplitude of the synchronous evoked component could be produced, which was additional to any effect of resonant-like filtering. Differently shaped peaks for different colours such as those presented in Figs. 1–4 could then result, while near the amplitude peaks, the phase versus frequency graphs remained unaffected by stimulus colour. This hypothesis is under investigation.



Figs. 1–3. Peak-to-peak amplitude of the synchronous component of the evoked response versus stimulus modulation frequency. Stimuli all of 9,000 trolands retinal illumination, 14° field with dark surround centrally viewed, and 100 per cent sinusoidally modulated in intensity. Fig. 1, blue 435 nm; Fig. 2, yellow 589 nm; Fig. 3, red 634 nm. Lines indicate range of each measurement

Fig. 4. Smooth curves drawn through Figs. 1–3 and superposed to demonstrate different amplitude-frequency characteristics for different colours

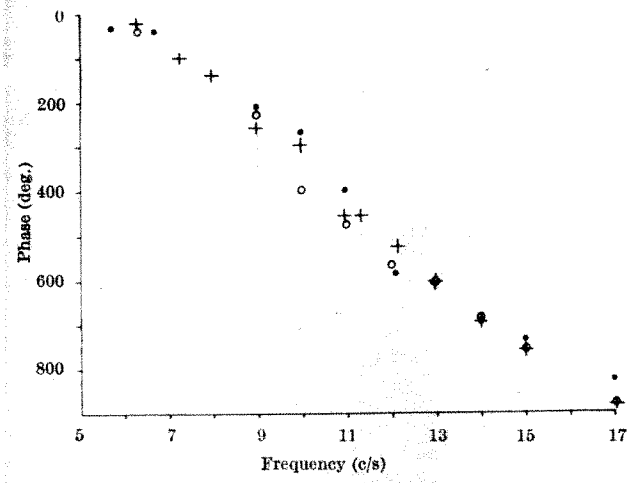


Fig. 5. Phase of the synchronous component of the evoked response versus stimulus modulation frequency for the same measurements as Figs. 1–3. Note the linear frequency scale. The zero of phase for each colour is arbitrary. ●, Blue; +, yellow; ○, red. Range of each measurement is typically ±10°

I thank Mr. G. Dickson for acting as my experimental subject, and Mr. R. Davis for constructing much of the apparatus.

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Evidence for Adrenergic Transmission of the Carotid Chemoreceptor Impulses in the Toad

RECENTLY when recording afferent impulse activity in the nerve supplying the carotid labyrinth in the toad, we have found chemoreceptor fibres which show increased discharge in response to hypoxia just as do those of the mammalian carotid body[1]. Although acetylcholine has been proposed as the normal chemical transmitter of the mammalian glomus nerve-endings[2–8] the evidence is not as yet wholly conclusive. Indeed, electron microscopy has revealed that the mammalian glomus epithelioid cells show numerous catecholamine-containing granules and the opinion has been advanced that the catecholamine may be the transmitter substance[3]. There is little physiological evidence for this, however, and even noradrenaline itself does not stimulate chemoreceptor discharge[6]. Based on the standpoint that the adrenaline-like substance produced in the body is not necessarily solely identifiable with noradrenaline, we have tried to stimulate carotid chemoreceptor discharge by electrical excitation of the cardiac sympathetic fibres. Our results indicate that an adrenergic mechanism is responsible for chemosensory transmission.

A pithed toad was laid on its back and the left carotid labyrinth was exposed. Afferent impulse activity was recorded from the cut 'carotid labyrinth nerve'. The left internal carotid artery was cannulated and all other branches of the left aortic trunk were tied, as was the external carotid artery. One of the large veins entering the sinus venosus was cannulated and all other cardiac veins were ligated. The right aortic trunk was cannulated and the cannula led to a reservoir *R* (Fig. 1) which in turn supplied the only patent cardiac vein. The heart, supplied with Ringer's solution from the reservoir, on contracting expelled the fluid into the carotid labyrinth and internal carotid artery, from which it escaped in drops, and simultaneously through the cannula in the right aortic trunk to return to the reservoir. The major part of the systolic volume was returned to the reservoir. Finally, the right vago-sympathetic nerve trunk and jugular ganglion were exposed and separated and the sympathetic nerve, cut at the second sympathetic ganglion, prepared for electrical stimulation.

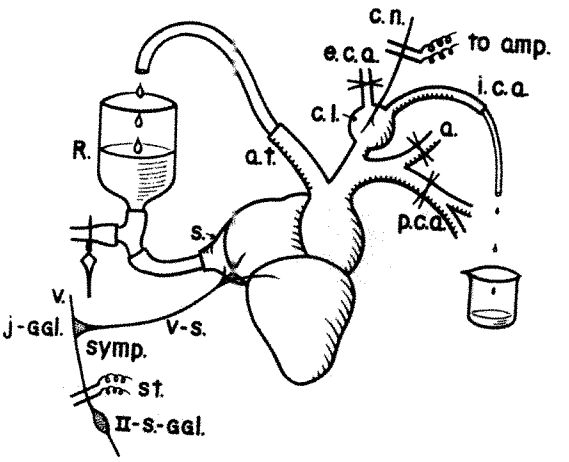


Fig. 1. Arrangement of the apparatus for stimulating the sympathetic nerve and also for recording the chemosensory impulses originating in the carotid labyrinth which was perfused with Ringer's solution through the heart. *c.l.*, Carotid labyrinth; *c.n.*, carotid nerve; *II-S-GGl.*, second sympathetic ganglion; *j-GGl.*, jugular ganglion; *symp.*, sympathetic nerve; *v.*, vagus nerve; *v-s.*, vago-sympathetic nerve trunk; *s.*, sinus of the heart; *a.*, aorta; *a.t.*, aortic trunk; *e.c.a.*, external carotid artery; *i.c.a.*, internal carotid artery; *p.c.a.*, pulmo-cutaneous artery; *R.*, reservoir of Ringer's solution (10 c.c.)

Faradic stimulation of the sympathetic fibres for 30 sec caused an increase in force and rate of the heart beat. There was an obvious increase in perfusate throughout the carotid labyrinth. On ceasing sympathetic stimulation, carotid chemoreceptor activity was reduced temporarily, whereas barosensory impulse discharge was transiently increased. After 1 min chemosensory discharge began to increase until it reached a maximum in about 3 min (Fig. 2)—this intensification of impulse discharge was sustained as long as there was evidence of increased cardiac rate. Afterwards, the impulse activity subsided to that of the control level. The return to the control level of impulse activity was hastened by replacement of the perfusate with fresh Ringer's solution.

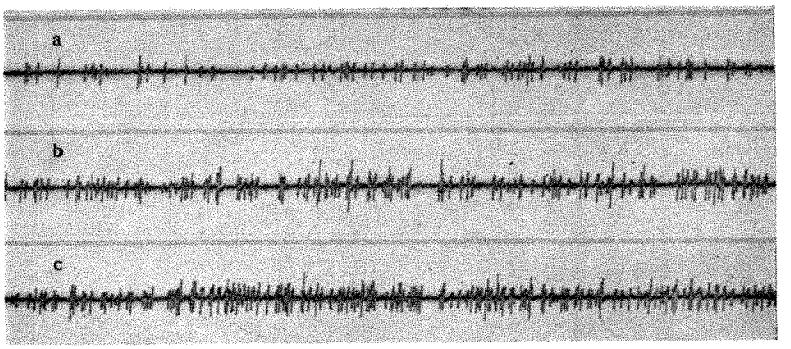


Fig. 2. Effect of adrenaline-like substance liberated from the sympathetic nerve endings on the chemosensory impulses. The impulses were recorded from the left carotid nerve. The repetitive electrical stimulation was applied for 30 sec to the right sympathetic nerve which innervates the heart. *a*, Before stimulation; *b*, about 90 sec after the cessation of stimulation; *c*, about 200 sec after. Time: 50 c/s

The initial decrease of chemosensory impulse activity is most probably due to both the increased flow of perfusion fluid through the labyrinth and the fact that the adrenaline-like substance liberated from the post-ganglionic sympathetic nerve endings has not yet had time to gain access to the fluid in the cardiac chambers. It seems probable that the subsequent intensification of chemoreceptor impulse traffic is due to the effect of this substance on the chemosensory nerve endings. The *p*H of the perfusion fluid remained at 7·40 before and after sympathetic stimulation.

These results support the possibility that the natural chemical transmitter of the chemosensory apparatus of the toad is of adrenergic nature.

We have recently found that the chemoreceptor cells in the carotid labyrinth possess many catecholamine-containing granules in their cytoplasm (unpublished). Thus, it can be concluded that this catecholamine is closely related to the mechanism of chemosensory transmission in the carotid labyrinth of the toad.

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Reflex Vascular Function in Unanaesthetized Rabbits

CHANGES in the calibre of vessels in the rabbit ear can be measured by a variety of methods. Assessment of the effect of various procedures on reflex vasomotor control requires direct stimulation of sympathetic nerves or ganglia, however, and is therefore unsuitable for unanaesthetized animals. It would be advantageous to induce predictable reflex vasomotor changes in intact animals which could be detected by a simple method. In man, reflex vasodilatation in the hand can be produced by heating the skin elsewhere[1]. This test depends on neural afferents from the heated area and sympathetic efferents to hand blood vessels and it has been used to measure autonomic function in diseases of the nervous system[2]. This test was adapted to the rabbit ear in order to investigate the effect on autonomic function produced by immunization with a variety of nervous antigens[3]. A diagrammatic representation of the test conditions is shown in Fig. 1. The animals were tested at constant environmental temperatures and silence was maintained throughout the procedure. To ensure that the rabbits were capable of vasodilatation at the time of the tests, 2 c.c. of 2 per cent procaine solution was injected subcutaneously around the base of the right ear. This produced vasomotor paralysis accompanied by a marked rise in temperature of the right ear. Reflex vasomotor changes in the left ear in response to heating the back for 40 sec were then monitored by thermocouples. When no vasodilatation occurred after the injection of local anaesthetic, the animal was presumed fully vasodilated and not testable. A normal record is shown in Fig. 2. Heating the shaved back produces an initial vasoconstriction in the ear vessels, of varying magnitude, which lasts 2–10 sec. This gives way to a marked vasodilatation which outlasts the stimulus by 10–30 sec before returning to preheating levels. Irradiation of the back can be repeated at 1-min intervals. The vasodilatation is associated with ear temperature changes ranging from 0·4°–1° C. This vasodilatation is independent of the rate of heating and the threshold is at about 225 W. Pretreatment with atropine, 2 mg/kg, abolishes reflex vasodilatation and 0·8 mg/kg of eserine enhances it. The pathways involved in these vasomotor changes are not fully known. It has been shown by Holton and Rand[4] that direct electrical stimulation of the superior cervical ganglion causes in rabbit ear vessels an initial vasoconstriction followed by vasodilatation and that the latter was abolished by atropine and enhanced by eserine. This vasodilator phase is presumably, therefore, due to postganglionic sympathetic cholinergic fibres. By analogy, brief heating of the back and direct electrical stimulation of the superior cervical ganglion utilize in the rabbit identical efferent pathways to the ear vessels. On the afferent side, heating the back produces its effect on ear blood flow probably by stimulation of receptors in the exposed skin rather than by warm blood. The short latency, the prompt return to baseline after cessation of the stimulus and the independence of the response on the rate of heating are all in keeping with this interpretation. This new test of vasomotor function in unanaesthetized rabbits was used in a large number of ani-

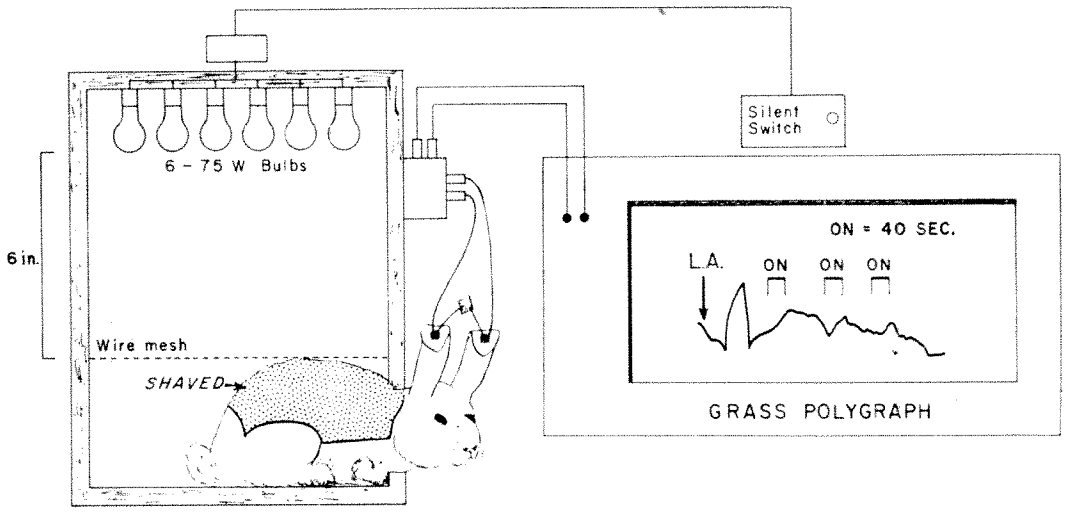


Fig. 1. Diagrammatic representation of the test conditions for reflex vasomotor function in the rabbit ear. Constantan-copper wire thermocouples were attached to each folded ear by loosely applied adhesive tape. They were so arranged that a change of 0·6° C in either ear produced a deflexion of 1·5 cm on the polygraph record. The wooden box fitted snugly around the animal's neck so that no light was visible when the bulbs were switched on by a noiseless switch

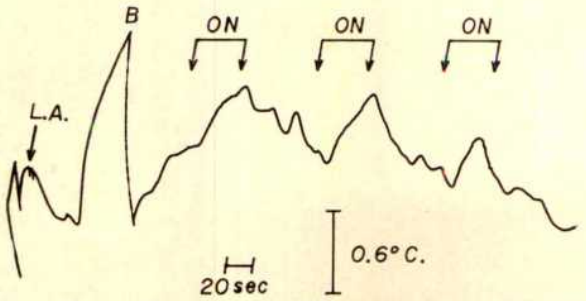


Fig. 2. Normal reflex vasomotor function in rabbit ears. After the subcutaneous injection of local anaesthetic (L.A.) around the base of the right ear, a marked rise in temperature occurred, necessitating the adjustment of the polygraph balance (B). When the lights were switched on for 40 sec (ON), an initial vasoconstriction followed by marked vasodilatation occurred as shown by the corresponding fall and rise in ear temperature

mals for a study of an experimental immunologically induced disorder of autonomic function.

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Spinal Cord Hypothermia by Localized Perfusion Cooling

A NEW technique has been developed which will rapidly and selectively cool the mammalian spinal cord while the temperature of the body core remains near normothermic levels. This becomes specially relevant in the light of the utilization of profound hypothermic levels[1] and isolated cerebral perfusion techniques in neurovascular surgery[2,3]. With this method both acute and chronic preparations can be used to investigate the neurophysiological and cardiovascular events associated with spinal cord cooling as well as to evaluate the efficacy of hypothermia in clinical situations associated with spinal cord trauma and disease.

Young, healthy dogs were used, anaesthetized with 25 mg/kg sodium pentobarbital and a flexible wire spiral endotracheal tube inserted. A midline skin incision was made over the spinous processes from T_8 to L_1, the spinal muscles divided and the posterior elements of T_{10} removed. The perfusion area or 'cold sink' is represented by the surgically exposed wound in depth over the cord and this will hold a volume of 35·0 ± 10·0 ml. of perfusate.

A volume of cold isotonic saline entering at 4·0°–6·0° C is continuously re-circulated over the area of the exposed cord using a pump, heat exchanger and cooling reservoir. The circuit (Fig. 1) consists of: (1) A variable speed, variable occlusion DeBakey roller-type pump. Using 3/16-in. 'Tygon' tubing, flows of 50 and 100 ml./min have been used.

(2) A highly efficient stainless steel Mayo pediatric heat exchanger with a priming volume of 65·0 ml.

(3) A small submersible pump placed in a plastic basin filled with ice water is attached to tubing coming from the inlet and outlet junctions of the heat exchanger. In this manner, the degree of cooling of the perfusate can be regulated by the temperature of the fluid in the cooling reservoir as it circulates in the heat exchanger water jacket. Since the heat exchanger and tubing in the perfusion circuit can be autoclaved, a sterile system can be employed for chronic experiments.

Temperature data were obtained using a '*YSI*' telethermometer system. Spinal cord, brain and paravertebral muscle temperatures were taken with 24 gauge needle thermistors. Oesophageal, rectal inflow and outflow perfusate and cooling reservoir temperatures were taken with appropriate thermistor leads. Arterial blood pressure and pulse rates were obtained from a catheter in a femoral artery connected to a strain gauge transducer. Electrocortical brain activity was recorded from silver disk electrodes placed extradurally and connected to a 'Grass' electroencephalograph.

Table 1. MEAN TEMPERATURE RANGES IN TEN ANIMALS SUBJECTED TO LOCALIZED PERFUSION COOLING OF THE THORACIC SPINAL CORD; PERFUSATE FLOW 100 ML. PER MIN

Perfusion time	Temperature (° C) Spinal cord	Brain	Rectal	Oesophageal
Pre-perfusion	35·0 ± 2·5	34·5 ± 2·5	35·0 ± 2·5	35·0 ± 2·5
30 min	11·8 ± 2·3	34·5 ± 2·5	35·5 ± 2·5	35·0 ± 2·5
150 min	11·8 ± 2·3	34·0 ± 2·5	34·0 ± 2·5	34·0 ± 2·5

Using this technique with steady perfusion flows of 50 ml. per minute, fifteen adult dogs were investigated. Within 30 min after initiation of cooling, mean temperatures in the spinal cord fell from 35·5° ± 2·0° C to 20·0° ± 1·5° C and remained at this level during the remaining 2 h of perfusion (Fig. 2). In another group of ten adult dogs, spinal cord cooling with steady perfusion flows of 100 ml. min were used (Table 1). Within 30 min the mean temperature in the spinal cord fell from 35·0° ± 2·5° C to 11·8° ± 2·3° C and was easily maintained at these hypothermic levels for a subsequent period of 2 h. In both groups of animals, brain and oesophageal temperatures remained

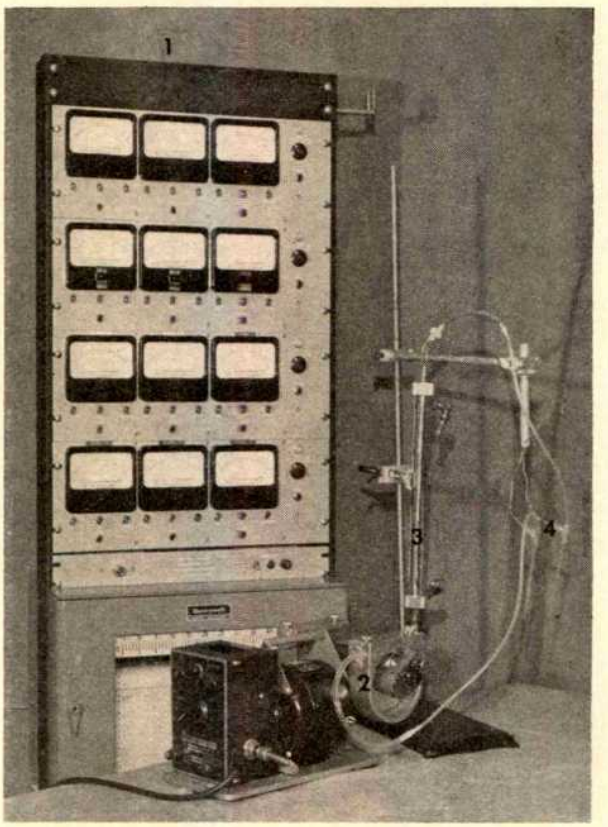


Fig. 1. Temperature recording and perfusion unit. Using the thermistors, temperatures are measured and recorded by the '*YSI*' module (1). Moved by an occlusive roller pump (2), the perfusate is cooled by the heat exchanger (3), pumped through tubing (4) into the area about the exposed spinal cord and then again returned to the heat exchanger by pump for recooling and recirculation

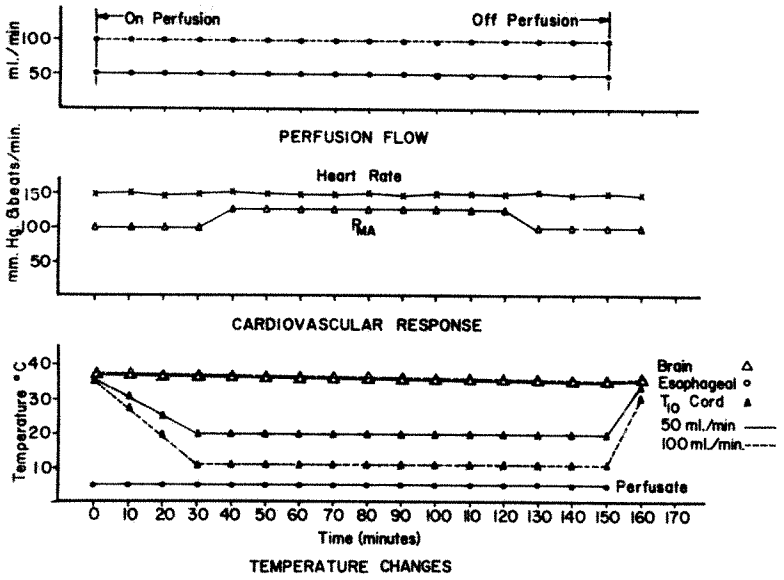


Fig. 2. Temperature changes, perfusion flows and cardiovascular responses during localized spinal cord hypothermia

near normothermic levels during perfusion. Within 10 min after termination of perfusion, spinal cord temperature in both groups returned to the pre-perfusion levels. In all animals, pulse rates remained stable during perfusion cooling and the electrocardiogram showed no abnormal changes. In both groups, the mean arterial blood pressure remained at the baseline level of 100 mm of mercury during the first 30 min of cooling, rose to and was sustained at 120 mm of mercury for 1·5 h and then returned to baseline. Electrocortical brain activity of the frontal, parietal and occipital areas monitored throughout the cooling period revealed no abnormal changes in wave forms in any of the animals during cooling.

In chronic experiments using a sterile technique, the spinal cord of five additional animals were perfusion cooled for 2·5 h at a flowrate of 100 ml./min, after which the surgical incision was closed and the animals were returned to their cages. No functional or neurological abnormality could be detected in any of the animals so cooled after repeated neurological examinations over a period of 2 weeks.

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Cyclic Activity in the Male Hypothalamus

IT is well known that the administration of testosterone to female rats shortly after birth induces the differentiation of an acyclic male type of hypothalamus, resulting in permanent sterility and cystic ovaries lacking in corpora lutea[1]. The role of testosterone in the genetic male rat has been examined by the method of castration during infancy[2,3]. The findings have shown that deprivation of endogenous testosterone within 3 days of birth is followed by the differentiation of a cyclic female type of hypothalamus as seen by the presence of corpora lutea in ovarian transplants made during adult life. Castration more than 3 days after birth does not prevent the differentiation of the male type of hypothalamus, for ovaries grafted into animals so treated contained no corpora lutea in adulthood. These and other investigations[4] raised the possibility that the findings would be different if the transplantation and examination of the ovaries were carried out in the immature animal.

In the present experiments male rats were castrated either less than 20 h after birth or when 5–6 days old. All animals received at the time of castration an abdominal transplant of a whole ovary, containing no ripe follicles or corpora lutea, from a littermate. The animals were killed at times varying from 34 to 117 days after operation and the ovarian transplants serially sectioned and examined for corpora lutea. The latter were carefully searched for entrapped ova but none was found.

All three animals castrated at 5–6 days from birth and killed when 114–122 days old showed transplants containing only well-developed follicles and no corpora lutea, thus confirming previous findings[2,3]. The two animals that were castrated on the day of birth and killed at 72 days of age had transplants containing ripe follicles and fresh corpora lutea, again in agreement with the observations of previous workers[2,3]. A new finding was that in all seven male rats castrated 5–6 days after birth and killed at between 39 and 61 days of age the ovarian transplants contained several fresh corpora lutea as well as ripe follicles.

The presence of the testis (and physiological amounts of testosterone) during the critical 1–3 days after birth did not therefore prevent ovarian cycles during the first 8 weeks of life, but induced lack of ovulation in adulthood. Hence it may be inferred that the genetic, intact male hypothalamus behaves in a cyclic female manner for some weeks after birth and then loses its cyclicity and functions in the stable male manner. It has been reported[4] that female rats treated with testosterone shortly after birth exhibit ovarian cycles for a few weeks before developing the acyclic "early androgen syndrome". It would appear, then, that all hypothalami are of the female type during a significant part of the life-span and that the action of androgen, natural or pharmacological, in the neonatal period does not take effect until some time after puberty.

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Changes in the Nervous System of Rabbits following the Administration of Sodium Diethyldithiocarbamate

IN 1956, Mûr, Zaruba and Charamza[1] reported myelin degeneration in twenty-one rabbits, of which sixteen had received repeated intravenous injections of α-benzoin oxime, and five had received repeated intravenous injections of diethyldithiocarbamate. The lesion, which was most marked in the spinal cord, was compared to that seen in swayback in lambs and disseminated sclerosis in man. They also reported a decrease in blood copper levels in nine out of twelve rabbits after intravenous admini-

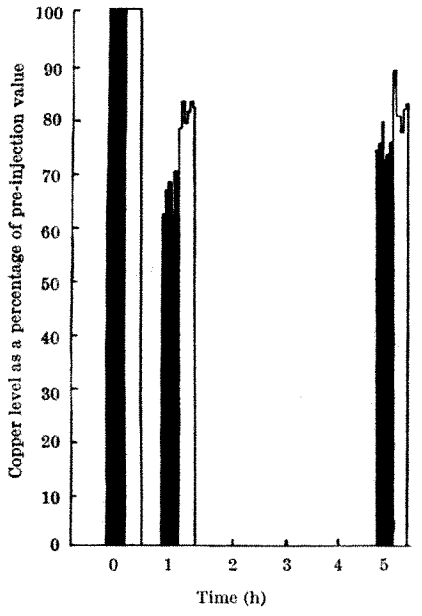


Fig. 1. Blood copper levels in six normal rabbits before, 1 h after, and 5 h after injection of NDDC. The effects of intravenous (■) and intraperitoneal (□) injections are compared

stration of α-benzoin oxime, and in two out of two rabbits following intravenous injection of diethyldithiocarbamate.

Howell[2] confirmed the depression of blood copper levels following the administration of sodium diethyldithiocarbamate (NDDC) and also reported that foetal resorption followed the administration of NDDC to pregnant rabbits. These were short-term experiments and the changes in myelin described by Mûr *et al.*[1] were not seen. Accordingly it was decided to give NDDC daily over a greater period of time in an attempt to produce a lesion in the myelin sheath. It is more convenient to give repeated injections intraperitoneally than intravenously. The effect of NDDC on blood copper levels when administered by the two routes was therefore compared in six rabbits. Methods of blood collection and analysis were as described previously[2]. The chelating agent was given as an isotonic solution, buffered after the method of Sunderman[3], and at a rate of 5 ml./kg body-weight. It can be seen (Fig. 1) that blood copper levels were depressed by either intraperitoneal or intravenous injection of NDDC.

One month before starting daily injections liver biopsies were taken from each of the eighteen Dutch and New Zealand rabbits and the copper content of the samples determined. Fifteen of the eighteen rabbits received daily injections of NDDC; three of these also received weekly subcutaneous injections of copper calcium EDTA; three also received, in addition to the weekly subcutaneous injection, a daily oral mineral supplement containing the elements iron, zinc, cobalt and manganese. The remaining three rabbits did not receive carbamate but were given a daily intraperitoneal injection of saline at a dosage of 5 ml./kg body-weight.

Daily injections for five months did not produce any significant change in pre-injection blood copper levels, nor could any sign of inco-ordination be detected. We therefore decided to kill six of the rabbits to determine the copper content of their tissues and to examine the peripheral and central nervous system histologically. The remaining twelve rabbits, two of which were receiving saline, were injected daily for a further two and a half months at a dosage of 10 ml./kg body-weight. Seven and a half months after the experiment began all the rabbits receiving NDDC showed varying degrees of inco-ordination. These rabbits were then killed and tissues again taken for histological examination and chemical analysis. The copper levels in the tissues of these twelve rabbits, together with the values of the initial liver biopsies, are recorded in Table 1. The copper levels of the liver and central nervous system of those rabbits which received carbamate were markedly increased whereas those of the rabbits receiving saline were not. This increase was in direct contrast to the postulation of Mûr and his colleagues[1]. The rabbits killed after five months' daily injections of NDDC showed a similar, though less marked, increase in copper levels.

Table 1

Treatment	No. of rabbits	Copper content in p.p.m. dry weight: Pre-injection Liver biopsy	Tissues taken at seven and a half months: Liver	Spinal cord	Kidney
Carbamate	7	11·6 (11·1–12·2)	235·4 (80·0–445·0)	28·2 (24·3–32·1)	15·5 (14·4–18·8)
Carbamate + copper EDTA	1	11·4	480·0	23·8	14·4
Carbamate + copper EDTA + minerals	2	10·8 (10·6–11·0)	857·0 (490·0–1,220)	45·9 (34·8–57·0)	17·1 (15·3–18·9)
Saline	2	11·3 (10·9–11·7)	16·4 (11·3–20·0)	3·1 (1·5–4·7)	11·5 (10·7–12·3)

The figures in parenthesis represent the range of values.

The spinal cord was fixed in Heidenhain's Susa while all other tissues were fixed in neutral formalin. Paraffin sections of peripheral and central nervous system were cut at 5μ or 15μ and stained by haematoxylin and eosin, luxol fast blue, the Nissl method, and Glees and Marsland's method for neuraxons. Histological examination of the white matter of the spinal cord of all rabbits that had received NDDC showed swollen axons and ballooned myelin sheaths (Fig. 2). In addition to these changes, which are characteristic of early Wallerian degeneration, several cords contained gitter cells. The lesion was found at all levels of the spinal cord and occurred predominantly in the periphery of the ventral and lateral columns. In six of the rabbits Wallerian degeneration was also seen in the dorsal columns. In the grey matter of the spinal cord some neurones had lost their structural detail and had a homogeneous eosinophilic appearance. This change was seen in ten of the fifteen rabbits receiving NDDC and occurred in Clarke's column and in ventral horn cells. Lesions typical of early Wallerian degeneration were also found in the sciatic nerves of eight of the rabbits receiving carbamate. None of these changes was seen in the tissues of the rabbits receiving injections of saline.

Although the lesion in the spinal cord was associated with a simultaneous accumulation of copper in the tissue, there was no evidence of pathological change in the corpus striatum of the brain, or of pigment deposits in Decemet's membrane, or of cirrhosis of the liver, or of permanently

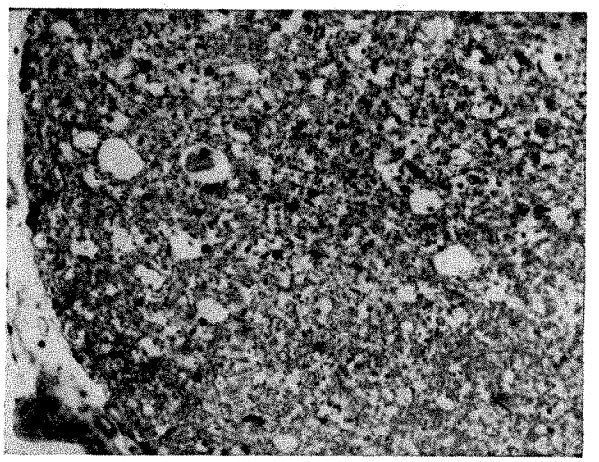


Fig. 2. Transverse section of lumbar spinal cord of a rabbit which had received daily intraperitoneal injections of NDDC for seven and a half months. The section shows axon swelling and ballooning of myelin sheaths. (Haematoxylin and eosin, ×150)

low caeruloplasmin levels. The condition cannot therefore be said to simulate Wilson's disease. The pathogenesis of the lesion produced in rabbits is not yet known. It may be associated with the ability of NDDC to inhibit enzymes[4].

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PHARMACOLOGY

Muscle Contraction produced by Sulphydryl Inhibitors

IT was recently reported[1] that the sulphydryl (—SH) agent, parachloromercuribenzoate (PCMB), causes superprecipitation of actomyosin gel. Masking of an —SH group appears to prevent interaction between ATP and some inhibitory site on the protein, thus enabling ATP to induce superprecipitation through reaction with another site. If this phenomenon is related to changes occurring in the actomyosin system during muscle activity, then —SH agents should be expected to produce contraction. Accordingly, several such agents were tested on skeletal muscle, and they were found to cause various types of contractile phenomena.

All experiments were done on the sartorius muscle isolated from the frog *Rana pipiens* during winter and spring months. The muscle was placed in a chamber containing 10 ml. Ringer's solution (110·88 mM sodium chloride, 2·0 mM potassium chloride, 1·8 mM calcium chloride, 0·1 mM sodium phosphate, 2·02 mM sodium bicarbonate; *p*H 7·2; 22°–24° C). Contraction was recorded isometrically with a strain gauge and ink-writing oscillograph, background tension being initially adjusted to 0·5 g. After a control period in normal Ringer's, during which the muscle was stimulated directly once per min with supramaximal square wave pulses, the muscle bath was changed to a Ringer's containing *d*-tubocurarine chloride (5×10^{-5} g/ml.), and stimulation was continued for another 10 min. The bath was then changed to a tubocurarine–Ringer's which contained the test agent. The presence of a paralysing concentration of tubocurarine in these experiments is necessary because initial tests showed that some —SH inhibitors facilitate neuromuscular transmission.

After a latent period of about 1 min, PCMB causes repetitive low-amplitude contractions and a moderate increase in muscle tension (Fig. 1). After this effect, stimulus-evoked contractions begin to decline, and within another 15 min the muscle becomes completely unresponsive to direct stimulation. The poor aqueous solubility of PCMB limited its test concentration to 1 mM. The more soluble sulphonated analogue, parachloromercuribenzenesulphonate (PCMBS), was tested at a 3 mM concentration. After a latent period of 2–3 min, this agent causes repetitive low-amplitude contractions with no accompanying increase in tension (Fig. 1). About 20 min after termination of this effect, there begins a very slow-rising and irreversible increase in tension which reaches an amplitude of about half the maximum stimulus-evoked twitch.

The modest effects of the organomercurials and their poor aqueous solubilities discouraged further experiments with this type of —SH agent. Instead, attention was focused on *N*-ethylmaleimide (NEM). This agent contains an activated double bond which forms addition compounds with reactive —SH groups of protein, and at *p*H 7·2 the reaction is rapid and highly specific[2]. Fig. 2*A* shows that NEM has a striking biphasic action on skeletal muscle. The initial contractile effect is immediate in onset, reaches a peak within a few seconds, and is short-lived. This is followed by a slow-rising increase in tension which is incompletely reversed by repeated washings in normal Ringer's. The magnitude of the initial response to 5 mM NEM is 60–125 per cent of the maximum stimulus-evoked twitch. The secondary response to the same concentration of NEM is more constant and is always close to the maximum twitch amplitude. Repetitive low-amplitude contractions, similar to those produced by the organomercurials, always appear during and immediately after recovery from the initial NEM response. The response to direct electrical stimulation is severely attenuated during the peak of the secondary contractile effect; but for several minutes after NEM application and even at the time when the secondary response begins, there is marked twitch potentiation. Dose-response relationships were not thoroughly studied, but the available data indicate that threshold NEM concentration (for the initial response) is 0·5–1·0 mM, and the maximum effect is produced by 5–10 mM. Treatment of a muscle with equimolar concentrations of *l*-cysteine and NEM has no effect on muscle tension or on amplitude of stimulus-evoked contractions. This suggests that the —SH binding group of the NEM molecule is required for pharmacological activity.

Further investigation indicated that absence of external calcium or membrane depolarization blocks the initial response to NEM. The role of calcium is suggested by the type of experiment shown in Fig. 2*B*, in which the muscle is initially immersed for 30–50 min in calcium-free Ringer's containing ethylenediamine tetraacetic acid (EDTA; trisodium salt at *p*H 7·2); the initial contractile effect of NEM is completely blocked by such treatment,

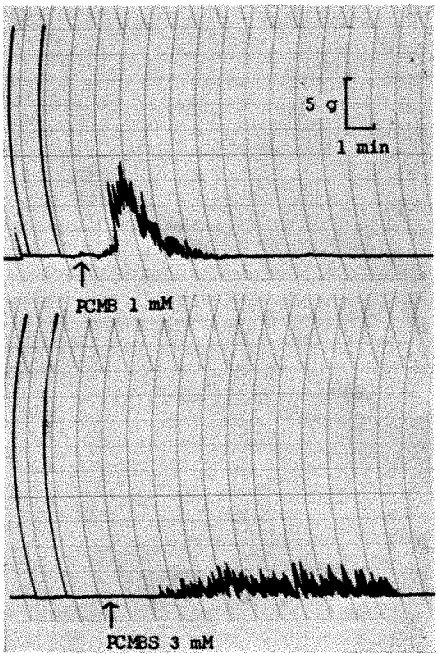


Fig. 1. Effects of PCMB and PCMBS on frog sartorius muscle. Arrows indicate time of application of test agent. Maximum stimulus-evoked twitches shown to left of arrows

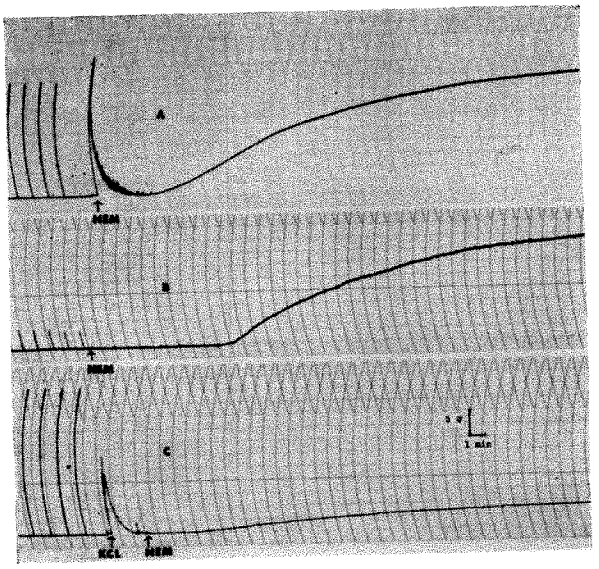


Fig. 2. Effects of NEM on frog sartorius muscle. *A*, Effect in normal Ringer's. *B*, Effect after 50 min of treatment in Ringer's containing 1 mM EDTA. *C*, Effect 3 min after application of 116 mM potassium chloride. Maximum stimulus-evoked twitches shown to left of arrows

but the secondary response is unaltered. Immersion of a muscle for the same amount of time in calcium-free Ringer's without EDTA has no effect on NEM action. Assuming that NEM produces its initial effect through binding of reactive —SH groups, it is interesting that low external calcium causes a pronounced decrease in the number of ionizable —SH groups in excitable tissue[3]. This suggests that protein configuration in EDTA–Ringer's is unfavourable for —SH group reactivity.

After pre-treating a muscle with isotonic potassium chloride, there is complete blockade of the initial NEM action, and this indicates a dependence of the NEM response on a normal resting membrane potential. Depolarization in isotonic potassium chloride may also be associated with changes in protein configuration which modify —SH group reactivity, for there is a marked loss of ATP and other organic phosphates from excitable cell membranes under these conditions[4]. In fact, this loss of organic phosphate also occurs in EDTA-treated tissues[4,5], so there may be a fundamental relationship between the two types of antagonism of NEM action, one by EDTA and the other by isotonic potassium chloride.

It seems unlikely that the exceedingly rapid and transient phase of NEM action is associated with a direct effect on contractile proteins. The secondary rise in tension may be related to such an effect and, if so, its resistance to EDTA is interesting. If the initial response involves a membrane action, NEM could serve as a useful tool in the study of molecular events underlying excitation and electromechanical coupling. Assuming that protein configuration changes are associated with these processes, the reactivity of cystein —SH groups could play a key part.

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HAEMATOLOGY

Haemoglobin Variants in a Species of Wild Mice—*Peromyscus maniculatus*

HAEMOGLOBINS from various strains of mice have been referred to as single (Hb^s) or diffuse (Hb^d)[1–3], according to the appearance of the electrophoretic patterns of the haemoglobin haemolysates. Popp[1] has suggested that two allelic genes are responsible for the single or double (diffuse) bands. Recently, two investigators[4,5] have shown independently that the diffuse patterns are the result of polymerization of mouse haemoglobins to eight-chain double molecules, and hence from 4*S* to 7*S* tetramers. This transformation can be enhanced by reduced glutathione (GSH) or oxidized glutathione (GSSG) and inhibited by iodoacetamide (IOA). These polymerization products, frequently encountered with inbred strains and with domestic mice (*Mus musculus*), are not often found with wild species.

In a sub-species of the white-footed deer mouse, *Peromyscus maniculatus rufinus*, three highly significant morphological differences were found in haematological studies. These mice were trapped alive from two distinct ecological and geographical locations, one from an area at Gothic, Colorado, at an elevation of 10,450 ft. and another from an area on the north rim of Black Canyon of the Gunnison National Monument, Montrose, Colorado, at an elevation of 7,500 ft. The haematological data are shown in Table 1. The results are in complete agreement with Gough and Kilgore[6], but differ markedly from those previously reported[7–11].

Table 1. HAEMATOLOGICAL STUDIES ON *Peromyscus maniculatus rufinus* FROM 10,450 FT. AND 7,500 FT.

	No. of mice	Hb (g per cent)	Hct (per cent)	RBC 10^6/mm^3	MCV $c\mu$	MCH $\gamma\gamma$	MCHC (per cent)
P.m.r. (Gothic) 10,450 ft.	81	15·5 ± 1·2	46 ± 3	11·2 ± 1·3	41 ± 4	13·8 ± 1·4	33·5 ± 1·7
P.m.r. (Black Canyon) 7,500 ft.	41	14·9 ± 0·9	47 ± 2	12·9 ± 0·8	37 ± 2	11·7 ± 1·0	31·6 ± 1·2

From the data it is apparent that the mice at the higher altitude have larger erythrocytes, but a lower erythrocyte count and a greater mean corpuscular haemoglobin concentration. The relationship of cell size and altitude in these studies is exactly the converse of the expectation that, as altitude increases, the erythrocyte count should increase with an accompanying decrease in erythrocyte size. As Wintrobe[12] points out, the polycythaemia is absolute; erythrocyte counts are markedly elevated with an associated increase in haemoglobin and packed cell volume. A slight macrocytosis was observed by Wintrobe, attributable to an increased reticulocytosis; the mean corpuscular haemoglobin concentrations were not found to be altered from the normal.

Haemoglobin electrophoresis on each of these mice on starch gel at *p*H 8·1 revealed only single bands although, in other species and sub-species of *Peromyscus*, among some sixty mice both single and double but no diffuse bands were seen (Figs. 1 and 2). The single haemoglobin band from Black Canyon was faster than the haemoglobin from Gothic. When a mixture of these haemoglobins was chromatographed on DEAE cellulose 'Sephadex 40', two separate components were again identified.

To test the possibility that diffuse bands could be produced experimentally, samples were subjected to 37° C for several days or allowed to age at room temperature for up to 30 days. No diffuse bands were produced. However, incubation of samples with reduced and oxidized glutathione produced the characteristic diffuse bands (Fig. 2). Incubation by dialysis with 6·8 mg iodoacetamide/ml. of haemoglobin for 12–24 h prior to addition of GSH or GSSG did not prevent (Fig. 2) polymer formation but tended to decrease it.

The solubilities of both Gothic and Black Canyon haemoglobin types are identical, and molecular weights are identical with ultracentrifuge sedimentation values of $4{\cdot}2 \times 10^{-13}$.

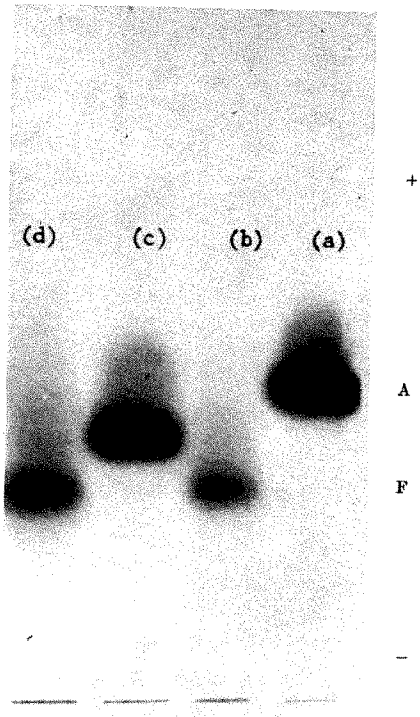


Fig. 1. Starch-gel electrophoresis at *p*H 8·1 of haemoglobin haemolysates from *Peromyscus maniculatus rufinus* at Gothic (10,450 ft.) and Black Canyon (7,500 ft.). Hb-A and F are used as markers. Hb-Gothic migrates slightly cathodal to Hb-Black Canyon. These samples were run before dialysis with iodoacetamide. (*a*) Hb-A; (*b*) Hb haemolysate-Black Canyon; (*c*) Hb-F; (*d*) haemolysate-Gothic

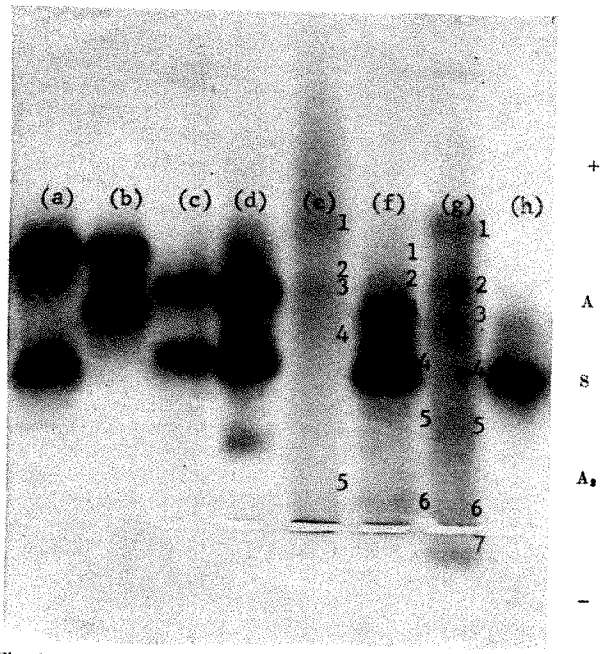


Fig. 2. Starch-gel electrophoresis (*p*H 8·1) of haemoglobin haemolysates from various sub-species of *Peromyscus maniculatus*. Hb-A and S are used as markers. The four samples on right of figure illustrate the diffuse six to seven banded components produced experimentally with GSSG and GSH mixtures from single banded *Peromyscus maniculatus rufinus* (Gothic) Hb haemolysate. (*a*) and (*b*) *Peromyscus maniculatus fulvus*; (*c*) *Peromyscus maniculatus blandus*; (*d*) Hb-A + S; (*e*) Hb haemolysate-(Gothic) + 10 mg GSSG and 20 mg GSH, after IOA dialysis; (*f*) Hb haemolysate-Gothic + 20 mg GSH after IOA dialysis; (*g*) Hb haemolysate Gothic + 20 mg GSSG—no IOA dialysis; (*h*) Hb-haemolysate-Gothic-untreated

Our findings, although still incomplete, suggest that natural selection has led to a more efficient type of haemoglobin, making possible the selection of the larger cell size. We thank Mrs. J. Odom for technical assistance and the Rocky Mountain Biological Laboratory at Gothic, Colorado, for the use of their facilities.

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Radioactive (Chromium-51) Heinz Bodies

HEINZ bodies are refractile granules appearing within erythrocytes and stainable with certain dyes such as crystal violet[1–3]. In man, their appearance is often associated with anaemia due to drug administration (for example, phenylhydrazine, menadione) or contact with other agents (for example, aniline dyes), particularly in certain sensitive individuals. Although the granules can be readily prepared experimentally by treatment of animals or isolated erythrocytes with various drugs, their chemical composition is only partially resolved and their metabolic disposition is unknown.

A prime difficulty in investigating Heinz bodies has been the absence of methods for quantitative evaluation. Most research workers simply estimated the number and size of granules observed under the light microscope[4,5]. Beutler *et al.*[6], in their study of Heinz body formation during exposure of erythrocytes to acetylphenylhydrazine *in vitro*, distinguished sensitive glucose-6-phosphate dehydrogenase-deficient cells from insensitive normal cells by counting the percentage of cells with five or more inclusion bodies. Jandl *et al.*[7] obtained more quantitative estimates by weighing the precipitate obtained on lysis of cells.

During experiments dealing with erythrocyte survival in rabbits treated with phenylhydrazine, we noted that the precipitate isolated from chromium-51-labelled erythrocytes was radioactive. This observation provides a convenient and simple method for quantitative analyses. A few experiments illustrating applications of the method follow:

Erythrocytes were labelled with radiochromium by incubating 3 ml. of rabbit or human, heparinized, whole blood with 30 μc. of chromium-51-labelled sodium chromate (Squibb, 100 mc./mg) for 1 h at room temperature[8,9]. Samples were washed twice with ten volumes of isotonic saline for re-injection into rabbits, or washed three times, admixed with three volumes of unlabelled cells from the same donor, and then suspended in isotonic phosphate buffer, *p*H 7·4, for *in vitro* investigations; the washing procedure eliminated radio-labelled proteins in the plasma as well as any residual chromate. The *in vivo* investigations were performed by perfusion of the ear vein of rabbits at a constant speed of 0·5 ml./min (Harvard infusion pump) for 50 min. On the other hand, *in vitro*

investigations were performed on a Dubnoff metabolic incubator at 37° C. Phenylhydrazine-hydrochloride (Eastman Organic Chemicals), recrystallized from ethanol, was dissolved in isotonic buffer and neutralized by titration with sodium hydroxide.

Heinz bodies were isolated by lysis of cells in from ten to twenty volumes of distilled water followed by centrifugation at 5,000*g* for 20 min. The insoluble stroma (controls) or Heinz bodies with stroma (experimental samples) were washed twice with 10 ml. water, at which point the residual radioactivity of the supernatant wash fluid was negligible (0–60 c.p.m. for the wash fluid versus 2,000–100,000 c.p.m. for the Heinz body preparation). Generally, 0·5–1 ml. of whole blood (*in vivo* investigations) or of cell suspension (*in vitro* investigations) was sampled; later, 2 ml. from a total of 4 ml. suspension of washed precipitate in water was analysed in a well-type scintillation counter.

The data of Tables 1 and 2 illustrate the formation of increasing amounts of radioactive precipitate in erythrocytes exposed to increasing doses of phenylhydrazine. Table 1 is from an *in vitro* experiment with human cells; Table 2 is from an *in vivo* experiment with a group of rabbits. Increasing amounts of Heinz bodies with increasing dose of stressor agent were observed microscopically[6] in these experiments. Other investigators[5,7] have correlated the increased Heinz body formation with increasing amounts of insoluble material obtained after lysis. The insoluble radioactive precipitate was undoubtedly derived from haemoglobin which has been shown to account for a minimum of 78 per cent of the chromium label[8], although contributions from other, as yet unidentified, proteins cannot be rigorously excluded. The activity associated with stroma, 3·1 per cent and 3·8 per cent for two samples of human erythrocytes, and 2·9 per cent and 3·3 per cent for two rabbits, corresponds to the amount of haemoglobin associated with stroma in water-washed preparations[10,11].

Table 1. HEINZ BODY FORMATION AND LOSS OF 'INTACT HAEMOGLOBIN' IN HUMAN ERYTHROCYTES *in vitro*

Phenylhydrazine (μmoles/ml.)	Radioactivity in precipitate (as percentage of total radioactivity)	Loss of 'intact haemoglobin' (per cent)
0	3·1 (stroma)	—
2·5	10·7 (7·6 corr.)*	11
5·0	15·3 (12·2 corr.)	17
10·0	23·5 (20·4 corr.)	31
20·0	35·5 (32·4 corr.)	54

A 25 per cent cell suspension in isotonic buffer was used. The combined total of oxyhaemoglobin and methaemoglobin (intact haemoglobin) was measured with a cyanomethaemoglobin method. Measurements were made at 3 h.
* Figures in parentheses have been corrected for stromal radioactivity.

Table 2. HEINZ BODY FORMATION IN RABBITS

Phenylhydrazine administered (mg/kg body-weight)	Heinz body formation at 24 h (as percentage of total radioactivity)*
10	1·8, 3·4
20	4·5, 4·7
50	12·6, 10·2
100	21·3

* Values have been corrected for stromal radioactivity. A correction of 3·1 per cent was used; this was the average (2·9 per cent + 3·3 per cent) for two animals before infusion. Results listed are for individual animals.

The term 'intact haemoglobin' was originally used by Harley and Mauer[5] to designate the combined total of oxyhaemoglobin and methaemoglobin. In Table 1 the loss of 'intact haemoglobin' gives an estimate of the conversion of haemoglobin to various breakdown products including Heinz bodies. The radioactivity incorporated into the isolated precipitate (corrected for stromal radioactivity) accounted for 60–72 per cent of the loss in 'intact haemoglobin'. In a second experiment with blood from another human donor, similar results were obtained, except that there was greater haemoglobin loss and proportionately greater radioactivity in the precipitate due to a longer incubation time of 24 h (for example, with 20 μmoles phenylhydrazine per ml., there is 66 per cent loss in haemoglobin against 47 per cent radioactivity in the precipitate). The haemoglobin lost but not appearing in the radioactive precipitate may comprise very small granules, for example, those observed in the electron microscope[12] which may not sediment at 5,000*g*, or other intermediates between haemoglobin and the larger coalesced granules.

There has been some controversy concerning the question of a stromal or haemoglobin origin for Heinz bodies. The granules are observed most frequently at the periphery of cells, closely associated with the plasma membrane, but the available evidence favours the suggestion that they are derived from haemoglobin[7,12–14]. Further support for this point of view can be found in: (1) the incorporation of chromium-51-labelled haemoglobin into the granules in amounts very much in excess of that initially bound to stroma; (2) the proportionality (Table 1) between the extent of incorporation and the extent of haemoglobin destruction. However, the possibility of stromal participation in Heinz body formation has not as yet been tested. The data in Table 3 are from an experiment in which Heinz body formation in erythrocyte lysates (with and without stroma) was quantitatively evaluated. The amount of radioactive precipitate was the same in the presence or absence of stroma and thus the importance of stromal participation appeared negligibly small. The association of Heinz bodies with stroma in intact cells is probably due, as suggested by Rifkind and Danon[12], to migration of the Heinz bodies to the cell periphery during the coalescence stage.

Table 3. HEINZ BODY FORMATION IN THE PRESENCE AND ABSENCE OF STROMA

Phenylhydrazine (μmoles/ml.)	Radioactivity in precipitate (as percentage of total radioactivity) With stroma	Without stroma
0	3·8	0·1
8	19·6 (15·8 corr.)	16·3 (16·2 corr.)
16	32·4 (28·6 corr.)	26·7 (26.6 corr.)

A 10 per cent erythrocyte lysate in distilled water was used. Stroma were removed by centrifugation at 16,000*g*. Samples were analysed after 3 h.

Hydrogen peroxide is a toxic intermediate in the drug-induced anaemias associated with glucose-6-phosphate dehydrogenase deficiency[15,16]. Heinz bodies formed by the action of hydrogen peroxide have been observed in erythrocytes pretreated with the catalase inhibitor, cyanide[4], as well as in the absence of catalase inhibitors[17,18]. A quantitative evaluation of Heinz body formation in normal human erythrocytes exposed to hydrogen peroxide vapour is presented in Table 4. The increase in radioactivity associated with the stroma of the control after incubation for 24 h is in keeping with the known formation of Heinz bodies after prolonged incubation in the absence of stressor drugs[7]. After correction for the control, the conversion of haemoglobin to Heinz bodies by roughly 100 μmoles hydrogen peroxide acting on 0·4 ml. of erythrocytes was 8·0 per cent and 8·2 per cent, respectively.

Table 4. HEINZ BODY FORMATION AFTER EXPOSURE TO HYDROGEN PEROXIDE

Sample	Radioactivity in precipitate (as percentage of total radioactivity) Subject 1	Subject 2
Zero time	3·8	3·1
Control at 24 h	6·3	5·8
H_2O_2	14·3 (8·0 corr.)	14·0 (8·2 corr.)

4 ml. of a 10 per cent suspension of cells in isotonic buffer were exposed to 4·5 μmoles of hydrogen peroxide per hour with a diffusion method (ref. 16).

The foregoing experiments demonstrate the isolation of radioactive Heinz bodies from chromium-51-labelled erythrocytes, and the usefulness of radioactive measurements for obtaining quantitative estimates of Heinz body formation both *in vivo* and *in vitro*. The method should find ready application for such studies as: (1) comparing the relative efficacies of the various oxidant drugs; (2) comparing the degree of reaction in the various sensitive cell types[6,17–19]; (3) evaluating the kinetics of formation and elimination of Heinz bodies *in vivo*. In addition, the chromium-51 tracer provides a tool with

which to approach problems such as the structure, pathway of metabolism, relationship to other erythrocyte inclusion granules such as Schmauch bodies[20], and involvement of various haemoglobin forms. (In normal erythrocytes, the chromium-51 label is located almost exclusively on haemoglobin A_3; however, labelling of haemoglobins F, H and Bart's has been observed[21].)

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I Antigen in Leukaemic Patients

FOLLOWING the report of McGinnis *et al.* that the red cells of fifteen out of forty patients with leukaemia tested by them had a diminution of I antigen[1], we tested red cells from fifteen patients with acute leukaemia using anti-I sera from the following three persons: (*a*) a group A patient with *M. pneumoniae* infection whose serum was obtained at the time of a transient rise of her cold agglutinin titre to 128 at 4° C; (*b*) a group A patient with the chronic cold agglutinin syndrome who had a cold agglutinin titre of 1,024 at 4° C; and (*c*) a healthy donor of group Ai who had a cold agglutinin titre of 8 at 4° C. These sera were used after neutralization of isoagglutinin anti-B with group B substance.

Fourteen of the patients investigated were children with acute leukaemia; five were investigated at the time of diagnosis or during subsequent relapse, five during partial remission and four during complete remission. Only three of the patients had been transfused during the 4 months before obtaining their red cells for testing. In addition, we were able to test red cells from an adult patient who initially came to us with a chronic myeloproliferative disorder and who 3 years later developed acute leukaemia.

The I status (agglutinability with anti-I) of red cells from the leukaemic patients was compared quantitatively with that of cells from normal subjects of I status, from a healthy donor of i status and from cord blood of $i_{(cord)}$ status. A 2 per cent saline suspension of washed red cells was prepared from each subject. Starting with a suitable initial dilution, serial two-fold dilutions of the anti-I sera were made. Then to one drop of each dilution of anti-I serum an equal volume of red cell suspension was added. Readings for the grading of agglutination were performed microscopically after 2 h incubation at 4° C.

When compared with normal I-positive red cells, none of the cells from the leukaemic patients showed reduction in agglutinability with any of the three anti-I sera. Initially the tests on the children were performed, a few at a time, using fresh red cells which had been taken into ACD. To achieve a more accurate comparison a sample of red cells from each donor and each leukaemic patient was stored in glycerol at −20° C (ref. 2) and the tests were repeated, all at one session, after storage times ranging between 3 and 10 months. Again no loss of I antigen was observed in the leukaemic patients. Some typical results are shown in Table 1. Red cells obtained from the adult patient during the myeloproliferative phase of his disease (stored at −20° C in glycerol for 7 months at the time of test) and those obtained when he entered the leukaemic phase (stored at −20° for 3 months) were tested at the same time using the same anti-I sera; both samples reacted strongly with all three anti-I sera, there being no diminution of agglutination in the second sample.

Our results are in agreement with those of Ducos *et al.* who, using a quantitative technique, also failed to detect loss of I antigen in leukaemic patients[3].

An observation worthy of note in Table 1 is that, under the conditions of these tests, the lack of I antigen in the cord blood cells was detected by the weak anti-I from the donor (*c*) but not by the stronger anti-I from donors (*a*) and (*b*), even though these latter two sera were suitably diluted for the test. It appears, therefore, that there may be differences in the ability of various anti-I sera to detect a partial lack of I antigen, as in $i_{(cord)}$ cells, whereas a severe lack of I antigen, as in adult i cells, is detected by all anti-I sera.

We thank Dr. W. J. Jenkins for the cells from the i donor, Dr. J. Wallace for the anti-I serum from donor (*c*), and Dr. W. Watkins for the group B substance.

Table 1. DEGREE OF AGGLUTINATION WITH ANTI-I OF RED CELLS FROM HEALTHY DONORS AND FROM PATIENTS WITH LEUKAEMIA
Tests performed at 4° C on cells stored in glycerol at −20° C

Source of red cells	Agglutination with anti-I* (reciprocal of serum dilution)								
	Anti-I serum (*a*)			Anti-I serum (*b*)			Anti-I serum (*c*)		
	32	64	128	512	1,024	2,048	3	8	16
Healthy controls									
I donor 1	+ + +	+ +	+	+ + +	+	−	+ +	−	−
I donor 2	+ + +	+	−	+ +	+ +	+	+ +	−	−
i donor	−	−	−	−	−	−	−	−	−
$i_{(cord)}$	+ +	+	−	+ +	+	±	−	−	−
Patients†									
(1) Terminal acute leukaemia	+ + +	+	±	+ +	+	−	+ +	−	−
(2) Acute leukaemia partial remission	+ + +	+ +	+	+ +	+	−	+ +	−	−
(3) Acute leukaemia in remission	+ + +	+ +	+	+ +	+	−	+ +	−	−
(4) Acute leukaemia in relapse	+ + +	+ +	±	+ + +	+	+	+ +	+	−
(5) Chronic myeloproliferative disease	+ + +	+ +	−	+ + +	+ + +	+ +	+ +	±	−
(5) At onset of acute leukaemia	+ + +	+	−	+ + +	+ + +	+ +	+ +	±	−

* Grading of agglutination: ±, two or three cells sticking together; +, agglutinates of four to twelve cells; + +, agglutinates of twelve to twenty cells; + + +, large agglutinates but some free cells.
† Not transfused during previous four months.

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Multiple Haemoglobins in Bats

MULTIPLE haemoglobins and variation in electrophoretic mobility have been reported for many animals. The physiological significance of these discoveries is not completely understood. Some investigators have compared haemoglobin electrophoretic properties and phylogeny. Dunlap, Johnson and Farner[1] analysed 21 species of birds and showed differences in haemoglobin apparently correlated with taxonomic kinship, and Marchlewska-Koj[2] noted that amphibian haemoglobin heterogeneity may serve as complementary data for taxonomy. Comparable investigations of fish haemoglobins have been conducted by Hashimoto and Matsuura[3,4], Smith[5], and others, and of mammalian haemoglobins by Blumberg, Allison and Garry[6], Foreman[7], Ewy and Wojcik[8], Buettner-Janusch[9], Buettner-Janusch and Buettner-Janusch[10] and others.

A search revealed no mention of electrophoretically distinct haemoglobin types in bats. Johnson and Wicks[11], using paper electrophoresis, found haemoglobin of the Mexican free-tailed bat (*Tadarida brasiliensis*) to be nearly isoelectric at *p*H 8·6. Foreman[12] published drawings of bat (identification uncertain) and human haemoglobin tryptic peptide chromatograms to show variations between distantly related taxa. It thus seemed of interest to investigate the existence of multiple haemoglobins in bats. Results showed that two of the five species investigated have electrophoretically heterogeneous haemoglobin.

Bat species and number used in this investigation were: big brown bat (*Eptesicus fuscus* (Beauvois)), 10; gray myotis (*Myotis grisescens* (Howell)), 9; big myotis (*Myotis lucifugus* (Le Conte)), 4; eastern pipistrelle (*Pipistrellus subflavus* (F. Cuvier)), 12; and big-eared bat (*Plecotus townsendii* Cooper), 4. Big-eared bats and big myotis were captured at Dillo cave, 2·1 miles north of Reed Springs, Stone Co., Missouri; some big brown bats were caught in a building in Kansas City, and all other animals were collected at Henson's cave, one-half mile west of Jane, McDonald Co., Missouri. Adult animals of both sexes were used.

Blood samples were drawn by heart puncture into a syringe wetted with cold 2 per cent sodium oxalate. From 0·1 to 0·5 c.c. of blood were received, added to equal volumes of cold isotonic saline (0·85 per cent sodium chloride) and centrifuged at low speed for 5 min. Washing with saline was repeated three times to remove all plasma constituents. Packed cells were lysed with 1·5 volumes of distilled water shaken for 5 min with 0·5 volumes of toluene, as recommended by Drabkin[13], and centrifuged at 3,000 r.p.m. for 15 min. The haemoglobin solution was removed from beneath the toluene layer with a fine capillary pipette. Some haemoglobin solutions were immediately analysed while others were frozen and stored at $-10°$ C until analysis. Freezing and thawing of samples up to three times resulted in no apparent difference in the ionograms.

Electrophoretic patterns were obtained with the Gelman electrophoresis apparatus (Gelman Instrument Co., Ann Arbor, Mich.) using Gelman 'Sepraphore III' cellulose polyacetate strips and barbitol buffer *p*H 8·6, ionic strength 0·05. Approximately 4 λ of haemoglobin solution was applied to the strip with the Gelman sample applicator. Separation was carried out using 300 V for 3 h. After separation strips were dried at room temperature, preparatory to staining, or placed immediately in Ponceau *S* stain (200 mg Ponceau in 100 ml. 5 per cent trichloroacetic acid) for 5 min, then in three consecutive rinses of 5 per cent acetic acid for 3 min each and then dried at room temperature. Immediate staining seemed to yield clearer ionograms.

Quantitation was accomplished by elution with 5·0 ml. of 0·1 N sodium hydroxide and measurement in a 'Spectronic 20' colorimeter (Bausch and Lomb Optical Co., Rochester, N.Y.) at 540 mμ. Hyland human haemoglobin electrophoresis controls AC (Hyland Laboratories, Los Angeles, Calif.) were subjected to electrophoresis simultaneously with bat specimens to serve as reference markers.

Alkali resistant haemoglobin was examined by the method of Singer, Chernoff and Singer[14], using the same colorimeter and wave-length. This portion of the investigation was severely limited by the small amount of material available.

Electrophoretic results showed that haemoglobin consists of two components in the eastern pipistrelle and big-eared bat, whereas haemoglobin of the big brown bat, gray myotis and big myotis are homogeneous. For sake of convenience the two haemoglobins of the eastern pipistrelle and big-eared bat have been designated, in the order of increasing electrophoretic mobility, Hb-PS_1, and Hb-PS_2, Hb-PT_1 and Hb-PT_2, respectively. Single haemoglobins from the big brown bat, gray myotis and big myotis have been designated Hb-EF, Hb-MG and Hb-ML, respectively.

Control human Hb-C migrated at a faster rate than human Hb-A. Hb-PS_1 and Hb-PT_1 were slightly slower than Hb-PS_2 and Hb-PT_2 (consistently 2 mm behind). Mobility of Hb-Ps_2, Hb-MG and Hb-ML was approximately equal to human Hb-A and Hb-PT_1 migrated at a rate equal to human Hb-C. Hb-EF was the fastest recorded, being appreciably in front of human Hb-C. All analyses were carried out at *p*H 8·6.

Quantitation of ionograms showed a relative distribution for the eastern pipistrelle of 34·5 per cent Hb-PS_2 (range 33·4 to 36·1) and 65·5 per cent Hb-PS_1 (range 63·9 to 66·6); for the big-eared bat 46·4 per cent Hb-PT_2 (range 46·3 to 46·5) and 53·6 per cent Hb-PT_1 (range 53·3 to 53·7). Pooled bloods gave comparable results to non-pooled samples. The minor component preceding the major has been shown by Matsuda *et al.*[15] for other mammals (monkey and human) using agar-gel electrophoresis, *p*H 8·6. Blood of every eastern pipistrelle and big-eared bat investigated contained haemoglobin of both varieties.

Alkali denaturation tests demonstrated small proportions of alkali-resistant pigments. These tests were difficult to conduct due to small samples of blood. The mean values for alkali-resistant pigment in the eastern pipistrelle, big-eared bat and gray myotis were 26 per cent, 12 per cent and 14 per cent, respectively. The amount of alkali-resistant pigment in the eastern pipistrelle and the presence of two electrophoretically separable haem components suggest that one of these bands may be the alkali-resistant pigment. All animals tested were adults and it was assumed that variation in resistance to denaturation and patterns after electrophoresis did not represent foetal haemoglobins. The difference in the proportions of alkali-resistant pigments between bats requires further study.

No sexual or age (as indicated by tooth wear) differences in haemoglobin distribution were observed. Blood from active big brown bats collected during July 1964 gave ionograms comparable with specimens in cold torpor collected during December 1964.

Conclusions with regard to specific problems in physiology and taxonomy are premature. A further detailed investigation will soon be conducted.

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IMMUNOLOGY

Antigenicity of Conjugates of Arsanilic Acid in Inbred Guinea-pigs

RECENT investigations have shown that the genetic constitution of the host is of critical importance as regards the immune response to hapten poly-L-lysine conjugates[1,2], random linear copolymers[3] and reactivity in certain hypersensitivity phenomena such as experimental allergic encephalomyelitis[4] and thyroiditis[5]. This information was obtained with guinea-pigs of inbred strains 2 and 13 which had been demonstrated by Bauer in 1958 (ref. 6) to be histocompatible. Investigations on arsanilic acid conjugates of amino-acid polymers[7,8] have shown that these conjugates are antigenic in random-bred guinea-pigs. Accordingly, experiments were designed to assess the immune response of the inbred strains 2 and 13 to arsanilic acid conjugates of poly-L-lysine (ars-L) and poly-L-glutamic L-lysine L-tyrosine (ars-GLT).

Conjugates were prepared by adding the appropriate amounts of diazotized arsanilic acid to ice-cold solutions of carrier maintained at *p*H 8–9 (refs. 7 and 8). Synthetic polypeptides lysine and glutamic-lysine-tyrosine were conjugated with small amounts of hapten in order to provide one azophenyl arsonate group 1 ars per three lysine or tyrosine residues. Bovine serum albumin (BSA) and polytyrosine (T) were conjugated with excess arsanilic acid for use as test antigen. (Poly-L-tyrosine of mean molecular weight 10,000 was purchased from Yeda Research and Development Co., Rehovot, Israel.) A sample of random linear copolymer glutamic-lysine-tyrosine (GLT Lot No. *M*-12; molar ratio of amino-acids 58 : 38 : 4, kindly provided by Dr. Maurer) was also used as test antigen.

Guinea-pigs weighing 400 g were immunized by injection in the four foot-pads of 100 μg of conjugate in 0·4 ml. of Freund's adjuvant emulsion containing 0·2 ml. of oil phase so that each guinea-pig received 2 mg of killed *H37Rv* human mycobacterium. Two weeks later, skin tests were performed by intradermal injections of 0·1 ml. of various antigens. Reactions were considered to be 'immediate' if present at 3 or 6 h after injection, and 'delayed' if they appeared at 24 h and remained still strong at 48 h. Passive cutaneous anaphylaxis tests were performed in random-bred Hartley guinea-pigs, as described by Ovary[9]. Blood was drawn for passive cutaneous anaphylaxis tests 1 day before skin tests. Sera were used in 1 : 10 dilutions and the amount of antigen injected was 500 μg protein in the case of ars-BSA and 250 μg for the GLT copolymer.

Table 1. RESULTS OF IMMUNIZATION WITH CONJUGATES OF ARSANILIC ACID

Immunizing antigen	Strain	Test antigen	Cutaneous reactions: Immediate	Cutaneous reactions: Delayed	PCA†
ars-L	2	ars-L 10 μg	0/3	3/3 (10)*	
		ars-BSA 20 μg	0/3	0/3	0/3
		ars-PT 10 μg	0/3	0/3	
	13	ars-L	0/5	0/5	
		ars-BSA	0/5	0/5	0/5
		ars-EA	0/5	0/5	
		ars-PT	0/5	0/5	
	Hartley	ars-L 10 μg	4/4	4/4 (14)	
		ars-BSA 20 μg	1/4 (7)	0/4	1/4
		ars-EA	2/4 (9)	1/4 (10)	
		ars-PT	1/4 (6)	0/4	
ars-GLT	2	ars-GLT 10 μg	0/3	3/3 (19)	
		ars-EA 20 μg	0/3	0/3	
		GLT 50 μg	0/3	3/3 (11·5)	3/3
		ars-BSA	N.d.	N.d.	2/3
	13	ars-GLT	0/5	0/5	
		ars-BSA	0/5	0/5	5/5
		ars-EA	0/5	0/5	
		GLT	0/5	0/5	1/5
	Hartley	ars-GLT	1/4 (14)	3/4 (20)	
		ars-BSA	1/4 (15·5)	1/4 (10)	3/4
		ars-EA	1/4 (15)	2/4 (16)	
		GLT	1/4 (9)	2/4 (18)	2/4

* Size of reaction (mm) in positive guinea-pigs.
† PCA, passive cutaneous anaphylaxis.
N.d., not done.

The results of immunization with conjugates ars-L and ars-GLT are shown in Table 1. Positive responses were obtained in skin tests only in guinea-pigs of inbred strain 2 (delayed type) or random-bred Hartley (immediate and delayed types) and in none of guinea-pigs of strain 13. Delayed skin reactions in guinea-pigs of strain 2 were directed only against the homologous conjugate or homologous carrier (GLT). Some of the Hartley guinea-pigs reacted also with heterologous (ars-BSA, ars-EA, ars-PT in group ars-L; ars-BSA, ars-EA in group ars-GLT) conjugates in immediate skin reactions and in delayed skin reactions (ars-EA in group ars-L and ars-BSA, ars-EA in group ars-GLT). Sera of guinea-pigs of strains 2 and 13 immunized with ars-L conjugate were negative in passive cutaneous anaphylaxis reactions against ars-BSA conjugate. One out of four sera from group ars-L, Hartley guinea-pigs, was passive cutaneous anaphylaxis positive. Sera from guinea-pigs of all three strains immunized with ars-GLT were positive against ars-BSA conjugate. Passive cutaneous anaphylaxis tests gave positive results in this system with GLT copolymer in strain 2 (3/3), Hartley (2/4) and strain 13 (1/5).

The results of this investigation provide further evidence for the view that the ability to mount an immunological response is genetically controlled. In the present work, poly-L-lysine azobenzenearsonate was immunogenic for the random-bred Hartley guinea-pigs and for the strain 2 but not for the other inbred strain 13 guinea-pigs. These findings are consistent with the view expressed by Levine, Ojeda and Benacerraf[1,10] that the ability of guinea-pig strains to develop an immune response to hapten-poly-L-lysine conjugates is of an all-or-none character, that is to say, either a response is evolved by all the hapten-poly-L-lysine conjugates or by none of them.

A different pattern of response emerged from our investigations with ars-GLT conjugate, where all strains were found to respond immunologically, although in different ways. In the case of strain 2 guinea-pigs, the immune response could be detected by delayed skin reactions and was directed against the carrier copolymer as well as against the whole conjugate. Hapten-specific and carrier-specific antibodies were also produced. However, in the case of strain 13, no skin reactions were detected, but hapten-specific antibodies were revealed in all cases (5/5) in passive cutaneous anaphylaxis tests with ars-BSA conjugate. The positive passive cutaneous

anaphylaxis reaction observed with GLT copolymer and one anti-ars-GLT serum of strain 13 may indicate that either injection of an ars-GLT conjugate in guinea-pigs of strain 13 can induce formation of anti-GLT antibodies or that GLT copolymer can react with antibodies directed against the whole conjugate ars-GLT. It is of interest to mention that GLT copolymer itself proved to be immunogenic for strain 2 but not for strain 13 (ref. 3). It seems, therefore, that while GLT copolymer cannot induce an immune response in strain 13 guinea-pigs, it is still able to function as a carrier for arsanilic acid hapten. To the best of our knowledge, this is the first example of a completely synthetic, lysine containing antigen, acting in strain 13 as an effective carrier, despite the fact that it was not immunogenic for the same strain. This situation is in contrast to that reported by Benacerraf *et al.*[8], with arsanilic acid conjugates of non-immunogenic copolymers of D-amino-acid preparations, which were also unable to act as carriers. In strain 2 guinea-pigs, the specificity of delayed type skin reactions was strictly confined to the intact homologous conjugate (ars-PLL, ars-GLT, and to the carrier portion of the conjugate GLT). In Hartley guinea-pigs some dermal delayed type cross-reactions with other arsanilic acid conjugates were also observed (in addition to hapten-carrier and carrier delayed-type specificity). These findings may be indicative of the presence of hapten-specific delayed sensitivity as has been postulated previously by Leskowitz[7]. The results obtained with ars-GLT in the inbred strains 2 and 13 suggest that the genetic differences between the strains may be expressed not only on the level of the ability to respond immunologically, but also in the type of immune response elicited by the same antigen. This situation is similar to that reported by Arquilla and Finn[11], who showed that inbred strain 2 guinea-pigs produced antibodies to portions of beef insulin to which strain 13 guinea-pigs could not produce antibodies.

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Differentiation of Diffusing Proteins according to their Geometrical Patterns

THIS communication draws attention to a phenomenon which appears in the simultaneous diffusion of two different reactants from two sources, if the diffusing substance reacts with the medium to form a precipitate. We have previously shown[1,2] that when two diffusion processes of one reactant take place simultaneously they may interfere and produce crossing or coupling phenomena, which manifest themselves in an altered rate of diffusion. It was concluded that the mobility of each diffusing reactant depends on the other concentration gradient as well as on its own. These effects can be described mathematically by adding to Fick's Law a term proportional to the other concentration gradient. Where two non-identical reactants are involved it is possible that the rate of diffusion in the area between the diffusing sources will increase with one and decrease with the other diffusing reactant. These different rates of diffusion result in different geometry of the precipitation patterns, and from these geometrical patterns it is possible to conclude whether the diffusing reactants are identical or not.

De-ionized water saturated with air was used throughout. 0·3 per cent purified Difco agar containing 0·05 per cent metaphosphoric acid as an indicator was used. We used the metaphosphoric acid as an indicator because Liesegang[3] showed that it precipitates proteins. 10 ml. of melted agar with indicator were poured into a Petri dish (9·2 cm in diameter). After solidification two wells were punched with a tube of 0·6 cm outer diameter. The distance between the centres of the sources was 20 mm. The experiments were carried out in a humid chamber at about 25° C. Readings were made 7 days after setting up the dishes. The identical diffusing proteins used were normal human sera or crystalline human albumin (Pentex Inc., Illinois). The non-identical proteins were normal and abnormal human sera, or human gamma globulin, '*FR*. II' (Pentex Inc., Illinois), and crystalline human albumin (Pentex Inc., Illinois).

Fig. 1 shows a diffusion experiment involving two sources of identical reactants. The precipitation patterns

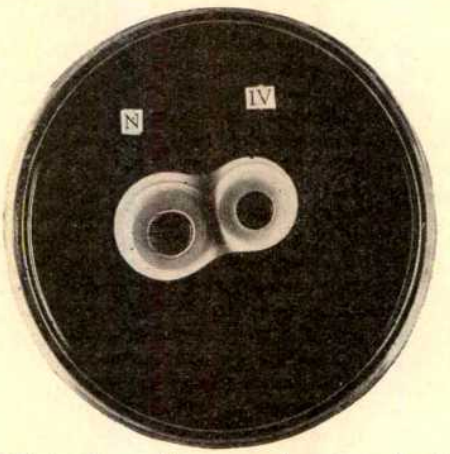


Fig. 1. Diffusion of normal human sera from two wells. Here the displacement of the precipitation patterns does not appear

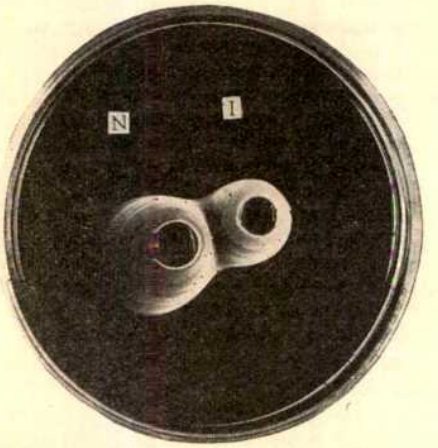


Fig. 2. The precipitation pattern of the normal human serum is displaced toward the opposite source, and the rate of diffusion of the pathological human serum is lowered toward the opposite source

are displaced toward the opposite source and between the two precipitation patterns a translucent zone without precipitate was produced. Fig. 2 shows that in the case of two non-identical diffusing reactants the precipitation pattern of one reactant is displaced toward the opposite source. This indicates that the rate of diffusion of this reactant is maximal along a line joining the centres of the wells and gradually decreases along other radii. The pattern of the reactant is not displaced, and the rate of diffusion of this reactant is minimal along a line joining the centres. From Fig. 2 it is evident that the boundary of the displaced pattern is concave toward the plane of separation. The boundary of the reactant which is not displaced is convex toward this plane.

Further research is in progress at the clinical department of this Institute on the differentiation of various pathological human sera by the method described here.

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Elevation of Cord over Maternal IgG Immunoglobulin: Evidence for an Active Placental IgG Transport

EXISTING evidence suggests that the concentrations of IgG immunoglobulin* in maternal and cord sera are essentially the same. In 1963 von Muralt[1] concluded that no significant difference existed in the absolute concentration of gamma globulin in paired maternal-cord sera after reviewing forty-three published studies in which gamma globulin was quantitated in 1,542 maternal-cord pairs by a variety of methods. Recently, Gitlin *et al.*[2] reported cord IgG to be equal to or slightly lower than maternal. They did observe, however, that IgG labelled with iodine-131 was present in higher concentrations in cord than in maternal serum if the iodinated protein had been administered to the mother 25 or more days before parturition. The higher cord ^{131}I-IgG levels were felt to be a consequence of tracer kinetics and not an indication of higher cord endogenous IgG concentrations[2].

The present investigation was undertaken to re-evaluate the quantitative relationship of IgG in maternal and cord sera using antisera specific for IgG heavy chains with the antibody-agar plate technique ('Immunoplates', Hyland Laboratories)[3,4]. IgG levels were measured in forty-six pairs of normal maternal-cord sera. Paired sera were tested in the same plate and the diameter of the precipitin rings, formed by interaction of IgG and goat antiserum specific for IgG, measured after 24 h incubation at 4° C. Reference samples containing known amounts of human fraction II gamma globulin, further purified by DEAE chromatography, were run simultaneously. The coefficient of variation for IgG quantitation by this method is 8·1 per cent in our laboratory.

In Fig. 1, the concentrations of IgG present in cord sera are compared with maternal levels. The mean cord concentration of IgG was 1,512·5 mg per cent (*S.D.* 323, range 640–2250) and mean maternal 1,260·1 mg per cent (*S.D.* 286, range 600–1,875). This difference is highly significant with $P < 0{\cdot}01$ (Student's *t*-test). A considerable variation in the ratio of cord/maternal IgG was observed (Fig. 1) which ranged from 2·04 to 0·45; mean 1·2. Ratios of paired sera which varied significantly from the 1·2 mean were consistently reproducible and the significance of these variations is at present under study.

* Immunoglobin nomenclature as suggested in *Bull. World Health Org.*, **30**, 447 (1964).

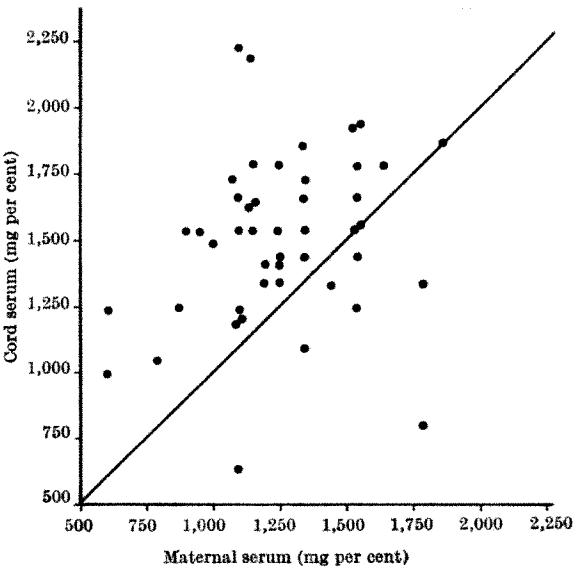


Fig. 1. Comparison of IgG concentrations in forty-six paired maternal-cord sera

Several experiments were carried out to verify the significant elevation of mean cord IgG as determined by the antibody-agar plate technique. First, agar plates were prepared which contained rabbit antiserum to purified (1) maternal IgG and (2) cord IgG from the same paired sera and to (3) heavy chains from pooled adult IgG. These preparations gave maternal-cord levels similar to those obtained with agar plates containing goat anti-IgG. Secondly, a spurious elevation of cord over maternal IgG might have occurred if cord IgG was a distinctly more antigenically homogeneous immunoglobulin than maternal IgG. For example, quantitation of isolated myeloma IgG by the antibody agar plate technique may give concentrations 60–90 per cent higher than by the Folin method[5]. This factitious elevation is the result of a lower agar concentration of antiserum capable of precipitating the monoclonal myeloma protein. IgG was therefore isolated from six maternal-cord pairs by DEAE chromatography and quantitated by the antibody-agar plate and Folin methods. The two methods were in close agreement and indicated that the foregoing consideration was not responsible for the observed elevation of cord IgG. Finally, absorption of rabbit antiserum to maternal IgG with cord IgG and vice versa completely removed the precipitating antibody.

The human placenta regulates the transfer of plasma proteins from mother to foetus in a highly selective manner and transfer of specific protein appears to be correlated with molecular configuration rather than molecular size[1,2]. The transplacental transfer of immunoglobulins is limited to IgG (7*S*) while the IgM (19*S*), IgA (7*S*–11*S*)[1] and IgD (7*S*)[6] immunoglobulins are essentially excluded from the foetal circulation. The present observations indicate that IgG is transferred from mother to foetus against a substantial concentration gradient: mean 252 mg per cent. It is highly unlikely that the mean difference can be attributed to foetal IgG synthesis. While IgG synthesis has been demonstrated in a 20-week-old foetus by sensitive *in vitro* techniques[6], it is not sufficient, even at term, to account for the differences observed in this investigation. Evidence for this is provided by the presence of extremely low IgG levels in two normal infants born to a mother with acquired agammaglobulinaemia[7]. The most reasonable explanation for the present observations is an active placental transport of IgG from mother to foetus.

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Effect of Antigen on the Structural Configuration of Homologous Antibody following Antigen–Antibody Combination

THE exposure of new antigenic determinant groups in rabbit antibody following combination with homologous BSA antigen has been reported[1]. The structural alterations occurring in the rabbit antibody molecule as a result of such combination are similar to those which Ishizaka has shown to be responsible for irritative skin reactions of soluble antigen–antibody complexes in guinea-pigs[2]. Nevertheless, in the case of the low molecular weight antigens already investigated (for example, BSA and ovalbumin), only certain ratios of antigen to antibody —three molecules of antigen to two of antibody, for example—are effective in the induction of such alterations in the antibody molecule.

The present investigation was undertaken to establish whether the size of the antigen molecule influences the degree of structural alteration induced in the homologous antibody molecule. To this end, the previous investigations have been repeated and extended, using soluble complexes consisting of a considerably larger antigen molecule (ferritin) and its homologous rabbit antibody.

Sandy-lop rabbits of allotype *As* 1,4 were injected subcutaneously with a crystalline (×5) horse ferritin preparation (3 × 5 mg ferritin in Freund's complete adjuvant at weekly intervals). The γG globulin fraction from one of the immune sera was isolated by DEAE cellulose chromatography[3]. Soluble complexes were formed by addition of a predetermined amount of homologous antigen, equal to three times the equivalence ratio. After centrifugation, the resultant complexes were separated from uncombined ferritin by zone electrophoresis, on a 'Pevikon' block at *p*H 8·6. The soluble complexes (obtained by pooling appropriate fractions) were injected into rabbits of the same allotype as the antibody donor. The immunization course consisted primarily of six subcutaneous injections of 2 mg material on alternate days. Precipitating antibodies to ferritin were demonstrable in serum obtained after the fourth injection. After an interval of one week, six 1 mg aliquots were administered intravenously on alternate days.

Gel-diffusion precipitin analyses of the donor serum (1) and the anticomplex serum after the subcutaneous (2) and intravenous (3) courses are shown in Fig. 1. As will be seen, both ferritin–antiferritin complexes and ferritin alone gave identical precipitin lines on testing with the antiferritin serum (1). However, similar tests (2) and (3) with the anti-(ferritin–antiferritin) serum revealed an additional precipitin line. It is possible that these observations reflect the exposure of new immunogenic groups within the ferritin molecule, following combination with its specific antibody. The distribution of both precipitating antigens throughout the ferritin peak eluted from 'Sephadex *G*-200' provides evidence in support of this suggestion.

As was found in a previous investigation[1] with complexes of BSA and anti-BSA, it proved to be difficult to demonstrate a specific anti-antibody reaction. Nevertheless, the reaction of antiserum (3) with rabbit and human γG globulins provides indirect evidence of the exposure of new immunogenic determinants within the specific antibody molecule following combination with ferritin. The precipitin reaction between heterologous complexes and the rabbit anti-(ferritin–antiferritin) complex serum (as shown in Fig. 2) supports this contention.

Gel-diffusion precipitin analysis and haemagglutination procedures both revealed that the reaction against γG globulins occurred more readily with human γG globulin than with rabbit γG globulin. This behaviour was not observed in similar tests with the rabbit anti-(BSA–anti-BSA) complex serum described previously[1]. It appears to be analogous to the effect produced in rabbits by Milgrom and Witebsky[4], and in guinea-pigs by McCluskey *et al.*[5] following administration of denatured autologous γG globulin. There, too, the antibody obtained was directed primarily against heterologous γG globulin, and, although reacting with isolated rabbit γG globulin (5 g per cent), it failed to react with the rabbit γG globulin contained in the donor rabbit serum (Fig. 2).

It may be relevant that optical rotation measurements have shown that a combination of antigen and antibody involving the relatively large ferritin antigen leads to structural alteration of the antibody molecule of appreciably greater magnitude than that observed with BSA as antigen. Moreover, the structural changes evoked in homologous antibody by combination with ferritin are of a similar order of magnitude to those observed on heat denaturation of non-antibody γG globulin (Table 1).

The demonstration that soluble complexes comprising a number of heterologous antigens and rabbit antibody also gave a precipitin line with the antiserum raised against ferritin–antiferritin complexes (Fig. 2) confirms earlier findings that similar structural alterations are induced in the rabbit antibody molecule following antigen–antibody combination, whatever the antigen.

Gel-diffusion tests, employing sub-units of γG globulin as reference substances, indicate that the new antigenic

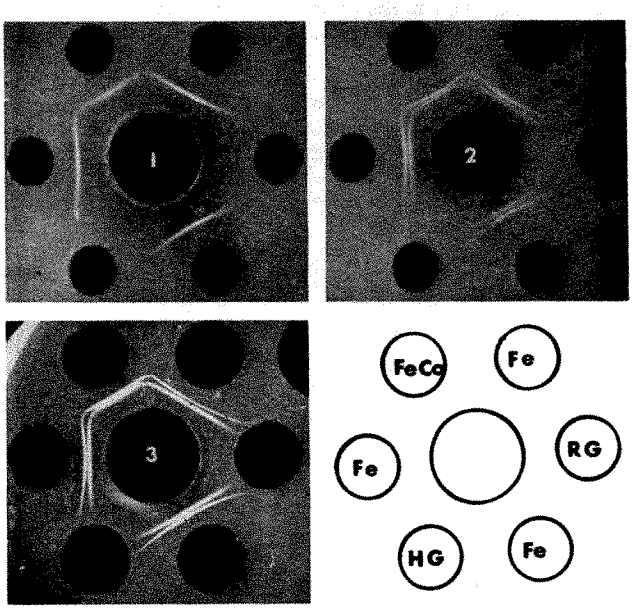


Fig. 1. Gel-diffusion precipitin analyses of donor rabbit antiferritin serum (1), and of rabbit anti-(ferritin–antiferritin) complex serum after subcutaneous (2) and intravenous (3) courses. Fe Co, homologous horse ferritin—rabbit antiferritin complexes; Fe, horse ferritin; R.G., rabbit γG globulin; H.G., human γG globulin

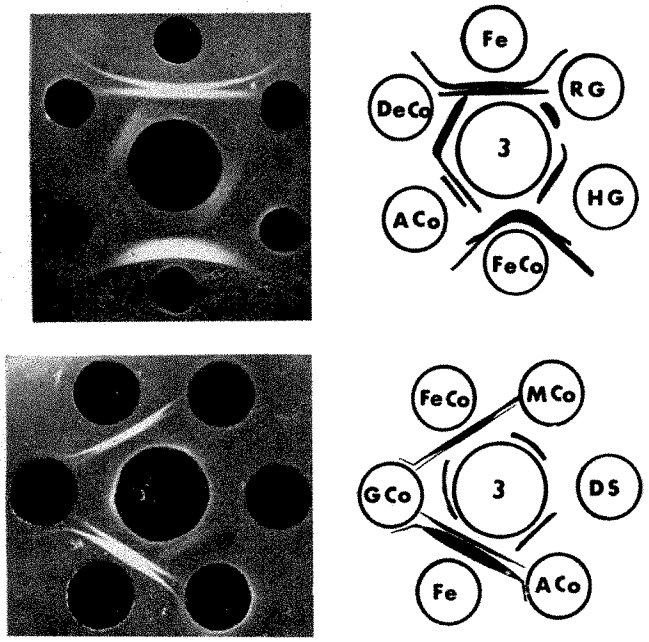


Fig. 2. Gel-diffusion precipitin analyses involving heterologous complexes. A Co, BSA–rabbit anti-BSA complexes; De Co, dextran–human antidextran complexes; M Co, human α_2 macroglobulin–rabbit anti-α_2 macroglobulin complexes; G Co, human γG globulin–rabbit anti-γG globulin complexes; D.S., donor, antiferritin, serum. Other symbols as in Fig. 1

determinants revealed on the antibody molecule were situated along the whole of the heavy chain. Preparations of Fab and Fc papain digestion fragments both react with the antiferritin complex serum. On the other hand, homogeneous light chain preparations, prepared by the method of Fleischman *et al.*[6], invariably failed to give a precipitin line. In the previous investigation[1] with soluble complexes of BSA and its homologous antibody, new structural determinants were apparent over only a fairly limited range of ratios of antigen to antibody (between 2 and 5 to 1). By contrast, soluble ferritin complexes comprising a far wider range of antigen/antibody ratios (up to 20 : 1) all reacted with the antiserum raised against ferritin complexes formed at five times equivalence.

The present findings provide further evidence that combination of antigen and antibody (within certain ratios) is accompanied by the exposure of new structural determinants in the antibody molecule. Although these groupings are demonstrable in all soluble complexes formed with moderate excess of antigen, their character appears to depend on the nature of the antigen. The implication that larger antigens cause greater structural alterations of the antibody molecule is significant in attempts to explain the formation of anti-antibodies when large antigens such as viruses may be involved.

Table 1. SPECIFIC OPTICAL ROTATION CHANGES INDUCED IN RABBIT ANTIBODY γG GLOBULIN BY COMBINATION WITH SPECIFIC ANTIGEN

Antigen	Antigen/antibody weight ratio	Specific optical rotation at 589·5 mμ: Immune complexes	Antigen–non-antibody γG globulin mixture	Difference
BSA	1·9	−64·6	−58·8	+5·8
	2·4	−61·0	−59·3	+1·7
	3·2	−60·4	−59·8	+0·6
	4·5	−60·6	−60·3	+0·3
	9·1	−61·0	−61·1	−0·1
Ferritin	2·7	−40·2	−31·9	+8·3
	4·1	−35·2	−30·9	+4·3
	5·4	−33·8	−30·3	+3·5
	6·8	−30·2	−29·9	+0·3
	13·6	−28·3	−29·0	−0·7
	19·0	−28·6	−28·7	−0·1

Heat denaturation of human γG globulin (50° C for 10 min) produced a rise in specific optical rotation at 589·5 mμ of 9·8°.

These observations are part of a continuing investigation into the influence of antigenic size and shape on the tertiary structure of homologous antibody, following antigen–antibody combination.

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PATHOLOGY

Normal Flora and Acute Inflammation

THE indigenous microbial flora has recently been recognized as being involved in the maintenance of the resistance of the host to infection. Oral administration of non-absorbable antibiotics produces a marked reduction in the enteric flora of the animal host, which is associated with an increased susceptibility of the host to parenteral infections[1]. Animals raised in the presence of a restricted gastrointestinal microflora likewise respond abnormally to experimental infections[2]. Animals reared in the germ-free state are also hypersusceptible to a variety of infectious agents administered by a number of different routes[3–6]. These findings leave no doubt that the normal flora somehow enhances the ability of the host to deal with infection, not only within the habitat of the flora, but systemically as well. The mechanism of this enhancement is obscure.

Because of the importance, in terms of host resistance, of events occurring during the early hours of acute inflammation, the effect of the normal flora on the mobilization of leucocytes and on the increase in vascular permeability to protein was assessed. This was accomplished by comparing the peritoneal inflammatory reaction elicited by a sterile starch tryptose irritant in mice harbouring a normal microbial flora with that elicited in mice having no demonstrable flora (germ-free mice). The number and types of leucocytes, and the total amount and electrophoretic pattern of protein appearing in the peritoneal exudates at various intervals after injection of the irritant, were determined. Parallel determinations were made on samples of peripheral blood from the same animals. The data relative to extravasation of protein are summarized in Fig. 1, and are compared with corresponding data concerning the emigration of leucocytes. Details of intravascular and extravascular mobilization of leucocytes have been presented elsewhere[7].

The average levels of total serum protein were virtually identical in germ-free and conventional mice, the values being 5·8 and 5·7 g per cent, respectively. As seen in Fig. 1, the mean amounts of protein extravasated into the peritoneal cavity in the two groups of animals were approximately the same at each of the intervals tested, none of the differences being statistically significant. In both groups, there was a marked outpouring of protein within the first hour of inflammation, with the level reached at 1 h being approximately maintained for the next few hours. Finally, in both germ-free and conventional mice, the values decreased sharply after 4 h, as vascular permeability began to return to normal. Thus, it was concluded that exudation of protein is not impaired in the absence of a normal microbial flora.

This, as shown in Fig. 1, stands in sharp contrast to the fact that exudation of leucocytes was found to be markedly deficient in germ-free animals. At the peak of the inflammatory process, almost twice as many leucocytes, on the average ($P < 0.01$), had been mobilized into the area of injury in conventional as compared to germ-free mice, this difference being independent of any difference in the availability of leucocytes from the circulating blood in the two groups of animals[7]. Another discrepancy between the outpouring of protein and that of leucocytes is that vascular permeability to protein began to wane after 4 h, at a time when emigration of leucocytes had yet to reach a peak. These findings are entirely consistent with other evidence indicating that the emigration of leucocytes and the increase in vascular permeability during acute inflammation are distinct phenomena, and are separately mediated[8]. It would appear that factors accounting for emigration of leucocytes are sharply influenced by the presence or absence of the flora, while the mediators of increase in vascular permeability to protein are not.

Cellulose acetate electrophoresis of the exudates revealed grossly similar patterns in germ-free and conventional mice. However, analysis by elution and spectrophotometry revealed the gamma globulin content of exudates to be significantly greater in conventional than in germ-free mice. In the former group, this fraction constituted 3·6 per cent of the total protein, as compared to 2·9 per cent in the latter group ($P < 0.05$). Not unexpectedly, this difference in the composition of the exudates was found to be the reflexion of an almost identical discrepancy in the level of serum gamma globulin in the two groups, the values being 3·4 per cent and 2·7 per cent, respectively.

At present, the biological significance of these results, in terms of reaction to living infectious organisms rather than to the sterile agents used in these experiments, is unknown. Unquestionably, however, cellular events and conditions prevailing in the immediate micro-environment of an invading organism, particularly in the first several hours of infection, are of vital importance in determining the ultimate course of the infection. It is reasonable, therefore, to predict that the enhancement of mobilization of leucocytes effected by the normal flora will prove to be a significant contribution to host resistance. Furthermore, although the total amount of protein exuded is unaffected by the flora, the amount of gamma globulin in the exudate is significantly influenced. Insofar as this larger fraction may contain a greater quantity of so-called natural antibody in conventional animals than in their germ-free counterparts (as it does in serum[9]) it may well act in synergy with the mobilized leucocytes, serving as yet another contribution of the normal flora to the defence of the host.

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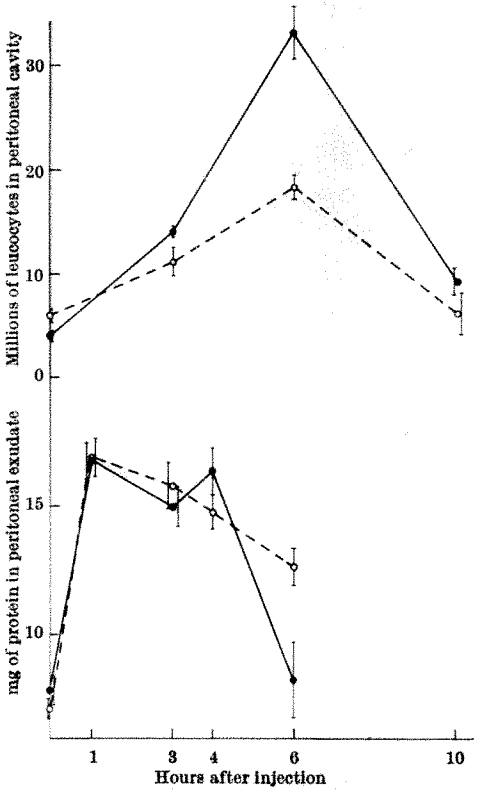


Fig. 1. Exudation of protein and of leucocytes induced by intraperitoneal injection of sterile starch-tryptose suspension in germ-free (- - - -) and conventional (——) mice. Each point represents the mean value for 4–6 animals, and each vertical bar the corresponding standard error

MICROBIOLOGY

New High-efficiency Light Source for Immunofluorescence Microscopy

THE light source usually employed in fluorescence microscopy is a high-pressure mercury vapour lamp; when Coon's antibody technique is used, this source has some disadvantages. The maximum absorption band of the fluorescein isothiocyanate which lies approximately between 440 and 510 nm (the continuous curve in Fig. 1) does not overlap with the mercury emission lines at 365, 405 and 435 nm used for exciting the fluorescence. Another inconvenience arises from the fact that there are two strong mercury lines (546 and 577 nm) in the fluorescence spectrum (the dotted curve in Fig. 1). This latter inconvenience is partially removed by the use of adecvated fluorescence and suppression filters.

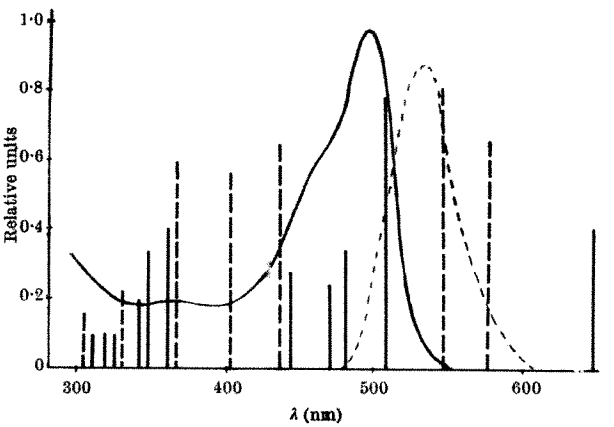


Fig. 1. The absorption curve of fluorescein isothiocyanate is continuous, the fluorescence curve is dotted; mercury and cadmium lines are dotted and full respectively

Among the elements, we have found that cadmium is the only element which has strong emission lines in the absorption band of the fluorescein isothiocyanate and, moreover, has no lines in the fluorescence band. The cadmium lamp can be used for transmitted and incident illumination. If a dark field condenser is used, only a suppression filter which would stop the waves under 510 nm is necessary. The red line of cadmium (644 nm) represents merely an advantage, because it ensures some illumination of the entire ground. This is useful for recognizing the sample. This red light contrasts strongly with the yellowish-green fluorescence, and if a bright field is used and the red line becomes annoying it can easily be suppressed using an adecvate filter.

The suppression of the ultra-violet radiations and the use of only the 468, 480 and 509 nm cadmium lines of maximum efficiency protect both the sample and the observer's eye. Hence it seems that the cadmium lamp is superior to the mercury lamp usually used.

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GENETICS

Deoxyribonucleic Acid Replication of Somatic Cells in the Chinese Hamster

OVER the past few years increasing attention has been focused on the relationship of gene inactivation to the phenomena of interphase DNA condensation and late DNA replication. It has been shown that the sex chromatin body of female mammalian cells appears during early embryogenesis[1] and is composed of one *X* chromosome[2]. In 1961 Lyon postulated that phenotypic variegation in heterozygotic female mice was a consequence of the inactivation of either the maternal or paternal *X* chromosome[3]. As well as being mostly metabolically inert, this *X* chromosome replicates late in the DNA synthetic period[4,5]. It has been assumed that the *Y* chromosome also becomes largely inactivated during early embryonic life, since it shows positive heteropyknosis in early prophase, is late replicating, and extra *Y* chromosomes produce no conspicuous phenotypic effects[6]. That late-replicating autosomal DNA may be related to gene inactivation is supported by the observation that the abnormal chromosome material found in most human autosomal chromosome abnormalities replicates toward the end of the DNA synthetic period. It was postulated that this late-replicating DNA is less deleterious than early replicating DNA when present in excess or deleted because it is relatively inert[6]. Furthermore, it has been shown that interphase heterochromatin is less active than euchromatin in that it produces less RNA[7,8], and that late-replicating chromosomes have less capacity for RNA synthesis[9].

Because of the obvious implications of DNA replication time as a monitor of cell differentiation, the investigation reported here attempted to compare the DNA replication patterns of somatic cells *in vivo* from the three germ layers of the adult Chinese hamster.

Chinese hamsters of both sexes were injected intraperitoneally with tritiated thymidine of specific activity 6·7 c./m mole (New England Nuclear Corporation) in a dosage of 10 μc./g weight. Two hours later 2 μg of 'Colcemid' (Ciba) were injected intraperitoneally, and after 4 h exposure to the tritiated thymidine the animals were killed and various tissues prepared. Bone marrow was prepared by a method similar to that described by Ford and Hamerton[10]. Corneal epithelial cells were prepared by the method of Fredga[11]. A cell suspension was prepared from a segment of jejunal mucosa by gentle trypsinization using 0·5 per cent trypsin at *p*H 8 for 30 min at 37° C. The cells were then washed and handled by a method similar to that used for bone marrow. Lymphoid cells from a hyperplastic popliteal node were prepared 48 h following the injection of about 0·1 c.c. complete Freund's adjuvant into the foot of the animal. The node was teased apart with dissecting needles in 1 per cent sodium citrate solution, and the cells were treated by the technique used for other tissues.

Orcein squash preparations were made and suitable mitoses selected and photographed. The coverslips were removed by the dry ice freezing technique and autoradiographs of the slides prepared[12]. Kodak '*NTB*$_2$' liquid emulsion diluted 1 : 1 with water was used to coat the slides. Slides were exposed for 30 days in the dark at 4° C, developed in Kodak '*D*19*b*' for 5 min at 4° C, and counterstained with Wright's stain. Previously photographed mitoses were relocated and post-autoradiography photomicrographs taken. Prints of mitoses before and after autoradiography were then made and analysis of radioactive label performed.

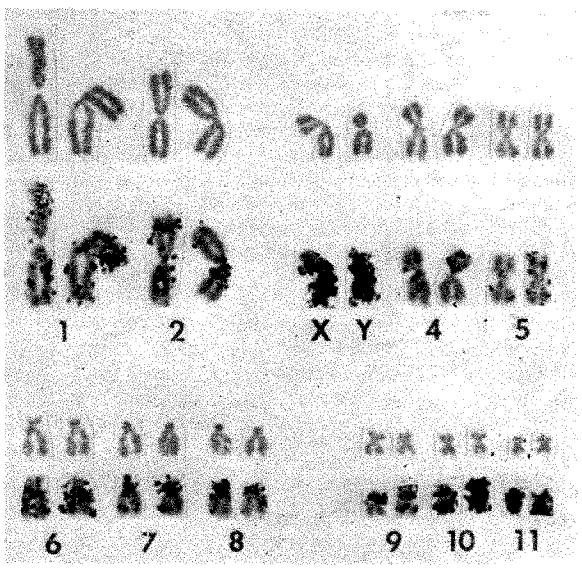


Fig. 1. Composite karyogram from a corneal epithelial cell of a male Chinese hamster exposed for 4 h to tritiated thymidine. In each chromosome group the upper and lower rows represent chromosomes before and after autoradiography. See text for description

Thirty-seven mitoses of corneal epithelium from two male animals, twenty-four mitoses of bone marrow from one female and two male animals, eighteen mitoses of lymphoid cells from one male animal, and five mitoses from jejunal crypt cells of one male animal were suitable for analysis. A characteristic, common labelling pattern was found for all tissues studied (Fig. 1). Late replicating chromosomes or chromosome segments were found to be the *Y* chromosome, the long arm of the *X* chromosome and chromosome pairs ten and eleven. In the female one entire *X* and the long arm of the other *X* replicated late. Relatively late replicating were the long arm of chromosome six, the short arm and centromeric region of chromosomes seven, eight, and nine, and the centromeric region of pairs four and five. In corneal cells, late replication was often observed in the distal portion of the short arm and near the distal end of the long arm of chromosome one, as well as the proximal and mid-portion of the long arm of

chromosome two. In other tissues, however, this pattern was less apparent.

Results from varying *in vivo* exposure times to tritiated thymidine indicate that the average G_2 period in corneal epithelium is about 3·25 h and that the average G_2 period of bone marrow and antigen-stimulated lymphoid cells is at least 1 h shorter. In this study the difficulty in analysing the labelling pattern of chromosomes one and two in cells other than corneal epithelium is probably a consequence of the difference in G_2 period observed.

The identification of Chinese hamster chromosomes has already been described[13]. This animal has twenty-two chromosomes of which all but pairs seven and eight and pairs ten and eleven can be easily distinguished by morphology. Since the late DNA replication pattern of these morphologically similar pairs is about the same, it is not essential to be able to distinguish them. In some mitoses, however, all pairs are morphologically identifiable. Frequently it is not possible to identify the arms of the nearly metacentric chromosome pair two. In Fig. 1 the arms of this pair were identified by autoradiography.

In this work no attempt was made to determine the bone marrow or lymph node cell type in mitosis. Because these tissues contain a heterogeneous cell population which is actively dividing, mitoses studied probably represent more than one cell type. However, the relative numbers of cell types and possible variations in the G_2 period might have favoured the selection of some of them.

The DNA replication pattern described here is similar to that described by Hsu in a strain of cultured fibroblasts of the Chinese hamster[14]. The results of this preliminary investigation suggest that this basic pattern of late DNA replication in the Chinese hamster is common to cells from the three germ layers *in vivo* as well. We are now trying to discern the subtleties of the DNA replication pattern of some somatic tissues in the hope that we can find some clues to the problem of cell differentiation.

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CYTOLOGY

Aggregation of Dissociated Embryonic Chick Cells at 3° C

WHILE investigations of the effects of environmental factors on aggregation *in vitro* of dissociated embryonic cells were under way in this laboratory[1], Curtis reported[2] that "extensive visible coherent re-aggregation" occurred at 3° C in suspensions of embryonic chick limb bud cells in a medium without serum. He concluded that the cells were able to aggregate normally at low temperatures, and that horse serum inhibited cell aggregation at sub-optimal temperatures. These conclusions differed from the previously demonstrated[3,4] and corroborated[5] temperature-dependence of aggregation of trypsin-dissociated embryonic cells. Because versene (EDTA) was the dissociating agent used in Curtis's experiments, the possibility arose that, in contrast to trypsin-dissociated cells[3–5], cells dissociated with versene might be able to aggregate normally at low temperatures; since such differences in aggregation behaviour between differently dissociated cells would be of obvious interest and usefulness in studies on cell adhesiveness, attempts were made to repeat and extend Curtis's results, using his experimental procedures.

Cells from 7-day-old embryonic chick neural retinae and from 5-day-old embryonic chick limb buds were dissociated by the standard trypsin dissociation procedure of this laboratory[4] (using Worthington's crystalline-lyophilized-sterile trypsin), or by treatment for 10 min with 0·002 M ethylenediamine tetraacetic acid (EDTA) at *p*H 8·0 in calcium- and magnesium-free Hanks's solution[2]. The treated tissues were dispersed in calcium- and magnesium-free Hanks's solution by flushing with a pipette of 0·5 mm inside diameter. The culture media used were those of Curtis[2]: (1) equal parts of Hanks's saline and medium 199 (Microbiological Associates); (2) equal parts of Hanks's saline, medium 199 and horse serum (Colorado Serum Co.); the media were sterilized by passing them through 'Millipore' filters. The dissociated cell suspensions were diluted into these media with 15 per cent calcium- and magnesium-free Hanks's solution[2] at concentrations of about 0·5–1·0 × 10^6 cells per ml.; 3-ml. portions of the cell suspensions were dispensed into 25-ml. Erlenmeyer flasks and rotated at 38° C or at 3° C on a gyratory shaker according to the standardized procedure for testing cell aggregability[4]. Aggregation was measured: (1) by counting with a Coulter counter[6,7] the number of cells remaining free in suspension after 30–90 min, and again after 24 h; (2) by microscopic examination of the 24-h cultures. Unlike treatment with trypsin, versene treatment did not result in complete dissociation, and produced mixed suspensions of cells and tissue fragments.

A summary of the experimental conditions and cell-counting results for four experiments is given in Table 1.

Table 1

Experiment No.	Tissue	Dissociation with	Culture medium*	Temp. (° C)	Time	C_0	C/C_0†
1	7-day retina	Versene‡	Hanks's-§ *NCTC*199¶ 1 : 1	38	90 min	1·79	0·298
					24 h	1·79	0·700
				3	90 min	1·79	0·352
					24 h	1·79	0·255
			Hanks's-*NCTC*199-serum 1 : 1 : 1	38	90 min	1·46	0·123
					24 h	1·46	0·065
				3	90 min	1·46	0·255
					24 h	1·46	0·182
2	7-day retina	Versene	Hanks's-*NCTC*199 1 : 1	38	60 min	1·49	0·500
					24 h	1·49	0·564
				3	60 min	1·49	0·630
					24 h	1·49	0·249
			Hanks's-*NCTC*199-serum 1 : 1 : 1	38	60 min	1·23	0·204
					24 h	1·23	0·063
				3	60 min	1·23	0·453
					24 h	1·23	0·204
		Trypsin	Hanks's-*NCTC*199 1 : 1	38	60 min	1·84	0·495
					24 h	1·84	0·128
				3	60 min	1·84	0·913
					24 h	1·84	0·565
			Hanks's-*NCTC*199-serum 1 : 1 : 1	38	60 min	3·14	0·084
					24 h	3·14	0·078
				3	60 min	3·14	0·057
					24 h	3·14	0·090
3	5-day limb buds	Versene	Hanks's-*NCTC*199	38	60 min	1·96	0·503
				3	60 min	1·96	0·838
					24 h	1·96	0·508
			Hanks's-*NCTC*199	38	60 min	1·86	0·126
				3	60 min	1·86	0·502
					24 h	1·86	0·203
4	5-day limb buds	Versene	Hanks's-*NCTC*199	3	60 min	1·79	0·737
					24 h	1·79	0·292
			Hanks's-*NCTC*199-serum	3	60 min	1·55	0·248
					24 h	1·55	0·112

* In all cases the serum used was pooled, unfiltered, sterile horse serum (Colorado Serum Co.).

† C_0 is the initial number of cells × 10^6 per 3 ml. of culture medium; C is the cell number at the designated cultivation time.

‡ Versene dissociations performed by a 10-min incubation in 0·002 M solution in calcium- and magnesium-free Hanks's solution.

§ Hanks's basic salt solution.

¶ *NCTC*199 tissue culture medium, Microbiological Associates Inc., Bethesda, Maryland.

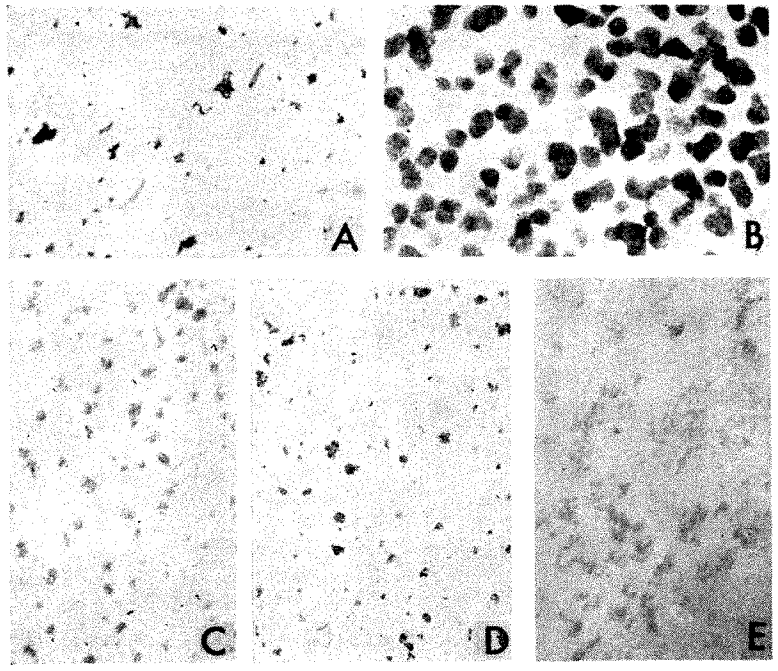


Fig. 1. Appearance of the aggregates after 24 h in rotation cultures (× *c.* 22): *A*, Retinal cells, trypsin-dissociated; medium with horse serum; 3° C. *B*, Retinal cells, trypsin-dissociated; medium with serum; 38° C. *C*, Retinal cells, trypsin-dissociated; medium without serum; 3° C. *D*, Retinal cells, versene-dissociated; medium without serum; 3° C. *E*, Limb bud cells, versene-dissociated; medium without serum; 3° C

The results clearly demonstrate that in both tissues, with both dissociation procedures and at either temperature, horse serum enhanced the formation of cell cluster as evidenced by the reduction in the number of free cells; in serum-free medium, the fraction of the initial cell number remaining at 30–90 min at 24 h was always larger than in cultures with serum. Therefore, the data do not support the statements[2] that cells aggregate normally at 3° C or that horse serum inhibits normal cell aggregation.

Turning now in more detail to the aggregation of retinal cells at 3° C (Table 1, experiments 1 and 2), it was found that at this temperature, both in the presence and absence of serum, trypsin-dissociated cells formed only small clusters of loosely adherent cells (Fig. 1*A*) and that their combination into normally sized, compact aggregates was prevented; this is consistent with the general results of previous investigations on cell aggregation at low temperatures[3–5]. In the presence of serum, the number of single cells remaining at 60–90 min was similar at 3° C and at 38° C (Table 1, experiment 2). The presence of the loosely adherent cell clusters at 3° C indicates that the re-adhesion of these dissociated embryonic cells may require both temperature-sensitive and temperature-insensitive processes; it further indicates that the suggested dependence of initial cell contacts on surface motility[6] is not the temperature-sensitive component of the aggregation process.

The results with versene-dissociated cells at 3° C were comparable with those already described, in that the formation of aggregates was inhibited by the low temperature both in the presence and absence of serum; only small, loosely packed clusters of cells were present at 24 h. In medium with serum, the number of free cells declined at a slower rate at 3° C than at 38° C, unlike following trypsin dissociation (Table 1, experiments 1 and 2). Since versene treatment did not produce a complete dispersal of the tissue into single cells, some of the clusters found at 24 h may have been residual tissue fragments (Fig. 1*C* and *D*); the increase in the number of free cells that occurred in serum-free medium between 90 min and 24 h at 38° C (Table 1, experiments 1 and 2) may have been due to disintegration of these fragments.

The results obtained with versene-dissociated 5-day-old chick limb bud cells were consistent with the data obtained for retinal cells and provided no evidence of the suggested[2] enhancement of normal cell aggregation at 3° C in serum-free medium (Table 1, experiments 3 and 4; Fig. 1*E*). Taken as a whole, the results show that under these experimental conditions both retina and limb bud cells, dissociated with trypsin or with versene, require temperature-dependent processes for the normal progression of aggregation. The difference in temperature-sensitivity between the initial and the subsequent stages of aggregation suggests the involvement of both temperature-independent and temperature-dependent processes in the aggregation of these cells[1].

The temperature- and serum-dependence of aggregation described in this report are not consistent with the findings[2] which prompted this series of tests. Recently, Dr. Curtis has informed me[8] that he has isolated a serum protein (mol. wt. 140,000) which inhibits aggregation and a second protein (mol. wt. 20,000) which promotes aggregation. The inhibitory protein is destroyed at incubation temperatures above 14° C (ref. 8). He reports that most sera tested have more inhibiting than promoting activity at 3° C, but that some have mostly promoting activity. Thus, the difference between the serum effect described by Curtis and that reported here may reflect the use of different sera. Our findings with respect to the temperature-dependence of aggregate formation are still not in agreement, and this disparity is at present unexplained.

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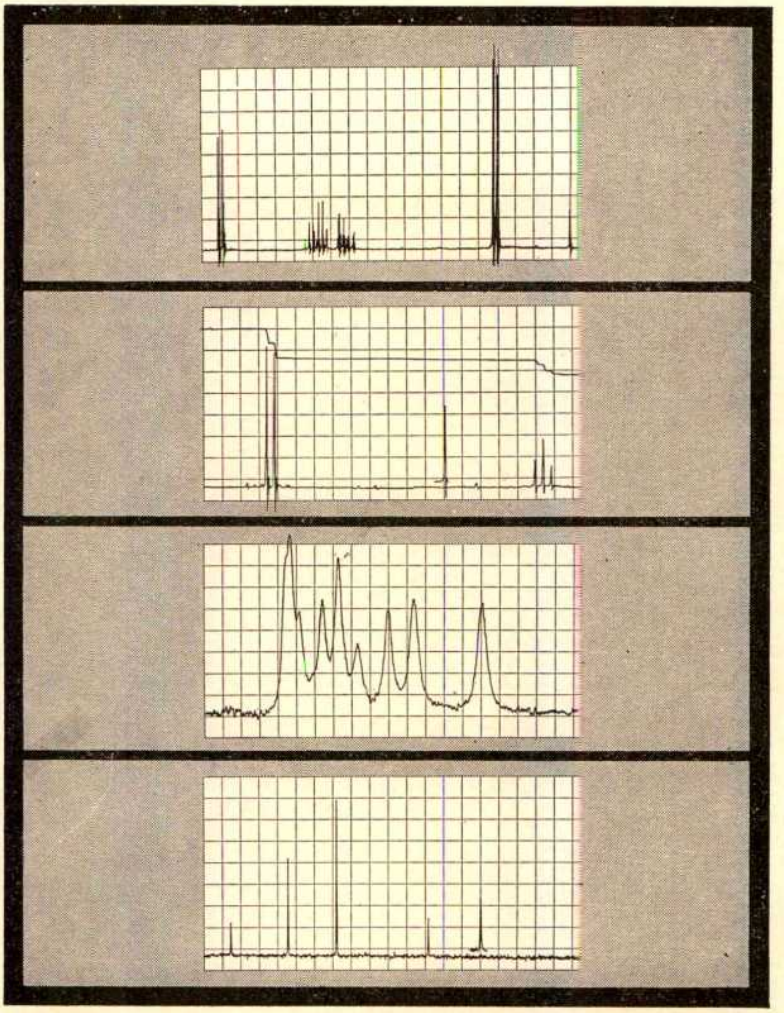
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RADIOBIOLOGY

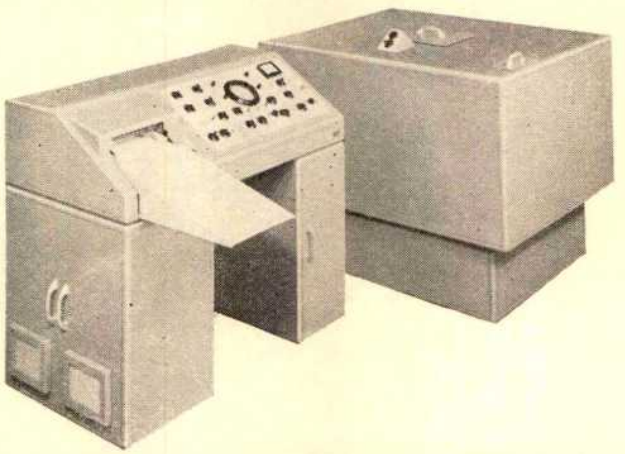
Exact Measurement of Gamma-ray Doses in the Ureter

WE have attempted to evolve a method for the accurate measurement of the radiation dose received by the ureter during radium therapy. Our radiation meter is a small rod-shaped piece of metaphosphate glass measuring 1 × 6 mm, which fits into a No. 6 ureteral catheter. In the course of a cystoscopy performed before the radiation therapy, one of us (O. W.) placed this measuring device in the tip of the ureteral catheter. A new hole was made in the catheter about 1·5 cm from its tip and the catheter as a whole inclusive of the glass was sterilized in formalin. The catheter was fixed to the thigh and the application of radium or cobalt was then carried out. The catheter or catheters were left *in situ* for about 30 min. The position

H F B P



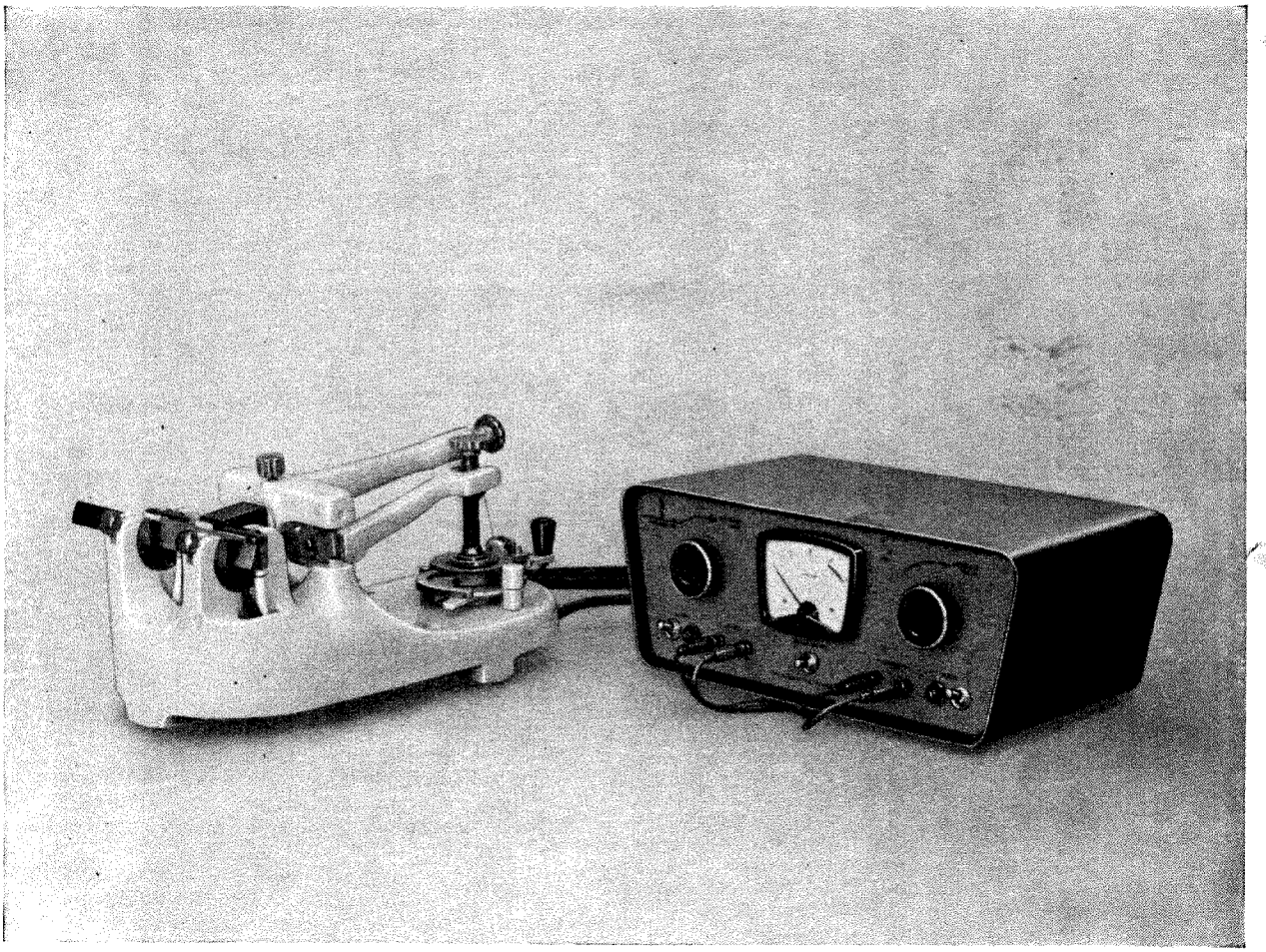
NMR with almost unbelievable simplicity for H and for F, B and P as well



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of the catheters was controlled using the routine radiograms taken for the isodose curves. The pieces of glass have a wide measuring range (50 mr.–10,000 r.) and a notable correlation with energy.

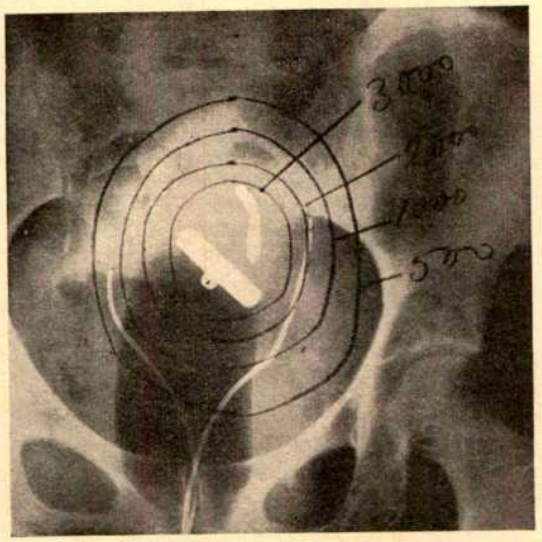


Fig. 1. X-ray picture showing catheters in the ureters and the applicators for γ-therapy. The isodose curves are drawn

Yokota *et al.*[1] investigated the energy-dependence of a corresponding glass on energy and found that the correlation with energies in excess of 110 keV was fairly small. We calibrated the glass for cobalt-60 radiation using doses of different magnitudes and the calibration was carried out with the glass in wax inside the catheter so that radiation scatter was also included. The correlation between the Toshiba readings and the radiation dose was linear. We used radium in addition to cobalt-60 in our radiotherapy. It has more than ten energies in the range 0·18–2·2 MeV, but the correlation with energy is smaller than 20 per cent. The glass was kept in the patient's ureters for about 30 min and ten patients were measured in this way. Comparison of the glass doses with those obtained by the isodose method showed a difference of under 20 per cent. The applicators in Fig. 1 were two cobalt tubes of 27 mgekV and a 100-mg radium plaque. The treatment time was 15 h. Using the glass method, the doses received by the ureters at the tips of the catheters during the whole period of treatment were 1,500 r. in the left ureter and 900 r. in the right. The corresponding doses according to the isodose curves were: left ureter, 1,800 r.; right, 800 r.

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HISTOCHEMISTRY

Demonstration of Ribonuclease and Deoxyribonuclease Activities by Substrate Film Methods

In a recent paper[1], Dr. B. D. Lake states that an important proportion of the enzyme activity revealed by substrate film methods may be attributed to the non-specific action of proteolytic enzymes dissolving the gelatine constituent of the film. Such a statement leaves the impression that the substrate film methods for RNase and DNase are unreliable and that results obtained so far by these methods have been erroneously interpreted.

The facts are that the authors who have used these methods have carried out several control experiments, did actually use gelatine–nucleic acid films resistant to proteolytic activity and thus specifically demonstrated RNase and DNase activities of tissue sections.

The point raised by Dr. Lake had been discussed before[2,3]. It is known that some varieties of gelatine remain digestible by proteases after formaldehyde fixation and it has been stressed in previous articles that such varieties of gelatine are not suitable for preparing films of substrate.

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SOIL SCIENCE

Contamination of Urban Garden Soils with Copper and Boron

Spectrochemical analyses of garden soils sampled in the Edinburgh and Dundee areas indicate that there is substantial contamination of urban soils with copper and boron.

This conclusion is based on the analysis of sixteen soil samples taken from gardens randomly selected in the Edinburgh area, and nine samples taken in the same way in the Dundee area. These soils were analysed spectrochemically with respect to total copper and water-extractable boron content with the view of comparing the levels obtained in urban areas with levels in arable soils in rural areas. The reference levels of copper and boron in arable soils were based on the analysis of one hundred soil samples representatively sampled from fields under cultivation in south-east Scotland. A modification of the method of Farmer[1], adapted for the analysis of soils, was used for the determination of total copper, and boron was determined by porous-cup spark excitation[2] following the hot-water extraction procedure of Truog[3]. 'Ultra' boron-free graphite preformed cups and counter electrodes were used in the sparking procedure.

The results (Table 1) indicate that urban garden soils tend to contain about four times as much copper and two to three times as much water-soluble boron as rural arable soils. The differences between the levels of copper and boron in the Edinburgh and Dundee soils were not statistically significant, but the levels in both urban areas were so much higher that statistical analysis of the results was almost superfluous ($P < 0{\cdot}01$). The existence of such a marked disparity between the levels of two potentially toxic elements in urban and rural areas is evidence of slow poisoning of the soil environment in built-up areas and is cause for concern.

Table 1. Mean Levels of Total Copper and Water-extractable Boron in Urban and Rural Soils

Sample	No.	Copper p.p.m.	*S.E.*	Boron p.p.m.	*S.E.*
Rural arable soils	100	15·5	± 1·5	0·70	± 0·03
Edinburgh garden soils	16	71·6	± 4·1	1·95	± 0·23
Dundee garden soils	9	56·2	± 7·6	1·56	± 0·21

All the copper levels encountered in the Edinburgh area (and, with two exceptions, in the Dundee area) were very high compared with levels generally encountered in arable soils, in which it is very unusual to find more than 50 p.p.m. total copper[4]. The boron levels in the urban soils were also abnormally high, not only in relation to

levels in arable soils in south-east Scotland but also in relation to levels reported by Lehr and Henkens[5] for arable soils in The Netherlands. It is likely that some of the levels of water-extractable boron encountered, for example, levels greater than 3 p.p.m., could be deleterious to plant growth.

While the major source of contamination of soils with copper and boron is still a matter for speculation, it is probable that the addition of soot to garden soils and the fall-out of sooty material in built-up areas where atmospheric pollution is a problem make a substantial contribution to the water-extractable boron content of urban soils. In this connexion, it is of interest that three samples of soot from domestic chimneys, obtained from independent sources, were found on analysis to contain 640, 650 and 555 p.p.m. water-extractable boron, and it is evident that the addition to soil of even small amounts of soot with a boron content of this order would have a marked effect on its water-extractable boron content.

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Determination of Total Potassium in Soils using the Gamma Emission of Potassium-40

Dunavant and Christian[1] have described a large-volume 4 π liquid scintillation detector, and Harris, Kessler and Christian[2] have described its use to determine total potassium in solids and liquids by measuring the gamma activity of the natural radioactive isotope potassium-40. The instrument is similar to the Armac liquid scintillation counter produced by the Packard Instrument Company.

The use of a large-volume scintillation detector, similar to that described, for the determination of total potassium in soils was considered attractive to us for a number of reasons: the use of a large sample reduces sampling error; cumulative errors and contamination due to handling in chemical methods of determination are eliminated; the time required for handling samples and processing results is small.

To assess the suitability of the above technique, an Armac scintillation counter capable of handling samples of about 1,500 g of soil was used to determine the total potassium content of six New South Wales soils. The potash chemistry of these soils is currently receiving detailed investigation at our laboratory. Total potassium determinations were carried out according to the semi-micro-chemical method of Jackson[3].

The gamma radiation emitted by potassium-40 has an energy of 1·46 MeV, but with the equipment used, the peak appears at 1·24 MeV, and the pulse height analyser was set for a channel of 1·0–1·5 MeV. Potassium as potassium chloride mixed with the soils in varying quantities gave a figure of 17·0 ± 1·7 c.p.m./g potassium.

This figure provided the calibration to enable calculation of soil potassium figures. Compared with chemical figures for total potassium, the potassium-40 count figures were found to be high as shown in Table 1.

Table 1. COMPARISON OF PERCENTAGE TOTAL POTASSIUM IN SIX SOILS BY CHEMICAL AND LIQUID SCINTILLATION METHODS

	Chemical potassium	Potassium by potassium-40 count
Soil 1	1·35	1·90
Soil 2	0·20	0·99
Soil 3	0·04	0·78
Soil 4	1·49	2·44
Soil 5	0·06	0·37
Soil 6	0·10	0·38

It was apparent that the discrepancies between chemical and potassium-40 count figures were due to interference from radioactive elements other than potassium.

In order to learn more of this interference, the six soils were counted on a 5 in. diam. × 4 in. sodium iodide crystal using a multi-channel pulse height analyser to group the counts into channels of 10 keV width. The sample container fitted over the crystal so as to give a layer of soil 1 in. thick around the side and over the top of the crystal. Figs. 1 and 2 are typical curves of counts versus gamma energy. The potassium count is superimposed on a continuum of gamma counts which results from the scattering of higher energy gamma radiation emitted by the uranium and thorium series elements. A number of small photo-peaks from gamma rays of the uranium and thorium series of elements are also present within this range.

It is apparent that the liquid scintillation technique described by Harris, Kessler and Christian[2] is unsuitable for the determination of total potassium in soils because the continuum interferes and it is not possible to correct for it when using a liquid scintillation detector. The

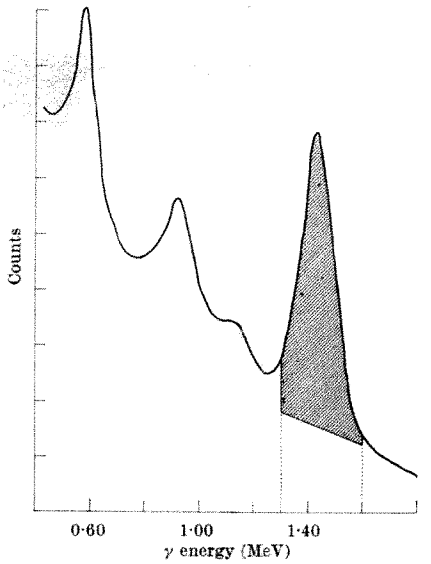


Fig. 1. Counts versus γ energy plot for a high potassium soil (Soil 1), using the sodium iodide crystal. The shaded area was taken to represent potassium counts. The remaining area on the plot represents the continuum and photo-peaks of the uranium and thorium series of elements

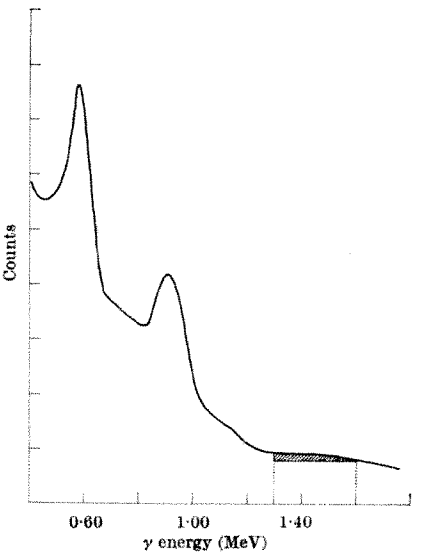


Fig. 2. Counts versus γ energy plot for a low potassium soil (Soil 3) using the sodium iodide crystal. The shaded area was taken to represent potassium counts. The remaining area on the plot represents the continuum and photo-peaks of the uranium and thorium series of elements

magnitude of the interference is not constant. It is about as great as the potassium count in high potassium soils, but many times as great in low potassium soils.

Estimates of total potassium obtained using the sodium iodide crystal in conjunction with the multi-channel pulse height analyser agreed well with the results of chemical analysis (Table 2).

Table 2. TOTAL POTASSIUM BY CHEMICAL METHOD AND POTASSIUM-40 COUNTING USING SODIUM IODIDE CRYSTAL

	Chemical potassium	Potassium by sodium iodide crystal
Soil 1	1·35	1·03
Soil 2	0·20	0·21
Soil 3	0·04	0 03
Soil 4	1·49	1·33
Soil 5	0·06	0·07
Soil 6	0·10	0·11

The determination of total soil potassium using the emissions from naturally occurring potassium-40 is possible using a sodium iodide crystal and multi-channel pulse analyser. The equipment is expensive and time spent counting samples of low potassium content is lengthy. On the other hand, the elimination of errors mentioned earlier is important and operator time using the equipment is short, so that where total potassium determinations on large numbers of samples are required the method may prove useful.

We thank the Prince Henry Hospital, Sydney, for making available the Armac liquid scintillation counter, Mr. Paix, Prince Henry Hospital, for his interest and assistance, and Dr. Duggleby and Mr. Parsons, Australian Atomic Energy Commission Research Establishment, Lucas Heights, for helpful discussions and for counting our samples on the sodium iodide crystal and multi-channel analyser.

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VIROLOGY

Growth of Foot-and-Mouth Disease Virus in Monolayer Cultures of Calf Thyroid Cells

THE most successful laboratory systems for the growth and assay of foot-and-mouth disease virus (FMDV) have been suckling mice[1], cell cultures of calf and pig kidney[2,3], and the cell line derived from baby hamster kidney (*BHK* 21)[4]. All these systems vary in their relative sensitivity for various strains of virus and none appears to effect an absolute determination of the titre of infective virus. Recently it has been found that, in the assay of unmodified cattle virus, higher titres are obtained in primary monolayer cultures of calf thyroid cells than in the systems enumerated and in some instances these cultures are 100–1,000-fold more sensitive. Calf thyroid cultures have been used previously in the isolation and study of the virus of malignant catarrhal fever[5] and human thyroid cultures for the isolation of human enteroviruses and adenoviruses[6,7].

The proximal part of the trachea, and the larynx with the thyroids attached, were obtained from calves 2–14 days of age which were killed at abattoirs. The thyroids were removed, washed in phosphate buffered saline containing antibiotics, cleaned of connective tissue and fat, and minced with scissors. For the production of trypsin dispersed cells from calf thyroids a trypsin concentration of 0·45 per cent was used. This is higher than the 0·25 per cent generally used for mammalian tissues and follows the work of Plowright and Ferris[8], who used 0·4 per cent, and of French, Snowdon and Southern (unpublished), who used 0·45 per cent. The cells were collected at intervals of 45–60 min, and tubes, 15 mm × 150 mm, were seeded with 1 ml. of a cell suspension containing 1×10^5 clumps of cells (a clump comprised aggregates of three or more cells). The growth medium was one part of Hanks's saline, containing 0·5 per cent lactalbumin hydrolysate and 0·01 per cent yeast extract, 1 part of Eagle's basal medium, modified to contain twice the normal concentration of amino-acids and vitamins, and 10 per cent of unheated bovine serum. For maintenance of cultures, the 10 per cent of bovine serum was reduced to 3 per cent.

The growth and appearance of the thyroid cultures were similar to those described by Plowright and Ferris[8]. Aggregates of cells attached to the glass but remained rounded for 48 h. After this period, flattened epithelial type cells appeared at the base of the aggregates and after a further 3–5 days had grown out and joined up with neighbouring islets to form a confluent monolayer. Larger, elongated fibroblastic type cells separated many of the islets of epithelial cells and also proliferated extensively around the edges of the monolayer.

To infect cultures, the medium was removed and in some experiments the cell sheets were given one or two washes in phosphate buffered saline before they were inoculated with 0·2 ml. virus suspension. The inoculum was allowed to adsorb at 37° C for 1 h, after which 2 ml. of maintenance medium were added to each tube and the inoculated tubes incubated at 37° C in roller drums. Cultures were examined daily for cytopathic changes and the final readings of titrations were made 48 h after inoculation.

After inoculation of calf thyroid cultures with FMDV, the first cytopathic changes were observed in single cells or groups of cells, which contracted, became irregular in shape but remained attached to neighbouring cells by long thin processes. As incubation proceeded, these cells became rounded and eventually detached from the glass. The cytopathic changes did not involve all cells in the monolayer. The susceptible cells appeared to be of the epithelial type, whereas the elongated fibroblastic elements were insusceptible. After the susceptible cells were destroyed and became detached from the glass, the remaining cells proliferated and rapidly filled the gaps that had appeared in the monolayer. The final appearance of an infected culture was a complete sheet of elongated fibroblastic type cells with cell debris scattered over the surface.

The multiplication of two types of FMDV is shown in Table 1. Cultures were infected with $10^{5.2}$ $TCID_{50}$ of virus and the first evidence of cytopathic change in infected cells was seen at 8–10 h and was completed at 24 h. Peak infectivity was reached in 24 h and in both instances was $10^{8.4}$ $TCID_{50}$ per ml. So far, strains of FMDV of types *A*, *O*, *C*, *SAT* 3 and *Asia* 1 have been tested in thyroid cultures and all have grown equally well.

The sensitivity of four other assay systems was compared with calf thyroid cultures, and the results are shown in Table 2. Higher titres were always obtained in thyroid cultures.

The increased sensitivity of calf thyroid cultures has been used to demonstrate virus when other methods have failed. In work at this institute on the susceptibility of marsupials to infection with FMDV (to be published), nineteen blood samples collected from red kangaroos previously inoculated with type *Asia* 1 were assayed for virus in both suckling mice and calf thyroid cultures. The virus titres in calf thyroid cultures exceeded those obtained in mice by $10^{0.5}$ to $\geqslant 10^{2.5}$ $TCID_{50}$ per ml. and

Table 1. GROWTH OF FOOT-AND-MOUTH DISEASE VIRUS IN PRIMARY MONOLAYER CULTURES OF CALF THYROID CELLS

Time post-infection (h)	Virus strains: *A Iraq* 24/64* Log_{10} $TCID_{50}$/ml.†	*Asia* 1 *Pak* 1* Log_{10} $TCID_{50}$/ml.†
0	3·6	3·6
2	3·2	3·2
4	3·2	3·0
6	4·7	5 4
8	6·2	5·7
12	6·7	6·7
24	8·4	8·4
32	7·7	7·4
48	7·4	7·4
72	6·4	6·8

* Unmodified cattle virus.
† Tissue culture ID_{50} when assayed in calf thyroid cultures.

Table 2. GREATER SENSITIVITY OF CALF THYROID CELLS IN THE ASSAY OF UNMODIFIED CATTLE FOOT-AND-MOUTH DISEASE VIRUS*

	No. of observations	FMDV No. of types	Increase in titre obtained in calf thyroid cultures (log_{10}) Range	Mean
Cattle: intradermal tongue inoculation (ref. 9)	4	3	0·5–2·9	1·5
Suckling mice: intraperitoneal inoculation	9	5	0 6–2·4	1·4
Secondary pig kidney cultures	4	3	1·4–2·3	1·5
BHK 21 cultures	10	5	0 8–3 2	1·8

* Virus suspensions, Seitz *E.K.* filtrates of cattle tongue epithelium.

five samples were positive for virus in thyroid cultures but negative when inoculated into mice.

We have also used calf thyroid cultures to recover virus from material in which it was present only in minimal amounts; for example, in specimens of oesophageal fluid from carrier cattle[10,11] and from specimens of cattle tongue epithelium collected from suspected clinical cases of the disease in the field. In both cases the infected tissue culture fluid was a satisfactory antigen in the complement-fixation test, and this technique is expected to be of great benefit in the rapid identification of virus in field samples. Another probable application is in the safety testing of inactivated vaccines, and preliminary work is in progress.

I thank Mr. P. B. Capstick, Mr. R. Burrows and Mr. R. H. Fagg for supplying some of the suspensions of unmodified cattle virus, and Mr. I. T. R. Barnett for carrying out the complement-fixation tests. The work described here was carried out when I was on secondment from the C.S.I.R.O. Division of Animal Health, Parkville, Victoria, Australia.

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Electrophoretic Separation of Two Plaque-type Variants of Encephalomyocarditis Virus

THE surface properties of large (*r*) and small (r^+) (formerly designated *l* and *s* respectively (ref. 1)) plaque-forming encephalomyocarditis (EMC) virus[1] have been compared using a sucrose-gradient electrophoretic technique[2]. A difference was suspected because the relative plaque sizes changed on the addition of polyanions or polycations to the agar overlay of the plaque-assay[1,3]. Both variants produced 'small' plaques in the presence of excess polyanions (for example, sulphated polysaccharides), and 'large' plaques in the presence of excess polycations (such as diethylaminoethyl-dextran)[1].

Preparations of cloned large (*r*) and small (r^+) plaque-forming EMC virus were concentrated by centrifugation and purified by treatment with 0·02 per cent trypsin and chromatography on calcium phosphate columns[4]. The virus was re-sedimented from the peak column fractions, suspended in 0·02 M *tris*, *p*H 7·4, and stored at −70° C.

The electrophoresis was performed in an apparatus previously described[5]. A 1 : 1 mixture of large and small plaque-forming virus containing 2×10^7 P.F.U. in 0·5 ml., *p*H 8·8, 0·05 M barbitone-buffered-sucrose of appropriate density, was introduced near the top of a 5–20 per cent (w/v) sucrose gradient column (45 cm × 1 cm) buffered with *p*H 8·8, 0·05 M barbitone. Electrophoresis was carried out for 16 h at 150 V and 3·3 m.amp at 18° C. Fractions corresponding to 1 cm of tube length were collected from the bottom of the column and plaque-assayed for large and small plaque-forming virus. Two such experiments were carried out under the foregoing conditions with similar results. The results of the first experiment are shown in Fig. 1. The peaks of infectivity for the large and small plaque-forming variants differed about three-fold in electrophoretic mobility, the small plaque-type being the more negatively charged. In the absence of independent criteria of virus purity, it is possible that the electrophoretic properties of the variants may have been modified by interaction with contaminating cell components. This does not, of course, affect the basic conclusion that the variants have a surface property difference.

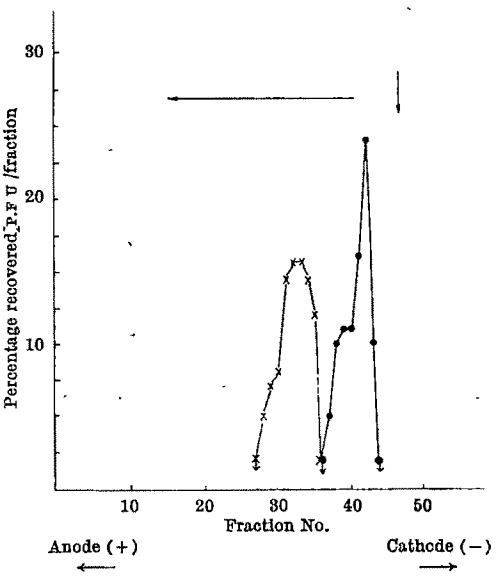


Fig. 1. Electrophoresis of small (×) and large (●) plaque EMC virus at *p*H 8·8. Horizontal arrow indicates direction of movement and vertical arrow the starting point

Following sucrose gradient electrophoresis in 0·02 M *tris* buffer at *p*H 7·4, the *p*H of the plaque assay, under conditions otherwise identical to the experiment first described, two separate bands of virus infectivity were not resolved. Nevertheless, a change in proportion of r^+ to *r* along the column, again demonstrating a higher negative charge for the small plaque virus, was clearly demonstrated (Fig. 2). For convenience the P.F.U./fraction as a percentage of the total recovered (about 20 per cent) is plotted on a log_{10} scale.

Although it cannot be concluded that the plaque-type difference is a consequence of differing charge-determined interactions of the two types of virion with polyanionic inhibitors in agar, much circumstantial evidence has accumulated in favour of this hypothesis[6–8]. Such

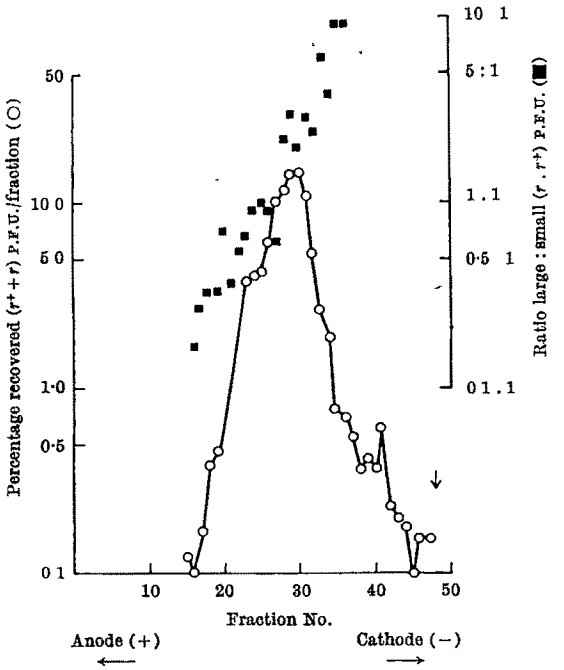


Fig. 2. Electrophoresis of small and large plaque EMC virus at *p*H 7·4. Vertical arrow indicates starting point

charge differences could occur following mutation and a consequent amino-acid substitution in the structural protein of the virus capsid.

We thank Miss Joyce Mason for technical assistance.

This work was performed during the tenure by one of us (D. C. B.) of a Medical Research Council scholarship for training in research methods.

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PSYCHOLOGY

A Local Sign for Depth

WHEN a human observer views a tridimensionally ordered scene, each of his eyes receives a slightly different perspective view of the scene. Differences in tridimensional location are coded on to the retinae as differences in bidimensional position, that is to say, as retinal disparities. As is well known[1], retinal disparity alone produces compelling perceived spatial separation. The way in which the nervous system decodes retinal disparity into perceived spatial separation is somewhat mysterious, since retinal disparity alone does not specify whether an object is nearer or farther than a fixated object, but merely that they are not in the same plane. (Disparities are geometrically ambiguous. For them to become unambiguous, additional information is required. Different authors have involved different sources of information, none of which is wholly satisfactory: see, for example, ref. 2).) The nervous system obviously can distinguish these two situations but the way in which it does this is not clear. Hering[3] solved the problem by giving each point on the two retinae a specific depth-value. On this model all points on the nasal hemiretinae of each eye have positive depth-values, so that any point imaged there will be seen as further than the fixation point, while points on the temporal hemiretinae have negative depth-values, so that points imaged there will be seen as nearer than the fixation point. This simple and elegant solution has never gained wide acceptance, largely because Hering never specified any mechanism which could add depth-values to inputs. The specification of a mechanism to do that is as great a problem as the specification of a mechanism to decode retinal disparity into perceived spatial separation. It is no wonder that the majority of investigators felt that Hering had solved one mystery by another mystery.

There have been a few attempts to explain depth-value. For example, Schön[4] suggested that we consider time of arrival at the cortex as the code mediating depth-value. It is well established that points on the retinae conduct at different speeds, and that points on the nasal hemiretinae transmit to the cortex much more quickly than corresponding points on the temporal hemiretinae[5]. Thus a depth-value would be given merely by the time of arrival of a signal relative to the time of arrival of other signals in the same 'message' in combination with its location in the bidimensional binocular field. This simple mechanism was tested and found to be valid in the following experiments.

The simplest test of the hypothesis seemed to be to vary the time of arrival of each eye's view of a tridimensionally ordered scene. (Such experiments are not new. However, previous authors have not been testing this hypothesis, and so have treated their data in ways which make them useless for this purpose, for example, by averaging near and far cases, by averaging, or not controlling for nasal and temporal hemiretinal position.) If Schön's hypothesis was correct, changing the time relations between signals should modify stereoscopic depth perception in a number of ways, depending on the stimulus situation in use. If the disparate images of an object fall, one on the temporal half of one eye and one on the nasal half of the other, presentation of the nasal image before the temporal image should not affect depth perception unless the delay is so long that the two signals cannot be seen as part of the same message. If, on the other hand, the temporal image is presented first, a number of phenomena should occur, dependent on the precise delay used. If the delay is such that both signals arrive at the cortex simultaneously, then zero depth should be perceived. If the delay is long enough for the temporal signal to arrive at the cortex before the nasal signal, then depth should be reversed, so long as the delay is short enough for both to be seen as parts of the same message.

Ten subjects with normal vision free from phoria served in the first experiment. Stimuli were presented at 1 m distance in a scientific prototype G.A. tachistoscope modified by the introduction of suitably crossed-'Polaroid' filters to serve as a stereoscope. In one field a continuous binocularly visible white fixation point was presented on a black ground. On test trials a white line was presented to each eye 2·5 cm to the right of the fixation point in one eye and 3·0 cm to the right in the other eye, so that the disparity was 18 min of arc. The lines were 10 cm long by 3 mm thick. The luminance of the white lines and fixation point was 31·8 equivalent lux, that of their black backgrounds 0·38 equivalent lux. By changing the orientation of the 'Polaroids', each line could be sent to either eye, thus reversing the direction of depth. The line going to the right eye always fell on the nasal hemiretina of that eye, that going to the left always on the temporal hemiretina of that eye. Stereo-lines were presented for 10 msec with the following delays between the onset of each: 0 msec, 0·50 msec, 1·00 msec, 1·5 msec, 2·5 msec, 3·5 msec, 5·0 msec, 7·5 msec, 10·0 msec, 15·0

msec. Each depth of direction was presented fifty times at each delay value, making a total of 1,000 presentations, given at a rate of one every 4 sec. Subjects were instructed to keep their eyes fixed on the fixation point and to report whether the white line was nearer or farther than the fixation point. Twenty-four hours later each subject was run through an identical experiment except that the lines were presented to the left of the fixation point. This was done in order to ensure that any effects were due to nasal-temporal differences, not right-eye left-eye differences.

Results are shown in Fig. 1. As can be seen there, there were no eye-specific effects, but there were strong nasal-temporal effects. The probabilities shown are uncorrected for guessing. They are the actual response probabilities obtained. With nasal preceding temporal the probability of a correct response falls to 0·5 monotonically and stays there. When temporal precedes nasal, probability of correct response falls to less than 0·5 ($P < 0{\cdot}01$), indicating a reversal of depth direction, and then climbs back to 0·5 with longer delays. These data obviously support Schön's hypothesis. From them we might infer that the difference in transmitting speed between temporal and nasal hemiretinae is about 1·5 msec, an estimate which is not at odds with the data cited here, and that the length of a visual message is probably not more than 7·5 msec.

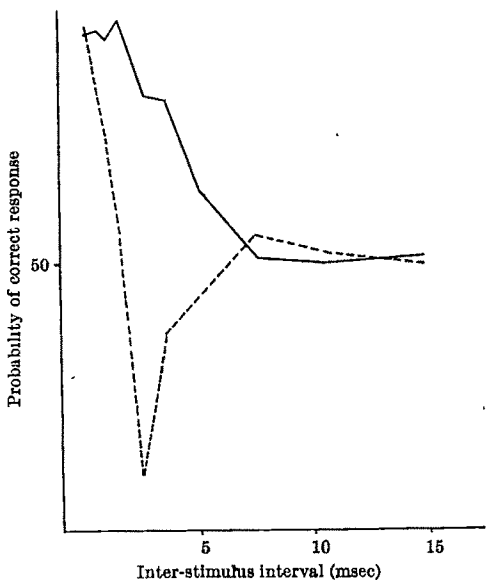


Fig. 1. Uncorrected probability of correct response at the inter-stimulus intervals. ——, Nasal→temporal, - - - -, temporal→nasal

Schon's hypothesis can thus provide a mechanism to explain Hering's depth-values. However, as it stands it is insufficient, since it does not explain the rigidity of stereoscopic depth. (Recent experiments have shown that the scale value of a given disparity may be rapidly modified; however, reversal of direction is not attained, even after some months of wearing right–left reversing prisms, which reverse disparity and thus lead to invalid stereo-depth judgments[6]. Were stereo-judgments the result of some kind of inferential process based on time of arrival only, one might expect that judgments would in time become valid once more.) If time coding was only a cue, stereodirection would be more reversible than it is. Hering thought that phenomenal depth-values were rigidly associated with retinal depth-values, and indeed carried out an experiment to test it[3]. He viewed a line the retinal representations of which were as disparate that both were seen; one fell on a nasal hemiretina, the other on a temporal hemiretina. He reported that the disparate images were seen at different distances. Stumpf[7] confirmed this, but Helmholtz[8] was unable to do so. Helmholtz suggested, as a critical experiment, that the nasal hemiretinae of each eye should be occluded. A flat wall viewed thus should then appear as a corner, with its two sides meeting at an angle. He reported that it did not. These experiments are obviously very poor tests of the hypothesis. In both cases there is too much supplementary information which contradicts the stereo-information, a fact which Hering noted. However, there are at least two experiments in the literature which indicate Hering's contention of fixed connexions as correct. Tschermak and Kiribuchi[9] required subjects to adjust a row of wires until it appeared to lie in their fronto-parallel plane. They used exposures so short that only information from the nasal hemiretinae could have been transmitted. In this case the apparent fronto-parallel plane was objectively a concave-semicircle, as Hering's theory would indicate. Katz and Schwarz[10] had subjects fixate a fixation point while attending to a light spot, moving in their fronto-parallel plane. 'Polaroid' filters were used to ensure that the light was seen only by one or other hemiretina of each eye. Vision was never binocular. The light spot was represented on only one retina at a time. When the light spot was seen by the temporal hemiretinae of the two eyes, the light spot was seen to move in a circular path in the third dimension, going from near to far to near as it went from periphery through fixation point to periphery. When the viewing conditions were reversed, so that only the nasal hemiretinae saw the spot, the direction of depth was reversed, the spot was seen to swing from far to near to far. These results are obviously just what Hering's theory would predict.

The experiment performed and the data reviewed here indicate that Hering's theory of stereopsis is probably correct, if time of arrival at the cortex of signals is introduced to provide a mechanism to account for depth-values. This modified version attributes phenomenal depth to a combination of bidimensional position and relative time of arrival. For this model to work, it is necessary to conceive of the visual system as sampling discrete, temporally defined messages from the available light. This conception is wholly plausible[11]. These data fill out the impressive array of evidence, assembled by von Békésy[12], which seems to show that spatial position is coded into the nervous system by way of differences in time of arrival of signals from paired receptor organs, the effectiveness of the code being independent of the sensory modality used. He has shown this for the auditory, olfactory and skin senses. Vision may now be added to the list.

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I thank Julian Hochberg and G. von Békésy for advice and criticism, and Miss A. Katz for invaluable library work.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, June 6

UNIVERSITY OF LONDON (in the Botany Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. Michael Sela (Rehovot) "Molecular Basis Antigenicity".*

Monday, June 6—Wednesday, June 8

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, W.C.2). Conference on "Design and Construction of Large Steerable Aerials".

Tuesday, June 7

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Prof. R. King. "Chemical Change and Physical Change" (afternoon lecture for Fourth Form and Sixth Form Boys and Girls from Schools in London and the Home Counties. To be repeated on June 8, 14 and 15).

UNIVERSITY COLLEGE, LONDON (in the Anatomy Theatre, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. Patrick Wall (Massachusetts Institute of Technology). "The Organization of the Dorsal Horn of the Spinal Cord".*

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 5 30 p m —Prof. J. G. Clunie: "The Theory of Functions in Complex Analysis" (Inaugural Lecture).*

UNIVERSITY OF LONDON (in the Botany Theatre, University College, Gower Street, London, W.C.1), at 5.30 p m.—Dr. Michael Sela (Rehovot): "Some Structural Aspects of Antibody Activity".*

Thursday, June 9

INSTITUTE OF MATHEMATICS AND ITS APPLICATIONS (in Lecture Theatre No. 2, Physics Department, Imperial College of Science and Technology London, S.W.7), at 4.30 p m.—Annual General Meeting. 5 30 p.m.—Prof. W. G. Bickley: "Mathematics for the Blind".*

MINERALOGICAL SOCIETY (at the Geological Society of London, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Scientific Papers

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Annual General Meeting.

CHEMICAL SOCIETY (in the Large Chemistry Lecture Theatre, Imperial College of Science and Technology, South Kensington, London, S.W.7), at 6 p m —Prof. W. G. Dauben (University of California): "Strained Ring Compounds".

BRITISH INSTITUTE OF RADIOLOGY (in the Reid-Knox Hall, 32 Welbeck Street, London, W.1), at 7.15 p.m.—Prof. Robley D Evans: "The Effects of Skeletally Deposited Alpha-Ray Emitters in Man" (Silvanus Thompson Memorial Lecture).

Thursday, June 9—Friday, June 10

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W 1), at 10.20 a.m. on Thursday and 10.30 a.m. on Friday—Discussion Meeting on "The Terrestrial Antarctic Ecosystem" organized by Dr. J. E. Smith, F.R.S.

Friday, June 10

INSTITUTE OF SCIENCE TECHNOLOGY, LONDON BRANCH (in the Manson Theatre, London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6.30 p.m.—Annual General Meeting.

Saturday, June 11

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C 1), at 5 15 p.m. —132nd Annual General Meeting. 6 p m.—Mr. L. H. C. Tippett. "Statistics in Manufacturing Industry" (Presidential Address).

Monday, June 13

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 3 p.m.—Annual General Meeting.

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER (with good academic qualifications and the ability and desire to prosecute research) IN METALLURGY—The Principal, Lanchester College of Technology, Priory Street, Coventry (June 8).

JUNIOR LECTURER (graduate with medical qualifications) IN ANATOMY—The Registrar, King's College (University of London), Strand, London, W.C.2 (June 10).

CHAIR OF STRUCTURAL ENGINEERING—The Registrar, University of Strathclyde, George Street, Glasgow, C.1 (June 11).

LECTURER IN BIOCHEMISTRY—The Registrar, King's College (University of London), Strand, London, W.C.2 (June 13).

LECTURER or ASSISTANT LECTURER (specialist in urban geography and planning) IN THE DEPARTMENT OF GEOGRAPHY—The Deputy Secretary, The University, Southampton (June 13).

LECTURER (with a good honours degree in mathematics) IN MATHEMATICS OR MATHEMATICAL STATISTICS; and an ASSISTANT LECTURER (with a good honours degree) IN MATHEMATICS—The Secretary, Chelsea College of Science and Technology, Manresa Road, London, S.W 3 (June 13).

RESEARCH ASSISTANT IN THE AGRICULTURAL CHEMISTRY SECTION OF THE DEPARTMENT OF AGRICULTURE—The Registrar, The University, Leeds, 2 (June 13).

ASSISTANT LECTURER (preferably with special qualifications in political geography) IN THE DEPARTMENT OF GEOGRAPHY—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (June 15).

RESEARCH WORKER IN THE DEPARTMENT OF BIOLOGY AS APPLIED TO MEDICINE, for the investigation of biochemical aspects of pattern formation in developing and regenerating systems—Prof. Lewis Wolpert, Middlesex Hospital Medical School, Mortimer Street, London, W.1 (June 15).

ASSISTANT LIBRARIAN in the Philosophical Library, to assist in co-ordinating library services in the scientific departmental libraries and eventually in the libraries of the Arts Faculties, with particular reference to the preparation of a union catalogue by computer—The Secretary, Philosophical Library Committee, University of Cambridge, Bene't Street, Cambridge (June 16).

ASSISTANT (Assistant Lecturer) (preferably graduate in veterinary medicine, pharmacology or physiology) IN VETERINARY PHARMACOLOGY—The Secretary of the University Court, The University, Glasgow (June 17).

LECTURER IN THEORETICAL PHYSICS—The Registrar, University of Kent at Canterbury, Canterbury, Kent (June 17).

LECTURER or ASSISTANT LECTURER (with a medical or scientific qualification) IN VIROLOGY IN THE DEPARTMENT OF BACTERIOLOGY—The Registrar, The University, Manchester, 13, quoting Ref 131/66 (June 17).

LECTURER IN MEDICAL BIOCHEMISTRY with responsibilities in the Rheumatism Research Centre—The Registrar, The University, Manchester, 13, quoting Ref. 132/66 (June 18).

LECTURER or ASSISTANT LECTURER IN THE DEPARTMENT OF PURE MATHEMATICS—The Registrar, The University, Liverpool, quoting Ref. CV/76 (June 18).

RESEARCH ASSISTANT IN THE DEPARTMENT OF NATURAL HISTORY to work under the direction of Mr. Blaxter, on the physiology and development of vision in marine fish larvae—The Secretary, The University, Aberdeen (June 18).

M.R.C RESEARCH ASSISTANT (with a Ph.D. degree, or equivalent research experience, in organic chemistry) IN CHEMISTRY, to work under the direction of Prof. H. N. Rydon on the synthesis of peptides related to the active centres of esterases—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devon (June 20).

RESEARCH ASSISTANT for work under the direction of the Honorary Curator of the Geological Collections—The Secretary of the University Court, The University, Glasgow (June 20).

GEOLOGISTS (2) (with a good honours degree) IN THE DEPARTMENT OF GEOLOGY, one for studies in the Upper Carboniferous Rocks of the North Midlands, and the other for work on the Triassic rocks of Cheshire—Prof. F. W. Cope, The University, Keele, Staffordshire (June 22).

UNIVERSITY SENIOR ASSISTANT BIOCHEMIST TO ADDENBROOKE'S HOSPITAL —T. D. Kellaway, Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge (June 23)

CHAIR OF OBSTETRICS AND GYNAECOLOGY at University College Hospital Medical School—The Academic Registrar, University of London, Senate House, London, W.C.1 (June 24).

LECTURER IN PHYSICAL CHEMISTRY—Prof. R. M. Barrer, F.R.S., Imperial College of Science and Technology, London, S.W.7 (June 24).

LECTURER (medicine or science graduate) IN THE DEPARTMENT OF PHYSIOLOGY—The Assistant Registrar, The Medical School, University of Birmingham, Birmingham, 15 (June 24).

SCIENTIST (preferably with pathological or immunological training or experience in related chemical techniques) IN THE DEPARTMENT OF EXPERIMENTAL PATHOLOGY, to work with a group on immunological aspects of connective tissue reactions in repair and inflammatory processes in adults and embryos—The Secretary, Institute of Animal Physiology, Agricultural Research Council, Babraham, Cambridge (June 24).

LECTURER IN EXPERIMENTAL PSYCHOLOGY IN THE DEPARTMENT OF AUDIOLOGY AND EDUCATION OF THE DEAF—The Registrar, The University, Manchester, 13, quoting Ref. 134/66 (June 25)

LECTURER or SENIOR LECTURER IN CLINICAL EPIDEMIOLOGY IN THE DEPARTMENT OF INFECTIOUS DISEASES at Ruchill Hospital—The Secretary of the University Court, The University, Glasgow (June 25).

LECTURER (with special qualifications in the quantum theory of molecular electronic structure) IN THEORETICAL CHEMISTRY—The Registrar, The University, Sheffield (June 25).

READER IN MEDICINE at the Royal Free Hospital School of Medicine—The Academic Registrar, University of London, Senate House, London, W.C.1 (June 27).

LECTURER or ASSISTANT LECTURER (qualified to lecture to honours degree standard and to supervise postgraduate research students) IN PHYSICAL CHEMISTRY—The Secretary, Sir John Cass College, Jewry Street, London, E.C.3 (June 30).

POSTDOCTORAL FELLOW (with an interest in cell behaviour and function) IN THE DEPARTMENT OF ZOOLOGY, to work on the physiology and biochemistry of cell surfaces—The Deputy Secretary, The University, Southampton (June 30)

READER IN MATHEMATICAL STATISTICS at the University of Adelaide, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, June 30).

UNIVERSITY LECTURER to engage in research and teaching, with special reference to the agriculture of the developing countries—The Director, The Institute for Research in Agricultural Economics, The University, Parks Road, Oxford (June 30).

SENIOR LECTURER (with a doctorate or equivalent achievement in the field of industrial research or of process design and development) IN CHEMICAL ENGINEERING at the University of Newcastle, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, July 23).

LECTURER/SENIOR LECTURER (with a distinguished academic record and able to produce evidence of strong research interest and experience) IN THE DEPARTMENT OF STATISTICS, SCHOOL OF MATHEMATICS, University of New South Wales—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, July 29).

LECTURER/SENIOR LECTURER (with a degree in veterinary science which is registrable in Victoria, and preferably experience in a rural practice) IN VETERINARY MEDICINE (Large Animals) at the University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W 1 (Australia and London, September 1).

CHAIR OF PHYSIOLOGY at the Institute of Advanced Studies, Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1.

EXPERIMENTAL OFFICER or ASSISTANT EXPERIMENTAL OFFICER (with a degree or equivalent qualification and some experience of analytical chemistry) for research on air pollution and atmospheric diffusion—Dr. M. J. G Wilson, Chemical Engineering and Chemical Technology Department, Imperial College of Science and Technology, London, S.W.7.

LECTURER (capable of teaching at degree level) IN THE DEPARTMENT OF MATHEMATICS—The Registrar (R/S), Portsmouth College of Technology, Hampshire Terrace, Portsmouth, Hampshire

LECTURER IN PSYCHOLOGY (Experimental Psychology/General Psychology) —The Registrar, Goldsmiths' College (University of London), London, S.E.14.

RESEARCH ASSISTANT (preferably post-doctoral, with a Ph.D. or at least a good honours degree in chemistry, and research experience in the field of inorganic or physical chemistry) to undertake research on the preparation and properties of a range of metallic compounds of interest in developing new processes for producing metals—Dr. J. H. E. Jeffes, Metallurgy Department, Imperial College of Science and Technology, London, S.W.7

SCIENTIFIC OFFICER or SENIOR SCIENTIFIC OFFICER (with, or expecting to obtain, a first- or second-class honours degree in biochemistry or chemistry or a related subject) IN THE DEPARTMENT OF NUTRITION, primarily to take part in investigations of the biochemical aspects of protein digestion and utilization—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, Berkshire, quoting Ref. 66/N/18.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Royal Observatory Bulletins. No. 106: Cape Catalogue of Circumpolar Stars for the Equinox 1950·0. Reduced by W. Gliese. Pp. A75–A94. 3*s.* 6*d.* net. No. 107: Discussion of Lunar Occultations Observed in 1958 and 1959 (including Treatment of Observations from 1943). By Flora McBain Sadley. Pp. A95–A102. 2*s.* net. (London: H.M. Stationery Office, 1966.) [253

Metropolitan Water Board. Forty-first Report on the Results of the Bacteriological, Chemical and Biological Examination of the London Waters for the years 1963–1964. By E. Windle Taylor. Pp. 152+9 plates. (London: Metropolitan Water Board, 1966.) 32*s.* 6*d.* [253

The Schools Council. Working Paper No. 4: Science in the Sixth Form. Pp. v+57. (London: H.M. Stationery Office, 1966.) 4*s.* 6*d.* net. [283

Association of Commonwealth Universities. Report of the Conference on Industry and the Universities—Aspects of Interdependence. Pp. 86. (London: Association of Commonwealth Universities, 1966.) 5*s* 6*d* [283

University of Leeds. Annual Report of the Librarian, Session 1964–65 (Leeds: The Brotherton Library, The University, 1965.) [283

Report of the Rugby School Natural History Society for the year 1965. Pp. 23+1 plate. (Rugby: Rugby School Natural History Society, 1966.) [283

Wool Industries Research Association. Report of the Director of Research for 1965. Pp. 43. (Publication No. 226.) (Leeds: Wool Industries Research Association, 1966.) [283

Ministry of Agriculture, Fisheries and Food. Annual Report of the Director of Fishery Research 1965. Pp. iv+90. (Lowestoft: Fisheries Laboratory, Ministry of Agriculture, Fisheries and Food, 1966.) [283

Shipbuilding Inquiry Committee 1965–1966: Report. Pp. 209. (Cmnd. 2937.) (London: H.M. Stationery Office, 1966.) 13*s.* net. [303

Geographical Abstracts. A: Geomorphology, 1966/1, Abstracts 66A/1–208. B: Biogeography, Climatology, and Cartography, 1966/1, Abstracts 66B/1–120. C: Economic Geography, 1966/1, Abstracts 66C/1–204. D: Social Geography, 1966/1, Abstracts 66D/1–163. Annual Subscriptions: A 36*s.*, 5 dollars; B 36*s.*, 5 dollars; C 54*s.*, 7·50 dollars; D 54*s.*, 7.50 dollars. Volumes A–D complete 180*s.*, 25 dollars. (London: *Geographical Abstracts*, Department of Geography, London School of Economics, 1966.) [303

United Kingdom Atomic Energy Authority: Research Group. AERE-R 4980. Silt Movement Investigation in the Oxcars Spoil Ground, Firth of Forth, Using Radioactive Tracers, 1961 and 1964. By D. B. Smith and T. V. Parsons. Pp. 30+19 figures. 4*s.* 6*d* net. AERE-R 5080: An Investigation Using Radioactive Tracers into the Silt Movement in an Ebb Channel, Firth of Forth, 1965. By D. B. Smith, T. V. Parsons and R. L. Cloet. Pp. 6+7 figures. 4*s.* net. (Wantage: Isotope Research Division, Wantage Research Laboratory (A.E.R.E.), 1965. Available from H.M. Stationery Office.) [303

The Overseas Development Institute. Aid in Uganda—Education. By Peter Williams. Pp. 152. (London: The Overseas Development Institute, 1966.) 20*s.* [194

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Latin America: An Introduction to Modern Books in English Concerning the Countries of Latin America. A list prepared by the Hispanic and Luso-Brazilian Councils. 2nd edition, revised. Pp. v+42. (London: The Library Association, 1966.) 10*s.* (L.A. members 7*s.* 6*d.*) [194

The Department of Education and Science. Report of the Land Use Study Group: Forestry, Agriculture and the Multiple Use of Rural Land. Pp. x+110. (London: H.M. Stationery Office, 1966.) 8*s.* net. [194

The Countryside in 1970. Proceedings of the Second Conference, November 1965. Pp. xi+178. (London: The Royal Society of Arts and The Nature Conservancy, 1966.) [194

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Trans-Antarctic Expedition 1955–1958. Scientific Reports, No. 8: Geology. 1. Theron Mountains, Shackleton Range and Whichway Nunataks. By P. J. Stephenson. With a Section on Palaeomagnetism of the Dolerite Intrusions by D. J. Blundell. Pp. 79+10 plates. (London: Trans-Antarctic Expedition Committee, 1966.) 45*s.* net. 194

The National Union of Teachers Annual Guide to Careers for Young People. Pp. 80. (London: The National Union of Teachers, 1966.) 4*s* [194

British Museum (Natural History). The John Murray Expedition 1933–34. Scientific Reports, Vol. 11, No. 1: A Review of the Cephalopod Family Sepiidae. By William Adam and William J. Rees. Pp. iv+165+46 plates. (London: British Museum (Natural History), 1966.) 140*s.* [194

Instruments—Electronics—Automation Year Book and Buyers Guide 1966. Pp. 688. (London: Morgan Brothers (Publishers), Ltd., 1966.) 60*s.* [194

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ONE WHITE ELEPHANT

THE decision of the British Government to withdraw from the current programme of the European Launcher Development Organization is at best a calculated indiscretion. Evidently it is intended that a sufficient show of intransigence shall leave no doubt that the British Government is washing its hands of ELDO. In diplomatic circles behaviour of this kind, though rare, is not unknown. General de Gaulle's rejection of the British case for joining the European Economic Community in January 1963 was in the same tradition. By making an unreasonably ferocious statement he erased, no doubt deliberately, all possibility of compromise. The British Government cannot be surprised if the position it has now taken on ELDO is reminiscent of that earlier intra-European squabble. It will have ensured that there can be no confusion about its intentions only by acquiring something of a reputation as a querulous and unreliable party to international agreements. It has done much harm and scarcely any good.

The unreasonableness of the British repudiation of ELDO is particularly regrettable because the case against the current programmes based on the *Blue Streak* rocket is even stronger now than it was in 1962, when the convention of ELDO was opened for signature by European countries. The truth is that the organization has from the start been an attempt to put a cart before a horse. Once it had become apparent that *Blue Streak* had no great future as a military weapon, chiefly because of its vulnerability to pre-emptive attack but also because of the inconvenience of liquid fuels in military rockets, it was natural that the British Government should seek other uses for it. After all, nobody likes to be left with a white elephant on his hands. And when the British Government was anxious to explore ways of making closer links with Europe, it was only natural that *Blue Streak* should seem potentially a makeweight in that exercise. The trouble, which has been apparent from the start, is that a rocket system developed as a military weapon of intermediate range is unlikely also to be the most effective means of launching scientific satellites and of exploring the potential usefulness of such things as communications satellites as well. To say this is not to suggest that *Blue Streak* is a bad rocket, but merely that one rocket cannot be as versatile as a dozen. The ELDO programmes based on *Blue Streak* have never made much sense to technical people. The British scientists most directly concerned have often been resentful that so much money has been spent on a white elephant when comparable projects have been short of funds. The first thing to be said about ELDO is that it was wrongly begotten.

ELDO has also been badly organized. The first estimates of cost were hopelessly optimistic. To have supposed that it would be possible to sustain a five-year programme of advanced research and development, involving tests of rockets in places as far apart as the north of England and the Australian desert, and the engagement of laboratories in a dozen European countries, was wishful thinking of an ostentatious kind. The surprise is not that the most recent estimate of the cost is twice the original, but that it is still thought feasible to design a complicated satellite launching system on what is, by all experience elsewhere, a shoestring.

Then ELDO has been overtaken by events. If the organization had started work soon after it was first mooted, five years ago, it might by now have accomplished something useful. In the event, however, diplomacy has been the thief of time. Only in 1964 did things begin to move, and only now has it really become apparent what technical difficulties lie ahead. In the meantime, the United States and the U.S.S.R. have made unexpectedly rapid progress towards the exploitation of communications satellites. The advantages of stationary satellites, which are for practical purposes beyond the scope of ELDO, have been made plain. And there is no reasonable basis for hoping that an independent European development can yield such prowess in rocketry that Europe will be able to act, or think of acting, independently of the United States and the U.S.S.R. in the design of communications systems. It is therefore pertinent to ask the question that ELDO itself has consistently run away from—what is ELDO really for?

These arguments are now so strong that it is hard to see why the British Government has not set out to persuade its ELDO partners of their force. For if those who conceived of ELDO five years ago are now convinced that it has no future, should it not be possible for them to persuade the others that it is time for a re-appraisal? This is what reasonable men elsewhere will ask. And it would have been entirely proper for the British Government to have insisted that other nations should form a realistic view of what they hope that launching *Blue Streak* rockets from the Australian desert will accomplish for the future prosperity of Europe. If the British Government had asked that its European partners should learn a few of the elementary lessons of cost-effectiveness, that would have been admirable, for there is still evidence that skill in rocketry is sometimes confused with national prestige. In other words, the circumstances required that the British Government should have used its influence to bring about a realistic reappraisal of the purposes of ELDO. If it had failed to do so, withdrawal might have been permissible.

Whether ELDO survives these troubles is in itself comparatively unimportant. To the extent that the British assessment of the situation has been accurate, it may be better for Europe if the whole thing comes to an end. Certainly the defects apparent with British membership will not now vanish if Britain becomes a sleeping partner until the ELDO convention comes to an end in 1969. It is, however, important to know how these events will affect the institutions for technical collaboration in Europe, and it is important to know what future there will be for rocket technology in Europe.

The first thing to be said is that a sudden shock of this kind unsettles confidence. Though the British decision on ELDO may be legally valid, and though it may have been heralded by all kinds of warnings within the working parties and committees, the members of other international organizations are likely to look out for early warning of possible withdrawal. ELDO is likely to have

a nervous time ahead. Future developments at CERN will be closely watched. Will there be a British contribution for the 300 GeV accelerator that the planners would like to contract? That is what people will be asking. If the British Government wishes to show that its behaviour over ELDO is not pure pique, it could not do better than make some clear promise about the future of CERN.

The future of rocket technology in Europe is more difficult to foresee, but some things are plain. The problems of ELDO have sprung from trying to accomplish in five years, and with small resources, developments which have taken three times as long, and which have cost enormous efforts elsewhere. There is a case for supporting modest programmes of rocket development in Europe in the decades ahead, but it is inevitable that European countries will have to look to the United States and the U.S.S.R. for such practical applications of rocket technology as may be feasible in the decades immediately ahead. But in the short run, a capacity to launch rockets is less important than fair access to whatever the benefits of rocket technology may be. In other words, what has happened in ELDO should be a spur to the negotiations of a lasting and equitable agreement on the operation of the international system of communications satellites now being established. The existing agreement with the United States COMSAT Corporation, like the ELDO convention, will have to be renewed in 1969, and it is not too soon to begin making plans for what comes next.

NO MORE MANDARINS

THE departure of Sir Solly Zuckerman from the post of chief scientific adviser to the British Minister of Defence is something of an occasion. For one thing, he has been there so long that he will be missed, to say the least of it. For most of the past decade his influence has been pervasive, and on the side of realism. He has helped to broaden the thinking of the defence people, and in particular has done much to expose them to the influence of academics of all kinds. He has been enlivening and stimulating. It is therefore good to know that he will not promptly disappear from Whitehall. The suggestion that Sir Solly will now concern himself with research and development on the civil side of government is particularly welcome, partly because the recommendations of the Committee on the Management and Control of Research and Development, of which he was chairman and which reported in 1961, are still only partially digested, but also because the British Government has not yet paid as much attention to the management of its own research and development as to the scrutiny of what is done elsewhere with public money.

The days are long since gone when ministries were dependent for scientific advice on such a small number of people that each adviser became a kind of mandarin, possessed, in the eyes of politicians and soldiers, of magic such as the differential calculus, and set apart from academic colleagues by having access to classified information. Instead, quite properly, there are now arrangements for making sure that scientific advice and criticism are an integral part of planning for defence and not an afterthought. At the same time, there has been a shift of interest from technology to economics. Cost effectiveness is the order of the day, in defence ministries everywhere. That, too, is as it should be.

MATHEMATICAL METEOROLOGY IN THE U.S.S.R.

Investigation of the Bottom 300-Meter Layer of the Atmosphere

Edited by N. L. Byzova. Translated from the Russian by A. Moscona. Pp. v+112. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1965.) 27*s*.

Long-Range Hydrodynamic Weather Forecasting

Edited by E. N. Blinova. Translated from the Russian. Pp. iv+124. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1965.) 27*s*.

Objective Analysis of Meteorological Fields

By L. S. Gandin. Translated from the Russian by IPST Staff. Edited by R. Hardin. Pp. vi+242. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1965.) 81*s*.

AFTER 20 years of comparative stagnation, relieved by only a few isolated bright spots, dynamical meteorology began to move forward soon after the end of the Second World War to become, what it can now claim to be, a coherent branch of quantitative fluid mechanics. For a number of years progress was much stimulated by close relations between American and European workers, while the work going on in Russia was unknown and ignored in the West, and it is only in quite recent years that representative literature has become available and readily accessible in translation. With still many gaps to be filled, the initiative of the Israel Program for Scientific Translation in publishing the three meteorological texts under review will be widely welcomed.

Investigation of the Bottom 300-Meter Layer of the Atmosphere is a collection of thirteen short research papers concerned with the data from a 300-m meteorological mast. The study of the planetary boundary layer is one in which the basic theoretical contributions from the Soviet Union have been of the greatest importance, and it suffices to draw attention to this translation to ensure that all workers in the subject will give it their attention.

Long-Range Hydrodynamic Weather Forecasting is also a compilation of research papers, seven in all, providing further examples of mathematical techniques and physical concepts which, in spite of the title, were largely anticipated by Kibel's[1] text-book which appeared in translation some years ago. Again, the mere notice of the work will ensure that it will be scrutinized carefully by the relatively few specialists engaged in mathematical studies of problems of meteorology on the largest scale. Special attention may, however, be drawn to a paper by M. B. Galin which attempts to set up a closed set of equations to determine certain correlation functions which emerge when the basic dynamical equations are averaged to produce relations between climatological or other time-mean values. The treatment of these functions, effectively of synoptic-scale turbulence, is perhaps the most fundamental problem of fluid mechanics for which no basic solution applicable to world-scale problems is yet in sight. It is doubtful whether purely mathematical manipulation will really overcome the physical difficulty, but the attempt will be studied with interest.

The third work, *Objective Analysis of Meteorological Fields*, is, by contrast, a valuable monograph on a limited theme, and prompts a little digression. The post-war application of basic fluid physics to atmospheric motions of weather systems on the largest scale and to the general circulation is particularly important and interesting because of its direct relevance to routine weather forecasting, more or less familiar to everyone. The techniques in use hitherto depended on the charting of weather patterns and particularly of barometric pressure patterns drawn freehand and extrapolated into the future by expert judgment—a highly subjective process. It should

perhaps be kept in mind that the same procedures are still in use almost everywhere in the world, and that the new mathematical techniques have as yet been introduced into routine practice only at a few weather centres in spite of 20 years' steady progress in a few leading countries.

The topic of L. S. Gandin's monograph is just one facet of the whole complex, and is concerned with methods whereby the raw data of meteorological observations received over telecommunication networks from all parts of the world may be fed directly into the high-speed computer to define the initial conditions of the dynamical problem without the need for expert analysis. The difficulties that arise are mainly specific to the problem and derive from the unevenness, sparsity and errors of the observations coupled with real variability on scales smaller than that of the problem. Where data are sparse (especially over the oceans), linear interpolation is hopelessly inaccurate; where data are numerous, smoothing is essential. For these reasons, there is no ready definition of a 'best fit', and quite elaborate techniques combining interpolation, smoothing and weighting have been developed and justified empirically. The main purpose of Gandin's book is to present a system of 'optimum interpolation'. It is essentially a method of least squares to determine grid-point values of the variables in terms of the data and the structure functions and correlation functions of the fields, statistical quantities much more familiar in turbulence theory than in world-scale dynamics. The book touches on a number of other problems which arise in weather analysis and, as a rare example of academic elegance and clarity applied to this field, will be welcome not only to research workers but also to teachers who struggle to provide students with an insight into what is now a rapidly developing technology. The bibliography, comprehensively covering 'western' as well as Russian literature, will be really useful, and the introductory chapter which gives an account of the non-Russian work is equally helpful.

In this monograph, structure functions and correlation functions are introduced as statistical concepts to be applied to a statistical problem, but the formal discussion is welcome, as their application to large-scale dynamics is of growing importance. Their value in the theory of smaller scale turbulence, including the atmospheric boundary layer, is, of course, well known, but we need look no further than a paper by Smirnov, included in Prof. Blinova's book, to find evidence of special complications. This paper shows the geographical patterns of covariance of pressure between selected positions and all other positions in the northern hemisphere. The inhomogeneous nature of large-scale turbulence is clearly shown and confirms that the climate of the real Earth, however it may be disguised, differs from place to place with longitude as well as with latitude, a geographical fact which some geophysicists still find uncongenial.

R. C. SUTCLIFFE

[1] Kibel, I. A., *An Introduction to the Hydrodynamical Methods of Short Period Weather Forecasting*, translation edit. by Baker, R., 383 (Pergamon Press, 1963).

ROCK METAMORPHISM

Controls of Metamorphism

Edited by Wallace S. Pitcher and Glenys W. Flinn. (A Symposium held under the auspices of The Liverpool Geological Society.) Pp. 368. (Edinburgh and London: Oliver and Boyd, Ltd., 1965.) 84*s*. net.

CONTROLS of Metamorphism is the proceedings of a symposium held in the Department of Geology at the University of Liverpool early in 1964, called to provide an opportunity for assessment of present understanding of the geological factors which bring about metamorphic changes in rocks and of the conditions in which such changes are effected. In the course of a re-examination of many familiar text-book statements concerning the significance of temperature, of pressure (both hydrostatic and non-hydrostatic) and of chemical environment, some time-honoured concepts were subjected to renewed and exacting scrutiny.

In an opening generalized survey J. Sutton adopts at once the view that regional metamorphism is essentially a thermal phenomenon. He holds that unusually great accessions of heat, in conditions which are geologically abnormal, are maintained for variable long periods, although such conditions are not widely developed at any one time. Other writers concur, but not all. M. P. Atherton examines the chemical significance of isograds and writes that recent work seems to indicate that pressure is relatively unimportant compared with temperature in relation to the definition of an isograd as a line joining points of similar pressure/temperature values. E. D. Lacy uses some striking examples of the interplay of temperature and activation energy factors to emphasize the importance of temperature in determining reaction rates. The view that there has been a tendency to minimize the importance of pressure relative to temperature is put forward by R. W. Rutland, who points out that recent experimental work at high pressures has emphasized a number of reactions which may properly be described as pressure-dependent. Harker's concept of stress minerals is rejected, the real importance of non-hydrostatic stress probably lying in its effect on the kinetics of reactions and the promotion of metastable growth. Rutland is mainly concerned with the problem posed by the often striking lack of agreement between geological estimates of depths of formation of some metamorphic minerals and the depths which seem to be indicated by experimental work. Likely tectonic overpressures appear inadequate to bridge the gap, and a chemical explanation (including the possibility of metastable growth) must be considered.

The textures of metamorphic rocks receive detailed attention from D. Flinn and N. Rast—many petrologists will fully endorse the opinion of the latter that preoccupation with the study of metamorphic facies has led to widespread disregard of textural patterns. Both these authors make frequent reference to the literature of solid-state physics, particularly metallurgy, and their contributions are followed by a helpful account of the science of metamorphism in metals presented by D. McLean, who applies the present development of these theories to a consideration of metamorphism in rocks. With the very different amounts of time available and very different temperatures prevailing, small variations in composition of a mineral may have a profound effect on equilibrium relationships—one example of many references to the way in which such relationships may come to depend a great deal on the particular composition of the rock in which a mineral is developed. Inherent in several contributions which are concerned with problems of host-rock composition and the chemistry of component minerals is the question whether chemical equilibrium is in fact commonly attained. Atherton, in his discussion of isograds, believes that in general it is. It has, of course, been recognized from the time of the earliest applications of the concept of index minerals that their conditions of genesis and their composition are often closely related to the bulk composition of the rock, and some "reversals" may thus be explained. Systematic variations in composition of micas, which are closely related in this way to the host-rock, are demonstrated by B. C. M. Butler, who points out that this situation may give rise to some difficulty in the determination of grade; and B. E. Leake reports similarly that over a wide range of temperatures and pressures it is the rock composition which mainly controls the composition of calciferous amphiboles.

Metasomatic metamorphism and the origin of migmatites provided yet another field for exploration. Discussions, opened by invited speakers, followed the presentation of the papers but are not recorded in full, though

there are frequent references to them in a general introduction to the volume by W. S. Pitcher. The reader is, in fact, occasionally tantalized by a remark such as "It is of interest to note that the validity of the basic assumptions [in isotopic dating procedures] could still be the subject of a vigorous debate". True to the original derivation of the word 'symposium', the meeting at Liverpool provided a feast of ideas (not all of which we have been able to mention here) as varied as the courses at a banquet and differing as widely in flavour and in nutritional value. They make a significant addition to the literature of metamorphism. The volume is described as *Geological Journal Special Issue No.* 1; we are encouraged to look forward to future special issues of similar quality.

F. C. PHILLIPS

ULTRASONICS IN MEDICINE AND BIOLOGY

Ultrasonic Energy

Biological Investigations and Medical Applications. Edited by Elizabeth Kelly. Pp. 387. (Urbana, Ill.: University of Illinois Press, 1965.) 12.50 dollars.

ULTRASONIC Energy brings together summaries of achievement and research in most aspects of ultrasonic technology as applied to medicine and the biological sciences, and is a good description of the subject as it stood three years ago. It is largely, if not entirely, the proceedings of the symposium on "Ultrasound in Medicine and Biology", held in the University of Illinois in June 1962. It is a very well produced book with excellently prepared diagrams, photographs and microphotographs, many in good colour.

In medical and biological research to-day, ultrasonics is providing useful assistance toward diagnosis by the delineation of structures as an alternative to X-rays for soft tissues or where radiation damage might be dangerous, for example, in the diagnosis of pregnancy. It is also being used therapeutically for producing discrete brain and nerve lesions, and in smaller doses for treatments in physical medicine. In biology it assists investigations of cell structure, helps to identify proteins and augments the destructive effects of X-rays on malignant tumours. It has the advantage that it can be focused and directed into the body with considerable precision.

Ultrasonic Energy will bring many aspects of the subject to the attention of workers whose main study is limited to a particular branch. Medical readers will find much of interest and ideas worth further study.

There are twenty-six sections or articles, each prepared by an expert research worker. Eight of these sections are on diagnostic techniques (for the eye, brain, heart and abdomen) by the pulse-echo technique; four are on surgical uses of high-intensity ultrasound, two on the effects of ultrasound on malignant cells, two on the neuromuscular system, one on the liver with emphasis on cellular effects, one on the effects on the ear and one is an abstract of work on physical medicine. There are five good articles on the action of ultrasound in biological tissues at different intensities and analysis of the effects of cavitation, of gas bubbles and streaming of cell protoplasm.

Practically the entire contents of the book can be found elsewhere by searching the technical and medical literature, but there is convenience in their concentration in a single volume. The expert reader should, however, be reminded that the book has scarcely been re-edited since the conference and, of the copious references, only four are dated after 1962. The article on physical medicine is given in a half-page abstract because the writer could not attend the conference; surely his full manuscript might have been obtained in the succeeding three years, especially as the book, in its title, does not purport to be a record of the conference but a text-book of the art. It contains verbatim reports of the discussions which followed each paper, even about films which the reader cannot see. As is usual in discussions in a large symposium, many of the remarks and questions are wide of the mark.

It surprises me that, with so much apparently known of the value of ultrasound coupled with X-rays for the destruction of malignant cells, this application has no advocates in the clinical field.

The contributors are from Europe and Japan as well as from many parts of the United States and it is internationally a comprehensive book.

C. N. SMYTH

FROM LIGHT TO SIGHT: THE METABOLIC BASIS

Biochemistry of the Retina

Edited by Clive N. Graymore. (1st International Symposium, London, September 1964.) Pp. xiv+172. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1965.) 60*s*.

VISION is a complex process involving a multitude of phenomena ranging from the relatively simple refraction of light to the interpretation of the nervous impulses in the brain. *Biochemistry of the Retina* is concerned with some of the intervening processes by which quantum energy is transformed into chemical and electrical signals, how this highly specialized tissue is maintained in a functional state and how diabetes, retinal degeneration and retinotoxic substances which each disturb metabolism and function may give a better understanding of normal processes.

Twenty papers were presented at the first international symposium on this subject and, although the approach and emphasis were extremely varied, the conclusions reached are entirely complementary. Discussion followed each paper, thus allowing alternative interpretations to be suggested and correlations with other findings to be emphasized. By including these, the editor has welded the individual reports into a coherent and valuable book.

Three papers deserve special mention, and a fourth, on the limitations in the use of tetrazolium salts for the histochemical localization of dehydrogenases, is important in view of the widespread use of this method in studying the retina. W. K. Noell examines the relationship between metabolism, light-evoked potentials (electroretinogram) and morphology of the retina, comparing normal animals, those with hereditary retinal degeneration and those with experimentally damaged visual cells. He concludes that the particular vulnerability of the visual cells to X-rays, hyperbaric oxygen, high light intensity and iodoacetic acid may be due to the presence of a light-activated energy transfer system which is highly susceptible to detrimental oxidations. A note at the end of the symposium on the role of ubiquinone is particularly relevant to this thesis. A defect in protective mechanisms could be a crucial factor in the genetic disease. L. Cohen, in conjunction with Noell, uses similar findings as a springboard for speculation on the molecular mechanism of the initial events of vision. Not all the facts fit the theory (or vice versa) but it is a worth-while exercise with stimulating ideas and a framework for new thought. Finally, M. Keen and C. Chlouverakis present an interesting hypothesis on the mechanism of diabetic retinopathy which evoked considerable discussion and approval.

Poor proof-reading of one paper detracts from the otherwise very high standard of editing and presentation, and two contributors have submitted inferior photomicrographs. It would have helped the wide range of non-retinal biochemists whom the book will surely interest if a consistent labelling of retinal sections had been adopted. Nevertheless the book begins to fill, most

admirably, a large gap in our knowledge of visual processes; it will be invaluable to the specialist and of absorbing interest to those with time to wonder.

M. V. RILEY

THE WORLD'S RICEBOWL

Rice

By D. H. Grist. Fourth edition. (Tropical Agriculture Series.) Pp. xxiii+548+74 photographs. (London: Longmans, Green and Co., Ltd., 1965.) 75*s*. net

THE importance of rice as a staple diet for many millions, particularly in the Far East, is underlined by the fact that nearly nine-tenths of the world production is in this area. Rice is also predominantly a peasant crop; only 6 million out of a total annual production of about 160 million tons enter world trade; much rice is consumed by the grower and most of the remainder in the country of production. This also highlights one of the difficulties in increasing yields per acre because, following the introduction of improved varieties, the chief means of doing this are by better water control and by the increased use of fertilizers, herbicides and pesticides. These improvements require a change from subsistence farming towards cash cropping. The heavily indebted peasant populations do not have the necessary money, so that social and financial as well as technical changes are involved in this very urgent struggle to increase world food supplies.

The present edition of the valuable book, *Rice*, by D. H. Grist will prove helpful to those engaged on all aspects of rice production—plant breeding, paddy growing, and rice processing and marketing. The book has been divided into three parts: 1, "*Oryza sativa* Linn.: The Plant"; 2, "Paddy Production"; and 3, "Rice: The Product". The arrangement of chapters between the parts does not appear entirely logical, because Part 1 contains chapters dealing with climate and soils, and water supply and control, and Part 3 covers the very wide range of subjects from milling and storage to production statistics and conditions of land tenure. The titles to the parts indicate the scope of the text, which occupies 463 pages. In Part 1, Mr. Grist starts with a short history of rice, digresses to soils and water supply and returns to his main theme with descriptions of the plant, and of varieties, and concludes this section with chapters on the genetics and breeding of rice. Part 2 covers the many facets of paddy production—methods of cultivation, fertilizers, weeds, diseases, and pests, and a useful description of fish farming in paddy fields. Part 3 describes the storage and milling of rice, factors affecting the nutritional value of rice, and finishes with chapters on the statistics of production and consumption and on economic conditions. Following the main text, there are three short sections: (i) seven appendixes relating to topics in seed testing, terminology and rice breeding; (ii) a bibliography with more than 700 entries, which are arranged alphabetically and numbered, so that reference to a particular author is easy and text references are exact; titles are also given, which is valuable in such a large and varied bibliography; (iii) the book concludes with a good index.

Mr. Grist has drawn his material from several standard works, from reports of the Food and Agriculture Organization and elsewhere, as well as from original work published throughout the world. He has quoted much that is not available to the normal literature searcher and therefore performs a most useful service. Unfortunately, his style tends to be indirect and at times slightly wordy, so that continuous reading of the book is something of an effort. There are one or two minor inconsistencies; for example, the columns in Table 2 do not have uniform units, and some of the headings do not allow the exact compound to be identified. The photographs and line drawings (which have been little altered since the first edition) complement the text admirably. There are very few misprints (and those noted are only single letters wrong). When reviewing the third edition of this book, the late Dr. H. Greene wrote a short article describing the current ideas on oxidation-reduction in paddy soils, and quoted some results by workers at the West African Rice Research Station (*Nature*, 186, 511; 1960). One would have liked to have seen Mr. Grist use these ideas and expand them with the work published between 1960 and 1964 for the present chapter on soils. However, these points are less criticisms of the present book than suggestions for any future revisions. There is no doubt that Mr. Grist's book has become a standard work on rice and is invaluable to anyone seeking a balanced opinion on any topic connected with the crop, or a guide to the original literature. At the price, the book represents good value for money and individuals as well as libraries should be able to afford to buy it.

J. K. R. GASSER

FINGERPRINT SCIENCE

Finger Prints

By Francis Galton. Pp. xxiii+xvi+216+15 plates. (New York: Da Capo Press, 1965. Originally published 1892.) 6.95 dollars.

THE scientific study of the dermal ridge patterns on the finger-tips has three aspects. First, it leads to a method of personal identification, secondly it forms the basis for genetical and anthropological investigations and, thirdly, it has significance in relation to certain malformations and growth disturbances. The most notable pioneer in the first and second fields was Francis Galton, whose chief work on the subject, *Finger Prints*, was originally published in 1892. This delightful book contains not only a very full description of the anatomy of variations in finger ridge patterns but it also gives some account of the historical background and initiates hereditary enquiries.

The main emphasis in Galton's work, however, is on the matter of personal identification. This application of dermatoglyphic science transcends all others in its scope and it chiefly concerns the small details of the ridge formations which seem to be chance variations rather than hereditary characters. As Galton says, "For instance, it would be totally impossible to fail to distinguish between the finger prints of twins, who in other respects appeared exactly alike". He explains how a system of identification by finger prints would be vastly more efficient than that proposed by Bertillon using an assortment of physical measurements and body markings. One great advantage of ridge configurations is that they do not alter with age—at least, their relations do not alter. "The pattern grows simultaneously with the finger, and its proportions vary with its fatness, leanness, usage, gouty deformation, or age. But, though the pattern as a whole may become considerably altered in length or breadth, the number of ridges, their embranchments, and other minutiae remain unchanged." On the general question of the use of these methods, Galton says that "in civilized lands and in peaceable times, the chief use of a sure means of identification is to benefit society by detecting rogues, rather than to establish the identity of men who are honest".

The reprint by the Da Capo Press is most welcome, since very few copies of the original are now to be found. An enlightening introduction is aptly supplied by the anatomist, Dr. Harold Cummins of Tulane University, who has been the pioneer in researches in the third field of dermatoglyphic studies, namely, their pathological applications. This addition amplifies considerably Galton's own historical account and directs attention to the world of Faulds, which was surprisingly neglected by Galton. Galton's failure to mention Nehemiah Grew might also have been

noted and perhaps the remarkable translation from Purkinje's paper might have been commented on.

Galton's ample illustrations are not so well reproduced as might have been desired. For example, Figs. 7 and 15 are too heavily printed and Figs. 9 and 19 seem to be fading away. Otherwise the new book is well produced. At the present time, when many new data are becoming available on chromosomal aberrations associated with deformities which include alterations in papillary ridge patterns, it will be particularly useful to have this classical work easily available. L. S. PENROSE

MODERN SURFACE COATINGS

Modern Surface Coatings

A Textbook of the Chemistry and Technology of Paints, Varnishes, and Lacquers. By Paul Nylén and Edward Sunderland. Pp. xiv+750. (London and New York: Interscience Publishers, a Division of John Wiley and Sons, 1965.) 147*s*.

A GOOD index of the rate of development of a branch of science or technology is provided by the number of books published on it. There has been a great flowering of books dealing with surface coatings, especially paints, in the past five years or so, rightly reflecting the active technological development and the growing orientation towards a systematic and science-based approach to the subject. In addition to specialist monographs on specific subjects, there have also been several general treatments of the field, and *Modern Surface Coatings* must be judged in relation to these, as well as on its own merits.

The important part in surface coatings played by developments in raw materials is illustrated by the allocation of space in the present book; there are 275 pages on polymers and polymerization, 50 on solvents and plasticizers and 170 on pigments—almost 500 in all on raw materials, compared with 120 pages on pigment dispersion, film properties and paint application, and 80 pages on specific types of paint. While accepting the importance of raw materials, one would have liked to see fuller treatment of the problems specifically related to paint making and using, the sections on surface preparation and application methods being particularly limited.

This having been said, it is difficult to find fault with the book as a general introduction to paint science and technology, and it should prove invaluable to students everywhere; the sound approach doubtless owes much to the first-hand experience of the authors in teaching the subject at the Royal Institute of Technology in Stockholm. Emphasis throughout is placed on the scientific principles behind the subject, and the authors have—rightly—little time for empiricism and the development of complicated formulations. As they say (p. 609) regarding the case of defects: "a thorough investigation and the use of a little physico-chemical imagination are much more likely to lead to a satisfactory and lasting solution". Readers need not expect, therefore, to find specific paint formulations in this book.

The general approach is to provide a balanced, critical and up-to-date survey; also a much more international outlook than usual is provided, with references to publications and standards drawn freely from the major contributory countries—mainly the United States, Germany and Britain, supplemented by specific Scandinavian contributors where appropriate. Another refreshing and useful feature is the use made of trade names and references to trade literature, whenever this seems the best source of information.

Minor omissions are the absence of any reference to polyvinyl fluoride polymers, and the curious omission of zinc silicates in the sections on inorganic and anti-corrosive paints, their treatment being restricted to anti-fouling paints. Only minor errors of fact were noted, and the whole book reads extremely well. References are numerous and up to date, one for 1965 being noted.

A better title would have been "Principles of Surface Coating Technology", but this was already pre-empted by the book by Parker from the same publishers; indeed, as Parker's book deals much more soundly with formulations than with principles, it is a pity the titles could not be reversed!

To sum up, *Modern Surface Coatings* should be of great value to students, lecturers and newcomers to the industry, and perhaps be educative even to established technologists. It is the best single-volume treatment of paint science and technology available to-day. L. VALENTINE

RUSSIAN FOR SCIENTISTS AND OTHERS

Complete Russian Course for Scientists

By M. Beresford. Pp. xvii+227. (Oxford: Clarendon Press; London: Oxford University Press, 1965.) 25*s* net.

COMPLETE Russian Course for Scientists is an excellent book and the key to its excellence lies in the fact that the title could easily have been "Complete Russian Course for Scientists and Others". There are a number of books in existence which claim to purvey Russian for scientists, and in most of them there is little or no attempt to teach the language. The books are useful—in a very limited way—for the particular scientists the authors have in mind (chemists, physicists, geneticists, etc.). They are rarely helpful to scientists in general.

The book under review is a very different affair—"a rapid, intensive course, designed to enable beginners to master the essentials of Russian for reading purposes". The author, obviously interested in philology, has produced a book which can be used not only by scientists and technologists, but by all students of Russian. It concentrates on the main features of Russian grammar and introduces scientific jargon gradually and progressively into the exercises, at first in the form of isolated phrases and then, in moderation, in the form of short texts.

The book contains twenty lessons (pp. 1–167), a supplement (pp. 171–193) containing eight divisions, including declension tables, tables of verbs, metric-English equivalents and a most useful list of Russian abbreviations, and finally (pp. 195–224) a select Russian–English vocabulary.

For the benefit of potential users of this book, here are a few notes on the contents of certain chapters. Lesson 1, as one would expect, deals with the Russian alphabet and includes numerous exercises. Lesson 2 deals in a masterly fashion with pronunciation, a most important feature of the Russian alphabet, with hard and soft vowels, stressed vowels and consonants and like topics. Lesson 3 deals with the structure and derivation of words. Lessons 4–12 cover the cases of nouns and the tenses and aspects of verbs. Lessons 13–20 deal with an important selection of topics—Russian names, pronouns, particles, participles, numerals, fractions, gerunds, compound words, suffixes and roots.

It will be clear from this brief review of the contents that this book gives all the grammar necessary to provide an adequate background for a reading knowledge of the language, and this is supplemented by appropriate exercises. Two final points for the reader who wants to get full value from the book—the Russian alphabet is very necessary for constant reference during the early stages of the course. A spare copy on a card, which could easily be moved from page to page, would facilitate such reference. The second point is in the nature of a warning. This is not a book to be read through. It calls for hard work and determination, but the author has done his best to make such hard work and determination a good investment.

S. I. TOMKEIEFF

EUROPEAN LAUNCHER DEVELOPMENT ORGANIZATION—BEGINNING AND END

THE European Launcher Development Organization (ELDO) began with the abandonment by the United Kingdom Government of the development of the *Blue Streak* military rocket in April 1960. The rocket, which had been intended as a ballistic weapon with a range of 2,000 nautical miles, was built around two rocket engines of a type similar to that used in the American *Thor* and *Jupiter* rockets, but constructed and developed in the United Kingdom by Rolls-Royce, Ltd.

Before the decision to abandon *Blue Streak* was announced, studies had already begun of the potential usefulness of *Blue Streak* for civilian purposes. In September 1960, the United Kingdom, after suitable diplomatic preparation, invited a number of European countries to participate in an organization for launching satellites. The conference met in Strasbourg on January 30, 1961, and was attended by ministers from the United Kingdom, France, Belgium, Denmark, Germany, Italy, the Netherlands, Norway, Spain, Sweden and Switzerland. Observers attended from Austria, Canada, Greece and Turkey.

At this time the British Government was separately concerned in discussions with members of the European Economic Community about the feasibility of British entry into the Common Market. At the same time, too, initiatives were being taken to establish a European organization for launching scientific satellites (ESRO) the existence of which was not affected by the discussions on the future of ELDO.

At the Strasbourg conference in 1961 it was agreed that if a European organization for launching satellites were created, the first programme would be the development of a three-stage rocket based on British and French experience. The first stage of the rocket would be built by the United Kingdom, the second by France, the third stage and any satellites to be launched by other European countries. Tests of the complete vehicle would be carried out at Woomera in Australia.

It was also agreed at Strasbourg that any organization would be exclusively pacific and that all technical information arising out of the developments would be placed freely at the disposal of member governments. The British and French Governments agreed that their own experience would be placed at the disposal of the joint organization. (This raised the question, which has never been cleared up, of whether the British Government was entitled to place at the disposal of other European Governments information acquired under bilateral military agreements with the United States.)

At the first meeting it was also agreed that the estimated cost of the first programme—£70 million—would be spread over five years and would be shared between the participating countries on the basis of a third share by the United Kingdom and a division of financial responsibility among other countries in proportion to national income.

The Strasbourg conference was followed by a meeting in London in October 1961 at which Australia joined the participants. By this time it had been agreed that the organization should undertake a study of other possibilities than those based on the *Blue Streak* rocket. At this early stage it was plain that the estimated cost of £70 million for the first programme might not be realistic. It was further agreed that any expenditures exceeding this estimate would be divided among the member countries of any organization set up on a basis which might have to be negotiated within the organization at a later stage.

The formal convention of ELDO was opened for signature on April 30, 1962, and was promptly signed by Australia, Belgium and France, West Germany, Italy, the Netherlands, and the United Kingdom. Technical and administrative committees were established as were headquarters in Paris. A number of legal difficulties arising from the lack of definition of the powers in international law of the preparatory committee was eventually overcome. Throughout 1963 plans were drawn up for an initial programme and finally it was agreed that the convention should become binding on its signatories on March 1, 1964. Because by this time it was plain that the Scandinavian countries, together with Austria, Spain and Switzerland, would not sign the convention, it was agreed that the financial responsibility of those countries would be shared between France, West Germany and the United Kingdom (which increased the British share of the total cost to 38·79 per cent, compared with 24 per cent for France and 22 per cent for West Germany).

By the beginning of 1965 it was plain that the original estimate of cost of £70 million would have to be exceeded. For one thing, the original plans were acknowledged to be over-optimistic, and in April 1965 it was recognized that expenditure of some £22 million more than the original estimate would probably be necessary. At the same time it was argued by the technical working group that the initial programme based on *Blue Streak* and upper stages with conventional fuel would have to be replaced by a programme called ELDO *B*, involving liquid hydrogen fuel, if desirable objectives were to be attained. It was estimated that the cost of this programme, in 1965, would bring the total to more than £140 million. Further refinements of the system suggested more recently by working parties of the organization would have involved costs rising to perhaps three or more times the original estimate of £70 million.

The implementation of the initial programme has involved so far the launching of one first-stage rocket (*Blue Streak*) from Woomera. The first launching of a rocket complete with upper stages had been planned for the end of 1967 at the earliest. The chances that the programme of satellite development and launching originally conceived could be completed within the five years set aside for the ELDO agreement have become increasingly remote.

British dissatisfaction with the rising cost of ELDO has been expressed openly in ministerial circles for at least eighteen months. It has also been pointed out that the time is long since past when the ELDO development could be usefully thought of as a basis for a European communications satellite system. The first ELDO ministerial meeting since the signature of the convention, held in Paris at the beginning of May 1966, ended inconclusively with a decision to allow a period for further consideration of proposed refinements of the ELDO system intended to increase the launching capability of the system, and for further consideration of the cost thereof. The British Government's decision to withdraw from the current ELDO programmes derives directly from that first ministerial meeting.

According to British sources, there is no reason why other European countries should be surprised at the British decision, which is expressed in the following form of words:

"For some time the Government has had serious doubts about whether or not it should continue to participate in the ELDO programmes. These doubts were centred on financial, technical and economic assessments of the initial programme.

"The Government has concluded after a very careful and detailed consideration of all the factors involved that the latest proposals for modifying the initial programme still do not constitute a sufficient basis for continuing United Kingdom participation in the activities of the Organisation, and it has so informed its partners."

THE FRANKS REPORT—AN INSIDE VIEW

By Prof. R. E. RICHARDS
Physical Chemistry Laboratory, University of Oxford

[This comment on the Franks Report by Prof. R. E. Richards, Dr. Lee's professor of physical chemistry at Oxford, differs in part from that which appeared in *Nature* on May 14 (**210**, pp. 667–8), particularly on admissions policy and balance between humanists and scientists. Its publication here should not be taken to indicate anything but a belief that public discussion of the Franks Report is in the public interest—or at least in the interests of the University of Oxford]

THE report of the Franks Commission on the University of Oxford was published on May 12, after 2 years of intensive work [*Nature*, 210, 661, 667; 1966]. Hasty judgments on the complicated issues raised in the report are not likely to be of much value, but the following personal impressions concern some of the matters raised and the preliminary reactions they have produced.

The character of a university is bound to be strongly influenced by its admissions policy, and the procedure at Oxford has been widely criticized on various, sometimes incompatible, grounds. Before 1945, most who had suitable qualifications and could pay the fees could come to Oxford. After the 1944 Education Act, however, applications for admission increased very rapidly and far exceeded the capacity of the colleges. For many years the colleges had been setting an examination for the election of Scholars and Exhibitioners, and this well-tried system soon came to be used for the selection of nearly all candidates for admission. The examination has proved to be very effective at selecting promising young men, and those who have had a third year in the sixth form have been at a great advantage over others. It has thus been responsible to some extent for the criticisms often made simultaneously that Oxford takes too much of the cream of talent among university entrants and at the same time favours the independent schools.

The Commission has recommended that the examination should be retained, but with special questions only for those who are in the second year of sixth-form work. This proposal has already been anticipated by the scientists, who are setting a special paper in the next examination, which may be taken only by those who are in their second year after Ordinary Level. It is hoped that this proposal will accelerate still more the trend towards a greater proportion of candidates from maintained schools (only 19 per cent of those admitted in 1938–9 as against 47 per cent in 1965). It is interesting to note that almost the same proportions of those applying from maintained schools, from direct grant schools and from independent schools were admitted in 1965.

The proposal to abolish closed awards will also be generally welcomed. Their origin is historical and they are anomalies at the present time; 45 per cent of the closed awards in 1965 were made to candidates from independent boarding schools. Opinion among university teachers and school teachers is divided on the value of Open Scholarships and Exhibitions. Some think that the incentive to study for an examination without a restricted syllabus is valuable, others that it distorts the school curriculum. The weight of evidence to the Commission strongly supported the view that the present Advanced Level Examination, rather than the Oxford Scholarship Examination, is the main cause of undue specialization in the sixth form. The report recommends that the number of scholarships should not exceed 10 per cent of those admitted, thereby saving about £100,000 a year for other educational purposes. On this basis a small college might be able to offer only five or six scholarships a year, so that comparison might have to be made between very different subjects. The choice between, say, a physicist and a historian is often likely to be a rather arbitrary one, and for this reason many tutors would prefer to see scholarships for undergraduates abolished altogether. One compromise might be to award scholarships for one year only, and then to re-allocate them each year on the basis of progress made that year. This is open to the objection that it depends only on the personal judgment of a tutor.

The award of scholarships to postgraduates by colleges is recommended, and many recommendations are also made on the admissions of postgraduates, but these seem to be more applicable to the arts than to the sciences.

The size of the University is discussed at some length. As expected, the Commission has concluded that the collegiate system has so many advantages that the University of Oxford should not be allowed to grow so much as to undermine this important feature. Furthermore, the essentially democratic constitution of the University is easier to maintain than it would be if numbers were allowed to rise by a large factor. The suggestion is that the present population of 9,800 should be allowed to grow gradually to about 13,000 during the next 15–20 years. Much of this growth is expected to be among postgraduates, whose numbers will rise from 2,200 to 3,500–4,000. The report recommends that the number of women should be increased by seven hundred. These increases in numbers must, of course, be accompanied by increases in accommodation and this is inevitably a slow process. There is already a desperate shortage of lodgings in Oxford and it would be foolish to imagine that these increases can be accepted without further building.

On the distribution among subjects, the report recommends that much of the growth in numbers should occur in the applied sciences; an undergraduate school of about five hundred engineers is envisaged. A major expansion in the pure sciences and social studies is also recommended and, because of the limitation of total numbers, a compensating contraction must occur in other subjects, within an agreed programme of development. Many exciting new developments in pure and applied science have already been planned and we all welcome the encouragement of the Commission.

The teaching in Oxford has traditionally depended on the tutorial, and although the Franks Commission heard some criticisms of this method, I am sure that the great majority of teachers have no doubt about its value. The Commission quite rightly points out that few undergraduates can prepare themselves adequately in one week for more than one tutorial, and it recommends that the tendency in some subjects to multiply tutorials and to use them as a means of "handing out" information

should be stopped. Among the interesting statistics on the tutorial is the fact that 90 per cent of all tutorials in science are given by Fellows or lecturers and only 6 per cent by graduate students.

The report makes it clear that "research, in the broadest sense, is not something which academics do in the spare time they have left over from teaching, but that it is the first priority in their lives, giving meaning to the rest of their activities". One of the most common complaints from the academic staff was lack of sufficient time for their research, and the detailed survey by the Commission clearly shows that the reason for this is the very heavy burden of teaching borne by the academic staff. For example, the ratio of students to teachers, weighted according to the formula of the University Grants Committee for science at Oxford, was 13·5 in 1964–65. In other British universities, excluding Oxford, Cambridge and six new universities, the average was 9·8. The report recommends that the ratio at Oxford should be brought down to the national average "as a first step". This would involve the appointment of 124 more senior academic staff in science alone, and it is plain that this could only be done over a prolonged period. If it took 20 years, it would correspond to an increase at the same average rate as in the period 1922–65. It is not enough to find the men and the salaries to pay them; laboratories and equipment would also be needed. The report points out that the Holford Plan for the science area could provide an additional 1,000,000 sq. ft. of buildings. This would be enough to cope with the expansion, but on even the most optimistic estimate it could only be developed over a period of 10–20 years. The first step must therefore inevitably be a slow one.

The solution offered by the Commission is to reduce the hours of contact with pupils (including research students) to 14 per week, which is still much higher than the national average. Even this seems a pipe-dream to some scientists at present, many of whom teach for an average of 18 h per week, not including graduate supervision.

Some feel that in the sciences it is unreal to separate personal research from that carried on in collaboration with research students, and that research students need not be included in "contact-hours". Within limits, graduate students provide a stimulating influence on research, and an original and imaginative supervisor can easily provide enough ideas to keep several research students busy; their interaction with him can often provide a remarkable spur to further work. I think it is a mistake arbitrarily to limit the number of research students and that it would be best to discount them altogether from teaching hours, thereby giving scope to the more energetic research workers, without requiring the load of undergraduate-teaching to be unequally shared among lecturers. The maximum flexibility should be retained, and as the student/teacher ratio is reduced over the next 10–20 years, the teaching load will decrease and adjust itself, provided the numbers of undergraduates are kept within the planned figure.

Despite the very adverse ratio of students to teachers, Table 330 shows that the average total number of publications of the academic staff at Oxford (and at Cambridge, which is about the same) is higher than at any other university in Great Britain and at Berkeley, California. Table 331 gives a comparison, for certain science subjects for the year 1962–63, relating publications to staff at lecturer level and above at Oxford, Manchester and University College, London. The average number of publications at Oxford was 40–60 per cent higher than at either of the other two universities. We all know that the number of publications is not necessarily a good indication of excellence, but the evidence does not support the view that the heavy teaching load has been allowed to interfere seriously with research.

The question of academic salaries has received much publicity. The total bill for academic salaries at Oxford in 1964–65 was found to be about 15 per cent higher than the average total bill for other British universities. However, the staff at Oxford are considerably older than those at other universities, and if allowance for this is made, it turns out that the average salaries at Oxford are only 4 per cent greater than the national average.

A not inconsiderable part of the income of many Fellow-lecturers at Oxford is derived from what the Franks Report refers to as "piece rate" teaching. In the sciences this arose in the 'fifties when Oxford responded very quickly to a national demand for more university places. For example, whereas the number of men taking finals in the physical sciences was 250 in the period 1951–54, in 1957 the number was 494, and in 1965, 644. Unfortunately this increase was not accompanied by a corresponding increase in staff. Everyone was therefore required to teach more, and teaching over the normal limit, which may vary from 14 to 18 h in different Colleges, was paid for "piece rate". The Franks Commission proposes that these payments should be abolished, and states that "Our proposals must be considered together with those which we make for restricting the amount of tutorial teaching given to each undergraduate, with those for limiting the total teaching load on the staff of Oxford, and that for bringing up the staff-student ratio to the national average". However, it expects the new arrangement to be implemented in 3–5 years, although there seems to be little hope of improving the staff situation for 10–20 years. Most Fellow-lecturers would expect their income to be cut only as their teaching burdens are reduced to something approaching the national average.

The most radical reforms proposed by the Commission are concerned with the administrative structure. The truly democratic nature of Oxford has often been the envy of members of other universities for the freedom enjoyed by the academic staff in the organizing of their research and teaching. Most of the power resides in the faculties, so that in a real sense the University is controlled by the whole academic staff. The present complicated structure of the administration is, however, in dire need of overhaul, and the Commission proposes a complete revision of the present system. There is to be an interlocking system of elected bodies, headed by Council, which is to have considerably greater powers than in the past to plan and act decisively and quickly when necessary. The system will have to be studied in great detail, however, and the many objections weighed up, before it is fully implemented. Certainly the proposal to elect the vice-chancellor, who is to be given greater responsibilities and a longer term of office, will be welcomed.

In the science departments, formal committees will be established, with six members and the head of department as Chairman, to evolve general policy on teaching, appointments, buildings, and allocation of facilities. Such committees already operate in most departments, but the formalization of this arrangement will be welcomed. It provides the academic staff of a department with an opportunity to take part in its government and would make possible the occasional relief of the head from his administrative duties.

These are only some aspects of the conclusions given in this very impressive document. It is easy to seize on particular recommendations and criticize them out of context of the report, but intensive study will be required during the coming months to evaluate its conclusions fully. No doubt the University will implement the recommendations, or modifications of them, when they have been adequately debated. It seems likely that some of the issues which have been raised may prove helpful to other universities, and it is also clear that some of the weaknesses which have been uncovered are the result of rather haphazard growth which is not entirely the fault of Oxford itself.

NATURAL LEVELS OF LEAD-210, POLONIUM-210 AND RADIUM-226 IN HUMANS AND BIOTA OF THE ARCTIC

By DR. RICHARD B. HOLTZMAN
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RELATIVELY high concentrations of some fission products in humans and biota from Lapland and Alaska have been reported[1–5]. These have been attributed to the continual accumulation of these products on the long-lived, slow-growing Arctic lichens and sedges. Such plants are an important source of forage for reindeer and caribou, the meat of which, in turn, constitutes a substantial portion of the diets of Laplanders and Eskimos. These high levels of fall-out suggest, together with the proposed mechanism of uptake and the similarities to strontium-90 in atmospheric distribution[6] and biochemistry, that in Arctic biota the naturally occurring airborne nuclides lead-210 and its decay product, polonium-210, may also be present in high concentration. This phenomenon has, in fact, been reported by me[7] and more recently by Hill[8].

These naturally occurring 'fall-out' activities are of special interest in studies of aerosol precipitation mechanisms because their levels are independent of bomb tests and consequently they have been constant over the years. Because of this constancy and the high energy of the particles emitted by the lead-210 series (a 5·3-MeV α-particle from polonium-210 and a 0·4-MeV [average energy] β-particle from bismuth-210 compared with β-rays with average energies of 0·4 MeV from caesium-137 and 1·1 MeV from strontium-90 [yttrium-90][9]), the radiation dose to Arctic biota from this source may be quite significant relative to that from artificial ones.

Samples and Experimental Procedures

In order to elucidate some of the properties and mechanisms related to the accumulation of the airborne activities, data are presented on the concentrations of ^{210}Pb, ^{210}Po and ^{226}Ra (the long-lived precursor of the ^{210}Pb) in specimens of the human food chain from Arctic regions. Specifically, these are: lichens, caribou muscle, bone and rumen (mainly lichens), and human soft tissues (placenta) from Alaska; lichens, fodder (horsetail, *Equisetum flaviatile*), and reindeer muscle and bone from Finnish Lapland; and lamb bone from Iceland. For comparison, some related plant materials from non-Arctic regions were included—grass from Chicago and lichens from New Hampshire (north-eastern United States). The origins and descriptions of the specimens are presented in Table 1.

The contents of ^{210}Pb and of ^{210}Po in the samples were determined by a previously described method[10]. The sample was first wet-ashed (1–10 g of bone, or plant material, or 10–100 g of dried soft tissue) in concentrated nitric acid until nearly complete dissolution. About 10 ml. perchloric acid was added and the mixture heated until it fumed. When the sample became colourless, concentrated hydrochloric acid was added and it was fumed again. This fuming procedure was repeated three more times to remove residual nitrate, after which the solution was diluted to about 200 ml., the *p*H adjusted to 0·3 and a portion taken for calcium analysis. The ^{210}Po was then plated on to a silver disk and counted in an internal flow proportional alpha counter. Subsequent replating of the sample after a delay of three or more months to allow grow-in of the ^{210}Po then made it possible to estimate both the ^{210}Pb and ^{210}Po in the sample at the time of collection.

The ^{226}Ra was determined by the emanation technique of Lucas[11].

Results and Discussion

The results are presented in Table 2. In order to facilitate the dosimetry *in vivo*, the specific activities in the soft tissues are given in picocuries per gram of wet tissue. However, because of the difficulties in defining a standard *in vivo* state for bone[12], antler and plants, the units are in picocuries per gram ash for bone and antler, and picocuries per gram dry material (at 100° C) for the plants. The overall uncertainties from counting statistics, chemistry, and handling are about 15 per cent, at the 90 per cent confidence level, except where specific values of uncertainties are given.

It was not possible to estimate the initial quantity of polonium in the Alaskan lichen samples because these samples had previously been dry-ashed at 425° C, a temperature at which this element volatilizes.

Vegetation. The lichens and rumen (composed mainly of lichens) contain specific activities of ^{210}Pb and ^{210}Po that are very high relative to those in the horsetails and grass. The Alaskan lichens, in turn, contain greater concentrations than those from Finland and New Hampshire. The rumen are lower than the other Alaskan samples, possibly because of contamination from other plant materials and stomach fluids.

Although the concentrations of ^{210}Pb and ^{210}Po in lichens are high relative to those in the grasses measured here, they are comparable with those in a number of grass samples observed by Hill which range up to 23 pc. ^{210}Po/g dry[13]. That these latter values are inconsistent with the relatively small amounts of ^{210}Pb–^{210}Po acquired by cattle[14,15], as compared with those observed in reindeer

Table 1. DATA ON SAMPLES

Sample	Region	Description
Vegetation		
Lichens	Inari, Finland	Species. *Cladonia alpestris*, March 1961
Horsetails	Inari, Finland	Species: *Equisetum flaviatile*, Sept. 1961
Lichens I	Chariot Site, Cape Thompson, Alaska	Species: predominantly *Cladonia sylvatica*, June 1961
Lichens II	Chariot Site, Cape Thompson, Alaska	Species: predominantly *Cornicularia divergens* Ach., *Alectoria nigricans* Ach. Myl., *Alectoria ochrolecua* (Ehrh.) Mass, and small amounts of other species, June 1961
Lichens III	Chariot Site, Cape Thompson, Alaska	Species: same as above, June 1961
Lichens IV	Lebanon, New Hampshire	Species: *Cladonia*, July 1965
Grass	Chicago, Illinois	Species: Kentucky blue grass, May 1962
Animals		
Reindeer	Finland	2–4-yr-old animals, female, composite of ten animals. Muscle: shoulder. Bone, marrow-free. Date of death: March 14, 1961
Caribou I	Denali Highway, Alaska	2-yr-old, male. Muscle: hind quarter. Bone: femur shaft. Rumen, antler. Date of death: Dec. 6, 1962
Caribou II	Denali Highway, Alaska	3–7-yr-old, male. Muscle: hind quarter. Bone: femur shaft. Rumen, antler. Date of death: Dec 6, 1962
Lamb	Iceland	Commercial lamb obtained in Chicago
Human being, Eskimo placentae		
Subject I	Barrow, Alaska	23-yr-old; date of parturition: June 1963
Subject II	Barrow, Alaska	39-yr-old; date of parturition: June 1963
Subject III	Barrow, Alaska	16-yr-old; date of parturition: June 1963

Table 2. LEAD-210, POLONIUM-210 AND RADIUM-226 IN HUMAN BEINGS AND BIOTA

Sample	Region	^{210}Pb	^{210}Po	^{226}Ra
Vegetation (pc./g dry)				
Lichens	Finland	4 5	13·1	0 0035 ± 0·0010
Horsetails	Finland	0·961	0 24	0 042 ± 0 005
Lichens I	Alaska	11·5	3 0	0 101 ± 0 006
Lichens II	Alaska	69 6	—	0·415 ± 0·017
Lichens III	Alaska	26 0	—	0·40 ± 0 02
Lichens IV	New Hampshire	7 61	7·32	—
Lichens IV A	New Hampshire	6 95	7 50	—
Rumen, caribou I	Alaska	5 86	14·28	—
Rumen, caribou II	Alaska	4·96	16·52	—
Grass, top	Chicago	0 128	0 053	—
Grass, centre	Chicago	0·230	0·064	—
Grass, lower	Chicago	0·717	0·431	—
Grass, roots	Chicago	0 819	0·939	—
Muscle: reindeer and caribou (pc./g wet)				
Reindeer	Finland	0 015	0 20	0 0003 ± 0 0002
Caribou	Alaska	0·0060	0·222	—
Caribou	Alaska	0 0083	0·163	—
Bone or antler: reindeer and caribou (pc./g ash)				
Bone, reindeer	Finland	5 00 ± 0 11	6 33	1·84 ± 0 022
Bone, caribou I	Alaska	11·0 ± 0 2	15·5	—
Bone, caribou II	Alaska	11·1 ± 0·21	14·6	—
Antler, caribou I	Alaska	7·75 ± 0 20	2 75	—
Antler, caribou II	Alaska	6·10 ± 0·25	4·63	—
Bone, lamb (pc/g ash)				
Rib	Iceland	0 314 ± 0·019	—	0 043 ± 0 007
Rib	Iceland	0 353 ± 0·012	—	0·104 ± 0·007
Femur	Iceland	0·337 ± 0·013	—	0 041 ± 0 005
Iliac crest	Iceland	0·269 ± 0·013	—	0 099 ± 0 005
Placenta: human being, Eskimo (pc./g wet)				
Tissue, subject I	Alaska	0·0037	0 027	—
Tissue, subject II	Alaska	0 0016	0·012	—
Blood, subject II	Alaska	0 00094	0 0040	—
Tissue, subject III	Alaska	0·0010	0 0095	—
Blood, subject III	Alaska	0 00059	0·0047	—
Umbilical cord, subject III	Alaska	0 00072	0·0040	—

and caribou (*vide infra*), may be due to the possibility that the grass measured by Hill is not representative of that consumed by the cattle. These samples were collected in December at which time they may have been growing slowly or have been dormant, so that, with a constant rate of ^{210}Pb fall-out, the concentration of this nuclide would increase at a constant rate. On the other hand, during the growing season, the quantity of grass would also have increased, thereby preventing increases in the concentrations of ^{210}Pb. Hill has recently demonstrated this phenomenon by comparing new with old grass from the same locations[8].

A similar phenomenon has been found in the Chicago grass collected in the spring as shown in Table 2. The average concentration was only 0·3 pc./g dry grass, in spite of the fair amount of precipitation (25 cm) in the 40 days before collection. The data in Table 2 do indicate an age effect, that is the older parts of the plant (or slower growing parts) nearer the ground show higher concentrations of ^{210}Pb. Moreover, correlated with this is an increase in the ^{210}Po/^{210}Pb ratio towards 1. Thus if "Grass, centre" is of recent origin, "Grass, lower" would appear to be about 3 months old.

The levels in reindeer may also be higher than in cattle because of the low ash content of lichens, less than 1 per cent as compared with 5 per cent for grass. Thus, if we assume the mineral requirements of reindeer and cattle to be similar, in order to obtain sufficient mineral the reindeer must consume much more material and consequently more of the radioactive nuclides.

The fact that the ^{210}Po/^{210}Pb activity ratios in the lichen and rumen are greater than 1 is of interest in that in a fall-out mechanism (neglecting other mechanisms) this ratio, as in the New Hampshire plants and Chicago grass, should be 1 or less, especially as in the atmosphere, at least in the temperate zone, it is approximately 0·1 (ref. 16). The ratio of two or more observed in the lichens implies either a high differential affinity of these plants for ^{210}Po from precipitation or rapid leaching of the ^{210}Pb from the plants after precipitation. The latter process also implies a non-constant mechanism, that is both nuclides build up and then the ^{210}Pb is leached preferentially. Continual leaching does not seem likely, since in this case the ^{210}Po could not build up to specific activities greater than those of its parent.

The specific activities of the ^{226}Ra were much lower than those of the ^{210}Pb and thus the ^{226}Ra does not appear to be a direct source of the ^{210}Pb. The correct specific activity of ^{226}Ra in lichens is closer to that of the Finnish lichens than to those of the others because this sample was carefully chosen to be dust free, having been collected from an area about 10 miles from the nearest road[17]. Consequently, its ash content was less than 1 per cent of the dry weight as compared with the values of between 5 per cent and 10 per cent for the others. The higher ^{226}Ra activities in the other lichens can, in the main, be attributed to contamination with dust.

Animals. Bone from animals, though not a direct source of ^{210}Pb for human beings, is of interest as, by integrating the intake, it may indicate the average ^{210}Pb content of the meat. The actual activity measured in muscle, on the other hand, represents the intake only immediately before slaughter (a week or two) and thus is not representative of the long-term average of meat. Investigations on bone may also lead to more subtle and better correlations in nutritional and metabolic studies of these animals because of the much higher specific activities of the nuclides of interest. For example, in reindeer bone the specific activity is twenty times that in bovine bone[14], whereas in muscle the respective ratio is only about twice as large[10].

The 11 pc. ^{210}Pb/g ash observed in the Alaskan caribou bone is about twice that in the Finnish bone. This may possibly be due to metabolic differences, but, because they are a very closely related species, the more probable cause seems to be the reduced dietary intake of this isotope by the semi-domesticated reindeer as a consequence of supplementary fodder feeds low in ^{210}Pb. Moreover, the Finnish lichens, because of their lower concentrations of ^{210}Pb, as indicated in the single sample measured, would supply less of this nuclide than would the Alaskan ones.

An analogous explanation also accounts for the differences in the ^{210}Pb concentrations between antler and femur of the Alaskan animals, that is, the antler is formed over a short period of time (3 months) in the spring[18] when, owing to the presence of other vegetation, lichens constitute a smaller fraction of the diet than that averaged over the entire year. A comparison of ^{210}Pb concentrations in femur with those in antler indicates that these concentrations do not vary much from animal to animal from a given region and that the concentration of ^{210}Pb in femur is about 1·8 times that in antler. Thus it is possible that a few animals, and even their antlers, alone, may well form a representative sample of this population.

The ^{210}Po activities in bone are somewhat higher than those of ^{210}Pb, possibly reflecting the equivalent situation in the lichens and rumen. In antlers, on the other hand, the ^{210}Po activities are lower than those of the ^{210}Pb which indicates a preferential deposition of the latter. Moreover, these deposits do not seem to be of sufficient age to allow attainment of radioactive equilibrium.

Although the ^{226}Ra activity in bone is much lower than the equivalent ^{210}Pb and is thus not a major source of this nuclide, it is comparable with DiFerrante's highest values in cattle[19]. The source of this high ^{226}Ra seems to be the lichens, as, although they contain little ^{226}Ra (per g), the ash content (calcium) is also small and consequently the ratio, ^{226}Ra/calcium, is about equal to that observed in bone.

The concentrations of ^{210}Pb in the muscle of reindeer and caribou are somewhat higher than those found in cattle, but only by factors of 2–4. However, the Finnish, rather than the Alaskan, animals in this case show the higher activities. This difference may be due to species or feeding habits, but it seems more likely to be seasonal variation. Hanson and Palmer[20] and Lidén and Svensson[21] observed very large seasonal variations in the ^{137}Cs content of animals and humans, which the authors attributed to the changes in feeding habits of the animal; they consumed lichens and dormant foliage with their high rates of accumulation versus growth only when necessary—that

is in the winter. Thus the Finnish animals, slaughtered in the early spring, contained the winter's accumulation of ^{210}Pb, whereas the Alaskan ones, killed in the late autumn, were somewhat depleted of this nuclide.

The ^{210}Po concentrations were comparable in all the animals and an order of magnitude greater than those in steer meat. These high concentrations may arise from the much greater intake of ^{210}Po by reindeer than by cattle and to the relatively higher ^{210}Po/^{210}Pb ratio in the food, it being about 0·2 in grass and 2 in lichens. Osborne has shown that at natural levels in humans the ^{210}Po/^{210}Pb ratio in soft tissue may be 3 or more[22]. Thus, in the case of reindeer where the ^{210}Po intake and the ^{210}Pb concentrations in bone are relatively high, ^{210}Po accumulation in the soft tissues may also be expected to be high.

In contrast to the reindeer and caribou, the concentrations of ^{210}Pb found in Icelandic sheep show that not all herbivores from this region contain relatively large amounts of these nuclides. In these animals, the ^{210}Pb and ^{226}Ra concentrations are comparable with those in bovine bone[14], suggesting that the ^{210}Pb content in its diet is comparable with that of cattle from the United States. By analogy with Puerto Rico, an oceanic island which probably has lower ^{210}Pb fall-out rates than do continental regions, and consequently lower ^{210}Pb concentrations (in humans the concentrations in bone are about 65 per cent of those in residents of continental regions)[23], the ^{210}Pb content of the diet of sheep in Iceland would be lower than that of reindeer and caribou even on a similar diet.

While muscle from caribou and reindeer has concentrations of ^{210}Pb low relative to those in bone, it still constitutes the major source of this nuclide in Lapps and Alaskans. Miettinen *et al.*[2] report that, in Finnish Lapland, male reindeer breeders consume about 12·4 kg/month or 400 g/day of reindeer meat which, if we use the ^{210}Pb content for the reindeer muscle (0·2 pc./g), implies that these people ingest about 8 pc./day. The rate is more than four times the mid-western United States rate of about 1·8 pc./day[10].

Other Lapps listed by Miettinen *et al.* consume from 1·2 to 12·0 kg/month which represents about 0·8–5 pc./day from meat alone. If the other food consumed is comparable with that from the United States, even these other Lapps ingest about twice that of the United States residents.

Similar dietary information on Alaskans[4] shows that in certain regions the average consumption of reindeer and/or caribou meat ranges up to 900 g/day (Anaktuvuk Pass Eskimos), so that even though the ^{210}Pb levels measured in the Alaskan caribou are lower than in the Finnish reindeer, the total intake by Alaskans is comparable with that of the Lapps.

Eskimo bone has not been measured here because of the difficulties in obtaining it. However, some comparison may be made from data on soft tissues in Table 2 from residents of Barrow, Alaska, who consume about 170 g/day of caribou or reindeer meat[4]. The values for placenta appear to range in wet tissue from 0·001 to 0·005 pc. ^{210}Pb/g and from 0·009 to 0·030 pc. ^{210}Po/g. These concentrations for both nuclides are somewhat higher than those for soft tissue found by Osborne[22] and by me[10].

Although the ^{210}Pb concentrations in placental tissue in Arctic residents are somewhat higher than in the soft tissues of non-Arctic residents, the ^{210}Po ingestion in the former group may be extremely high, 40–160 pc./day. These levels are 20–100 times those of others if we assume a concentration of 0·2 pc./g wet meat and a consumption of about 200–800 g/day[2,4]. Thus, the steady-state amount of ^{210}Po in the body acquired from a constant daily intake may be estimated from equation 48 of the report[9] issued by the International Commission on Radiological Protection, derived from the exponential model for the uptake and excretion of nuclides:

$$\frac{mC}{I} = \frac{T_e f}{0{\cdot}693} \qquad (1)$$

where m is the mass of the body, C is the concentration of the nuclide of interest, I is the daily intake, T_e is the effective half-life of the nuclide in the body, and f is the fraction of the nuclide going from the food to the body.

If for ^{210}Po $T_e = 25$ days and $f = 0{\cdot}06$, then the portion of the whole-body content from this source is 2·2 times the daily intake. In the case of interest it is 90–350 pc. The ^{210}Po, which deposits mainly in the soft tissues, is then quite significant, being 125–350 per cent of that in non-Arctic populations in whom the ^{210}Po content of the soft tissues is estimated to be about 230 pc. (ref. 24). In bone, the ^{210}Po levels from this source would be increased only a few per cent.

The large increase in levels of ^{210}Po is indicated in the data on human placentae which show that, if the metabolic properties of this nuclide in this tissue are assumed to be similar to those in the lung, skeletal muscle or spleen, Arctic dwellers accumulate six to ten times that found in the soft tissues of Britons[22]. The concentrations in the placentae are as much as three times those found in the liver and kidney of people living outside the Arctic. In addition to this comparison, it should be noted that because the placentae studied were obtained in the summer, they probably represent only the lower limit of concentrations that could be found. These tissues were formed during the winter when the main food was caribou killed in the autumn, which after summer forage (little intake of lichens) are known to contain minimum amounts of ^{137}Cs (refs. 20 and 21), and thus probably also of ^{210}Pb and ^{210}Po.

Dosimetry. In the mid-western United States, ^{210}Po from the deposited ^{210}Pb has been shown to contribute 20 per cent of the total skeletal dose in the human (50 per cent of that from internally deposited nuclides)[10]. The data presented here show that in the Arctic these nuclides exist in substantially greater concentrations in reindeer and caribou than in mid-western cattle and in Icelandic sheep. The consequent intake by humans consuming the meat from the Arctic animals is also large, but by a smaller fraction than previously estimated[17]. (C. R. Hill, in a personal communication, has pointed out that the previously reported values of ^{210}Pb in reindeer muscle were mainly values of ^{210}Po.) The data on the Eskimo placentae give some support to this conclusion, but to a lesser extent than Hill's data on the rib bone of Canadian Eskimos. In two cases, high activities of ^{210}Po were observed and probably also of ^{210}Pb, as in bone the ^{210}Po/^{210}Pb ratio is near unity, at least in people living outside the Arctic region[10,22]. The result of 2·3 pc. ^{210}Po/g ash found by Hill[25] is more than fifteen times greater than the average for people living in the mid-western United States. (*Note added in proof.* Since submission of this paper, Hill has reported this in more detail. The values measured were actually ^{210}Pb (*Nature*, 208, 423; 1965).)

These findings imply that in users of reindeer and caribou meat the total radiation dose rates from the natural sources are somewhat higher than in non-users. The total skeletal rates to people living in the mid-western United States are about 100 mrad/yr[26]. If the relative biological effectiveness for α-particles is assumed to be 4, the effective dose is about 130 mrem/yr, of which about 20 per cent is contributed by the ^{210}Pb–^{210}Po pair. Thus, populations ingesting four times the average level of ^{210}Pb would experience dose rates about 30 per cent greater than those living in the mid-western United States. (It should be noted that in this latter group only about half is acquired by ingestion, the other half coming from inhalation, the absolute amount of which would be about the same in Arctic residents[10]. Thus, quadrupling the ingestion rate would increase the total amount only by a factor of 2·5.) In the case of Hill's Eskimo, the bone would experience a total dose of more than 400 mrem/yr, three times that of people living in the mid-western United States. The ^{210}Pb–^{210}Po dose alone may then be comparable with the high fall-out doses from bombs as measured in the Arctic.

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Although data from Arctic placentae show a dramatic increase in ^{210}Po levels over those in other tissues from non-Arctic residents, the actual increase in dose at natural levels is small (about 10 per cent), based on the assumptions that the normal level is 100 mrem/yr, that 0·01 pc. ^{210}Po/g wet tissue produces a dose of 1 mrad/yr, and that the relative biological effectiveness for α-particles is 4. (With the usually assumed relative biological effectiveness of 10, the dose would be increased to about 130 mrem.) However, these numbers may be significant relative to the much larger values in bone because, for instance, the radiation effects to soft tissue may be greater than to bone, or the relative biological effectiveness at low dose rates may be much different than that used here.

Conclusion

Further investigations of this type, together with the epidemiology, would provide information of value in assessing the effects of low-level radiation on human and animal populations. (The reindeer and caribou are exposed to skeletal doses of 1–10 rems/yr (relative biological effectiveness = 4) which is fifty times that of cattle and 500 times that of humans.) Natural 'fall-out' is also of particular use in tracing food chains and in studying the metabolic properties of the nuclides and elements involved. Moreover, similar studies of this type appear to be feasible elsewhere, as the high levels of natural (and artificial) 'fall-out' are, with our present knowledge, only by chance characteristic of the Arctic, that is, the lichen is a slow-growing plant eaten only by reindeer and caribou which, in turn, form a substantial part of the human diet. Similar conditions might obtain in other regions, such as deserts, where vegetation, for lack of water, may grow only slowly. This phenomenon is indicated in Hill's data on camel bone[8] and in DiFerrante's ox bone[14], in which the specimens with the higher activities came from the more arid regions.

I thank the many people who contributed many ideas and specimens to this research, particularly R. A. Dudley of the International Atomic Energy Agency, J. N. Wolfe and J. J. Davis of the U.S. Atomic Energy Commission, J. K. Miettinen of the University of Helsinki, L. A. Viereck of the State of Alaska Department of Fish and Game, and R. T. Light of the U.S. Public Health Service. Special thanks are due to L. D. Marinelli and J. E. Rose for their help and encouragement. The work was performed under the auspices of the U.S. Atomic Energy Commission.

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OBITUARIES

Prof. R. O. Kapp

Reginald Otto Kapp, professor emeritus of electrical engineering in the University of London, died suddenly on February 20 at the age of 80, four days after the death of his wife.

Kapp was born at Brentwood in 1885. After early education in Germany, he graduated in electrical engineering at the University of Birmingham, where his father, Prof. Gisbert Kapp, was head of the department. He then spent four years with Messrs. Brown Boveri in Baden, Switzerland, working on the development of electric traction. In 1913 he returned to Britain to join the staff of Messrs. Kennedy and Donkin, engineering consultants, but his new work was soon interrupted by the outbreak of the First World War in which he served with the Royal Engineers in Salonika and elsewhere.

After the War, Kapp returned to consultancy and, under Sir John Kennedy, began detailed studies of electrical power supplies and demands in Britain. These studies provided load forecasts and financial estimates which formed the technical basis of the Electricity Act, 1926, the legal instrument which established the grid system. The grid encouraged an increase in the size of generators, and Kapp worked on the associated design and switching problems. The integration of power generating systems also aroused an early interest in the economic and technical advantages of standardization.

In 1935 Kapp was appointed to the Pender chair of electrical engineering at University College, London. At the beginning of the Second World War, the College departments were evacuated to other universities, and Kapp, as Dean of the Faculty of Engineering, went with the engineering departments to Swansea. On their return to London, he was mainly concerned with the post-war rehabilitation of his department.

In 1950 Kapp reached the academic retiring age of sixty-five but, still full of vitality, returned to part-time consultancy with his former employers. He was chairman of a committee appointed by British Railways to reduce inductive interference between railway telecommunications and power lines. He also worked on energy storage by compressed air and on tidal systems as sources of power. As he had taken advantage of his early training on the Continent to equip himself as a linguist—his German, Italian and French were impeccable—he was much in demand as a chairman at international conferences. His linguistic abilities combined with his interest in standardization, of terminology as well as of equipment, led him to the chairmanship of committees of the International Standards Organization and the International Electrotechnical Commission as well as of the British Standards Institution. Consultancy work

requiring the critical assessment of proposed systems of generation and distribution of electrical power took him in this later period to Egypt and to Argentina. He enjoyed his travels immensely, making many friends abroad, taking great delight in trying exotic foods and in solving new problems of linguistic communication.

But, full as it was, his career as a professional engineer was only part of his public life. For many years he had pondered two fundamental problems, one philosophical—the relation of 'mind' to 'matter', and one cosmological—the creation and evolution of the universe. His approaches to these problems, adumbrated in *Science versus Materialism* (1940), were developed with characteristic tenacity in later works. The philosophical problem was further discussed in *Mind, Life and Body* (1951) and in his Riddell Memorial Lectures on *Facts and Faith: the Dual Nature of Reality* (1955). He then returned to cosmology. By the ruthless application of Occam's Razor, which Kapp later elevated to 'The Principle of Minimum Assumption', he disposed of the 'big bang' and other contemporary theories of the origin of the universe and, in 1940, concluded that creation is continuous: matter is continuously being created at random throughout existing space and is continuously being destroyed at random throughout existing matter. Inferences from this hypothesis, including the idea that the continuous destruction of matter is the cause of gravitation and the prediction that lunar rock will be found to be micro-porous and mechanically weak, are worked out in *Towards a Unified Cosmology* (1959). This book, his final work, is about to be published in a German edition. Kapp was disappointed by the reception of his cosmological theories and regretted that he lacked the mathematical skill to exploit fully the implications of his physical insights.

He immensely enjoyed tackling new huge problems, technological or administrative, obscured by detail and complicated by conflicting interests or economic constraints. His invariable technique was, first, patiently to seek the relevant guiding principles and then, having established them, to apply the principles rigorously and without fear or favour; Kapp abhorred the *ad hoc*. He also enjoyed writing, especially as a means of solving intellectual problems: successive drafts on a given thesis were for him a means of self-communication, each new draft being not merely a polishing but a significant development of its predecessor—a process which continued until a solution, usually of a problem much more fundamental than the one he began with, was obtained. His interest in writing, and his long experience of the inadequacies of the working papers on which his consultancy work depended, led him to establish a lectureship in the presentation of technical information at University College and to found a Group, of which he was the very active president for seventeen years, and, more recently, a Research and Education Trust, all concerned with aspects of scientific and technical writing. He also led the British Society for the Philosophy of Science to its present autonomy and founded the Occam Society.

He served on the governing bodies of Middlesex Hospital Medical School and of Northampton College of Advanced Technology where, as chairman of the academic council, he recently devoted much thought to the transition of the College to university status. His committee work, always thoroughly prepared, gave the impression to those who shared any one committee with him that that particular committee had commanded his whole attention since the previous meeting. But at one time in the 1950's he was an active member, if not chairman, of fifty assorted committees.

Yet he found time also to enjoy the arts—the ballet, the theatre and music especially—and to publish occasional compositions for the piano.

He died of a heart attack, physically tired by the long illness and death of his wife, but in full possession and control of his formidable faculties to the last, and leaving, as his friends would have expected, all his affairs and papers in perfect order. A son and daughter, both married, survive him.

B. C. BROOKES

Prof. A. Fraenkel

PROF. ADOLF ABRAHAM HALEVI FRAENKEL, emeritus professor of mathematics at the Hebrew University of Jerusalem, died on October 15, 1965, at the age of 74, after a short illness.

Prof. Fraenkel was born in Munich in 1891, of an illustrious Jewish family. He studied mathematics at the Universities of Munich, Marburg, Berlin and Breslau. In 1922, he started teaching at the University of Marburg, later obtaining there the chair of the renowned German algebraist, Hensel. In 1928, he was called to the University of Kiel. Three years later, he received an invitation from Dr. Chaim Weizmann, who was to become the first president of the State of Israel, to join the recently created Hebrew University of Jerusalem. Having been an ardent Zionist since his youth, Fraenkel accepted this invitation and, alternating with Prof. Fekete, became chairman of the Mathematical Institute of the Hebrew University. It was mostly due to him that the Institute quickly achieved a world-wide reputation. Almost all the present professors there are his former students, while other students occupy important positions in other scientific institutions.

During the years 1938–40, Fraenkel served as rector of the Hebrew University. For many years, he headed its Institute of Adult Education, spending on it much time and fruitful effort. He was a member of the National Council of the Jewish community in (then) Palestine and was active in the establishment of the State of Israel in 1948. He became one of the founding members of the Israel Academy of Sciences and Humanities.

Fraenkel started as an algebraist and was one of the first to study the theory of abstract groups, thereby paving the way for the investigation of abstract algebraic structures in general. His main contribution, however, was in the field of abstract set theory, a contribution which is still very much alive and finds its external expression in the fact that one of the major axiom systems of set theory is known as 'ZF', the Zermelo–Fraenkel system. Zermelo was the first to publish, in 1908, a workable axiom system for set theory in order to fortify the foundations of that discipline which had become shattered through the discovery of paradoxes at the turn of the century. Fraenkel, in 1921, just a few months before a similar accomplishment by the late Norwegian mathematician, Th. Skolem, was able to increase decisively the logical rigour in the formulation of one of the axioms, the so-called axiom of subsets (or *Aussonderungsaxiom*), and to add considerable strength to the system by the addition of another axiom, the axiom of replacement. As a result of the life-long interest in the axiom of choice, an interest which was of combined mathematical and philosophical origin, he made significant contributions towards the final proof of independency of this axiom, achieved only three years ago by P. J. Cohen and hailed as one of the major achievements of this century.

Fraenkel's philosophy of mathematics was an unabashed Platonism, and he stuck to this view even when it was no longer fashionable. But his deep convictions in this respect did not hinder him from becoming one of the main interpreters of intuitionism in his text-books, which have long since become classics in their fields.

Fraenkel was deeply religious but of extraordinary tolerance and liberalism towards his environment. He combined his religious and mathematical interests in a thorough investigation of the Jewish calendar.

For many of his students Fraenkel was the decisive factor in shaping their lives, and they will never forget this man who combined ice-cold scientific rigour with the warmest interest in their scientific and other progress,

who applied the highest standards to himself and tried, successfully, to live up to them, but took the foibles of his environment smilingly in his stride.

Among Fraenkel's hobbies were mountaineering and swimming, both of which he pursued until his last days. Having served during the First World War as a meteorologist with the German Army, he continued to remain interested in the field for the rest of his life.

Prof. Fraenkel is survived by his wife and four children.

Y. BAR-HILLEL

NEWS AND VIEWS

Acceleration at the Rutherford

THE £11 million 7·2 GeV proton synchrotron at the Rutherford High Energy Laboratory at Chilton, Berkshire, has at last begun to resemble the white elephant which is the Thai symbol of prosperity and good luck. Scarcely 10 months after *Nimrod* was opened in April, 1964, disintegration of the power supply motors caused a 10-month halt for repairs that were only completed 5 months ago. Now the operation of the laboratory is only interrupted every 3 weeks when ultrasonic tests are made in order to assure the laboratory that the stable door at least is guarded.

In practice, *Nimrod* has been running for 80 per cent of the time allocated to experiments—60 per cent of the total clock time—the other 20 per cent being lost in periodic breakdowns lasting from 30 min to a few hours. At this rate, Dr. Thomas Pickavance, the director of the laboratory, thinks it will be economic to run for another 15–20 years. Considering that the machine is in nearly continuous operation, Dr. Pickavance is pleased with the present level and, in fact, hopes within the year to equal the 90 per cent record at CERN—which, according to him, has had much longer breakdowns. Dr. Pickavance's colleagues, such as Dr. G. H. Strafford, head of the High-Energy Physics Division, believe that brief interruptions do not affect most experiments, and that increased beam extraction and greater beam intensity would be more important improvements. On another scale the laboratory hopes soon to circumvent, by introducing portable units, the bottleneck caused when an experiment is dismantled. Already twice as many experiments have been submitted as there is floor space available; the waiting period is often one to two years, though much of this is profitably used for preparation. Twelve to fifteen university physics departments make regular use of *Nimrod*, though only Oxford, Cambridge, and London are convenient for commuting.

Pickavance believes that as larger machines are built small ones such as *Nimrod* will not be put out of business; there is still a great deal of work to do and a large number of machines will be needed to do it.

Nimrod's future role will depend largely on international plans; Pickavance mentioned that sites for a 300 GeV European accelerator have been considered but nothing definite decided. The United States is planning a 200 GeV accelerator for early in the 1970s; France and the U.S.S.R. have recently agreed to co-operate on the 70 GeV electron accelerator being built in Serpukhovo by the Russians.

Electrostatic Generator at Oxford

A TANDEM electrostatic machine for 20-MeV protons in coupled operation has been installed in the Nuclear Physics Laboratory, University of Oxford. The system consists of a vertical injector—the pressure vessel of which is 42 ft. long and 13 ft. internal diameter—developed by the Electrostatic Generator Group of the Rutherford High Energy Laboratory, coupled to a horizontal 6-MeV (type E.N.) tandem, of which the generator was provided by High Voltage Engineering Europa of Amersfoort, Holland; the commissioning was carried out by the Oxford Electrostatic Generator Group. In single operation, the injector has delivered microampere beams of negative hydrogen ions at 10·2 MeV, negative oxygen ions at 9·5 MeV and He^{++} at 15·6 MeV, corresponding to 9·7 MeV on the terminal.

In operation, when coupled with the tandem, protons of 20 MeV energy have been delivered on target. The transmission efficiency has varied between 12 per cent and 30 per cent: this is illustrated in the case of hydrogen by 0·25 μamp analysed proton beam for 1·5 μamp of hydrogen negative beam injected into the tandem, and for oxygen by 0·9 μamp of oxygen analysed in the 6+ charge state for 1·3 μamp of negative oxygen injected.

Earthquakes and Buildings

THE book *Earthquake Investigations in the Western United States, 1931–64*, published by the U.S. Department of Commerce, Coast and Geodetic Survey (Publication 41–2), is a notable record of activity in the fields of earthquake recording and engineering and seismology.

Some of the material was first published in 1936 (U.S.C.G.S. Special Publication 201), including papers by R. S. McLean and W. W. Moore, R. R. Martel and the late Frank Neumann. New papers include several on instruments and instrumental studies and on damage studies.

The paper by John A. Blume on "The Building Vibrator" describes three vibrators at present in use, together with the methods of testing. In buildings, the vibrator is securely braced between two columns. A 4 in. × 4 in. strut is placed horizontally between the end of the vibrator and the adjoining column and is screwed tightly into place by means of two large bolts attached to the frame of the machine, or by a jack. To steady the apparatus, ballast is usually placed on skids bolted to the base of the machine. On structures in which there are no columns or walls for bracing, it is necessary to resort to other methods of transmitting the disturbing forces. On the Colorado Street Bridge, the vibrator was held in place mainly by piling a large number of sacks of cement on the skids. For ground shaking tests a heavy wooden framework was constructed to enclose a large vibrator. For horizontal forces, the frame may be jacked between curbs of a roadway, and it may be weighted down for vertical shaking. Resulting vibrations in structures or in the ground are measured by portable seismographs. For buildings a magnification of about 200 may be used, but for dams or on the ground the magnifications may be 1,500 to 10,000.

The tests have included buildings, dams, bridges, rock, and hard and soft ground. In each case, records showing forced vibration have been obtained. The test results indicate that such machines can be used to obtain valuable information that otherwise would be difficult to determine regarding the vibration characteristics of structures. This is especially true when simultaneous timing and recording are available from several sections of the structure. The use of the vibrator enables the positive identification of several of the highest natural periods of vibration in each translational direction of multi-storey buildings, and also the plotting of forced vibration

resonance, curves, and mode shapes for these periods. In addition, torsional modes of vibration have been established together with the centres of rotation. It is also possible to obtain viscous damping factors from the shapes of the resonance curves.

Seismologists hope that the work of recording strong earthquake motion with the related activities of questionnaire and vibration programmes will continue, so that, like statistics on precipitation, wind, temperature and tides, reliable data on ground motions will be available. In the meantime, it is hoped to have accumulated sufficient material in two years to warrant the publication of another volume of papers.

Phosphorus in Soil

SINCE it became apparent many years ago that a determination of the total nutrients in the soil gave little indication of what would become available to the plant during a growing season, soil chemists have devised numerous relatively simple laboratory techniques to assess availability. Based on convenience and local experience, these methods have played a useful part in assisting the farmer with his fertilizer programme, but inevitable difficulties arose in comparing the results of work in different areas. Soil phosphorus has been a particularly complex problem because crop responses to applied phosphorus have varied widely, not only between soils but also between seasons, and correlations of laboratory and field data have been burdened by anomalies. It was fitting, therefore, that a serious attempt should be made to examine techniques and experimental results from a large number of soils. At a conference in October 1962, organized by the National Agricultural Advisory Service, fifteen papers covering various aspects of the subject were presented and discussed, and five syndicates considered and reported on several specific questions confronting the adviser: (*a*) the concept of intensity and capacity factors; (*b*) the possibility of selecting one routine method for determining available phosphorus; (*c*) the mode of expressing soil phosphorus; (*d*) the classification of soil phosphorus figures; and (*e*) the factors concerned in the interpretation of the results. A full record of the proceedings has now been published in *Soil Phosphorus* (Ministry of Agriculture, Fisheries and Food. Technical Bulletin No. 13. Pp. v+159. London: H.M.S.O., 1965. 37*s*. 6*d*. net). It is a most valuable guide to the present position with respect to the measurement of fertility levels, the difficulty of assessing the influence of soil organic matter, the grouping of crops in manuring recommendations and the kind of investigations still required to improve the advice given to farmers.

Water in Living Systems

Forms of Water in Biologic Systems comprises a series of papers delivered at a conference of the New York Academy of Sciences in October 1964 (*Ann. N.Y. Acad. Sci.*, 125. Article 2. By H. J. Berendsen and 39 other authors. Pp. 249–772. New York: New York Academy of Sciences, 1965. 10 dollars). Only a month before, a similar British symposium, organized by the Society for Experimental Biology, was held at Swansea, under the general title "The State and Movement of Water in Living Organisms". Both these symposia have now appeared in print, and they testify to the considerable interest which exists at the present moment in the properties of the fluid which is undoubtedly the most important single chemical compound in living things. Inevitably the two symposia will be compared. What emerges from the comparison is that there is little overlap, for whereas the Swansea symposium was oriented towards what might be called the larger-scale aspects of plant and animal water relations (though it deals also with the theoretical and physical background), the New York symposium is concerned rather more with physico-chemical and macromolecular aspects of the subject. Particularly interesting is a contribution from Prof. Fernandez Moran appearing in the discussion at the end, in which he outlines some exciting developments in the direction of high-resolution electron microscopy, at liquid helium temperatures, of structures involving water on the molecular level. This seems to indicate a line of advance from which much may be expected.

Document Preservation

THE preservation of paper is a recognized necessity—sometimes, it may be thought, an unfortunate one. Micro-filming, the obvious solution to the problem of storage, has some practical drawbacks. Much thought has therefore been given to the subject, but much of it is pointless unless the resistance of a paper to ageing can be forecast numerically.

In 1928 the National Bureau of Standards, Washington, D.C., evolved such a test. It was based on the determination of the decrease in folding resistance after heating for 72 h at $105° \pm 2°$ C. As a result of evaluations of stored papers at intervals during the following years[1], it is considered that the conditions of this test are equivalent to natural ageing for 25 years under normal room storage conditions. On the relatively short view of a century or so, a recent article entitled "Archives Document Preservation" by Raff, Herrick and Adams of the Washington State University, Pullman, Washington[2], is of particular interest. These workers started with the accepted fact that acidity found in paper on storage is the principal cause of deterioration. Treatment with calcium and magnesium bicarbonates is a recognized antidote. The authors carry this principle a stage further by using the alkali salts of organic polyacids; on impregnation with this mixture, a paper will be both neutralized by the buffering effect and also reinforced. Preliminary experiments justified these expectations, but showed the necessity for the addition of a plasticizer in order to improve the folding resistance.

Such papers, when subjected to the standard accelerating ageing test, showed little or no decrease in *p*H value or folding resistance; the wet strength was actually increased by the test. The quantitative significance of these results must be assessed with caution, however, since the accepted ageing test referred to above is based on paper fibres alone and may not necessarily apply to paper treated by the authors' method.

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Medical Sciences at Toronto

THE University of Toronto has completed plans for its new Medical Sciences building, the largest and most complex structure planned for any Canadian university. By this means Toronto hopes to become one of the largest medical schools in North America, increasing its class to 250 a year by the time the main facilities are completed in 1969.

The complex of buildings will comprise a teaching block of five floors and a research tower of eight with an adjacent three-storey auditorium; the cost of the building is estimated at 29·5 million dollars and equipment will cost an additional 6·5 million dollars. The University plans to break ground this June, to complete the teaching and research blocks by September 1969, and to finish the auditorium building the following year.

The stimulus for the project was a committee report in May 1964 which said that "inadequacies of space have frustrated the development of research and the increasing demands of administrative duties and teaching have markedly reduced the effectiveness of the existing staff and hindered the acquisition of new staff of high calibre".

This new building should enable Toronto to keep pace in the future with the rapidly increasing demands of medical teaching and research.

Lenin Prizes

THE 1966 Lenin Prizes for Science and Technology have now been announced by the Soviet Government. In science, 21 research workers were awarded prizes for work on eight projects. These included the theory of superconductive alloys; theoretical and experimental research into excitons in crystals; research on the origin of peculiarities in surfaces of negative curvature; work on a mathematical theory of the synthesis of control systems; a cycle of works on irregular problems; investigations into elemental-organic compounds; a chromosome theory of heredity and a theory of mutations; and the book, *X-Ray Diagnosis of Diseases of the Bones and Joints*.

The technologists carried the day by sheer weight of numbers. Altogether 103 research people working on ten projects were awarded prizes in technology. These were for developing a new process for manufacturing a large module gear wheel; for developing and introducing automatic die-casting production of machine components; for developing the means for welding train rails in field conditions; for designing and introducing the mass production of a 100,000 kW steam heating turbine; for research into complex structures with *p-n* junctions and developing and introducing the mass production of power silicon rectifiers; for discovering oil and gas deposits in South Mangyshlak and prospecting two other deposits; for scientific verification and practical application of block systems for working oil deposits in the Kuibyshev region; for providing the scientific basis and the practical mechanisms for boring in underground conditions; for developing a practical ladle treatment by liquid synthetic slags for the manufacture of high-quality steels; and for introducing new industrial methods in building the Norilsk town and Combine in the permafrost soil and harsh climatic conditions of the Arctic Circle.

U.S. Science Advisory Committee

THE following new members have been appointed for four-year terms to the United States President's Science Advisory Committee: DR. I. BENNETT, director, Department of Pathology, Johns Hopkins University; PROF. S. DRELL, professor of physics, Stanford University; W. HEWLETT, president, Hewlett-Packard Company; PROF. C. SLICHTER, professor of physics, University of Illinois; DR. C. TOWNES, provost, Massachusetts Institute of Technology.

University News:

Massachusetts Institute of Technology

DR. HERBERT S. BRIDGE has been appointed professor of physics at the Massachusetts Institute of Technology. Dr. Bridge has worked with the Cosmic Ray Group of the M.I.T. Laboratory for Nuclear Science since 1946 and has been a principal investigator in NASA's solar wind experiments. Dr. Jerome Y. Lettvin has been appointed professor of communications physiology with faculty association in both the Department of Biology of the School of Science and the Department of Electrical Engineering in the School of Engineering.

Strathclyde

DR. PETER J. HEALD, head of the Department of Animal Biochemistry at the Twyford Laboratories, Ltd., Park Royal, London, has been appointed to the chair of biochemistry in the University of Strathclyde with effect from September.

Sussex

DR. R. J. TAYLER, at present Fellow of Corpus Christi College, Cambridge, has been appointed professor of astronomy.

Appointments

COUNCIL of the Scottish Marine Biological Association has appointed Mr. R. I. Currie, of the National Institute of Oceanography, to be director of the Millport/Oban Laboratory in succession to Dr. C. H. Mortimer. At the same time Mr. R. S. Glover will become director of the Oceanographic Laboratory, Edinburgh. Mr. Currie and Mr. Glover will act as joint secretaries to the Association.

ADMIRAL of the Fleet the Earl Mountbatten of Burma has been elected a Fellow of the Royal Society, under the statute of the Society which provides for the election of persons who have either rendered conspicuous service to the cause of science or are such that their election would be of signal benefit to the Society.

PROF. E. R. LAITHWAITE, professor of heavy electrical engineering at the Imperial College of Science and Technology, University of London, has been awarded the S. G. Brown Award and Medal of the Royal Society for 1966, for his work on the linear induction motor.

Announcements

A NEW *Journal of Computational Physics* is to be published in July by Academic Press.

A NEW journal, *Chemistry and Physics of Lipids*, is to be published quarterly by the North-Holland Publishing Company. The first issue is scheduled for July/September 1966.

A SYMPOSIUM on "Polymer Degradation" will be held at the Welsh College of Advanced Technology on July 1. Further information can be obtained from the Organizer of Short Courses, Welsh College of Advanced Technology, Cathays Park, Cardiff.

THE 1966 summer meeting of the British Society for the History of Science will be held at St. Anne's College, Oxford, during July 1–2. Further information can be obtained from M. P. Earles, Chelsea College of Science, Manresa Road, London, S.W.3.

THE seventy-second annual conference of the Museums Association will be held in Sheffield during July 4–8. Further information can be obtained from the Secretary, the Museums Association, 87 Charlotte Street, London, W.1.

AN international conference on "Lens Design with Large Computers" will be held in Rochester, New York, during July 5–8. Further information can be obtained from the Institute of Optics, University of Rochester, Rochester, New York.

THE Water Pollution Research Laboratory, at Elder Way, Stevenage, has published a list of substances for which information on their toxicity and biodegradability is available. This list and any enquiries about the properties of a specific substance may be obtained from the director of the Laboratory.

A RESIDENTIAL advanced summer school on "Molecular and Cellular Aspects of Immunobiology", sponsored by McGill University and the National Research Council of Canada, will be held near Montreal during July 4–15. Further information can be obtained from Prof. A. Sehon, Department of Chemistry, McGill University, Montreal, Quebec.

A CONFERENCE on "The Economics of Automated Materials Testing", arranged by the Institution of Engineering Inspection in collaboration with the Materials and Testing Group of the Institute of Physics and the Physical Society, will be held at the Imperial College of Science and Technology, London, during July 4–6. Further information can be obtained from the Secretary, Institution of Engineering Inspection, 616 Grand Buildings, Trafalgar Square, London, W.C.2.

LUNAR OCCULTATION AND PROVISIONAL IDENTIFICATION OF *MSH* 19-27

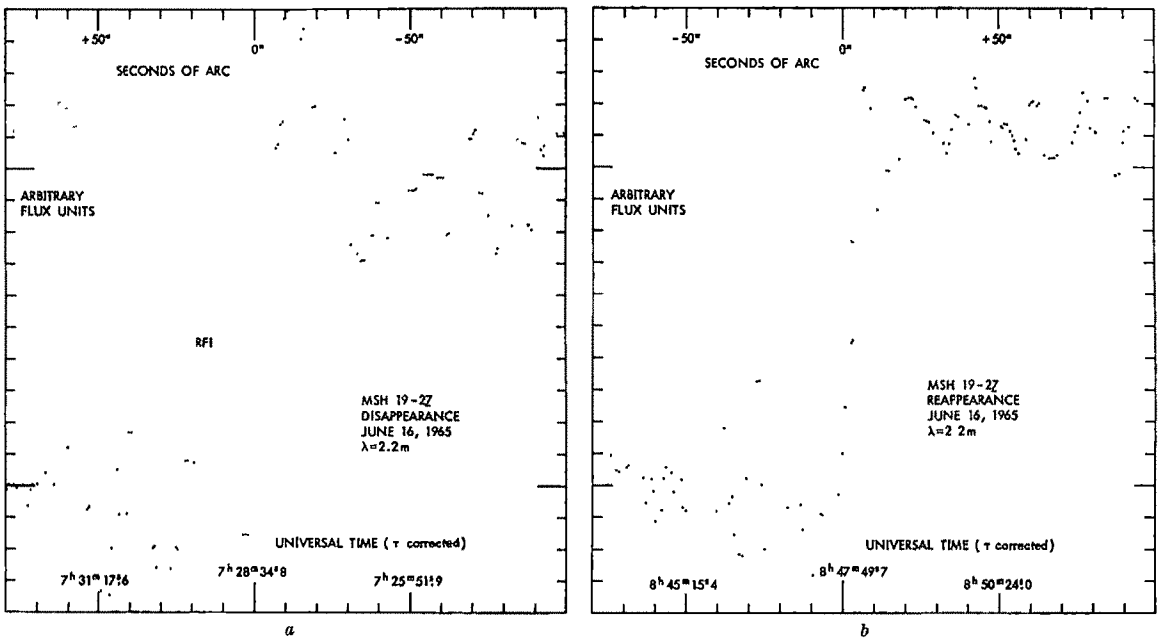
By LARRY W. BROWN

Radio Astronomy Section, Laboratory for Space Sciences, National Aeronautics and Space Administration, Goddard Space Flight Center

A LUNAR occultation of the radio source *MSH* 19–27 was observed on June 16, 1965. The observation was made at the National Aeronautics and Space Administration's Data Acquisition Facility located in the western North Carolina mountains (latitude 35° 12′ 0·05″ N., longitude 05h 31m 29·294s W.). In addition to position and diameter measurements, the occultation has provided a possible optical counterpart to the radio source.

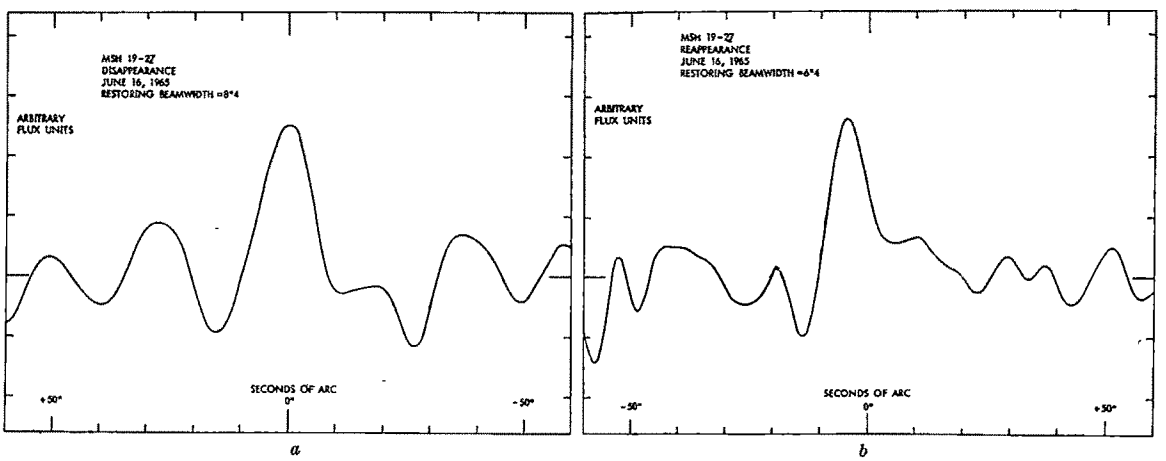
The observation was made with a parabolic antenna 85 ft. in diameter, which tracked the centre of the Moon for the duration of the occultation. The receiver was a switched-type system using gain modulation. The receiving frequency was 137 Mc/s with an intermediate frequency of 30 Mc/s, bandwidth of 2·5 Mc/s and RC integration time of 3 sec. Digitized records of the disappearance and reappearance of the source are presented in Figs. 1*a* and *b*, respectively.

The records were analysed in an IBM 7094 computer with a programme adapted from the work of Von Hoerner[1], which was based on the restoration programme discussed by Scheuer[2]. This programme convolves the digitized data with various restoring functions to produce the brightness distribution as it would be observed with a Gaussian fan beam scanned along the position angle of the occultation. Examples of the restored data are presented in Fig. 2*a* and *b*.



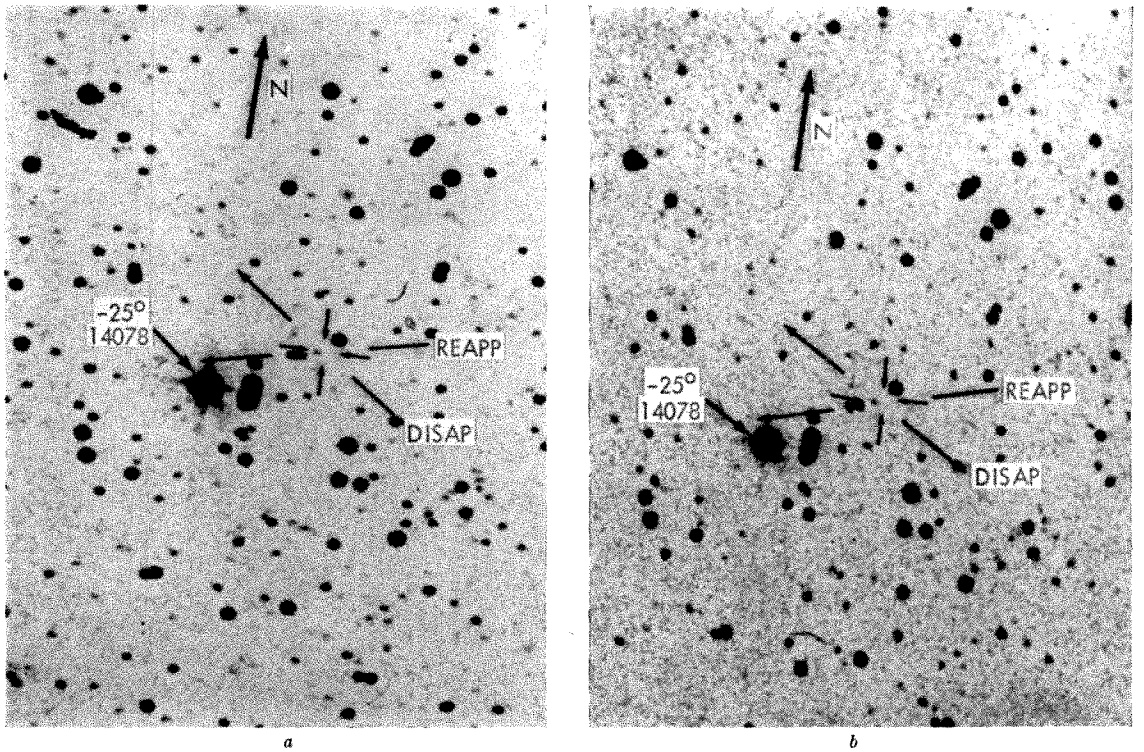
a *b*

Fig. 1. *a*, Digitized data disappearance; *b*, Digitized data reappearance



a *b*

Fig. 2. *a*, Restored data: disappearance restored with a beamwidth of 8·4″; *b*, Restored data: reappearance restored with a beamwidth of 6·4″



a *b*

Fig. 3. 20× enlargement of National Geographic—Palomar Survey Plates. *a*, Red plate; *b*, blue plate

The data have been restored with seven different beam-widths and a gaussian curve fitted to the resulting brightness distribution. Analysis of the fit provides the best values for the position of the intensity peak and the diameter of the source along the scan. The diameters and corresponding position angles are given in Table 1.

Table 1

Phase	Position angle	Diameter	Time of occurrence U.T.	Restoring beamwidth used
Disappearance	58°	8·0″ ± 1·7″	07h 28m 35·0s ± 1·6s	4·6″ 8·0″
Reappearance	104°	5·9″ ± 1·5″	08h 47m 38·0s ± 1·5s	3·4″ 5·9″

The position of the source has been computed from universal times (corrected for RC integration time[3]) corresponding to the peak intensities of the restored brightness distributions. There are two possible positions for the source corresponding to these times:

Position 1: R.A. (1965·5) = 19h 26m 58·46s ± 0·03s
Dec. (1965·5) = −25° 55′ 32·4″ ± 0·5″

Position 2: R.A. (1965·5) = 19h 26m 51·18s ± 0·03s
Dec. (1965·5) = −25° 44′ 1·4″ ± 0·5″

In calculating these positions, it has been assumed that the difference in universal and ephemeris time is 35·0 sec.

The ambiguity in position can be resolved by considering the position given in the *MSH* catalogue[4].

Table 2

	Position 1	*MSH*	Position 2
R.A. (1950·0)	19h 26m 1·62s ± 0·03s	19h 25·6m ± 0·3m	19h 25m 45·42s ± 0·03s
Dec. (1950·0)	−25° 57′ 26·6″ ± 0·5″	−25° 44′ ± 5′	−25° 45′ 55·4″ ± 0·5″

Because position 2 lies within the quoted error limits of the catalogue while position 1 lies considerably outside the declination limits, the source is assumed to be at position 2.

The radio position of the source was located on the National Geographic Society—Palomar Observatory Sky Survey Plates from comparison with a number of 8th and 9th magnitude stars in the area. The optical positions were obtained from the *Cordoba Durchmusterung Catalogue* which has positions given to the nearest 0·1′ of arc. Within the error limits of the plate measurement, the radio position was found to coincide with the position of an extremely faint object ($\Delta\delta \approx 0''$ $\Delta\alpha \approx 1''$). Owing to the fact that in most cases the optical and radio positions do not coincide exactly, an area twice the radio diameter and several times the optical diameter was examined for other possible objects. The probability of the identification was increased when no other objects were found in this area.

Because of the extreme faintness, any interpretation of the nature of the object was seriously affected by the photographic limits of the survey plates. Figs. 3*a* and *b* are 20× enlargements of the source area on the red and blue plates, respectively. In red light, the source appeared to consist of a faint object with two centres of light concentration; while in blue light, there was only one centre (apparently brighter than the red) surrounded by a faint halo. A number of different types of objects were examined on the survey plates in an attempt to find similarities. While no conclusions could be reached, the blue image was found to be similar in some ways to a faint galaxy with a luminous nucleus. However, the nature of the object was not identified and must await more optical evidence.

I wish to thank Dr. S. Von Hoerner of the National Radio Astronomy Observatory for supplying his computer programme and H.M. Nautical Almanac Office for predicting the occultation.

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ASYMMETRICAL STOP ZONES IN JUPITER'S EXOSPHERE

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A VARIETY of mechanisms has been suggested to account for the bursts of radio noise which are received sporadically from Jupiter at decametre wavelengths. For example, there are the Čerenkov emission model of Warwick[1], the Doppler-shifted cyclotron emission model proposed by Ellis and McCulloch[2], and the "escaped-whistler" model, based on the ideas of Carr[3], Field[4], and Chang[5]. In these three models and in others, emission occurs in the extraordinary mode near or well below the electron gyrofrequency, and the radiation reaching the Earth is initially guided by Jupiter's magnetic field (although propagation is not strictly along the field lines in some of the models). The asymmetry which is observed in the pattern of emission probability as a function of central meridian longitude at a given frequency is assumed to be due to asymmetrical beaming of the radiation. The beaming asymmetry, in turn, is assumed to be caused by field distortions resulting from a tilt of the magnetic dipole axis with respect to the rotational axis, from a displacement of the dipole centre relative to the centre of the planet, and from local departures from a true dipole field configuration. It is the purpose of this article to point out that in addition to altering the beaming pattern, another effect of field distortions is to produce a variation with longitude in the thickness of the stop zone for frequencies in the vicinity of the gyrofrequency. Čerenkov radiation, Doppler-shifted cyclotron radiation, and whistlers would all be expected to escape most often from regions in which the stop zone is thinnest (although it is not entirely clear in any of the models how the stop zone is traversed). This effect may be partially responsible for the structure of the observed emission probability pattern.

For quasi-longitudinal propagation in a plasma in which the collision frequency is relatively low, the Appleton–Hartree equation reduces to:

$$n^2 = 1 - \frac{f_p^2}{f(f \pm f_H)} \qquad (1)$$

where f is the wave frequency, f_p is the plasma frequency, and f_H is the electronic gyrofrequency associated with the magnetic field component parallel to the direction of propagation. The positive and negative signs refer to the ordinary and extraordinary modes respectively. The magnitude of the propagation vector, k, is related to the refractive index, n, by the relation:

$$k = \frac{2\pi f}{c}\sqrt{n^2}$$

For positive values of n^2, k is real, and normal propagation occurs. For negative values of n^2, k is imaginary, and in the steady state only evanescent waves exist. A stop zone is a region in which $n^2 < 0$. At the boundaries separating stop zones from regions in which propagation is possible, either $n^2 = 0$ or $n^2 = \infty$. Booker[6] has discussed these forbidden regions, and has plotted stop zone boundaries for both modes in the terrestrial magnetosphere at specified frequencies. His assumption of a field due to a centred dipole aligned with the rotational axis and of a radically symmetrical electron density distribution leads to stop zones which are independent of longitude and are symmetrical about the equator. However, in the case of Jupiter, the field is not so symmetrical. This, we will show, leads to an asymmetrical forbidden region. For the extraordinary mode, which is the only case we are considering, n^2 is positive wherever $f_H > f$ or wherever $f(f - f_H) > f_p^2$.

Let us first consider a hypothetical planet having a centred axially aligned magnetic dipole and a radially decreasing electron density. There will be only one stop zone for longitudinal propagation in the extraordinary mode at a given frequency, f. The equation of the inner boundary, determined by the condition that the gyrofrequency equals the wave frequency, is given by:

$$R_1^3 = \frac{K}{f}\sqrt{1 + 3\sin^2\theta} \qquad (2)$$

where R_1 is the radius vector measured from the dipole centre, θ is the magnetic latitude, and K is a constant dependent on the strength of the dipole moment. If f is in Mc/s, then $K = 2{\cdot}80\, H_0 R_0^3$, where H_0 is the magnetic field strength in gauss at a point on the magnetic equator at a distance R_0 from the dipole centre. On the outer boundary of the stop zone, $f(f - f_{H_2}) = f_{p_2}^2$, where f_{H_2} and f_{p_2} are the gyrofrequency and the plasma frequency, respectively, at this boundary. The radius vector, R_2, to a point on the outer boundary at magnetic latitude θ may be found from:

$$R_2^3 = \frac{R_1^3}{1 - f_{p_2}^2/f^2} \qquad (3)$$

where R_1 is given by equation (2) and f_{p_2} is itself a function of R_2. Equation (3) can be simplified if it is assumed that $f_{p_2}^2 \ll f_{H_2}$, implying that $10^{-5}\, N_2^2 \ll H_2^2$. This is not unreasonable for a model of Jupiter's magnetosphere, since for a value of H_2 on the order of 10 G, it is required only that N_2 be smaller than, say, 10^6 electrons cm^{-3}. Then the expression for R_2 can be approximated by:

$$R_2 = \left[1 + \frac{f_{p_2}^2}{3f^2}\right] R_1 \qquad (4)$$

The stop zone thickness is given by:

$$(R_2 - R_1) = \frac{f_{p_2}^2}{3f^2}\, R_1 \qquad (5)$$

The term $f_{p_2}^2$ can be replaced by $80{\cdot}6 \times 10^{-6}\, N_2$, where N_2 is the electron density in cm^{-3}. Equations (2) and (4) indicate that the location of the inner boundary of the stop zone depends only on the magnetic field, while that of the outer boundary depends on both the magnetic field and the electron density.

The variation of plasma frequency (or electron number density) with radial distance must be known before an explicit expression for either R_2 or $R_2 - R_1$ can be found in terms of independent variables. Thus, if we assume that $N = N_0 \frac{R_0^m}{R^m}$, the expression for the stop zone thickness becomes:

$$R_2 - R_1 = 2{\cdot}7 \times 10^{-5} N_0 R_0 (2{\cdot}8 H_0)^{(1-m)/3} (1 + 3\sin^2\theta)^{(1-m)/6} f^{(m-7)/3} \qquad (6)$$

However, even without precise knowledge of the electron distribution, it is possible to draw certain conclusions regarding the variation of stop zone thickness with longitude. It is apparent from equation (5) that a necessary condition for the stop zone thickness to remain constant is that $f_{p_2}^2\, R_1$ (or $N_2 R_1$) be constant. Thus, for an axially symmetrical dipole field and a radially symmetrical electron distribution, the stop zone thickness varies with latitude but not longitude, as suggested by Fig. 1*a*. On the other hand, if the field is not symmetrical, either because of a displacement or a tipping of the dipole or

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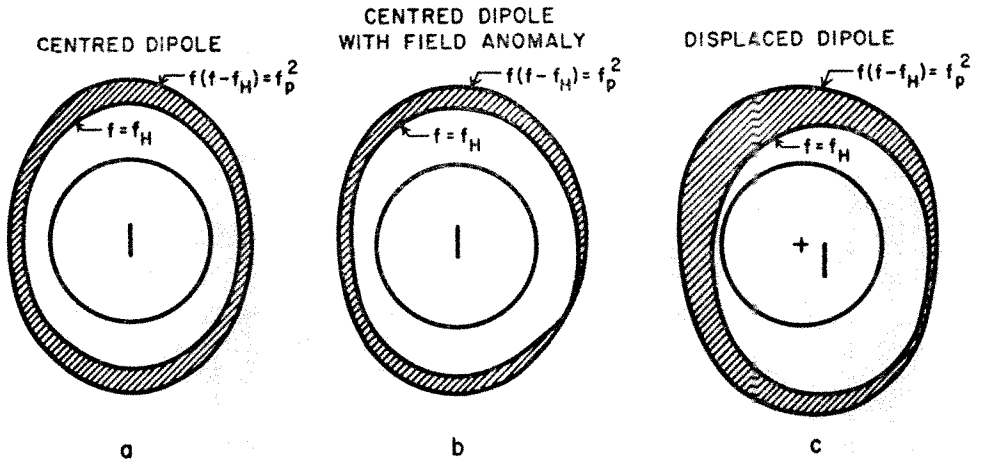


Fig. 1. Variation of thickness of the stop zone (represented by the shaded region) with latitude for various magnetic field models. The thickness has been greatly exaggerated to illustrate its variation

because of field anomalies, R_1 now depends on longitude as well as latitude, and the stop-zone is no longer symmetrical. It is thinnest at longitudes for which the field is strongest. A variation of electron density with longitude would also destroy the symmetry of the stop zone, which would in this case be thickest at longitudes of highest electron density. The effect of a localized field anomaly is suggested by Fig. 1*b*, and of a displacement of the magnetic dipole by Fig. 1*c*.

It appears very likely that if any of the aforementioned mechanisms is indeed responsible for the Jupiter radiation, stop zone asymmetries must be one of the factors producing the characteristic variations of occurrence probability with central meridian longitude. Beaming of the escaping radiation into thin sheets is probably another contributing factor. Thus, if the probability of escape of radiation from a given region of Jupiter toward the Earth is high, then the stop zone near this region must be relatively thin and the field orientation must be suitable to produce a beam in the right direction.

It is possible that the striking correlation of Jupiter activity with the position angle of the satellite Io, which was discovered by Bigg[7], is due at least in part to the influence of the satellite on Jovian stop zones. The effect might result either from perturbations by Io of the magnetic field or of the electron density in any of a number of ways.

If Io is directly involved in the emission of radiation, it would seem most likely that the radiation must originate either near the satellite or near a region where field lines passing close to the satellite intersect the surface of Jupiter. In either of these cases, initial propagation toward the Earth would be more or less transverse to the field. Longitudinal propagation has been assumed in the preceding equations for the sake of simplicity. However, the effects of changes in field strength and electron density on stop zone thickness are not qualitatively different when propagation is not longitudinal, and the foregoing general conclusions would still apply.

This investigation was supported in part by the National Aeronautics and Space Administration, the National Science Foundation, the Office of Naval Research, and the Army Research Office Durham).

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BRIGHTNESS DISTRIBUTION OF ANTARES FROM LUNAR OCCULTATIONS

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THE first direct measurements of the angular diameters of stars were made by Michelson and Pease[1], using an interferometer which yielded an effective angular resolution of about 0·02 arc sec. More recently, Hanbury Brown *et al.*[2] have used an intensity interferometer to measure the diameter of Vega to a resolution of 0·004 arc sec. The present article discusses a third method of measuring stellar diameters and brightness distributions, namely the method of lunar occultations.

During the years 1950–53 there occurred a series of lunar occultations of the star Antares, at least five of which were observed with fast photo-electric equipment[3–6]. On the assumption that the star appears as a uniformly illuminated disk, the resulting light curves were shown to indicate a diameter of about 0·040 arc sec for Antares, the same value as that deduced by Pease[7] from interferometer measurements. A more complete analysis of these occultations has now been performed. The process required a convolution of the photo-electric data with a suitable restoring function and gave an effective angular resolution of 0·0084 arc sec. The results suggest that Antares (a star of magnitude 0·9 and spectral type *M*1 Ib) consists of a bright central component about 0·019 arc sec in radius, surrounded by an outer shell extending to about 0·028 arc sec

It is further shown that the diameters of a surprisingly large number of stars can be measured by the same method. The limiting resolution for stars of seventh magnitude observed with a 50-in telescope is of the order of 0·001 arc sec, which would permit the resolution of twenty or so stars at a given location in a year.

Scheuer[8] has derived an elegant solution to the mathematical problem of recovering one-dimensional brightness distributions from occultation observations. The method

was developed for the purpose of studying radio sources occulted by the Moon and it has been used by Hazard[9], von Hoerner[10,11], and others. The restoring process yields the brightness distribution of the occulted object as it would appear if scanned with a fan beam of Gaussian shape, oriented parallel to the limb of the Moon. So long as the lunar limb can be regarded as a smooth diffracting screen, the minimum effective beamwidth of the restoration is determined by the signal-to-noise ratio, the band-width, the time resolution of the observations, and the diameter of the telescope. The method is applied here for the first time at visual wave-lengths.

The problem of recovering full two-dimensional brightness distributions from fan beam observations has been discussed by Bracewell[12]. If the object under investigation is known to possess circular symmetry, then a single fan beam observation suffices to determine the radial distribution of brightness. In the present case, while observations were obtained with the fan beam oriented in different position angles, the differences in the resulting data were not significant, and circular symmetry has been assumed. This assumption is supported by previous observations of Antares made with a rotating Michelson interferometer[13].

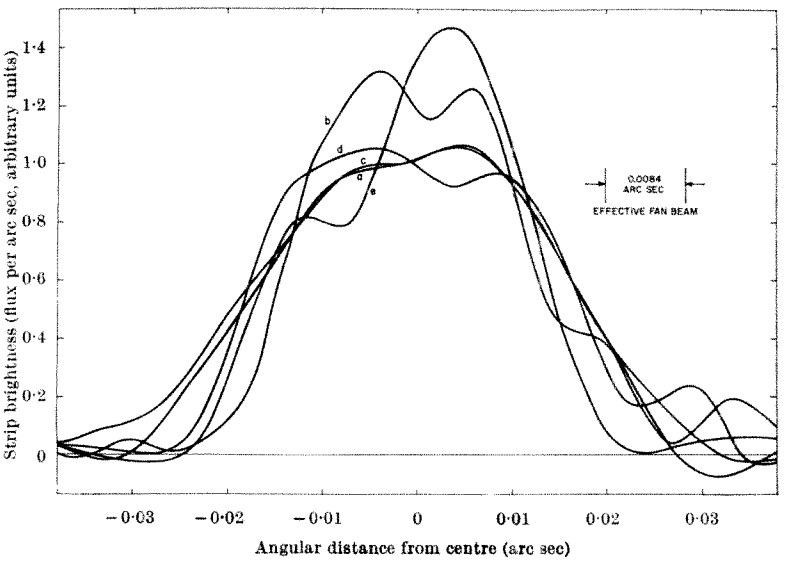


Fig. 2. Strip-integrated brightness distribution of Antares derived from five different lunar occultation observations

Table 1 lists the circumstances of the five occultations of Antares which provided the observations used in this article. Two other observations available in the literature[3,6] were rejected because of reported non-linearities in the electronic recording equipment. A sample of the observed data is reproduced in Fig. 1. The restorations to strip-integrated brightness distributions were performed digitally with a modification of von Hoerner's computer programme. An effective wave-length of 5100 Å was assumed for all of the observations. The results for a restoring beamwidth of 0·0084 arc sec are plotted in superposition in Fig. 2, normalized so that the areas under the curves are equal.

Antares, like many red giant stars, varies slightly in luminosity, but its variations over the period of the observations are believed to have been small and have been neglected. If one also rules out the possibilities of an appreciably elongated star, or an unresolved double star, then the differences among the five curves in Fig. 2 must be attributed to errors. The principal source of systematic error is the uncertainty in v_r, the velocity of approach of the lunar limb. This uncertainty results from our lack of knowledge of the precise angle between the mean lunar limb, assumed in the analysis to be smooth and circular, and the actual limb at the point of occultation. The uncertainty causes a proportional ambiguity in the horizontal scales of the strip-integrated brightness distributions. These effects are minimized in the case of head-on occultation, and conversely are greatest in the case of a grazing event. It is easy to show that the root mean square error of v_r, namely Δv_r, can be expressed approximately by the relation:

$$\Delta v_r/v_r = [(1-\cos\alpha)^2 + \tan\varphi \sin\alpha)^2]^{1/2}$$

where φ is the angle between the normal to the mean lunar limb and the direction of the Moon's motion, and α is the root mean square slope of the lunar surface over the relevant distance scale of a few tens of metres. If one assumes that α is approximately 8°, as indicated by radar investigations of the Moon[14], then the uncertainties in v_r for the occultations in question range from 3 per cent for event d to 35 per cent for event b. These uncertainties are sufficient to explain the discrepancies in the widths of the curves of Fig. 2.

Corrections to the values of v_r were made on the assumption that the brightness of the central region of the star is constant in time. The adopted values of v_r were each multiplied by the factor necessary to equalize the average central ordinates of the strip brightness distributions. The corrections are of the order of 10 per cent or smaller, and have the effect of reducing by 15 per cent the root mean square scatter of the points that determine the mean strip distribution. The corrected strip scans then agree to within their own random uncertainties.

For the purpose of deriving the radial brightness distribution of Antares, the strip scans were folded and averaged and then corrected for instrumental smoothing by the method of successive substitution[15]. The inverse Abel transform[12] of the mean strip scan was computed numerically, the result being the radial brightness distribution $I(r)$ plotted in Fig. 3. The error bars were derived from the root mean square deviations of the five corrected strip scans from their mean.

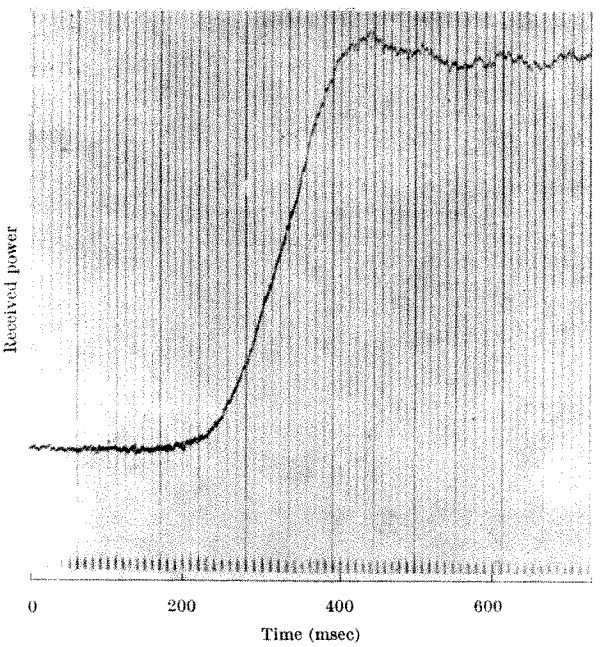


Fig. 1. Photoelectric light curve for occultation of Antares 1952 April 13 (after Evans *et al.*, ref. 5)

For comparison with limb darkening laws predicted for model stellar atmospheres. the radial brightness distribution has been replotted in Fig. 4 as a function of $\mu=[1-(r/R_0)^2]^{1/2}$, where r is the angular distance from the centre of the star, R_0 is the overall radius of the star, taken as 0·028 arc sec, and μ is the cosine of the angle between the line of sight and the normal to the surface of the star at r. The observed distribution is not well described by a law of the form $I(\mu) \approx a + b\mu$, as might be expected if the outer layers of the star were in radiative equilibrium, or by a law of the form $I(\mu) \approx \mu^c$, as might be expected if convective equilibrium prevails.

Figs. 3 and 4 suggest that Antares is composed of a central component of approximately uniform brightness, 0·019 ± 0·003 arc sec in radius, which accounts for 80 ± 10 per cent of the flux at 5100 Å, and an additional component—perhaps a semitransparent shell or enormous prominences in the outer atmosphere of the star—which extends to at least 0·028 ± 0·004 arc sec from the centre and accounts for the remainder of the flux.

On the assumption that the radiation from Antares comes from an overall disk of radius 0·028 arc sec, the

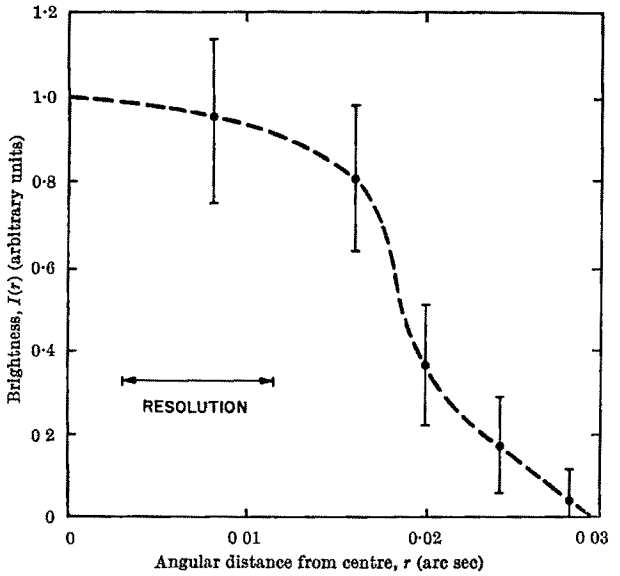


Fig. 3. Radial brightness distribution of Antares, plotted as a function of angular distance from the centre of the star

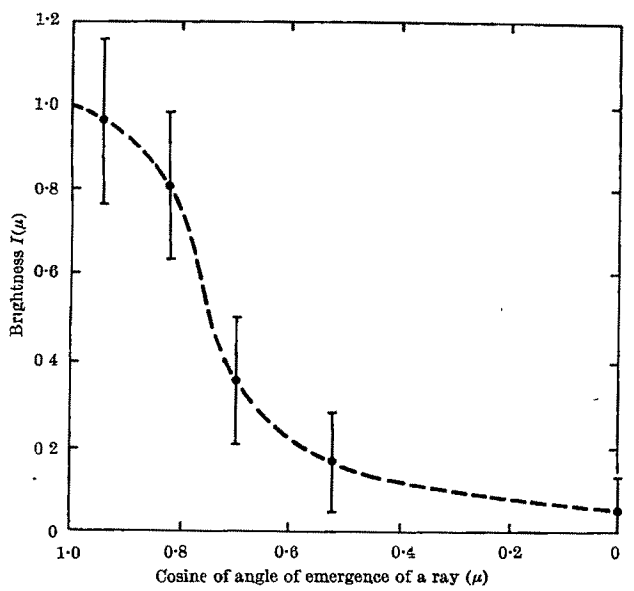


Fig. 4 Radial brightness distribution of Antares, plotted as a function of μ, the cosine of the angle between the line of sight and the normal to the surface of the star

Table 1. CIRCUMSTANCES OF THE OCCULTATIONS OF ANTARES

Event	Date	Position angle	Angle between normal to limb and Moon's motion, φ	Velocity of approach, v_r (arc sec per second)	Reference
a	1950 May 4	267°	14°	0·414	Fig. 3, ref. 4
b	1951 July 15	43°	68°	0·181	Fig. 3, ref. 4
c	1952 April 13	349°	58°	0·241	Plate 12, ref. 5
d	1952 April 13	301°	12°	0·459	Fig. 3, ref. 4
e	1953 March 8	235°	52°	0·233	Fig. 3, ref. 4

effective temperature of the star is estimated to be 3,200° K. For this calculation we have used 1,919 arc sec and 5,800° K for the angular diameter and effective temperature of the Sun, +0·94 and −26·78 for the apparent magnitudes of Antares and the Sun, and 2·5 for the difference between the bolometric corrections for Antares and the Sun (all numerical values from Allen[16]). The temperature estimate for Antares should, however, be regarded with caution, since the uncertainty in the bolometric correction for Antares is large.

The limits of resolution by the lunar occultation method have not been reached in the foregoing Antares example. Two observations[5] of occultations of Antares *B* (a companion star of magnitude 6·6 and spectral type *B*4 *V*) were restored with an effective beamwidth of 0·003 arc sec. The star was not resolved; nor would one expect it to be, since the calculated diameter, based on the known colour and apparent magnitude, is only 0·0001 arc sec[17]. The limiting resolution was in this case determined by the optical bandwidth, which was of the order of 30 per cent. Upper limits to diameters found in this way are quite valid, since the unknown roughness of the lunar limb cannot diminish the apparent angular diameter of an object.

The limits of resolution set by noise and bandwidth have been formulated by von Hoerner[18]. They are respectively:

$$\beta_r \geqslant 52/q_0^2$$

and

$$\beta_r \geqslant 0{\cdot}616\sqrt{B\lambda}$$

where β_r is the fan beam resolution in arc seconds, q_0 is the root mean square signal-to-noise ratio for integration over 1 arc sec of the Moon's motion, λ is the wave-length in metres, and B is the fractional bandwidth as a percentage. The resolution limit set by telescope size is a consequence of the fact that the diffraction pattern of the occulted star is not scanned by an infinitely narrow slit, but by the finite aperture of the telescope. If a is the aperture (in inches) of the telescope perpendicular to the limb of the Moon, then this limit is given by the relation:

$$\beta_r \geqslant 9a \times 10^{-6}$$

In some cases, where occultations of small, bright stars are observed with large telescopes, it will be advantageous to use a rectangular diaphragm over the telescope aperture.

Atmospheric scintillations typically have a period of the order of 0·1 sec. Since the motion of the Moon relative to the stars is of the order of 0·35 arc sec per sec, scintillation noise will adversely affect stellar diameter measurements only for stars larger than about 0·01 arc sec, for which the occultations last for longer than about 0·03 sec.

Fig. 5 shows graphically the resolution limits for occultation observations carried out with telescopes of 50-in., 100-in., and 200-in. circular aperture, if the observational noise-level is equal to that experienced by Evans *et al.*[5]. The horizontal branches of the curves represent the limits set by telescope size, while the steepest portions are determined by the noise-level with the assumption that the maximum practical bandwidth is 30 per cent.

The diagonal lines in Fig. 5 give the calculated diameter of a star as a function of its spectral type and apparent visual magnitude[17]. It is clear that a number of stars should be resolvable by the method of lunar occultations. An indication of the number of stars available is shown

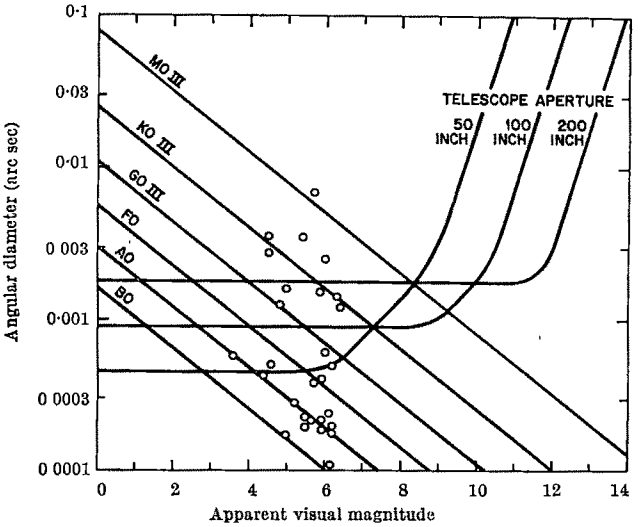


Fig. 5 The limits to resolution of stellar diameters by the method of lunar occultations are shown for three telescope sizes. The diagonal lines give calculated diameters of stars as a function of spectral type and apparent visual magnitude. The circles represent stars brighter than magnitude 6·5, which will be occulted during the latter half of 1966 for observers in the south-west United States

by the circles in Fig. 5, which represent the stars brighter than magnitude 6·5 subject to occultations visible in the south-west United States during the second half of 1966 (ref. 18). Only occultations occurring at the dark limb of the Moon are included.

Fig. 5 indicates that the diameter of the brightest quasi-stellar radio source, 3*C* 273 (visual magnitude 12·6), could be measured to a limit of about 0·01 arc sec, if a lunar occultation were observed under favourable conditions with a 200-in. telescope. Such a measurement would be of great astrophysical interest.

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CRANIAL CAPACITY OF THE OLDUVAI BED I HOMININE

PROF. TOBIAS[1] has replied to my communication[2] regarding the cranial capacity of the 'pre-Zinj' hominid, Bed I, Olduvai Gorge. Tobias has presented statistical data regarding variation of this metrical character in higher primates, and claims that the 'pre-Zinj' volume exceeds significantly by 3·79 *S.D.*s the mean volume (502 c.c.) for a sample of seven australopithecines. He has further claimed that since the variation of metrical characters varies in different primate taxa, one cannot *a priori* assume that australopithecine variation was of the same order. While I am certainly in basic agreement with Prof. Tobias's comments, I think it is important to make a few points regarding his arguments, and the relation of his data to the question of taxonomic placement of the Olduvai hominid.

My original communication[2] was not offered with any aim of making taxonomic inclusions or exclusions. The evidence I presented was solely meant to support Prof. Tobias's[3] figure of 680 c.c. for the 'pre-Zinj' endocast. I merely added the last paragraph to forewarn students against accepting the confirmation of his values as supporting any taxonomic conclusion. While I agree with Tobias's argument regarding the fallacy of *a priori* assumptions of comparable variation in all taxa of higher primates, I think it only fair to point out that Tobias has used the *a priori* assumption that if the difference between means is significant, one has evidence suggesting taxonomic separation. It is hard to escape the conclusion that Tobias has made the *a priori* assumption that the forms are taxonomically distinct at both generic and species levels, and is using the statistical data to back his position. I have no quibble with his heuristic procedure, provided that the statistical data are comparable. In this case they are not, and I fail to see the relevance of his arguments regarding primate variability in cranial capacity.

Tobias[3] has shown that the capacity of the 'pre-Zinj' (680 c.c.) exceeds by 3·79 *S.D.*s the australopithecine mean of 502 c.c. He then discusses extant higher primate variability, noting that gorilla show a coefficient of variation (*C.V.*) of 13·6 per cent (the *C.V.*s for modern *Homo* being 12·8 per cent; 6·8 per cent in chimpanzee, and 9·35 per cent in australopithecines, $n=7$). Two points must be made regarding this procedure: (*a*) If the mean for a large sample of gorillas ($n=113$) (mean = 510 c.c.)[4] is compared with Schultz's[5] value of 752 c.c., the difference between means exceeds 3·48 *S.D.*s. (*b*) Comparison of different taxa based on completely different sample sizes is a dubious matter. It makes little sense to compare *C.V.*s and *S.D.*s for large samples ($n > 100$) with those based on very small samples, for example, chimpanzee and gorilla with australopithecines, particularly when the latter are reconstructions based on fragments, and the reconstructions in turn based on other members of the same sample. In short, there is the fallacy of sample size, as well as the fact that in the australopithecine sample the members are not wholly independent.

To illustrate this matter, I have calculated the *S.D.*s and *C.V.*s for a number of fossil hominids. These are shown in Table 1.

It is interesting that the *C.V.* for australopithecines given by Tobias as 9·35 per cent is larger than the values for Solo (7·8 per cent), and neandertals (8·6 per cent). The Chinese material ('Pekin man') is somewhat larger (10·7 per cent). The very high values for the East Asiatic pithecanthropines reflects the outcome of low sample size ($n=3$). The equation for *S.D.*, using ($n=3$), reduces the denominator in the radical to two ($n-1$), thus increasing *S.D.* and *C.V.* If Woo's[6] recent discovery is excluded, the *S.D.* becomes 141 c.c., and the *C.V.* approximately 16·1 per cent. Surely these values are meaningless in terms of illustrating variability in this taxon? There is

no sound basis for comparing *C.V.*s of Solo (7·8 per cent) and *Pithecanthropus* (13·6 per cent) with the chimpanzee or gorilla and conclude anything regarding taxon variability. If values for 113 gorillas or chimpanzees were written on pieces of paper, placed in a hat, and seven drawn, the statistics would be comparable with the australopithecines only with respect to sample size. Even this procedure would not wholly equalize the situation, since one would have to assume *a priori* that variability was comparable in the two taxa, and that is what one is trying to ascertain. Furthermore, after drawing seven values, one has recourse to the remaining population to see how the selection went. This cannot be done with the fossils. In addition to Tobias's listing of factors contributing to taxon variability, one might include the possibility that in a species with larger absolute brain size and body-weights, more variability might be expected. In fact, both Keith[7] and Hagedoorn[8] have shown that this is the case. The ranges approximate absolute values.

Thus any arguments regarding different taxon variability in metrical characters should be based on comparable samples. It may well be the case that taxon variability in higher primates is itself variable, as Tobias argues. His figures do not establish this as absolute fact, since the samples are not comparable. The question is still open. Consequently, for Tobias to use these figures he must implicitly be taking the *a priori* assumption that variability is comparable in different taxa of higher primates.

It should also be pointed out that the sample size of australopithecine endocasts, $n=7$, includes the 'Zinjanthropus' or *robustus* member. Apart from this inclusion, the $n=7$ statement *does not* also imply that the reliability of each value of capacity is the same for all seven. This is another difference between the hominid sample and those of the apes based on Ashton and Spence's[4] data. In the latter case, the reliability should be the same for all members of the sample.

If any *a priori* argument can be advanced, it should be in the direction of granting variability for the australopithecine taxon. After all, this is the first experiment in hominid evolution, This 'experiment' did involve a new behavioural adaptation, cultural behaviour, surely based to some extent on the organization of the brain. Logically, one might expect greater variability in this unstable beginning phase, which soon led to the more advanced pithecanthropine stage, present in Bed II. Thus far, the evidence as given by Tobias does not decide one way or the other in this case.

In any event, the fate of taxonomic designation for the 'pre-Zinj' specimen cannot be decided by reference to *S.D.*s or *C.V.*s, unless the sample size is expanded. Should that occur, there would be much more total morphological evidence than is at present available, and decisions of taxon placement could be based, then, not on cranial capacity but on the total morphological configuration.

Table 1

Fossil group	Cranial capacity	Mean c.c.	*S.D.*	*C.V.* per cent	Ref.
Pithecanthropus		843	114·6	13·6	
I	975				(9)
II	775				,,
Woo's (1965)	778				(6)
'Pekin man'		1,043	112	10·7	
II	1,030				(9)
III	915				,,
X	1,225				,,
XI	1,015				,,
XII	1,030				,,
Solo-Ngandong		1,096	86 3	7·8	,,
I	1,035				,,
V	1,255				,,
VI	1,035				,,
IX	1,135				,,
X	1,055				,,
XI	1,060				,,
Neandertals		1,425	123 2	8 6	,,
La Chapelle	1,620				,,
La Quina♀	1,350				,,
Le Moustier Juv. ♂	1,560				,,
Gibraltar	1,300				,,
Rhodesian	1,325				,,
Neandertal	1,370				,,
Ehringsdorf	1,450				,,

$$S.D. = \sqrt{\frac{\Sigma(x-\bar{x})}{n-1}} \qquad C.V. = \frac{100\,(S.D.)}{\bar{x}}$$

In sum, I agree with Prof. Tobias's remarks regarding possible taxon variability in metrical characters, but do not find his arguments and application to the australopithecines as necessarily convincing. The 'pre-Zinj' case is comparable with the gorilla in terms of *S.D.*s, and similar arguments could be advanced to end members of the 1,000-c.c. variation in modern *Homo*. While Tobias may be correct regarding australopithecine variability, and the 'pre-Zinj' significant difference in terms of means, this position still remains to be validated empirically. If the extant higher primates are excluded on the basis of sample size, Table 4 indicated that australopithecine variability is comparable, if not more, than that for other hominid groups.

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WHEN Holloway[1] ingeniously corroborated my estimate of 680 c.c. as the cranial capacity of *Homo habilis*, I commented briefly on the state of knowledge arising from this confirmation[2]. My remarks were not a reply, since his paper did not call for a reply. In one sentence, I pointed out that the corroborated value of 680 c.c. exceeded the newly re-computed australopithecine sample mean of 502 c.c. by 3·79 *S.D.*s. This simple statement of fact made no reference to the taxonomic implications of this significant difference. The comment was in my view justified because: (*a*) the value of 680 c.c. had previously been doubted by some, but was now shown quite independently to be valid; (*b*) the sample mean and variance for the australopithecines had been re-computed, based on my new estimate of 562 c.c. for the 'adult value' of the Taung cranial capacity, instead of the 600 c.c. previously accepted.

It is therefore difficult to see how Holloway could read into my comments "the *a priori* assumption that if the difference between means is significant, one has evidence suggesting taxonomic separation". In any event, the difference between means was not referred to by me, but the difference between a single value (680) and the australopithecine sample mean.

It is equally difficult to follow Holloway's reasoning when he goes on to say: "It is hard to escape the conclusion that Tobias has made the *a priori* assumption that the forms are taxonomically distinct at both generic and species levels, and is using the statistical data to back his position". This would suggest that my brief comments following Holloway's previous paper constitute the whole case for the distinctness of *Homo habilis*! Yet, this case is based not on a single criterion (cranial capacity), but on several criteria[3–11], and it has been carefully built up over a long series of some eight or more papers. An investigation of these papers shows that the analysis came first, and the taxonomic conclusions thereafter. For example, in a paper prepared as long ago as April 1964 for the thirty-third annual meeting of the American Association of Physical Anthropologists, and published in October 1965 (ref. 9), I stated: "Right up until December 1963, I had believed that I could explain the features of

the juvenile mandible of 'pre-Zinjanthropus' simply as those of an extreme variant of *Australopithecus*. But at that time, I turned my attention from the dentition of this mandible to the cranial capacity and other features of the cranial bones, as well as to remains from other levels above and below the 'pre-Zinjanthropus' level. It was then I realized that the hominizing departures were not confined to the one feature, the teeth, of one individual, 'pre-Zinjanthropus', but were consistent throughout other analysable parts of the morphological pattern and were maintained from the lowest hominid site in Bed I (*MKI*), through the 'pre-Zinjanthropus' level (*FLK NNI*), where they characterized at least two different individuals, and even apparently co-existed on the same level with *A. boisei* himself. It was this dual consistency that made me realize we were not dealing with an isolated extreme variant of the australopithecines, but with a sustained morphological trend existing alongside a persistent australopithecine line". Seen in the light of these earlier publications, Holloway's claim that I was using the statistical data to back a previously adopted position is a complete *non sequitur*.

The relevance of my comments regarding primate variability is as follows: Several workers, including Holloway, have suggested, or implied, that australopithecine variability would have been comparable with that of modern primates. Thus, he pointed out in his earlier paper that "The parameter of cranial capacity is quite variable in the higher primates", and that "The end-points of the greater 1,000 c.c. variation between members of modern *Homo* are not placed in separate species, nor are gorillas, the volumes of which vary between 752 c.c. and 320 c.c.". He went on to claim: "Thus, it would not be unlikely that on the basis of cranial capacity, at least, the 'pre-*Zinj*' materials are within the Australopithecine taxon of early hominids".

Such statements, by both Holloway and others, are valid only in very general terms, since they disregard the fact that the degree of variability of cranial capacity in modern higher primates is itself highly variable. Some living species have high variability and some low, as determined by measurements of large samples. If we are to infer australopithecine variability from that in modern species, it is therefore pertinent to enquire: which modern species are we to select?

When Holloway speaks of "the fallacy of sample size" and the dubious value of comparing the variability of large samples of chimpanzee and gorilla with that of a small sample of australopithecines, he is simply tilting at windmills. In fact, I stated that the estimated australopithecine coefficient of variation is "very likely . . . an imperfect estimate". This was emphasized by the statement, apparently overlooked by Holloway, that "We simply do not know if *Australopithecus* was a highly variable taxon in general, or whether cranial capacity in this group was of low or high variability"[2].

Holloway's statistical exercise on cranial capacity is of little relevance to my warning about varying variability, for time and again he conveys the misleading impression that I have based taxonomic conclusions on comparisons between the variability of the fossil group and that of living primates, when this is precisely what I have warned against doing. Thus, he states that "the samples are not comparable", which is true only if one includes the fossil samples. However, for living hominids, the samples are adequate and comparable. Thus, my statements were based on 144 chimpanzee, 260 orang-utan, 400 male gorilla and 653 gorilla of both sexes. These large samples yield widely differing coefficients of variation. Hence, it seems reasonable to infer that the cranial capacities of living higher Primates differ appreciably in variability. Therefore, the variability of cranial capacity in them provides no basis from which to infer the variability in extinct higher Primates like *Australopithecus*; *a fortiori*, it provides no adequate basis from which to infer—without other evidence—that the brain-size of *H. habilis* is within the range of variation for *Australopithecus*. It is actually closer to the smallest cranial capacity of *Homo erectus* than to the largest cranial capacity of *Australopithecus*: a case could therefore be made out for a null hypothesis that *H. habilis* and *H. erectus* are not drawn from separate populations! Whether or not the capacity of *H. habilis* proves to be within the australopithecine range can be answered only by more discoveries, not by *a priori* considerations, such as the variability in gorillas and modern man, or, still less, the assumption that the australopithecines represent "the first experiment in hominid evolution" and should therefore have had a more variable brain-size.

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INTER-RELATIONS OF ACTIVATION, DEACTIVATION AND DESTRUCTION STEPS IN CHEMICAL KINETICS

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TEXT-BOOKS universally discuss the dependence of the order of a unimolecular reaction, in the homogeneous gaseous phase, on the partial pressures of reactant and inert species. It is instructive to consider whether analogous transitions in order can occur for bimolecular and termolecular reactions, and (if possible) to formulate criteria for such transitions.

In what follows, the term 'molecularity' is taken to mean the number of molecules, n, simultaneously coming together (participating in an n-body collision) to form the activated complex. It is not taken to mean the total number of individual reactant molecules which together constitute the activated complex.

In this sense of the term, it cannot be asserted without further argument that a bimolecular reaction is necessarily of the second order, nor is a termolecular reaction necessarily of the third order.

It is fundamental to modern theories of chemical reactions that, before reaction can occur‡, reactant species must acquire a certain critical energy, activation, and

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‡ There are two exceptions to this: (i) where there is tunnelling; (ii) for example, atom-atom recombinations where the energy is already sufficient for reaction and activation is superfluous.

that, once this energy has been attained, the rate of reaction can be expressed as the rate of passage of activated complexes through the critical configuration conventionally represented as the top of an energy barrier. A rate-determining step, however, may be either the rate of attainment of the critical energy, or the rate of destruction of the activated complexes by reaction; or both steps may be significant. The precise details depend on whether the reaction is unimolecular, bimolecular or termolecular, and also on the experimental conditions.

Activation, when it occurs, and deactivation are both binary collision processes, while destruction by reaction has a molecularity dependent on the process involved. The order of the reaction and its dependence on the total pressure are thus very much concerned with the interplay of activation, deactivation and destruction by reaction.

Inter-relations for the various mechanisms. The salient features of the mechanisms to be discussed are summarized in Tables 1 and 2. The schemes considered are the simplest ones capable of exhibiting the features of interest. Thus, for example, the unimolecular mechanism is presented in drastically simplified form. More complete schemes (such as the long-known treatment of Kassel[1] for unimolecular reactions, and such recent discussions of termolecular reactions as those of Porter, Szabo and Townsend[2] and Benson[3]) resemble those exhibited here in the main qualitative features. A^* denotes an activated molecule which has acquired the critical energy from a favourable collision or successive favourable collisions, and which can then pass, without further acquisition of energy (though, in relevant cases, after encounter with another molecule—or other molecules), through the critical configuration known as the activated complex, and then form products. The kinetic expressions follow from a consideration of the stationary-state concentration of activated molecules or from the procedure, more directly related to the present subject-matter, outlined in the *Appendix*. The partitioning of the activated molecules A^* between their two possible fates is represented by the ratio:

$$\chi \equiv \frac{\text{chance of deactivation (from } A^*)}{\text{chance of conversion to the activated complex (from } A^*)}$$

The dependence of χ on pressure may be inferred from the molecularity of the steps resulting in destruction of the activated molecules A^*.

Mechanistically, group IV is fundamentally different from groups I, II, III*A*, III*B* in that all four schemes outlined in group IV involve the initial bimolecular formation of an associated species. Nevertheless, IV*A* is kinetically equivalent to III*A*, and only by independent evidence could the two be distinguished. Within group IV, however, there are dissimilarities: in contrast to *A*, no activation is required for *B*, *C* (ref. 4) and *D* (ref. 4). In a more specific fashion, *C* and *D* differ from each other in respect of the type of bimolecular associated species. In *C* there is prior formation of an associated AA^*, the excess energy of which, with respect to A_2, must be removed by M before formation of the stable A_2 molecule; where in *D*, an associated species is formed from one reactant molecule and the third body M. It is also probable that the reaction:

$$A + A + M \rightarrow A_2 + M$$

can occur by simultaneous operation of both mechanisms *C* and *D*. The conclusions for such a scheme are very similar to those drawn for *C* and *D*.

Criteria for a transition in the kinetic order. Two classes of mechanisms are to be distinguished: (i) groups I, II and III*A*; (ii) group IV. For the former, if the destruction and deactivation steps are not of the same molecularity then the rate constant is, in principle, pressure dependent. If these steps are of the same molecularity, then kinetics of order equal to the molecularity will be observed for all pressures. This occurs only for the bimolecular mechanism II.

Table 1

	I Unimolecular	II Bimolecular	III*A* Termolecular activated	III*B* Termolecular unactivated, for example, $2NO + O_2 \rightarrow 2NO_2$ ($\rightleftharpoons N_2O_4$)
Elementary steps by which reaction proceeds	$A + A \xrightarrow[\text{bimolecular activation}]{k_1} A^* + A$ $A^* + A \xrightarrow[\text{bimolecular deactivation}]{k_{-1}} A + A$ $A^* \xrightarrow[\text{spontaneous dissociation}]{k_2}$ products	$A + A \xrightarrow[\text{bimolecular activation}]{k_1} A^* + A$ $A^* + A \xrightarrow[\text{bimolecular deactivation}]{k_{-1}} A + A$ $A^* + A \xrightarrow[\text{bimolecular reaction}]{k_2}$ products	$A + A \xrightarrow[\text{bimolecular activation}]{k_1} A^* + A$ $A^* + A \xrightarrow[\text{bimolecular deactivation}]{k_{-1}} A + A$ $A^* + A + A \xrightarrow[\text{termolecular reaction}]{k_2}$ products	$A + A + A \xrightarrow[\text{termolecular reaction}]{k}$ products
Rate of reaction	$\frac{k_1 k_2 [A]^2}{k_2 + k_{-1}[A]}$	$\frac{k_1 k_2 [A]^2}{k_{-1} + k_2}$	$\frac{k_1 k_2 [A]^2}{k_{-1} + k_2 [A]}$	$k[A]^3$
Order	Neither first nor second. Rate constant is pressure-dependent	Strictly second order	Neither third nor second Rate constant is pressure-dependent	Strictly third order
χ	Necessarily pressure-dependent	Function of rate constants alone	Necessarily pressure-dependent	——
Deactivation Destruction	Bimolecular Spontaneous	Bimolecular Bimolecular	Bimolecular Termolecular	—— Termolecular
Transition in order occurs 'at'	$[A] \sim k_2/k_{-1}$	[Not found]	$[A] \sim k_{-1}/k_2$	——
Rate-determining step in high-pressure region.	First order kinetics imply that destruction of activated complexes is rate-determining $k_{-1}[A]/k_2 \gg 1$; $[A]$ large	Under all conditions of pressure second order kinetics imply that reaction is rate-determining.	Second order kinetics imply that activation is rate-determining $k_2[A] \gg k_{-1}$	
Consequent state of supply of activated molecules	Adequate	Adequate	Inadequate	——
Rate-determining step in low-pressure region.	Second order kinetics imply that activation is rate-determining $k_{-1}[A]/k_2 \ll 1$, $[A]$ small	As above	Third order kinetics imply that destruction is rate-determining.	——
Consequent state of supply of activated molecules	Inadequate		Adequate	——

For groups I, II and III*A* there seem to be no universal criteria in terms either of adequacy of the supply of activated molecules, or of conditions in which there is the possibility of a complete Maxwell–Boltzmann distribution of energies in the reaction system or vice versa, except perhaps the following. If, under all conditions of pressure, the rate-determining step is destruction by reaction and an adequate supply of A^* is always present, then the kinetic order is equal to the molecularity. If, on the other hand, there are regions of pressure such that the supply of A^* becomes inadequate and activation becomes rate-determining, then a pressure-dependent rate constant will be observed.

If active intermediates are formed and the mechanisms are as listed in group IV, then, even though destruction by reaction and deactivation have the same molecularity, a transition in order with pressure for the given reaction may be obtained. Similarly, a criterion in terms of adequacy of supply of activated molecules is not strictly applicable to IV*A* and for IV*B*, IV*C* IV*D*, where activation is not required, is irrelevant.

Rate-determining steps and activation equilibria. The rate-determining step may vary with pressure, and from the kinetics inferences may be drawn regarding the possibility of setting up an equilibrium:

$$\text{reactants} \rightleftharpoons \underset{\text{molecules}}{\text{activated}} \rightleftharpoons \underset{\text{complexes}}{\text{activated}}$$

Such an equilibrium is not, of necessity, implied by the establishment of stationary state concentrations of activated molecules.

Since there is no activation step in III*B*, IV*B*, IV*C* and IV*D*, only groups IV*A*, I, II and III*A* need to be considered.

For the simple strictly second-order bimolecular mechanism II, the kinetics imply that, at all pressures, reaction is the rate-determining step. Consequently, there is always an adequate supply of activated molecules and of activated complexes. Kinetic investigations cannot by themselves demonstrate decisively whether this adequate supply corresponds to a complete Maxwell–Boltzmann equilibrium distribution (of translational, rotational and vibrational energies) for the processes written down here. If there were such a distribution, the number of very energetic molecules A^* would be small, and it is normally taken as plausible that the kinetically adequate stationary concentration of A^* is approximately equal to the 'equilibrium' concentration. There is, however, no *a priori* justification for such an assumption[6]. Similar situations are implied for the high-pressure first-order regions of a unimolecular reaction, for the 'low'-pressure third-order region of a termolecular reaction, and the third-order kinetic region of the 'bimolecular' reaction exhibiting variable order. In this last-mentioned case, the pressure of A is low relative to that required for the second-order region, though in absolute terms it often is found to be relatively large.

In contrast, the second-order, low-pressure region of a unimolecular reaction, and the perhaps hypothetical second-order high-pressure region of a termolecular reaction imply that activation is rate-determining. This, in turn, implies an inadequate supply of activated complexes, that is to say, a complete Maxwell–Boltzmann distribution is not attained. For the 'bimolecular' mechanism exhibiting second- and third-order kinetics when the pressure is sufficiently high for second-order kinetics, very little can be asserted regarding the possibility or otherwise of setting up an equilibrium between

Table 2

IV. 'Bimolecular' exhibiting second and third order kinetics

	Reaction of stoichiometry $A+A+A \rightarrow$ products, but with no chemically distinguishable step of molecularity greater than 2		Association of $A+A$, where A is an atom or free radical which involves third body M	
	A	*B*	*C*	*D*
Elementary steps by which reaction proceeds	$A+A \xrightarrow[\text{bimolecular activation}]{k_1} A^*+A$ $A^*+A \xrightarrow[\text{bimolecular deactivation}]{k_{-1}} A+A$ $A^*+A \xrightarrow[\text{bimolecular association}]{k_2} AA^*$ $AA^* \xrightarrow[\text{spontaneous dissociation}]{k_{-2}} A^*+A$ $AA^*+A \xrightarrow[\text{bimolecular reaction}]{k_3}$ products	$A+A \xrightarrow[\text{bimolecular association}]{k_1} AA^*$ $AA^* \xrightarrow[\text{spontaneous dissociation}]{k_{-1}} A+A$ $AA^*+A \xrightarrow[\text{bimolecular reaction}]{k_2}$ products	$A+A \xrightarrow[\text{bimolecular association}]{k_1} AA^*$ $AA^* \xrightarrow[\text{spontaneous dissociation}]{k_{-1}} A+A$ $AA^*+M \xrightarrow[\text{bimolecular deactivation}]{k_2} A_2+M$	$A+M \xrightarrow[\text{bimolecular association}]{k_1} AM^*$ $AM^* \xrightarrow[\text{spontaneous dissociation}]{k_{-1}} A+M$ $AM^*+A \xrightarrow[\text{bimolecular reaction}]{k_2} A_2+M$
Rate of reaction	$\dfrac{k_1k_2k_3[A]^3}{k_{-1}k_{-2}+k_3(k_{-1}+k_2)[A]}$	$\dfrac{k_1k_2[A]^3}{k_{-1}+k_2[A]}$	$\dfrac{k_1k_2[A]^2[M]}{k_{-1}+k_2[M]}$	$\dfrac{k_1k_2[A]^2[M]}{k_{-1}+k_2[A]}$
Order	Neither third nor second. Rate constant is pressure-dependent.	Neither third nor second. Rate constant is pressure-dependent.	Neither third nor second Rate constant depends on $[M]$	Neither third nor second. Rate constant depends on $[A]$
χ	Necessarily pressure-dependent	———	———	———
Deactivation Destruction	Bimolecular Bimolecular	——— Bimolecular	(Bimolecular) Bimolecular	——— Bimolecular
Transition in order occurs 'at'	$[A] \sim \dfrac{k_{-1}k_{-2}}{k_3(k_{-1}+k_2)}$	$[A] \sim k_{-1}/k_2$	$[M] \sim k_{-1}/k_2$	$[A] \sim k_{-1}/k_2$
Rate-determining step in high-pressure region	Reaction is no longer rate-determining	Association		
Consequent state of supply of activated molecules	———	———		
Rate-determining step in low-pressure region.	Third-order kinetics imply that $[A]$ is low compared with value required for second-order kinetics. Reaction is rate-determining.	Reaction	Deactivation	Reaction
Consequent state of supply of activated molecules.	Adequate	———	———	———

reactants and activated complexes. It can merely be stated that destruction by reaction is no longer the rate-determining step.

For the three mechanisms I, III*A*, IV*A* which exhibit a pressure-dependent overall rate constant, for a given pressure region the rate-determining step depends critically on the mechanism. This is in direct consequence of the relative efficiencies of the processes of activation, deactivation and destruction. As seen in Tables 1 and 2, there are distinct contrasts between the three cases. For the termolecular reaction III*A*, since reaction requires the simultaneous collision of three molecules while deactivation requires only two molecules, destruction by reaction is, at 'low' pressures, the less efficient process, and thus is rate-determining. In contrast, at low pressures, activation for the unimolecular process I is inefficient relative to decomposition (activation requires the simultaneous presence of two molecules, decomposition is spontaneous), while for the 'bimolecular' process of variable order IV*A*, activation and destruction should be of comparable efficiency. On the other hand, at sufficiently high pressures, for the termolecular reaction III*A*, activation (bimolecular) becomes less efficient than reaction (termolecular) with the rate-determining step now being activation; but for a unimolecular reaction I, activation and deactivation, being bimolecular, are more efficient than spontaneous decomposition which thus is rate-determining.

In contrast to gas phase reactions, analogous transitions in order with concentration are never found for reactions in solution. Reaction is always the least efficient process (for reactions where diffusion is not rate-determining), solvent molecules being able to maintain an adequate concentration of activated molecules.

Appendix. The following kind of argument[6], based on considering the relative chances of alternative fates of the species produced in an initial step, leads in a quite direct way to expressions for rates. As an illustrative example, the treatment for the simplified unimolecular mechanism of Table 1 is presented.

The rate of production of A^* from A, that is, the rate of the activation step, is $k_1[A]^2$. Once A^* is formed it will either: (*a*) be deactivated, with the rate of deactivation proportional to $k_{-1}[A]$; (*b*) decompose, with rate of destruction proportional to k_2.

Thus the fraction deactivated is proportional to $k_{-1}[A]$ and the fraction decomposed is proportional to k_2; therefore the fraction decomposed

$$= \frac{k_2}{k_2 + k_{-1}[A]}$$

and the rate of formation of product = (rate of activation) × (fraction of A^* which are destroyed by reaction but are not deactivated)

$$= k_1[A]^2 \frac{k_2}{k_2 + k_{-1}[A]}$$

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ISOTOPICALLY LABELLED MACROMOLECULES IN BIOLOGICAL RESEARCH: DEXTRAN LABELLED WITH RADIOACTIVE IODINE

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MACROMOLECULES labelled with radioactive isotopes have several potential uses. Two applications of interest in biological research are firstly for measuring *in vivo* the permeability of membranes to solute molecules of various sizes and secondly to serve as standards in the comparison of samples of a polydisperse substance. The radioactivity provides rapid and accurate measurement, susceptible to automation, and avoids the subtraction of an arbitrary 'blank' value from chemical measurements due to the presence of interfering substances in biological fluids.

Inulin, a polysaccharide with a molecular weight of about 5,000, is widely used for measurement of renal glomerular filtration rate. In a previous article the labelling of inulin with radioactive iodine isotopes by introducing an occasional allyl group into the molecule and adding iodine at the double bond was described[1]. Satisfactory measurements of glomerular filtration rate have been made using labelled inulin[2], with some reservations on the stability of the compound.

Dextran fractions of known molecular weight distribution are available and greatly extend the range of molecular sizes accessible for permeability investigations[3,4]. Dextran has been labelled[1] with radioactive iodine isotopes in the same way as inulin was labelled.

For the higher molecular weights, which are of greater biological interest, fractions which are sufficiently narrow are difficult to prepare. This has been overcome by the injection of polydisperse dextrans and determination of the molecular weight distribution of dextran recovered from the biological fluids by the automatic turbidimetric method developed by Wallenius, Waschewsky, Aronsson and Hegedüs[5]. The advent of the 'Sephadex' range of cross-linked dextrans greatly facilitates the analysis of polydisperse materials in this higher range of molecular weights and makes it possible to investigate a very wide range of permeability from a single injection in one experiment. When also isotopically labelled macromolecules are used the amount of material injected is so small that disturbance of the physiological osmotic equilibrium is avoided.

The present communication reports some extensions of the work with labelled dextran. Introduction of allyl groups was carried out exactly as described for inulin, and allyl dextrans were freeze-dried and stored as powders. Addition of iodine was carried out on a small scale (0·1–1 g) as required. It was found that solutions of iodine (^{131}I or ^{125}I)-labelled dextran could be stored at −20° C for several months without increase in free iodide ions. It was convenient to expose the solution to a small portion of the anion exchange resin 'Amberlite *IRA*-400' in chloride form to remove any iodide ions present immediately before use in an experiment.

Columns of 'Sephadex *G*.200' provide some degree of fractionation of dextran according to molecular size. Use of radioactive dextran facilitates assessment of this separation. The allyl groups and therefore the isotopic iodine atoms are randomly distributed among dextran molecules, consequently the measured radioactivity is proportional to the weight of labelled dextran present. This is true for each fraction from a 'Sephadex' column even though the fractions may contain dextran of different molecular weights. (In contrast iodine-labelled polyvinyl pyrollidone is labelled by an end-group reaction so that the

radioactivity is proportional to the numbers of molecules present in a sample and this number varies inversely with the molecular weight.)

Fig. 1 shows the distribution of radioactivity due to a sample of ^{125}I-labelled dextran of molecular weight (M_w) 150,000, prepared from a dextran fraction (Pharmacia, Ltd.). The sample was mixed with serum and the separation of the protein peaks shows the efficient working of the column. Fig. 2 shows a similar distribution for another sample of radioactive dextran. When fractions from near the ends of the distribution were mixed and re-chromatographed two peaks were obtained showing that genuine separation was occurring, and not simply spreading due to diffusion and flow.

In a further experiment, ^{125}I-allyl dextran was mixed with an excess of the dextran fraction M_w 150,000 from

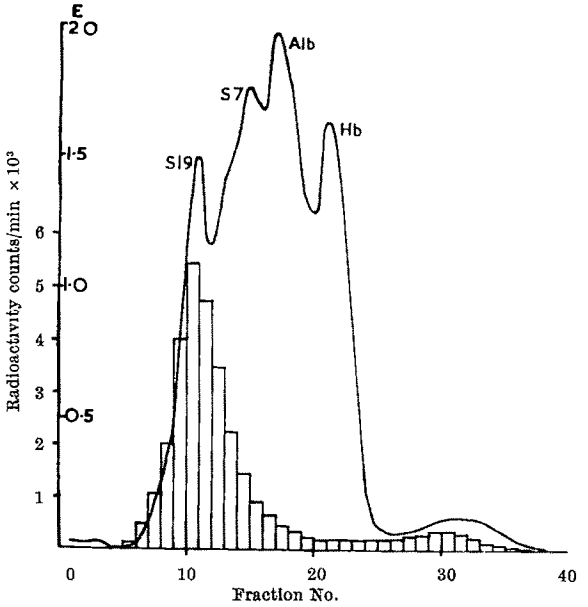


Fig. 1. Showing the distribution of radioactivity due to a dextran fraction of M_w 150,000 labelled with ^{125}I, plotted as a histogram, and the simultaneous resolution of serum proteins, measured as peaks of absorption at 280 mμ. A mixture of 2 ml. serum and 0·05 ml. dextran was separated on a column of 'Sephadex *G* 200' 35·5 cm long and 2·5 cm diameter using 0·9 per cent sodium chloride solution for elution into 5 ml fractions

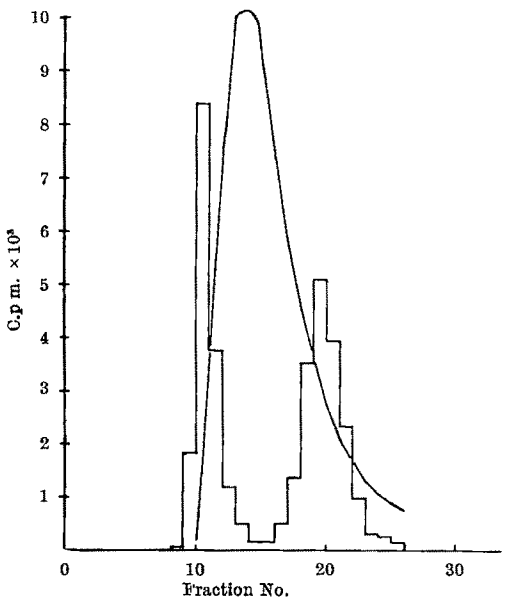


Fig. 2. Showing the distribution of radioactivity due to a reference sample '*R*' of dextran labelled with ^{125}I, plotted as a solid line, and the re-chromatography of a mixture of fractions 11 and 21+22, plotted as a histogram of c.p.m. 'Sephadex' column 45 cm long, other conditions as in Fig. 1

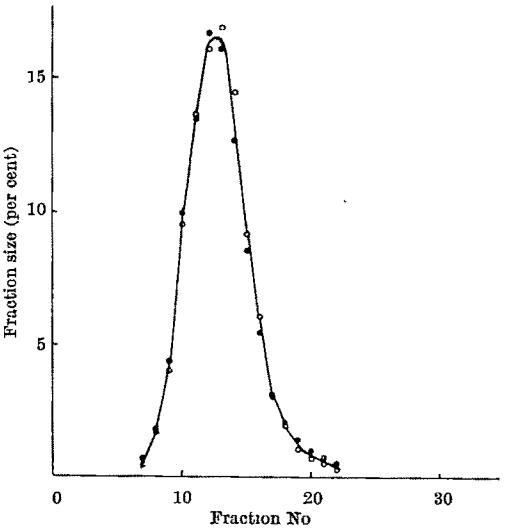


Fig. 3. Dextran labelled with ^{125}I was mixed with an excess of the dextran fraction M_w 150,000 from which it had been prepared and the mixture was run on a column of 'Sephadex *G*.200' as in Fig 1. The diagram shows the distribution of dextran (○), determined by the anthrone estimation and the distribution of radioactivity (●) The distributions are very similar showing that molecular weight distribution has not altered appreciably during the process of labelling

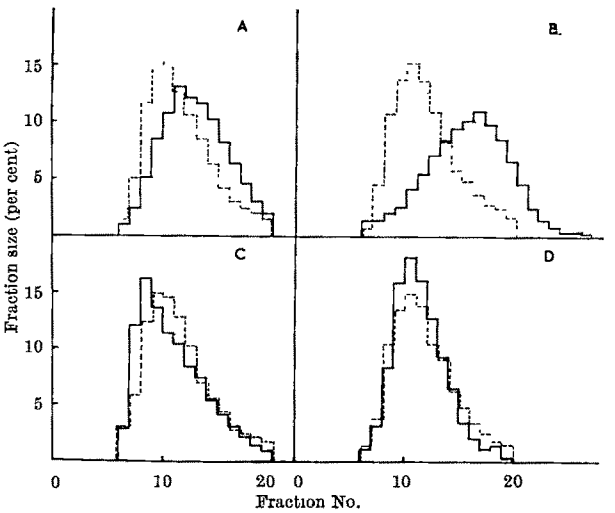


Fig 4 Comparison of four clinically used dextrans with the radioactive reference preparation of dextran '*R*' shown as dotted line. *A*, M_w 75,000, *B*, M_w 40,000; *C*, dextran B P.1963; *D*, dextran conforming to a new specification, B.P. *Addendum 1966*

which it had been prepared. The mixture was separated on a column of 'Sephadex *G*.200' and the radioactivity and dextran content of each fraction were measured (Fig. 3). The ratio of radioactivity to dextran was approximately constant over a range of fractions corresponding with 95 per cent of the whole material. This experiment shows that introduction of allyl ether groups has not been accompanied by hydrolysis sufficient to cause a detectable alteration in molecular size distribution.

The preceding experiment shows the practicability of using radioactive dextran as a reference substance when examining the distribution on 'Sephadex *G*.200' of other samples of dextran which may have quite different distribution of molecular size, as in the examples shown in Fig. 4. The reproducibility of separation of the labelled dextran into fractions was ± 1 per cent of the total radioactivity. The dextran distributions illustrated are those of various dextrans in clinical use. The radioactive reference preparation was also prepared from a clinical dextran conforming to a new specification.

The permeability of a biological membrane may be deduced from the concentrations of serum proteins present on each side of it[6]. For example, the proteins

present in serum and urine in certain diseases have been separated according to molecular size by 'Sephadex' columns and their concentrations have been determined, enabling the permeability of the renal glomerulus to these proteins to be calculated[7].

Columns of 'Sephadex *G*.200' enable a distribution according to molecular size for small samples of dextran, such as may be recovered from serum, to be readily obtained. Radioactive dextran was used in the initial experiments. Fig. 5 shows the distribution on a 'Sephadex *G*.200' column of radioactive dextran from the serum and from the urine of a rabbit. Fig. 6 shows the urine/plasma (*U*/*P*) ratio for the dextran of each fraction. Clearly it is not necessary to use radioactive dextran and Fig. 7 shows similar data for a patient transfused with clinical dextran conforming with the new specification mentioned here. In these experiments radioactive dextran has been mixed *in vitro* with the dextran recovered from biological fluids to act as a reference substance for checking the reproducibility of 'Sephadex' column separations.

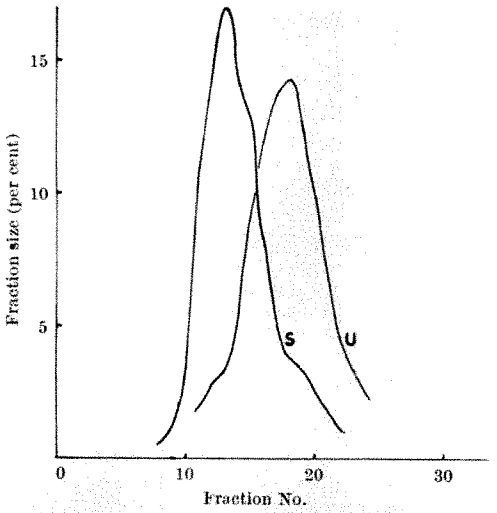


Fig. 5. The distribution of dextran recovered from the serum (*S*) and urine (*U*) of a rabbit given the radioactive reference preparation of labelled dextran '*R*' with 4 ml. 6 per cent dextran of similar molecular weight distribution as 'carrier'. The serum sample was taken in the middle of a 2-h urine collection period

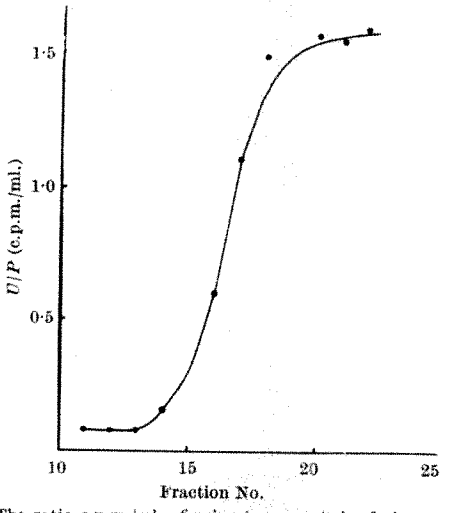


Fig. 6. The ratio c.p.m./ml. of urine to c.p.m./ml. of plasma, termed *U*/*P*, for each fraction, calculated from the data of Fig. 5. The sigmoid shape of the curve is related to the permeability of the renal glomerulus

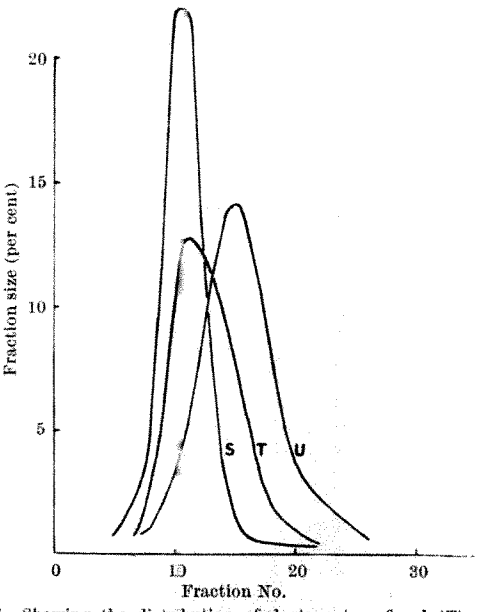


Fig. 7. Showing the distribution of dextran transfused (*T*) and recovered from urine 24 h after transfusion (*U*) and serum 72 h after transfusion (*S*). Dextran was determined by the anthrone method on each fraction from the 'Sephadex *G*.200' column. The patient, who had a burn of 37 per cent of the body surface, was transfused with 4 l. 6 per cent dextran

The further development of this technique and the application to the investigation of renal permeability in normal and diseased man and animals is being actively pursued in collaboration with members of the Department of Experimental Pathology, University of Birmingham[8].

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In vitro DESTRUCTION OF SKELETAL MUSCLE BY SENSITIZED CELLS

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THE aetiology of polymyositis remains obscure even though it is nearing a century since the original descriptions of the disease were reported[1–4], and despite considerable more recent interest[5–8]. The disorder is non-hereditary and shows itself as weakness of the limb girdle muscles and often involves the pharynx and neck. In approximately two-thirds of patients there are cutaneous lesions[7] and in these the disease is better designated as dermatomyositis. A remarkable feature is the high incidence of associated visceral malignancy, which is as much as 20 per cent in some series[8]. Microscopically the lesions consist of segmental muscle fibre necrosis, phagocytosis of necrotic sarcoplasm by macrophages and regenerative changes with which is associated a variable

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leucocytic infiltrate, chiefly lymphocytes, larger mononuclear cells and plasma cells, and fewer neutrophils and eosinophils. Similar alterations occur in skeletal muscle in a wide variety of circumstances[6], but especially in the nutritional myopathies of some animals[9]. From a study of the histopathology it may be reasonably inferred that the pathogenetic mechanism taking place in polymyositis is fundamentally muscle fibre necrosis and the other changes occurring in the lesions are a secondary and non-specific response to selective injury of sarcoplasm.

The cause of polymyositis is suggested to be related to a disorder of immune mechanism because of the sharing, not uncommonly, of clinico-pathologic features with other diseases, such as rheumatoid arthritis or systemic lupus erythematosis, where disordered immunity is also strongly suspected to be of importance, as well as the high incidence of underlying carcinoma in polymyositis, the sensitivity to tumour extracts in some cases[10,11], the elevation of serum globulins and the improvement which follows the administration of corticosteroids[7]. Up to the present, there has been little experimental evidence to support an immunopathogenic hypothesis of aetiology, principally because attempts to devise an experimental model have been hampered by difficulties in interpretation of the lesions[12,13] related to the use of Freund's adjuvant which causes a severe arthritis and adjacent myositis[14], so that adequate experimental control has not been possible. Other workers report 'dystrophy-like' muscle lesions induced by immunological methods[15], but the reported changes do not resemble the histological findings in cases of polymyositis.

In order to explore the possibility that an appropriately sensitized cell may be detrimental to skeletal muscle in a manner related to delayed or cellular hypersensitivity mechanisms[16], an *in vitro* experiment following the methods employed to induce demyelination in cultures of peripheral nerve was utilized[17] and promised to surmount some of the difficulties encountered in *in vivo* models.

To this end, 2-mm cubes of skeletal muscle were excised aseptically from the limb girdles of foetal Sprague–Dawley rats, 19–20 days gestation, and planted on 22-mm coverslips coated with reconstituted rat tail collagen[18]. The explants were fed with a drop of medium consisting of equal parts of human placental serum, 50 per cent nine-day chicken embryo extract, and balanced salt solution with glucose added to give a final concentration of 600 mg per cent. The preparations were placed in a Maximov double coverslip assembly[19] and incubated at 35·5° C. The cultures were re-fed twice weekly and frequent microscopic observations of the living cultures were made. The cultures grew rapidly and many cross-striated young muscle fibres were present in one to three weeks.

Sensitization of young (150 gm) female Sprague–Dawley rats was achieved by the use of homogenates of skeletal muscle, obtained from the pelvic girdles of adult New Zealand white rabbits, in Freund's adjuvant (2 gm of muscle to 3 ml. of adjuvant, containing 3 mg AFB/ml.) prepared in a 'Virtis' homogenator run at alternating fast and slow speeds for about 30 min and with care taken to avoid heating the mixture. Injections of 0·05 to 0·1 ml. of the antigen were made intradermally in both hind footpads of ten rats, each receiving three injections at intervals of one week. Controls consisted of ten normal, untreated rats, ten rats injected once a week for three weeks with adjuvant alone (0·5–0·1 ml. into both hind footpads), and ten rats injected with homogenates of rabbit kidney in adjuvant in the same proportions, dose and site of injection as in the test group. Rats were examined daily for signs of disease.

The rats were killed with chloroform at intervals of three to six weeks after injection, and inguinal lymph nodes from both sides were excised under sterile precautions and followed by necropsy examination. Muscle blocks from upper and lower limb girdles were chosen for microscopy and fixed in Heidenhain's Susa for 24 h, embedded in paraffin and stained by haematoxylin and eosin. Portions of sciatic nerve and of main viscera were also selected.

The excised lymph nodes were placed in a few drops of balanced salt solution and repeatedly incised with a sharp blade. Clouds of cells liberated into the fluid were then washed and centrifuged at 700 r.p.m. for 5 min. The supernatant was decanted, and the cells resuspended in nutrient medium containing penicillin 1,000 u./ml., dissolved in the balanced salt solution, and the concentration of cells adjusted so that one drop of this suspension, fed to a culture, contained approximately 4×10^6 cells. Six cultures containing differentiated skeletal muscle were tested with cells or serum from every animal. Four cultures were tested with one drop of the cell suspension and two comparable cultures were 'fed' with a drop of medium in which the human placental serum was replaced by serum from the test animal obtained by means of cardiac puncture, prior to the excision of the lymph nodes. The living cultures were carefully examined throughout the experiment and observations continued for up to six days following the addition of the lymph-node cells or serum. At the end of the observation period cultures were fixed in 10 per cent formalin and stained by Mallory's phosphotungstic acid haematoxylin method and mounted whole.

Of the forty cultures treated with lymph-node cells from the ten donor rats given three injections of rabbit muscle and adjuvant, thirty-eight showed destructive changes (Fig. 1). Briefly, these consisted of thinning of individual muscle fibres, with vacuolation and granularity of cytoplasm progressing by further attenuation to separation from the collagen substrate and becoming fragmented. Such changes appeared 'suddenly', and required 12–24 h for their development and often first appeared in the most mature area of the outgrowth which was nearest to the original explant, resulting in 'clear spaces' in this area. These destructive changes began from 2 to 6 days after the addition of sensitized lymph-node cells. In phosphotungstic acid haematoxylin stained preparations architectural detail was lost in the fragments and clumps. Nuclei were dark, irregular and often disrupted. Numbers of large macrophages accompanied the breakdown of muscle fibres and many were observed to contain sarcoplasmic debris deeply staining with phosphotungstic acid haematoxylin, and although many lymphocyte-like cells were present at the conclusion of the 6-day period of observation it was apparent that a significant proportion had increased in size when compared to the cells in control cultures.

Lesions were present in the muscles of the hind limbs in all ten rats in the group sensitized to rabbit muscle

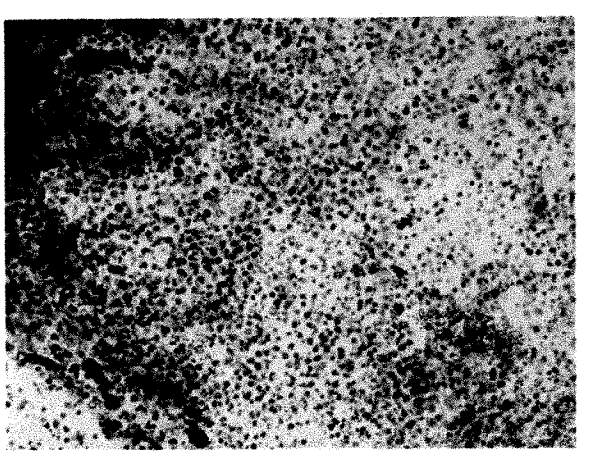


Fig. 1. *In vitro* destruction of muscle fibres following addition of lymph-node cells of rat (*MF8*) sensitized to rabbit muscle. Note dark fragments of fibre remnants, phagocytic cells and fewer lymphocyte-like cells. Culture 12/13-11*L*, phosphotungstic acid haematoxylin. Whole mount (×75)

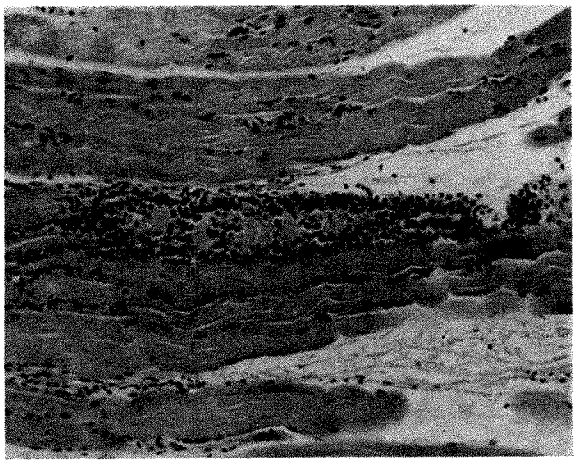


Fig. 2. *In vivo* muscle fibre necrosis and leucocytic infiltration. Pectoral muscle in longitudinal section, rat (*MF*22). (Haematoxylin and eosin. × 140)

and consisted of round cell infiltrates, macrophages, neutrophils and giant cells. There was associated frank necrosis of tissue in some instances. Individual muscle fibres in the vicinity of these lesions, which were most prominent in the neighbourhood of joints, were infrequently necrotic, showing myophagic activity. In nine of the ten rats in this group, muscle lesions were present as well in the fore limbs where arthritic changes were much less severe and in pectoral muscles, and consisted of focal muscle fibre necrosis and interstitial infiltrates of small numbers of polymorphonuclear leucocytes and round cells (Fig. 2). Destructive changes appeared in four of the twenty cultures to which serum was added from animals in this group. This rate was only a little higher than in the controls, as follows. Normal (untreated rats): 1 spontaneous 'breakdown'; 1 contaminated by bacteria; 18 unaffected. Rats injected with adjuvant alone: 3 'breakdowns'; 17 unaffected. Rabbit kidney and adjuvant-treated rats: 3 'breakdowns' and 17 unaffected cultures.

Of the forty cultures to which lymph-node cells were added from healthy untreated rats, seven showed 'spontaneous breakdown' of a small proportion of fibres in the culture. The changes were less severe and differed in appearance from those which occurred in cultures treated with cells from the group sensitized to muscle. There were fewer large macrophages, and there was a greater proportion of small cells. Myotubes and young muscle fibres in those cultures became granular and very gradually decreased in size. The morphological changes were similar to those observed in cultures which 'spontaneously' degenerate in the laboratory. Thirty-three of the cultures to which cells were added from normal rats remained intact at the conclusion of the 6-day period of observation (Fig. 3). Muscles were examined histologically from both pelvic and shoulder girdle regions in nine of the ten rats in this group and none showed lesions.

In the control group of cultures to which lymph-node cells from animals treated by three injections of adjuvant alone were added, six of forty broke down after the addition of the cells. The changes in the positive cultures were similar to those which broke down with cells from the untreated group. Muscle sections from hind and fore limbs of all ten animals were examined. In three rats the hind limb muscles, and in two of the three the fore limb muscles, showed lesions similar to those observed in the test group.

In the group of control cultures treated with lymph-node cells from animals sensitized to rabbit kidney and adjuvant by three injections, eight showed destructive changes, two became contaminated with bacteria, and thirty were normal at the end of six days. Hind and fore limb muscles obtained from the ten rats revealed muscle fibre necrosis in only one from a hind limb group. In contrast, all these rats showed kidney lesions. Microscopically, hypercellularity of glomerular tufts, due to epithelial and endothelial cell hyperplasia, was present together with numbers of round cells and polymorphonuclear leucocytes. Adhesions in Bowman's space were numerous and proteinaceous material was found in many renal tubules.

All groups which received adjuvant developed arthritis, mainly of the hind limb joints. Because this process caused impairment of mobility by itself and the fact that no animal became fully paralysed, it was not possible to perceive a difference, during life, between animals which later showed severe microscopic muscle lesions and those which did not. The much higher incidence of necrotic lesions in fore limb muscles of animals sensitized to rabbit muscle was considered a valid sign of effectiveness of the sensitization method. The fore limb muscle lesions did not bear a relationship to the proximity of joints as was the case with the hind limbs, and as a rule arthritic changes were much less severe in the fore limbs than in the hind limb joints.

Table 1

Groups of ten rats	No. and effect on cultures treated with Cells +	Cells −	Serum +	Serum −	Muscle lesions at necroscopy: Hind limb	Fore limb
MF	38	2	4	16	10	9
NR	7	33	1;1*C*	18	—	—
F	6	34	3	17	3	2
KF	8;2*C*	30	3	17	1	—

+, *in vitro* destruction of muscle; −, no effect. *C*, Bacterial contamination; *MF*, group of rats sensitized by intradermal injections of rabbit muscle in Freund's adjuvant; *NR*, untreated rats; *F*, group of rats given intradermal injections of adjuvant alone; *KF*, group of rats given intradermal injections of rabbit kidney in adjuvant.

The results summarized in Table 1 show that lymph-node cells obtained from rats sensitized to muscle are able to destroy muscle fibres, *in vitro*, at a rate much greater than in controls. The findings are presented as evidence that a cell, in certain circumstances, may cause necrosis of muscle and therefore would support a postulate that muscle fibre necrosis, the fundamental lesion of human polymyositis, may also be the result of the action of a cell which has developed this property because of a pathological disturbance of immune mechanisms.

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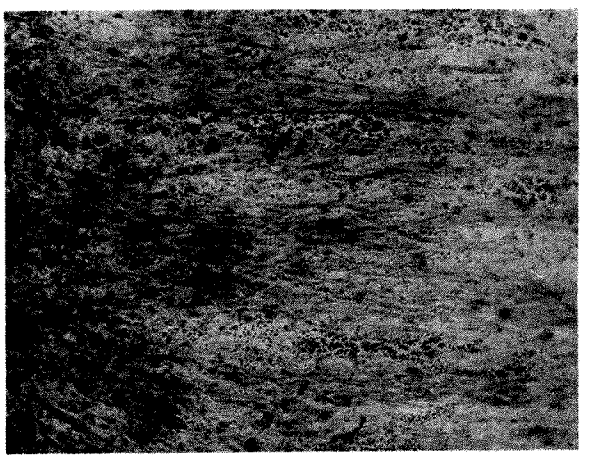


Fig. 3. *In vitro* muscle fibres unaffected by lymph-node cells of untreated rat (*NR*5). Culture 3/17-4*L*. phosphotungstic acid haematoxylin. Whole mount (× 60)

(1963–64) and C. J. Martin travelling fellowship of the National Health and Medical Research Council of Australia (1964–65), while on leave from the Department of Pathology of the University of Western Australia.

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RESTORATION OF THYMIC FUNCTION IN NEONATALLY THYMECTOMIZED MICE BEARING XENOGENEIC THYMIC GRAFTS

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DIRECT evidence has been presented to show that the thymus produces a humoral factor which plays a significant part in the development and maintenance of normal immune responses. Neonatally thymectomized mice of several strains carrying thymic tissue in cell-impermeable 'Millipore' diffusion chambers have their capacity restored to reject skin homografts, to form antibodies, both IgG and IgM, to sheep erythrocytes, and to repopulate their lymphoid organs[1–4]. Growth failure has also been prevented in neonatally thymectomized recipients of thymic tissue in 'Millipore' chambers[2].

Adequate control experiments have been carried out to show that these restorative effects are not the result of an adjuvant or of non-specific tissue influences. Lymph node and splenic tissue in 'Millipore' diffusion chambers[5], for example, have been shown to be ineffective in preventing immunological deficiencies in thymectomized mice. Mice with empty diffusion chambers are not restored[5,6].

It has recently been reported that allogeneic (*C57BL*) thymic tissue, enclosed within diffusion chambers, has the capacity to restore immune reactivity in *CBA* mice, showing that thymic humoral factor (THF) is not strain specific[5].

The work reported in this article was designed to determine whether thymic grafts from other species could prevent the growth failure, lymphoid depletion and immunological deficiencies of neonatally thymectomized mice.

F_1 hybrid mice, $(C57BL/Ka \times C3Hf/Lw)F_1$, were thymectomized under ether anaesthesia within 18 h of birth. Either sham-operated or intact litter mates were kept in each litter. Within 24 h of thymectomy one lobe of a thymus from a new-born Osborn–Mendel rat or from a Syrian hamster (*Mesocricetus auratus*) was grafted into the subcutaneous tissues of the lower back. Thus each litter comprised intact or sham-operated controls, thymectomized mice only and thymectomized mice bearing thymic grafts. In our experiments thirty thymectomized mice received rat thymic grafts and thirteen received hamster grafts. Since no particular differences were noted between the groups they were combined for convenience and referred to as mice with xenogeneic thymic grafts.

Body-weights were taken weekly. The absolute lymphocyte levels were determined at 2 months of age, before any other experimental procedures were undertaken. Mice were bled from the retro-orbital sinus. Immunization with sheep erythrocytes was accomplished at 3 months of age by the method described previously[3,7]. Haemolysin antibodies were determined at 7 days and agglutinin antibodies at 10 days.

Allogeneic skin grafts from *AKR* donors were carried out shortly after 2 months of age. Full-thickness homografts were performed using the method of Billingham and Medawar[8]. All mice dying in the course of the experiment or killed at 7 months of age at the end of the experiment were necropsied. Lymphoid tissues were taken routinely and the superior mediastinum was examined for thymic remnants. Histological sections were obtained on all suspected tissue in the retrosternal area.

Neonatally thymectomized $(BL \times C3H)F_1$ mice are subject to the 'wasting' syndrome but the onset is relatively late and the condition relatively chronic. The most striking sign is a loss of subcutaneous fat. The cumulative mortality for the several groups of mice is shown in Table 1. There were seven incomplete thymectomies in the non-grafted group and these were detected at necropsy at 7 months of age. These mice did not 'waste'. On the other hand, there were also six mice bearing thymic remnants which 'wasted' and died from 54 to 150 days of age. Among the thymectomized mice bearing xenogeneic thymic grafts, nearly 50 per cent lived to necropsy at 7 months and did not 'waste'. Seven of the twenty-one restored mice had thymic remnants which were disclosed only on microscopic examination of the retrosternal tissues. It is of interest that six of the twenty-two grafted mice which died from wasting at ages 53 to 115 days also had thymic remnants. Only one intact or sham-operated control died during the period of observation.

Fig. 1 shows the mean weight curves for intact or sham-operated and neonatally thymectomized F_1 mice compared with thymectomized mice bearing xenogeneic thymic grafts which did not waste and die (restored) and the group receiving thymic grafts which wasted and died (not restored).

The survival of skin homografts in neonatally thymectomized F_1 mice in sham (intact) thymectomized and in the thymectomized group grafted with xenogeneic thymuses is shown in Table 2. All the intact group completely rejected *AKR* skin by 20 days, whereas 80 per cent

Table 1. MORTALITY IN THYMECTOMIZED, INTACT AND RESTORED $(BL \times C3H)F_1$ MICE

Group	No. of mice	Number dying (cumulative percentage) at days: 30–60	61–90	91–120	121–150	151–180	181–200
Thymectomized	54	8 (15 per cent)	21 (54 per cent)	6 (65 per cent)	3 (70 per cent)	4 (78 per cent)	5 (87 per cent)
Sham or intact	40	—	—	—	—	1 (2·5 per cent)	0 (2·5 per cent)
Thymectomy plus xenogeneic graft	43	6 (14 per cent)	5 (26 per cent)	5 (37 per cent)	1 (47 per cent)	2 (51 per cent)	1 (51 per cent)

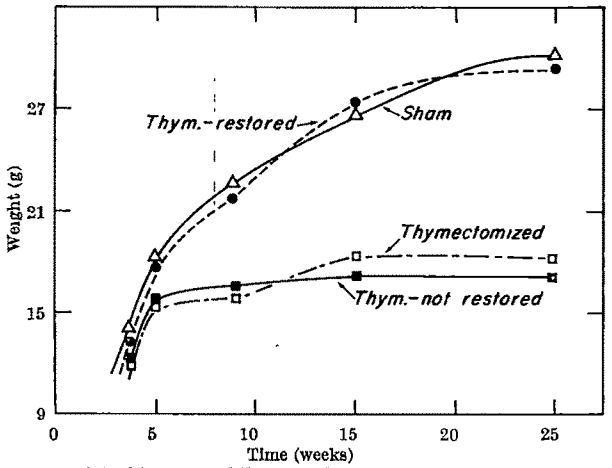


Fig 1. Weight curves of the several groups described in text Both sexes are included but are weighted equally. At the beginning of weighing there were twenty-three in the thymectomized group, eighteen in the sham-operated group, twenty-three in the thymectomized-restored group, and sixteen in the thymectomized-not restored group

of the thymectomized mice retained viable grafts beyond this time, the majority until death. Some of these thymectomized mice which rejected the allogeneic grafts early in the experiment were found at necropsy to be incomplete thymectomies (see Table 1). The presence of xenogeneic thymic tissue in the subcutaneous connective tissues apparently restored the capacity of a large number of the neonatally thymectomized F_1 mice to reject *AKR* homografts; fourteen (64 per cent) of these rejected skin grafts by 20 days. Among the group retaining *AKR* skin grafts until death, four 'wasted' and died and were thus not, in fact, restored.

The antibody responses of the several groups of mice to intraperitoneal challenge by sheep erythrocytes at 3 months of age are shown in Table 3. Very few neonatally thymectomized mice showed haemolysin or agglutinin responses within the range of the sham-operated controls and seven of these were found to have thymic remnants at necropsy. On the other hand, the majority of these neonatally thymectomized F_1 mice grafted with xenogeneic thymic tissue, which did not 'waste' and die, exhibited antibody titres within the range of the sham controls, although the mean titres were somewhat lower.

The mean total lymphocyte count of fifteen sham-operated or intact F_1 mice obtained from peripheral blood at 2 months of age was found to be 8,700/c.c. This represents 70 per cent lymphocytes. Thymectomized litter mates, on the other hand, had a reduced total lymphocyte count of only 3,800 (34·3 per cent lymphocytes), while the mean lymphocyte count of those thymectomized mice with xenogeneic grafts fell between these two figures: the mean total lymphocyte count was 6,250, which represents 47 per cent of the total peripheral leucocytes. The distribution of the individual counts is shown in Fig. 2.

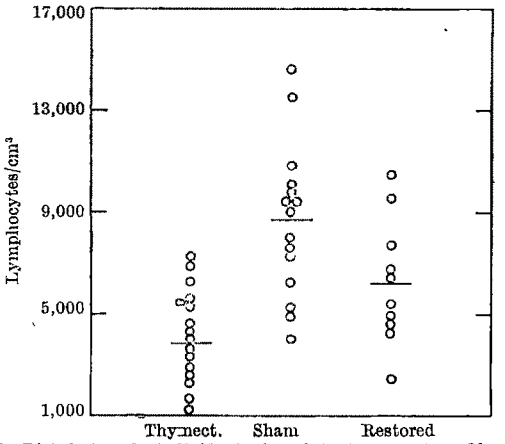


Fig. 2. Distribution for individual mice of absolute number of lymphocytes in neonatally thymectomized F_1 mice, in sham-operated controls and in thymectomized mice bearing xenogeneic thymic grafts (restored)

For the most part, an appreciable difference between the lymphoid tissues of the neonatally thymectomized group and their sham-operated litter mates was found on microscopic examination. There was a reduction in the size of lymphoid follicles and particularly of the germinal centres of the follicles in thymectomized mice dying at various times. The deficiency was extreme, showing nearly complete absence of follicles, in those mice exhibiting severe 'wasting'. In those F_1 mice classed as 'restored' by the several criteria used in this experiment variable histological patterns in the lymph nodes, spleen and Peyer's patches were found. In few of these mice was there a return to a completely normal picture. Lymphoid follicles tended to be smaller but the germinal centres were usually distinct and recognizable. In no case did the tissues of the restored mice show the extreme lymphoid deficiency characteristic of neonatally thymectomized mice.

Xenogeneic thymic grafts were not recovered from the subcutaneous connective tissues of any of the F_1 mice dying from 3 to 7 months of age or from those which lived and were killed at 7 months. These findings are similar to those reported by Miller[9] and Leuchars *et al.*[10]. These investigators found that thymectomized mice bearing allogeneic thymic grafts had their immunological capacity restored but that the graft of thymic tissue was not regenerated and was rejected by the restored host within 10 or 11 days or at the latest within 3 weeks[9] after grafting. Since both groups of investigators observed a persistence of epithelial-reticular cells within the allogeneic thymic graft, they concluded that restoration of immunological function could result from a humoral factor liberated by these cells which acted on host-type lymphoid cells.

Recovery of grafts of thymuses from Osborn–Mendel rats and hamsters into neonatally thymectomized $(BL \times C3H)F_1$ mice was therefore undertaken in a special group of mice at earlier periods, that is at 5-day intervals

Table 2 SURVIVAL OF ALLOGENEIC (*AKR*) SKIN GRAFTS ON NEONATALLY THYMECTOMIZED ($BL \times C3H)F_1$ MICE AND THEIR LITTER MATES GRAFTED WITH XENOGENEIC THYMUSES

Group	No. of mice showing skin graft survival for: 15 days	16–30 days	31–60 days	61–200 days
Thymectomized	6*	2*	6*	16
Sham thymectomy	22	1	0	0
Thymectomy + Xenogeneic thymus	12	2	4	4†

* Six mice were found to be incomplete thymectomies at necropsy, one incomplete retained *AKR* graft to 23 days, another to 53 days.
† All these mice which retained *AKR* grafts eventually 'wasted' and died before 180 days.

Table 3 HAEMOLYSIN AND AGGLUTININ PRODUCTION TO SHEEP ERYTHROCYTES IN ($BL \times C3H)F_1$ MICE NEONATALLY THYMECTOMIZED, SHAM-OPERATED AND RESTORED WITH XENOGENEIC THYMIC GRAFTS

Group	No. of mice	No of mice showing $\log_2$ titres of: 0	1	2	3	4	5	6	7	8	9	Mean $\log_2$ titre
					(haemolysins)							
Thymectomy	29	—	20	3	1	1	1(2)*	—(1)*	—	—	—	1·44
Sham	28				—	4	5	7	10	2	—	6·04
Thymectomy + xenogeneic graft	21	—	1(6)†	1(1)†	1	4	1	3	3	—		4·70
					(agglutinins)							
Thymectomy	30	—	12	2	4	5	1(2)*	2(2)*	—			2·50
Sham	22	—	—	1	—	3	3	4	7	2	2	6·18
Thymectomy + xenogeneic graft	21		(4)†	(1)†	1	3	5	5	2	—		4·73

* Had thymic remnants in retrosternal tissues microscopically at necropsy
† These mice died from 'wasting'

from 5 to 40 days after grafting. The grafts were removed at these times for histological study. The histological picture was similar in both types of grafts. There was degeneration of lymphocytes, but a few persisted until approximately the tenth day. Regeneration into a typical structure was not observed. There was, however, a persistence of epithelial-reticular cells in many grafts to as late as 30 days. Polynuclear cells eventually invaded the periphery, and there was central necrosis and invasion by fibrous tissue. In contrast to these results we were able to confirm the findings of Yunis *et al.*[11] that Holtzmann rat thymuses grafted into neonatally thymectomized mice are regenerated into a typical thymic structure. Neonatal thymic grafts from Holtzmann rats grafted into these F_1 mice were, in most cases, completely reconstituted by the fifteenth day and were found persisting as viable thymic fragments with lymphoid differentiation and organization into cortex and medulla for as late as 50 days. The results of attempts at restoration with these donor Holtzmann thymic grafts will be reported later.

The results reported here show that a considerable number of neonatally thymectomized $(BL \times C3H)F_1$ mice were restored toward normalcy when they were grafted with neonatal xenogeneic thymic tissue. It has been reported that allogeneic thymic grafts are capable of restoring the normal immunological function in thymectomized mice yet viable grafted tissue could not be recovered after 3 weeks[9,10]. Allogeneic thymic tissue in 'Millipore' diffusion chambers has also been shown to promote immunological recovery; splenic tissue and lymph nodes were unable to do so[5]. These findings would seem to indicate that the restoration observed in the present investigation was the result of a humoral factor, THF, dependent on the presence of surviving epithelial-reticular elements of the xenogeneic thymic graft and capable not only of inducing the maturation of residual host (F_1) lymphoid cells but of re-establishing lymphopoiesis. Obviously an unequivocal explanation for restoration cannot be given since adequate controls were not included in this experiment.

If this is the explanation for the restoration seen in our F_1 mice, the thymic-dependent humoral-mediated mechanism is not species-specific.

The lack of effect observed by Yunis *et al.*[11] in *C3H* mice in which there occurred successful rat (Holtzmann) thymic tissue grafts has no ready explanation. The syndrome following neonatal thymectomy in *C3H* mice is rapid and acute, whereas signs of deficiencies in the $(BL \times C3H)F_1$ mice used here appear later and have a more chronic form. It is likely that the residuum of potentially competent precursor lymphoid cells in our F_1 mice represents a more sensitive assay technique.

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WATER-SOLUBLE FACTORS WITH VITAMIN K ACTIVITY FROM PIG LIVER AND FROM *Fusiformis nigrescens*

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SINCE its discovery as a blood-clotting factor by H. Dam[1], vitamin K has been accepted as a fat-soluble vitamin of the 2-methyl-1.4-naphthoquinone series which in natural forms are substituted in the 3 position with a phytyl group (in vitamin K_1) or with multiples of isopentoid (in K_2) series. These forms can be readily extracted with fat solvents from plants and from certain aerobic and anaerobic bacteria[2–4]. Recently a 2-dimethyl form of vitamin K_2 has been found in *Haemophilus parainfluenzae*[5].

Many workers have been unable chemically to detect vitamin K in animal tissues such as liver[6,7]. Those who claim to have done so, for example Martius[8], detected extremely low levels—far below those found in biological tests. The paper by Pitt and Morton[9] and the discussion of Martius's paper in a Ciba symposium[10] illustrate the considerable controversy on this subject.

Lev[11,12] reported that an organism, *Fusiformis nigrescens* (*Bacteroides melaninogenicus*), isolated from the bovine rumen, has a specific growth requirement for vitamin K. Various analogues, both natural and synthetic, of vitamin K were assayed microbiologically and the spectrum of activity of these compounds for *F. nigrescens* and animals was found to be close enough to warrant the use of this organism for the assay of vitamin K. Gibbons *et al.*[13] later worked with strains of this organism from the human mouth and studied their vitamin K requirements. Recently they demonstrated[14] the usefulness of the microbiological assay in detecting vitamin K synthesis by *Serratia marcescens*. Martius and Leuzinger[15] using the vitamin K requiring strain of *F. nigrescens* have shown that, when supplied with methionine, the bacterium causes the methylation of 1,4-naphthoquinone to make menadione. The menadione is further converted to vitamin K_2 (45) by the bacterium. Glick, Zilliken and Gyorgy[16] have described a strain of *Lactobacillus bifidus* the growth of which is stimulated by vitamin K. A mutant of *Haemophilus parainfluenzae* has been isolated[17] which requires menadione under certain microaerophilic conditions; the narrow range of specificity of the requirement makes this organism unsuitable for vitamin K assay work.

Although vitamin K activity in the liver has been known for some time on the basis of biological assay in animals on vitamin K-deficient diets[18] the chemical nature of the vitamin in the liver has not been determined. Experiments with a liver faction showing vitamin K activity are described here.

(1) *Liver extracts.* Fresh pig liver from an abattoir was used. For satisfactory results, it was found essential to use liver which was fresh and kept on ice during transportation.

Water-soluble factor was obtained by a procedure summarized in Fig. 1: 100 g liver was emulsified in a Waring blender under an atmosphere of nitrogen with 400 ml. of 0·1 M phosphate buffer, *p*H 8·0, heated at 60° C for 10 min and centrifuged at 8,000*g* for 30 min. The red-coloured fluid thus obtained contained the water-soluble liver factor (LF); when used directly for assay, it was sterilized by filtration through a membrane of pore size 0·45μ.

Lipid extractions for detection of vitamin K in liver (or in bacterial cells) were made by first disrupting the cells (2–3 g dry weight) in a French pressure cell and

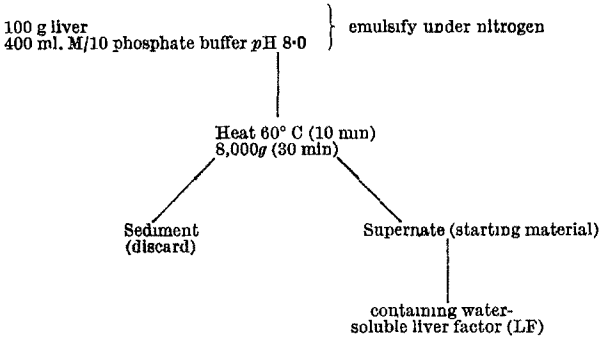


Fig. 1. Extraction procedure for liver factor

lyophilizing the product. The lyophilized samples were then extracted with greater than 100 × volume of dry ethyl ether for 24 h at 5° C. The ether extract was decanted, filtered and taken to dryness under reduced pressure. The residue was taken up in a small volume of ethanol and the solution reduced under vacuum to a final volume of 1 ml. A 1 in 10 dilution of the ethanol extract in distilled water was made and the resulting emulsion used for microbiological assay.

(2) *Bacterial cells.* *F. nigrescens* was cultured for 2 days in trypticase-yeast extract broth (see later) containing 0·1 μg/ml. vitamin K_1, after which the cells were collected. Vitamin K-deficient cells were obtained from 10 l. of culture in trypticase-yeast extract medium which was not supplemented with vitamin K but which was inoculated with 6 ml. of a 48-h culture of *F. nigrescens* grown in trypticase blood broth containing vitamin K_1 (see later); the cells were gathered after 2 days and in some instances 4 days incubation. For all large volume cultures vessels were aseptically filled to the top with medium to exclude oxygen.

(3) *Assay.* The basal medium for *F. nigrescens* consisted of 3 g trypticase (BBL), 0·3 g yeast extract (Difco), 0·3 g and 0·5 g sodium chloride (per 100 ml.); *p*H 7·0. This was tubed in 10-ml. amounts, sterilized by autoclaving and, immediately prior to inoculation, heated in boiling water for 15 min and cooled. For stock culture maintenance, 0·1 ml. of a 1/200 dilution of horse red blood cells in sterile distilled water and 0·1 μg vitamin K_1 were added to each 10 ml. of medium. For assays of growth factor from liver, an inoculum of 0·1 ml. of a 1/10 dilution of a 48-h culture of *F. nigrescens* was used for each 10 ml. medium. Assay cultures were incubated anaerobically in Fildes-McIntosh jars (A. Gallenkamp and Co., Ltd., London) for 2 days under an atmosphere of 95 per cent hydrogen and 5 per cent carbon dioxide, at 37° C.

Because most contaminating micro-organisms are aerobic and many aerobic bacteria synthesize vitamin K_2, all assay tubes showing growth were routinely subcultured in trypticase broth and incubated aerobically for 2 days. The rare flask or tube of medium showing growth aerobically was excluded.

(4) *Results.* Good growth of *F. nigrescens* occurred after addition of 1·0, 0·5, 0·25 and 0·1 ml. of the watery liver extract to the assay system, Inoculated but unsupplemented assay medium failed to support growth. Growth was ascertained by turbidimetric measurement relative to control tubes and by microscopic examination. *F. nigrescens* could be cultured serially in broth containing the water-soluble liver factor. A precipitate was formed in addition after 3 days incubation in all tubes, including the uninoculated control tubes.

Vitamin K_1 and liver factor were compared microbiologically. The minimal amount of vitamin K_1 giving a response was found to be 0·025 μg/10 ml. tube. Since 0·1 ml. of extract containing liver factor give a similar growth response, the extract had the activity equivalent of about 0·25 μg of vitamin K_1 per ml.; thus, 1 g of fresh pig liver contains a soluble growth factor for *F. nigrescens* equivalent in activity to 1 μg vitamin K_1. It is of interest to note that Dam and Schoenheyder[19] found fresh hog liver contained the equivalent of 0·6–0·9 μg of menadione per gram by chick assay and Green *et al.*[18] found beef liver contained 0·71 μg/mg of menadione activity by chick assay.

The heat stability of liver factor was examined by heating aqueous extracts at 60° C for 10 min, 80° C for 10 min and 120° C for 15 min. The first condition produced a heavy precipitate, but essentially undiminished activity remained in the red-coloured supernatant. Preliminary 'pasteurization' at 60° for 10 min, therefore, was adopted as a routine final step in the extraction procedure (see sections 1–3). Heating at 80° C for 10 min diminished activity by more than 50 per cent; autoclaving for 15 min abolished the activity.

Extracts containing liver factor were dialysed against distilled water (100 × volume of extract) for 18–24 h. The dialysate and remaining fraction were brought to original volumes under reduced pressure, sterilized by filtration and assayed. The active component did not penetrate the dialysis membrane.

Attempts were made to purify liver factor by ammonium sulphate fractionation. Aqueous liver extracts were first defatted with ether, and fractions successively precipitated by 20, 30, 45 and 50 per cent saturated salt were prepared at 5° C; together with the supernate these were dialysed against 0·1 M phosphate buffer *p*H 8·0. All fractions were made up to the original volume of liver factor, sterilized by filtration and assayed microbiologically. The main activity was found to be associated with the material precipitated between 20 per cent and 30 per cent saturation. This material was used for certain experiments (see later) because it was easily sterilized by filtration. Large volumes (*c.* 1 l.) of crude extract containing liver factor were difficult to sterilize satisfactorily.

In order to gain information on the nature of the active component, portions of extracts containing liver factor were incubated individually with 0·1 per cent twice recrystallized trypsin, 0·1 per cent 'Pronase' (Calbiochem Grade *B*: 'Pronase' is a proteolytic enzyme from *Streptomyces griseus*), 0·1 per cent pancreatic lipase (Mann), 0·1 per cent twice recrystallized chymotrypsin and 0·01 per cent ribonuclease (Worthington). All solutions used were previously sterilized by membrane filtration and all enzyme-extract mixtures were incubated for 2 h at 37° C at *p*H 8·0. A comparison of the properties of liver factor with vitamin K_1, and the results of assay of the digest mixtures are summarized in Tables 1 and 2.

'Pronase' and pancreatic lipase completely abolished activity of liver factor, although in control assays these enzymes at this concentration did not diminish the growth-stimulating effect of vitamin K. Proteolytic action was found in the preparation of pancreatic lipase. Incubation with trypsin reduced the activity of liver factor; that is, growth occurred when 1·01 ml. or 0·5 ml. but not when 0·25 ml. of trypsinized extract was included in 10 ml. medium. Untreated liver factor showed growth at 0·25 and 0·1 ml. levels.

Table 1. Comparative Properties of Liver Factor and Vitamin K_1

	Liver factor	Vitamin K_1
Solubility in lipid solvents	−	+
Solubility in water	+	−
Heat stability (121° C, 15 min)	−	+
Sensitivity to certain hydrolytic enzymes	+	0
Stimulation of growth of *F. nigrescens*	+	+
Activity in blood-clotting mechanism	Not tested	+

Table 2 Effect of Enzymes on Growth-stimulating Property for *F. nigrescens* of Liver Factor

Enzymes	Activity after digestion
'Pronase'	0
Pancreatic lipase	0
Trypsin	Reduced *c.* 50 per cent
Chymotrypsin	Undiminished
Ribonuclease	,,

Chymotrypsin had no apparent effect on the activity of liver factor although treatment of liver factor with it prevented formation of precipitate in the uninoculated controls (as did trypsin treatment). Ribonuclease had no effect on the growth-stimulating effect of liver factor. The inclusion of either trypsin or pronase in levels greater than 0·1 per cent were inhibitory for *F. nigrescens*.

The foregoing experiments suggest that liver factor is a protein, possibly a protein containing vitamin K bound in some manner. Several attempts were made to dissociate vitamin K from liver factor. As mentioned previously, when 250 ml. of liver factor was lyophilized and extracted with ether (5° C, 24 h) no growth stimulatory activity for *F. nigrescens* was found in the ether extract; full activity remained in the extracted water-soluble fraction. Moreover, after incubation of aqueous extract containing liver factor with trypsin, pronase or pancreatic lipase, no activity was found in the ether-soluble fractions. Finally, liver factor was incubated with a cell-free extract of *F. nigrescens*; no active ether-soluble form was detected after this treatment. Thus all attempts to dissociate a lipid form of vitamin K from extracts containing liver factor have so far been unsuccessful.

It seemed of interest to determine whether *F. nigrescens* grown with vitamin K is able to produce a water-soluble protein resembling that from liver in capacity to replace vitamin K as a growth factor for *F. nigrescens*. Experiments were made using cells (2·43 g dry weight) from a 2-day growth in 3 l. of vitamin K-supplemented medium. The cells were broken in a French pressure cell, lyophilized, ether extracted continuously in a Soxhlet apparatus for 18 h, and resuspended in distilled water to their original volume. After 'pasteurizing' at 60° C for 10 min and centrifuging, the resulting turbid grey solution was sterilized by membrane filtration; it was highly active for *F. nigrescens*.

Vitamin K-deficient cells were similarly prepared by inoculating 10 l. of unsupplemented medium. The yield of dry cells was 0·34 g and these were treated in the same way as the cells grown with vitamin K. The lyophilized and ether-extracted deficient cells were made up to the same concentration as the supplemented cells, that is, 0·034 g/ml. After pasteurizing, the supernate from the deficient cells yielded a straw-coloured clear solution quite distinct from the grey turbid extract of the supplemented cells. The extract of the deficient cells was not active in the microbiological assay.

It was of interest to determine in what form vitamin K activity was represented in cells of *F. nigrescens* grown with vitamin K_1, without vitamin K (that is, deficient), and with liver factor. Cells from 3 l. of medium containing 0·1 μg K_1 per ml., from 10 l. without K, and from 10 l. of culture grown with a fraction of liver extract precipitated between 20 and 30 per cent ammonium sulphate equivalent to 100 ml. of liver extract were used in this experiment. The cells were broken, ether extracted as already described and assayed. Activity associated with ether-soluble material was detected only in the case of cells grown with vitamin K and, although a smaller weight of deficient cells (1 g) was used than of K_1-grown cells (2·6 g) and liver factor-grown cells (3·25 g), this does not account for the lack of activity. (The liver factor cell weight is not meaningful as a precipitate is formed in the medium and is collected with the cells during centrifugation.) Activity associated with ether-insoluble material was produced by cells grown either with vitamin K or with liver factor but were not detected in deficient cells. The grey-coloured extract from the K-grown cells was highly active while the material from the liver factor-grown cells was similar to that from K-grown cells and was active although considerably less so than the K material. These results are summarized in Table 3.

The pig liver factor described here is radically different from classical vitamin K. A similar factor has been found in rat and chick liver too, suggesting a wide distribution.

Table 3. VITAMIN K ACTIVITY (MICROBIOLOGICAL ASSAY)

Cells	Ether extractable	Water extractable
K grown	+ +	+ + + +
Deficient	−	−
LF grown	−	+ +

The pig liver factor is water soluble, heat labile and sensitive to certain hydrolytic enzymes. This may explain why it has not been possible to detect vitamin K chemically in tissue such as liver although K activity is readily assayed by the use of vitamin K-deficient animals. This liver factor may be the active form of vitamin K in animals—possibly a conjugated form of vitamin K. Although preliminary experiments failed to detect vitamin K in ether extracts of liver factor or of enzyme-treated liver factor, possibly more refined techniques may be successful.

There is an interesting relationship between substances with the microbiological assay properties of vitamin K in pig liver and in *F. nigrescens*. In liver, vitamin K microbiological activity appears to exist entirely in the protein form. In *F. nigrescens* substitution of liver factor for vitamin K in culture medium stimulated the synthesis of a water-extractable factor but not of an ether-soluble factor. A factor resembling liver factor has been found in cells of *F. nigrescens* grown with vitamin K_1, but it is usually possible to extract a lipid form of vitamin K from these cells also. When vitamin K-deficient cells of *F. nigrescens* were tested, no liver factor-like material was detected, and no lipid-soluble form of vitamin K found. The liver factor-like protein from *F nigrescens* is very active for the micro-organism and it is possible that some degree of specificity exists, that is to say, that the factor from liver may be more active for animals than for the micro-organism.

These proteinaceous substances with vitamin K activity for *F. nigrescens* may be stages in the metabolic pathway of vitamin K_1. It is interesting that the only known function of vitamin K in animals is in the synthesis of specific proteins, prothrombin, factors VII and X.

Tests of the ability of liver factor to restore the blood-clotting picture of animals are in progress. Since it is difficult to deplete animals such as rats of vitamin K due to the contribution of the bacterial flora, experiments have been instituted to test the vitamin K activity of liver factor using germ-free rats fed a vitamin K-depleted diet. The possibility exists that liver factor-like substances may be present also in tissues other than liver since the feeding of irradiated beef has resulted in vitamin K deficiency.

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PERITONEAL FLUID VOLUME AND THE OESTRUS CYCLE IN MICE

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IT has recently been reported that the amount of fluid found in the peritoneal cavity following the intraperitoneal injection of saline is greater in female mice than in males[1]. It has since been found that the peritoneal fluid volume is greater in normal female mice than in normal males[2]. At the same time the scatter in peritoneal fluid volume is great, particularly in the females.

As changes in body water related to the female sex cycle are well documented in women, these observations suggested to one of us (F. H.) that the variations in peritoneal fluid volume in female mice might be related to the oestrus cycle. As the volume of peritoneal fluid originally present may be of importance in assessing the reaction to substances injected intraperitoneally, and not least in determining the degree of scatter in the values obtained within a group of females, the following investigation was set up to test this hypothesis.

Mice of the closed colony kept at this Institute[3] were used. A group of ten males (mean weight $\pm$ *S.D.* = 30·1 $\pm$ 2·2 g) and a group of thirty virgin females (mean weight $\pm$ *S.D.* = 25·0 $\pm$ 1·9 g) of similar age were set up.

All the mice were killed by cervical dislocation. The peritoneal cavity was opened, the fluid removed and its volume measured directly.

In the females, vaginal smears were taken using the lavage method. Short Pasteur pipettes with rounded ends were used. One drop of saline was taken up in the pipette which was then inserted into the vagina. The fluid was sucked in and out three times. The character of the fluid was recorded. It was then placed on a slide and spread out with the pipette. The smear was air-dried and stained with Leishman's stain. The vagina was removed and fixed in formalin. Sections were made and stained with haematoxylin and eosin and with alcian blue–periodic acid–Schiff. The stage in the oestrus cycle was determined according to a modification of the criteria outlined by Snell[4], being briefly as follows:

Prooestrus smears were characterized by a smear containing few cells, mainly epithelial, some of which were cornified, and a few leucocytes; oestrus by a more cellular smear containing mainly cornified epithelial cells; metoestrus by a very cellular smear made up of cornified cells and dioestrus by a cellular smear consisting mainly of leucocytes and some epithelial cells.

Prooestrus sections were characterized by an epithelium with many layers, the outer four nucleated and a distinct stratum granulosum. Oestrus was characterized by about 12 nucleated layers covered by a cornified layer. In metoestrus the cornified layer had delaminated and leucocytes were present. In dioestrus only a few epithelial cell layers were present and these were infiltrated by leucocytes.

For both smears and sections prooestrus, oestrus, metoestrus and dioestrus were recorded as stage 4, 3, 2 and 1, respectively.

The amount of mucus in the vaginal sections was judged from the alcian blue–periodic acid–Schiff stained sections, and recorded as present in more than the top three layers of the epithelium, present in the top two or three layers, present in the top layer, or absent. These different amounts of mucus were recorded as 4, 3, 2 and 1, respectively.

The peritoneal fluid volume in both male and female mice is detailed in Fig. 1, which shows the sex difference and the scatter in the values. The mean peritoneal fluid volume ($\pm$ *S.D.*) was greater in the female mice (0·10 $\pm$ 0·05 ml.) than in the males (0·02 $\pm$ 0·01 ml.). This difference is statistically significant ($P < 0·05$). The standard deviation in the female values was five times as great as that in the males.

The vaginal lavage fluid was found, macroscopically, to be either cloudy (containing fine particulate matter; type 1), or flocculent (containing coarse particulate matter; type 2) or else the specimen consisted mainly of mucus (type 3). It was found that eleven of the specimens were cloudy, four flocculent and fifteen consisted of mucus.

Fig. 2 shows the results of correlating the macroscopic appearance of the lavage specimen to the peritoneal fluid volume. The positive correlation between these two characteristics is statistically significant ($P < 0·001$), less fluid being present in the peritoneal cavity when the lavage specimen was cloudy than when mucus was present. The flocculent specimens lay between these two extremes.

The quality of the vaginal smears containing mucus was not all that could be desired, but air-drying proved

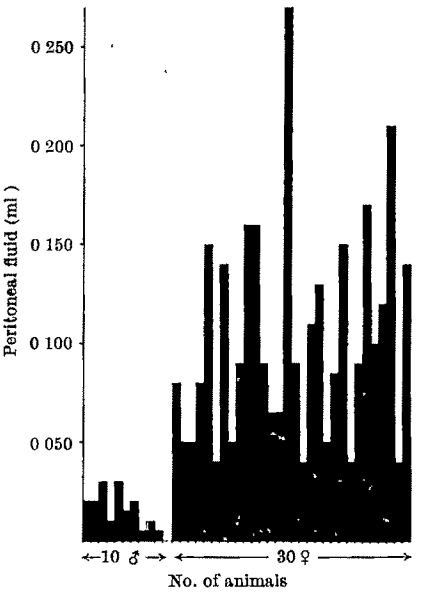


Fig. 1. The peritoneal fluid volume in male and female mice

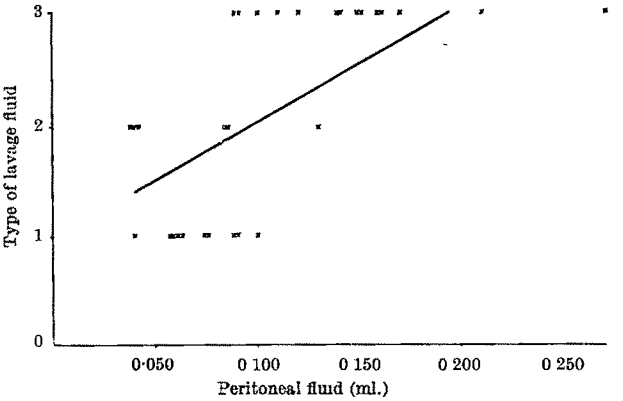


Fig 2. The correlation between the peritoneal fluid volume and the macroscopical appearance of the vaginal lavage fluid (see text)

otherwise satisfactory. In the sections stained with haematoxylin and eosin the stratum granulosum was at times difficult to define. It was, however, clear in the alcian blue–periodic acid–Schiff sections, and these were therefore used for the final classification. In some specimens the vaginal epithelium presented a combination of two adjacent stages and occasionally different stages were seen in different sections from the same vagina. In such borderline cases the predominant stage was recorded. The result of these classifications is shown in Table 1.

Table 1. NUMBER OF MICE IN DIFFERENT STAGES OF THE OESTRUS CYCLE AS DETERMINED BY VAGINAL SMEARS AND HISTOLOGICAL SECTIONS OF THE VAGINA

Stage	Smears	Sections
Proœstrus	15	12
Oestrus	2	4
Metoestrus	5	4
Dioestrus	8	10
Total	30	30

Fig. 3 shows the results of correlating the stage of the oestrus cycle as judged from smears to the peritoneal fluid volume. The positive correlation between these two characteristics is statistically significant ($P < 0{\cdot}001$), least fluid being present in dioestrus and most in proœstrus.

Fig. 4 shows the correlation between the stage of the oestrus cycle judged from the sections and the peritoneal fluid volume. A statistically significant positive correlation was found here also ($P < 0{\cdot}01$), though the correlation was not so high as that obtained with the smears.

Fig. 5 shows the correlation between the amount of mucus present in the vaginal sections and the peritoneal fluid volume. Most mucus was present when the peritoneal fluid volume was highest. This positive correlation was also statistically significant ($P < 0{\cdot}01$).

Fig. 6 shows the result of correlating the macroscopical appearance of the lavage fluid to the stage of the oestrus cycle as determined from the smears. The positive correlation in this case was also significant ($P < 0{\cdot}01$), cloudy fluids being clearly related to dioestrus and the presence of mucus to proœstrus.

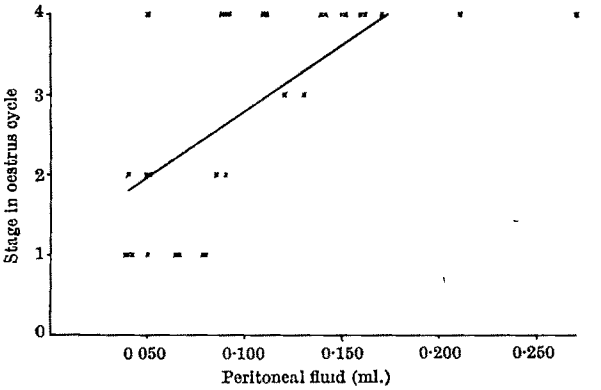


Fig. 3. The correlation between the peritoneal fluid volume and the stage of oestrus cycle judged from vaginal smears (see text)

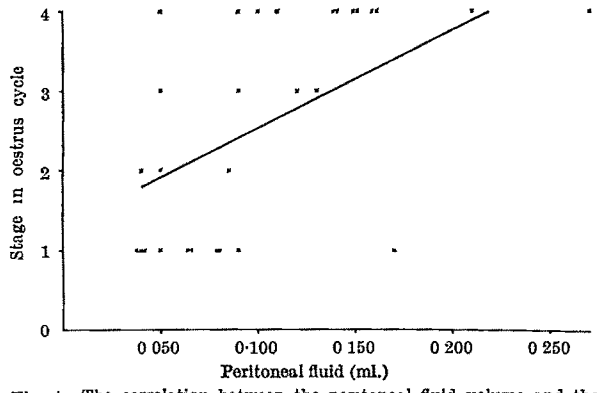


Fig. 4. The correlation between the peritoneal fluid volume and the stage of oestrus cycle judged from vaginal sections (see text)

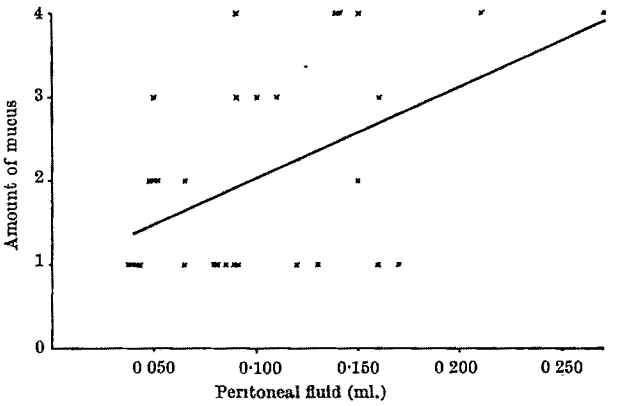


Fig. 5. The correlation between the peritoneal fluid volume and the amount of mucus present in the vaginal sections (see text)

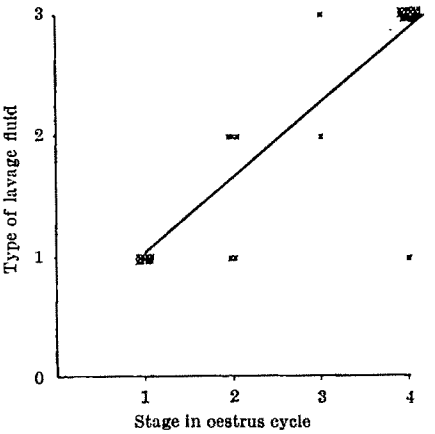


Fig. 6 The correlation between the stage of oestrus cycle judged from vaginal smears and the macroscopical appearance of the vaginal lavage fluid (see text)

The present investigation confirms our previous impression that the volume of peritoneal fluid is greater in female than in male mice of our closed colony. It also confirms that the scatter in the female values is greater than that in the males. These variations in peritoneal fluid volume in the females are clearly shown to be related to the different stages in the oestrus cycle. The correlation obtained from the vaginal smears was higher than that obtained from vaginal sections. The explanation here may be that the sections were taken from the vagina immediately after lavage had been carried out. Thus the possibility that the top layer of cells had been washed off cannot be excluded. Indeed it may be this that has accentuated the variations in histology from section to section that are normally to be expected.

The finding of a high correlation between the macroscopical appearance of the lavage fluid and the peritoneal fluid volume provides a quick and simple way of sorting a group of females into those in which the peritoneal fluid volume can be expected to be high and those in which it can be expected to be low. This may enable us to reduce the scatter in experimental results in female mice following, for example, the intraperitoneal transplantation of ascitic tumours, and thus facilitate the assessment of differences due to treatment.

The finding that the presence of mucus in the lavage fluid coincides with proœstrus in our mice may also be of practical use in breeding experiments. It is, however, at variance with data obtained from other strains in which mucus is said to be present in dioestrus smears[4]. However, the histology in this investigation, the findings of which

are in full agreement with those outlined by Snell for other strains, shows that most mucus was present when the peritoneal fluid volume was greatest, that is, in pro-oestrus. It is suggested on the basis of the present investigation that as the presence of "epithelial cells sometimes more or less filled with mucus"[4] is characteristic of prooestrus, it is more reasonable to expect mucus in the lavage fluid and in smears in prooestrus than in dioestrus, when the epithelial cells are quiescent.

The present findings therefore support the hypothesis that water retention as evidenced by an increase in peritoneal fluid volume occurs in prooestrus. It is tempting to suggest that this fluid may play its part in facilitating the transport of the ova to the oviducts when ovulation occurs in early oestrus. The mechanism of this increased fluid production may lie in the increased vascularity that accompanies the proliferative changes characteristic of prooestrus.

We thank Prof. E. Waaler for his interest in this work.

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ANTI-TRANSFERRIN-DEPENDENT HAEMAGGLUTINATION IN ADULT C_3H MICE DURING INJECTIONS OF CASEIN AND IN UNTREATED SUCKLING C_3H AND *DBA*/2 MICE

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THE attachment of serum proteins to the surface of red blood cells may be detected by use of a direct haemagglutination test (HT), based on principles outlined as early as 1908[1]. The anti-globulin test was developed in 1945 by Coombs *et al.*[2] with the purpose of detecting the presence of incomplete Rhesus antibodies on red blood cells. It has since been shown[3,4] that both gamma and non-gamma globulins may cause a positive reaction, when the test is performed with wide-spectrum antiserum produced in animals by immunization with whole serum. The work of Jandl[5] and Nelken[6] showed that the reticulocytes in adult humans, when present in large numbers, could be agglutinated with wide-spectrum antiserum, and the same was later shown to be the case in mice[7]. This agglutination was at least in part due to attachment of transferrin to the reticulocytes[8]. It was shown earlier that circulating antibodies against liver, spleen and kidney[8,9] can be demonstrated after injections of casein. In the course of investigations on the agglutinability of mouse red blood cells in an anaemia of unknown aetiology[10], the present investigation was undertaken in order to find out, using the direct HT, if the anaemia which accompanies the development of amyloidosis in casein-treated mice[11] may be caused by antibodies against their own red blood cell antigens. The possible role of reticulocytes in the positive HT in casein-treated anaemic mice was evaluated in suckling mice, in which a high percentage of reticulocytes is known to exist[12].

Twenty-six 2-month-old C_3H mice of both sexes were injected daily subcutaneously with 0·5 ml. of a 5 per cent casein solution; eleven mice were injected for 3 weeks, seven for 6 weeks and eight for 8–12 weeks. During the first 2–3 weeks of treatment and for 2–3 weeks after cessation of treatment the animals were tested every third day with a direct HT. Red blood cell and reticulocyte counts were made before treatment and after 3 and 6 weeks of treatment. The animals were autopsied at the end of the scheduled investigations. In addition, untreated C_3H and *DBA*/2 mice, five in each group, were bled at 1 day and 1, 2, 3, 4 and 5 weeks of age for HT and reticulocyte counts.

The procedure for the HT was as follows: Blood was taken from the retrobulbar venous plexus in glucose citrate. The red blood cells were washed four times with saline and made into a 5 per cent suspension in saline. One drop of this suspension was added to one drop of antiserum on a glass tile, and the tests were read macroscopically after 20 min at room temperature. The antisera used were pure rabbit mouse-gamma-globulin antiserum (batch *CS*-1498 Colorado Serum Company, Denver), diluted 1 : 50, and a wide spectrum rabbit mouse-serum antiserum diluted 1 : 7 and 1 : 50. The wide-spectrum antiserum was produced by immunization with mouse whole serum. After inactivation of complement by heating to 56° C for 40 min the antiserum was absorbed twice with two volumes of packed washed normal mouse red blood cells, first 1 h at 37° C and then overnight at 4° C. Controls in each series of tests included the use of saline in place of antiserum and testing of both positive and negative reacting cells. The positive cells were prepared by coating normal red cells with isoimmune sera as described by Garfein *et al.*[7]. Isoimmune sera for coating C_3H and *DBA*/2 red blood cells were obtained by immunizing *CBA* and $C_{57}Bl$ mice with C_3H and *DBA*/2 spleen cells. *CBA* and *DBA*/2 red cells incubated with non-immune sera served as negative cells to this latter test. Serial dilution experiments with the antisera diluted in saline from 1/1 to 1/1,024 were used to observe the prozone and to find the optimal dilutions.

Results of casein injections. All twenty-six adult mice showed a negative HT when tested with the pure anti-mouse-gamma-globulin antiserum irrespective of the duration of the casein treatment, whereas the HT performed with wide-spectrum antiserum became positive during the injection period. Thirteen of fourteen mice showed a positive HT after 14 days of treatment (Table 1) and the reaction remained positive so long as the injections were continued. Regardless of the duration of treatment the agglutinability disappeared about 2–3 weeks after the last of the casein injections (Table 2).

The number of red blood cells decreased during the casein treatment to an average of $6{\cdot}9 \pm 1{\cdot}1 \times 10^6$ with a range of $5{\cdot}2$ to $9{\cdot}2 \times 10^6$. After 8–12 weeks of casein treatment all eight mice of this group showed less than $6{\cdot}2 \times 10^6$ red cells. The average of red blood cells of thirteen untreated adult (2–4 months) C_3H mice was $9{\cdot}6 \pm 1{\cdot}0 \times 10^6$ with a range of $7{\cdot}9$ to $11{\cdot}5 \times 10^6$.

Table 1. RESULTS OF DIRECT HAEMAGGLUTINATION TEST WITH WIDE-SPECTRUM ANTISERUM IN 2–4 MONTH-OLD C_3H MICE DURING DAILY SUBCUTANEOUS INJECTIONS OF 0·5 ML. CASEIN

Duration of treatment (days)	0	3	6	9	13
Results (positive/negative)	0/14	0/14	0/14	3/11	13/1

Table 2. RESULTS OF DIRECT HAEMAGGLUTINATION TEST WITH WIDE-SPECTRUM ANTISERUM AFTER CESSATION OF CASEIN INJECTIONS

	Day after cessation							
Duration of treatment	0	3	6	9	12	16	19	22
3 weeks (positive/negative)	11/0	11/0	11/0	9/2	4/7	1/10		
6 weeks ,, ,,	7/0	7/0	7/0	4/2	0/6			
8–12 weeks ,, ,,	7/0	7/0	4/3	4/3	4/3	2/5	2/5	0/7

Table 3. PERCENTAGE OF RETICULOCYTES IN BLOOD OF MICE INJECTED WITH CASEIN

Duration of treatment in weeks	0	3	6
Number of mice tested	15	15	6
Mean value	1·8	3·7	1 3
Range	1·0–4·0	0·6–7·6	1·0–2·8

Table 4. RESULTS OF DIRECT HAEMAGGLUTINATION TEST AND RETICULOCYTE COUNTS IN NEW-BORN MICE

Age in weeks	*C₃H* mice 0 (1 day)	1	2	3	4	5
HT (positive/negative)	5/0	5/0	5/0	5/0	0/5	0/5
Percentage of reticulocytes*	21·6	15·0	10 8	6 5	1·4	0 7
Range	18·9–24·0	14·9–16 0	7·4–24 0	2·2–9·0	0 6–2 2	0·2–1·2
	DBA/2 mice					
HT (positive/negative)	5/0	5/0	5/0	5/0	0/5	0/5
Percentage of reticulocytes*	24·0	16·3	10 7	8·9	2·8	2·4
Range	9·8–36·0	6·0–23·0	7·6–13·4	6·0–11·0	0·2–5·6	0·4–4·2

* Mean value in five mice.

Reticulocyte counts determined in fifteen animals before onset of treatment were $1·9 \pm 0·9$ per cent; this was slightly increased after 3 weeks of casein injections and was normal again after 6 weeks of treatment (Table 3). Immediate microscopic examination of red blood cells agglutinated in the antiserum usually showed half the cells in the agglutinates.

When the mice were killed, the microscopic examination revealed a slight haemosiderosis in the liver and spleen. After 8 weeks of treatment, amyloidosis occurred in the liver, spleen and kidneys in all eight mice of this group. At this time a definite atrophy of the thymic cortex was noted. The peripheral lymph nodes showed no abnormalities.

Results in suckling mice. The influence of an increased percentage of reticulocytes on the result of the HT was examined on red blood cells of untreated suckling *C₃H* and *DBA*/2 mice with the wide-spectrum antiserum. A positive HT was present in the neonatal period so long as the percentage of reticulocytes was in the range of 5–10, that is, in the first 3 weeks of life (Table 4). The mothers of these mice showed negative HT.

Inhibition tests were performed to identify the antigen on the red blood cells with which the antibody in the wide spectrum antiserum was reacting. It was found that the agglutination of red cells from casein-treated as well as from suckling mice was only slightly inhibited by the addition of one drop of 1 per cent solution of mouse gamma globulin to one drop of antiserum diluted 1 : 7 before the test cells were added, whereas the agglutination of isoimmune-serum-coated red cells was clearly inhibited under these circumstances. This gamma globulin solution also inhibited a positive Coombs test in seven mice of the *NZB* strain, which is known to develop a Coombs positive anaemia spontaneously[13]. The results of the inhibition tests made a sensitization of the red blood cells of the casein-treated and suckling mice with ordinary warm gamma auto-antibodies improbable. Therefore, the possible presence of cold agglutinins was investigated. Tests for complete cold agglutinins were negative, and the HT was not potentiated at 4° C. HT carried out on blood collected into 37° C glucose citrate to avoid sensitization with incomplete cold antibodies gave the same results as when carried out at room temperature. Since no evidence could be found for cold agglutinins, further inhibition tests were made. The addition to the wide-spectrum antiserum of an equal quantity of a 5 per cent casein suspension in saline, of a 1 per cent solution of mouse gamma-*A* myeloma globulin[14], of a 1 per cent myeloma macroglobulin[15] or of undiluted guinea-pig serum as a source of complement did not show more than a slight inhibitory effect on the HT. The addition, however, of an equal quantity of a 1 per cent solution of mouse transferrin inhibited the positive HT of the red cells from casein-treated and suckling mice but had no effect on isoimmune-serum-coated red cells.

The transferrin and the gamma globulin used in inhibition tests were isolated by DEAE-cellulose column chromatography and the mixture separated by 1·6 M ammonium sulphate. The purity of the various serum proteins was examined in agar immune diffusion experiments using the technique of Wadsworth[16]. The transferrin solution was found to contain a slight contamination of gamma globulin and the gamma globulin solution a slight contamination of transferrin. The pure mouse-gamma-globulin antiserum reacted only with gamma globulin, while the wide-spectrum antiserum reacted with several mouse serum proteins, including transferrin. After absorption with red blood cells from casein-treated or suckling mice the wide-spectrum antiserum no longer reacted with transferrin, but still reacted with gamma globulin. The results of both the absorption and the inhibition tests point toward transferrin as the substance coating the red cells. The wide-spectrum antiserum gave no precipitation lines in agar gel diffusion experiments with casein, which would appear to be evidence against casein as the source of the antigen in the HT. Agglutination with the wide-spectrum antiserum was found to be optimal in a dilution of 1 : 7 against the red blood cells from casein-treated and suckling mice; it was, however, also marked with undiluted antiserum, confirming that the HT was not an ordinary anti-gamma globulin reaction[17].

The observed correlation between agglutination in the wide-spectrum antiserum and the high percentage of reticulocytes in the suckling period of mice is in accordance with the findings of Garfein *et al.*[7] in mice with reticulocytosis caused by X-ray treatment. It is unlikely that the agglutination of red blood cells from casein-treated mice is caused by the reticulocytes, since their percentage in the majority of these mice was normal, and the agglutinates were found to contain considerably more red cells than the reticulocytes. The antigen coating the red cells from casein-treated mice seems to be identical with the one responsible for haemagglutination of the reticulocyte-rich blood from suckling mice, since the HT was negative in both cases, when carried out with antiserum absorbed with either type of cells. Since anti-transferrin is known to agglutinate reticulocytes[5,6] the agglutination of red cells from casein-treated mice may also be caused by coating with this protein. The lack of anti-transferrin activity in the absorbed sera and the inhibitory effect of transferrin on the HT also point toward transferrin as the protein causing the haemagglutination by reaction with the anti-transferrin in the wide-spectrum antiserum. The high sensitivity of the HT[18] does, however, leave open the possibility that the factor acting in this test is not made evident by the less sensitive agar diffusion method. Coating the red cells with bovine transferrin contained in the casein solution and thereby causing the agglutination reaction is unlikely judging from the weak inhibitory effect of casein on the HT and the lack of precipitation in the agar gel diffusion experiments. The reason for the observed adsorption of transferrin to the red cells after casein treatment is unexplained. The present article, however, supports the view that the positive HT in casein-treated mice is due to transferrin.

Red blood cells from adult *C₃H* mice during injections of casein and from suckling untreated *C₃H* and *DBA*/2 mice were found to be agglutinable by a rabbit wide-spectrum mouse-serum-antiserum, but not by a pure mouse-gamma-globulin antiserum. This haemagglutination was inhibited by addition of mouse transferrin, but not by addition of mouse gamma globulin to the antiserum, and in the casein-treated mice it was found to be independent of the number of reticulocytes.

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DEPENDENCE OF SYNAPTIC TRANSMISSION ON PROTEIN METABOLISM OF NERVE CELLS: A POSSIBLE ELECTROKINETIC MECHANISM OF LEARNING?

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BY analogy with the mechanism of the genetic code, the suggestion has frequently been made that nucleic acids of brain tissue may be the repositories of the permanent trace of memory. According to this hypothesis, learning is accompanied by an altered metabolism of DNA and RNA which, in turn, affects the general metabolism of the brain[1]. A specific aspect of the hypothesis, for which considerable experimental evidence has been gathered, is the correlation with learning of changes in protein metabolism of the brain[2–4]. Thus, for example, it was found that learning is made easier after administration of exogenous RNA[5] and, conversely, that learning is impaired by substances interfering with protein metabolism[6–8]. However, such experiments provide no clue as to how metabolic processes may affect the elaboration of information in the nervous system[3].

It is known that information is encoded in sensory receptors as trains of nervous impulses, the frequency of which is modulated by oncoming stimuli[9,10]. These impulses converge on neurones in higher centres only after having been transmitted through several relay stations. Because each relay involves synaptic transmission, which may or may not activate the next neurone in the chain, a profound modification in the pattern of response may follow even a modest increase or decrease in efficacy of these synapses. For this reason it is generally supposed that a modification of the response to synaptically transmitted impulses must be an important step in learning. If changes in protein metabolism are indeed related to memory, it must be expected that modification of DNA and RNA structure will be coupled with changes in synaptic activity, but there are no experimental findings reported to corroborate this postulate.

This article reports some observations on electrokinetic displacement of nerve cells and proposes a mechanism whereby a regulatory influence on synaptic transmission may be exerted by movement with such an origin. It is also shown that changes in DNA and RNA structure can be expected to modulate the electrokinetic effect on the synapse, and the mechanism proposed here may thus provide a link between the neurochemical and electrophysiological aspects of learning. It should be clear that the hypothesis that DNA in brain cells may become imprinted by experience is not considered here from a critical point of view, and the reader is referred to the reviews cited[3–5] for an evaluation of the experimental evidence. Instead, with imprinting as a starting point, the present article is concerned with tentatively providing a read-out mechanism by which imprinting might influence nervous activity.

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Electrokinetic Displacement in the Synapse

The displacement of nerve cells in a focal electric field has recently been demonstrated in tissue-culture experiments[11,12]. A localized electric field was established by passing current from a micropipette placed near a cell. The resulting movement is apparently related to the extensively studied electrophoretic migration of cells in a uniform electric field[13,14], and results from the interaction of the field with a polarized ionic layer around the cell surface[15]. Besides the study of the displacement of the entire cell, however, the use of a focal field also makes possible an exploration of the electrokinetic behaviour of small segments of the cell membrane. Of particular interest is the situation in which cultured cells have become attached to the walls of the culture chamber and to one another, and are therefore unable to migrate under influence of the field. As shown in Fig. 1, under these conditions the electrokinetic force can produce a local deformation of the cell membrane. The effect of the electric field may be seen even more clearly with isolated cells (Fig. 2). (Photomicrographs can unfortunately provide only a static representation of the effect; a ciné film has, however, been prepared in which the development and time-course of the electrokinetic movement may be observed[62].) Passage of current out of the electrode (that is, electrode tip positive in respect to surface of the cell) usually causes attraction, while current flowing into the electrode results in movement of the membrane in the other direction[11]. The current necessary to cause this effect varies from 5×10^{-7} to 5×10^{-6} amp, and because the electrode is 5–10μ away from the nearest point of the cell, the current density at the surface of the cell may be calculated as 4×10^{-10}–$1{\cdot}7 \times 10^{-7}$ amp/μ^2 (ref. 11). A direct measure is thus available of the current density required to deform the membrane of intact living cells.

Although this effect is not limited to nerve cells (Fig. 2)[11,12], it seems that synapses between neurones or between neurones and muscle cells are the only places at which a current of such a density is generated in most living organisms. (The exceptions are some electric fish.) After the release of transmitter substance from the presynaptic terminal, the conductance of the subsynaptic terminal is briefly increased about 100 times[16]. Since the interior of the cell is negative with respect to the outside, current flows into the cell through the conducting region of the sub-synaptic membrane. Direct measure-

ments on spinal motoneurones have revealed a synaptic current of 2×10^{-8}–$1{\cdot}5 \times 10^{-7}$ amp[17,18]; in the neuromuscular junction the current intensity has been estimated at $1–3 \times 10^{-6}$ amp[19–21]. A low-resistance path for the flow of this current is available only at the fraction of the cell surface activated by transmitter substance, and a remarkably high current density across the synapse is therefore attained. From the data given by Eccles and Jaeger[20], the current density at the neuromuscular junction can be calculated as 3×10^{-10} amp/μ^2; the current density estimated for motoneurone synapses is even a little higher[22].

From these estimates it would appear that the current density during normal synaptic activity approaches that required for the displacement of cells in tissue culture, and an analogous displacement due to the flow of synaptic current is therefore postulated. The duration of the synaptic current (1–2 msec)[16] is also comparable with the 3-msec duration of the pulses used in the tissue culture experiments. Admittedly, the estimates of current density at the synapse may be too high because of the absence of direct experimental data on the area of membrane involved in synaptic activity, but the measurements of current required for this displacement of cells in tissue culture should also be regarded as an upper limit because of the necessarily limited sensitivity of this optical viewing system (2–3μ). Indeed, because of the linear relationship between current density and displacement[11], even if the density of the synaptic current is smaller than the aforementioned by two orders of magnitude, a displacement of several hundred Å may still reasonably be expected during synaptic activity.

These considerations permit a consideration of electrokinetic displacement within the synapse. Electrokinetic displacement results from interaction of electric field with the polarized ionic envelope formed by adsorption around any organic or inorganic surface placed in an electrolyte solution[15,23]. In excitatory synapses, current drawn to the sub-synaptic membrane from the extracellular space interacts with the ionic envelope of the presynaptic component of the synapse as it flows through the synaptic cleft. So far as the presynaptic component is concerned, the situation is analogous to that when a negative electrode is placed in its vicinity; it should therefore be displaced away from the sub-synaptic element, thus increasing the width of the synaptic cleft. The synaptic cleft is quite narrow (100–500 Å (ref. 24)), but still far thicker than the ionic envelope responsible for electrokinetic movement which, for physiological ion concentrations, is estimated at 5–10 Å (refs. 25 and 26). Extrapolation of the findings on electrokinetic movement in tissue culture should therefore be valid even for this comparatively narrow space.

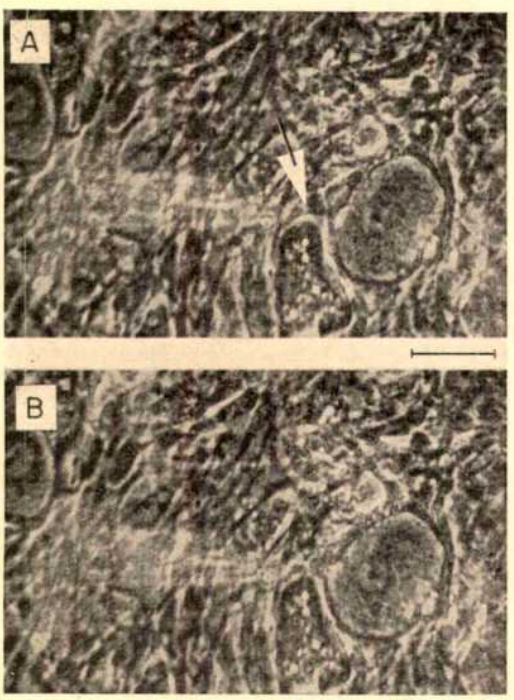


Fig. 1. Deformation of the membrane of a neurone in tissue culture by a focal electric field. Chick dorsal root ganglion culture, twenty-third day. Phase contrast optics, no staining. Electrode tip slighly out of focus (arrow); current pulses (3 msec, 30/sec) passed out of electrode in *B*. Current intensity 1×10^{-6} amp, electrode tip positive. Calibration, 50μ

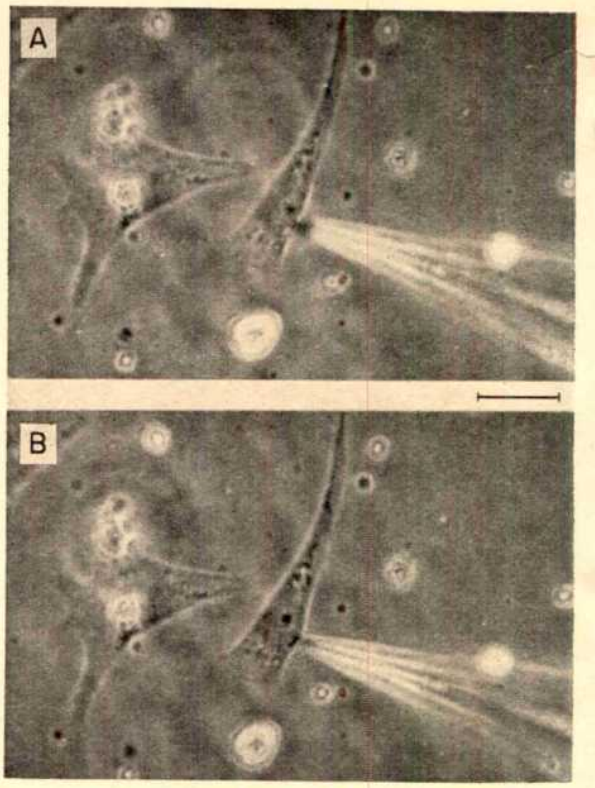


Fig. 2. Deformation of fibroblast membrane by a focal electric field. Seven-day rat, cerebellum culture, fifteenth day. Fibroblast shown has migrated from main explant and is now attached to floor of the chamber. *A*, Control photograph. *B*, Photograph taken during passage of current pulses as in Fig. 1*B*, electrode tip positive, current intensity 5×10^{-4} amp. Phase contrast. Calibration, 50μ

With regard to the sub-synaptic element, the situation is more complex: the effect of current flow is similar to that of a positive electrode, tending to constrict the synaptic cleft, and thus counteracting the movement of the pre-synaptic element. However, because the electrokinetic force is diminished in conducting, as compared with insulating, particles[27,28], the displacement of the sub-synaptic element, through which flows the synaptic current, will be far smaller than that of the non-conducting pre-synaptic membrane. It has been suggested that activity of the synapse might give rise to osmotic pressure across the sub-synaptic membrane[22], but this force would not be in the direction required to compensate for electrokinetic displacement of the presynaptic ending, nor is it very probable that its magnitude would be similar.

For these reasons, a net increase in width of the synaptic cleft can be expected in excitatory synapses; in inhibitory synapses, where the current flow is opposite, the synaptic cleft should become narrower. Even if the reaction to electric field of the specialized synaptic surfaces differs from that of non-synaptic neurone membrane, flow of synaptic current should still cause movement, except that the width of the cleft may perhaps decrease where it was expected to increase and vice versa. For the argu-

ments presented here, however, the direction or magnitude of the change in the synaptic cleft is irrelevant; the only assumption required is that the width of the synaptic cleft is altered during synaptic activity.

Electrokinetic Displacement and Synaptic Efficacy

Operation of the synapse is critically dependent on the dimensions of the synaptic cleft. According to the analysis reported by Eccles and Jaeger[20], two opposing factors are at play: stricture of the cleft should reduce the distance of diffusion of the released transmitter, thus allowing additional transmitter to reach the sub-synaptic membrane and to augment the synaptic potential; on the other hand, stricture of the cleft should increase the impedance to flow of synaptic current of the extracellular path, resulting in reduction of the postsynaptic potential. Although information on the relative importance of each of these factors in various synapses is not as yet available, theoretical reasoning indicates that the net effect of a given change in width of the synaptic cleft might significantly differ in synapses of 1 and 10μ diameter[20]. It is likely, therefore, that electrokinetic effects will at least be present in synapses of some types.

It might be argued that the synaptic potential will be well under way before the beginning of the electrokinetic movement, which is due to the flow of synaptic current; thus the potential cannot be much affected by the movement. This criticism is certainly valid for an isolated synaptic event but, as already mentioned, messages in the nervous system are transmitted as trains of closely spaced impulses, at a rate close to that used in our tissue-culture experiments (30/sec). Because a sustained displacement without relaxation was found in tissue culture at this repetition rate, it is likely in the synapse also that complete recovery of the cleft from preceding deformation would not take place before the onset of the next impulse. Electrokinetic deformation of the synapse would thus influence not only the flow of synaptic current, but also diffusion of the transmitter generating subsequent synaptic events. Although further consideration shows that this process must tend towards an equilibrium, because excessive stricture of the synaptic cleft depresses the current and thus also proportionately diminishes the electrokinetic force, the mechanism is important in so far that it allows simultaneous control by the electrokinetic effect of transmitter diffusion as well as synaptic current, both of which determine the amplitude of the postsynaptic potential, and thus also triggering of spikes.

Protein Metabolism and Electrokinetic Displacement

The electrokinetic effect has been discussed up to now solely as a function of density of the current. There are, however, several other factors which also determine the extent of electrokinetic movement, and which must be considered in relation to its effect on the synapse. These factors explicitly appear in the equation of electrophoretic velocity[15]:

$$\text{electrophoretic velocity} = \frac{\varepsilon\zeta E}{\eta} A$$

from which displacement may be found as the product of velocity and duration of the current. The intensity of the electric field E essentially is a function of the current density[29] (see also ref. 11) which has already been considered; A is a constant for any given surface configuration[14,15,23]; the viscosity η is also generally assumed to be constant, although it may increase when the width of the synaptic cleft falls below 100 Å (ref. 30) and could thus appreciably attenuate the electrokinetic effect; the dielectric constant ε is determined by physical properties of the extracellular fluid and may also be treated in the present context as a constant.

On the other hand, the electrokinetic potential ζ, known also as zeta potential, varies markedly with changes in protein metabolism. The zeta potential is an expression of the preferential adsorption from the solution of particular polar groups[15,23], and obviously the chemical nature of the surface determines its affinity for specific ions, and the quantity of ions adsorbed. Since cellular membranes are apparently coated with protein[31], it is most probable that changes in the composition and configuration of cellular proteins exert a dominant influence on the zeta potential. It is at present believed that nucleic acids serve as a template for formation of cellular protein[32–34]; and it is easy to see that changes in DNA or in RNA might lead to alteration of the zeta potential, and thus to augmentation or reduction of the electrokinetic effect on synaptic potentials.

Learning and Electrokinetic Displacement in the Synapse

The two main premises on which is based the proposed mechanism of memory read-out can now be stated: (1) The electrokinetic force generated by synaptic current flow alters the width of the synaptic cleft during repetitive activity of the synapse. Because spike firing is determined by the amplitude of synaptic potentials, and these in turn depend on the width of the synaptic cleft, electrokinetic movement could modulate the firing of the postsynaptic neurone. (2) Modification during learning of DNA and RNA has been shown by other investigators; changes in membrane structure, which is under DNA control, can therefore be expected following learning in neurones. Such changes in pre- and post-synaptic cells would affect the magnitude of electrokinetic force, and hence also the width of the synaptic cleft and the amplitude of synaptic potentials. As a result, the effect on the postsynaptic neurone of a given synaptic input would differ from that prior to learning.

Experimental evidence in support of proposition (1). Numerous investigations have been undertaken of the displacement of isolated living cells in a uniform electric field[13,14]. The current density required is of the same order as that necessary for deformation of cell membranes by focal electric field in tissue culture[11,12]. It would seem, therefore, that the mechanism in both cases is identical—local deformation of the membrane ensues when displacement of the cell *in toto* is impeded. It may be questioned whether extrapolation of these data to cell membranes *in situ* is permissible. Experiments bearing directly on this point would encounter obvious difficulties, but some suggestive evidence is available. Thus, in the spinal ganglion of the toad and also in brain-stem of the cat, pulses of 2×10^{-8} amp and 100 msec duration delivered through a micropipette may often result in penetration of neurones close to this electrode[35,36]. Penetration of cortical neurones in the cat also is facilitated by brief pulses (5 msec, 3–5 $\times$ 10^{-8} amp), and reversal of current polarity before completing penetration may lead to withdrawal of the electrode from the cell membrane[37]. The most satisfactory interpretation for these observations is that cell membranes are either attracted or repelled from the electrode during passage of current, according to the direction of current flow.

The data presented here indicate that in normal synaptic activity the current density might be sufficient to reproduce the effect of an extraneous field on synaptic membranes; also the rate of repetition of synaptic discharge is adequate to prevent relaxation of the displaced membranes between impulses. The feasibility of such changes in the spatial relationship of the pre- and postsynaptic elements *in vivo* has already been noted: based on the electron microscope observations by Palay[38] and on Ranck's theoretical considerations[22]. However, even though the width of the synaptic cleft may change electrokinetically, it does not necessarily follow that this mechan-

ism has any functional importance. Experiments bearing directly on this question have yet to be designed and carried out, but some circumstantial evidence for electrokinetic effects in the synapse may be mentioned.

Of special interest in this context is the post-tetanic potentiation found in many synapses and in the neuromuscular junction. Since repetitive synaptic activity results in a maximal electrokinetic effect, it is tempting to suggest that such potentiation may be due, at least in part, to electrokinetic deformation of the synapse. One of the principal mechanisms invoked in relation to post-tetanic potentiation is hyperpolarization of presynaptic terminals[39], but although this mechanism plays an important part in certain synapses[16,40,41] post-tetanic potentiation is equally present in synapses which do not show it[42]; for these synapses, the possibility of electrokinetic post-tetanic potentiation cannot be rejected. Another concept which has been successfully used to explain certain features of post-tetanic potentiation is mobilization of transmitter substance[43,44], but again some observations cannot be accounted for by this postulate[45]. Another example is the effect of calcium: increase in the extracellular concentration of calcium is associated with increased synaptic current[46]; since calcium changes the configuration of proteins[47], the resulting electrokinetic force could alter the resistance of the synaptic cleft. Investigations of the effect of extraneous polarizing currents on synaptic transmission are also relevant. With a focal electric field, a slowly developing facilitation (20–120 sec time-course) was found[48,49] even when the frequency of the spontaneous miniature end plate potentials was not changed[48]. It should be borne in mind that the neuromuscular junction, which features an exceptionally wide synaptic cleft (500 Å) (ref. 24), may scarcely provide an ideal background for detection of electrokinetic displacement, because a change in width of the cleft would have a far less critical effect on transmission than in narrower synapses[20]. Indeed, results recently obtained in the cerebral cortex with focal currents were far more dramatic; changes in firing pattern of neurones were found which outlasted the current by more than 3 h (ref. 50).

Experimental evidence in support of proposition (2). Variations under various environmental conditions in the electrokinetic potential have been extensively explored[51,52]. The relationship of protein configuration to the electrokinetic potential will therefore be illustrated only briefly. The mobility of proteins, which is linearly related to their zeta potential, can be varied over a 1 : 3 range by changing the *p*H (ref. 53). Similarly, mobility of viruses[14], bacteria[54] and blood cells[55] varies with the *p*H. Even more suggestive are the experiments in which addition of a virus which adsorbs on the cell surface reduced the mobility of erythrocytes to half its previous value[56]; tumour cells were similarly affected by homologous serum[14]. Changes in the chemistry of the surface of the cell thus result in substantial changes in electrokinetic force.

Conclusion

One of the important attributes of the present hypothesis is its generality: it does not require a complex synaptic membrane, which undergoes highly specific structural modifications during learning; neither does it necessitate correlation of firing of the pre- and post-synaptic cells. In fact, almost any change in protein structure, even if not restricted to the synaptic area, might serve the requirements of this hypothesis. Also, so long as additional modifications in protein metabolism have not occurred, the effect should persist regardless of the frequency of activation of the synapse. However, the very generality of this mechanism makes it impossible to interpret learning in terms of the present hypothesis alone. Clearly, any change in the electrokinetic potential affects all synaptic endings of the same cell to an equal degree; neuronal connectivity patterns must therefore be relied on appropriately to channel the adaptive changes.

It cannot be over-emphasized that the hypothesis outlined here may only account for the read-out of information previously stored in nucleic acids and in proteins. A complementary mechanism has to be postulated which alters the composition of DNA and thereby of the membrane, and the principle of operation of this imprinting process obviously must be quite distinct from the read-out mechanism outlined here. Certain data suggest imprinting in protein metabolism[57–59], but they shed no light on the mechanism involved. The recent report of the effects on protein synthesis of changes in potassium concentration[60,61] raises the possibility that potassium extrusion associated with the action potential may affect the protein metabolism of neurones, but the practical importance of this mechanism remains to be determined.

At the basis of the present hypothesis are the experimental observations on displacement and deformation of living cells by focal electric field[11,12]. It is necessary to introduce a series of accessory assumptions, extrapolated from other data, however, and the conclusions reached are far from being unequivocal. Thus, it cannot be claimed that the hypothesis is unique. It is hoped, however, that compared with certain other theoretical approaches to learning, it is more accessible to direct experimental testing of each individual underlying extrapolation. Even though such experiments may fail to confirm the hypothesis, some purpose may be served in directing attention to a hitherto neglected class of biological phenomena—that of electrokinetic processes.

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PROTECTION OF PHAGOCYTIZED BACTERIA FROM THE KILLING ACTION OF ANTIBIOTICS

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PHAGOCYTIC cells ingest and destroy a variety of micro-organisms[1–3]. Many of the analyses of these activities are made *in vitro*, in culture media prepared with antibiotics to prevent multiplication of extracellular organisms. The killing of extracellular bacteria with antibiotics facilitates long-term investigation of the fate of intracellular bacteria and would reveal a defect in ability to digest intracellular organisms which ordinarily would be obscured by extracellular multiplication. Some antibiotics are known to be relatively ineffective against intracellular parasites such as tubercle bacilli and brucella[4–6], bacteria which survive intracellularly but are killed by the same antibiotics when not within the cells. Despite this evidence, the use of antibiotics is often deemed disadvantageous[1]. The interpretation of results obtained in investigations where antibiotics are used and the evaluation of the contribution of antibiotics to bacterial inactivation are particularly difficult when the bacterial strain used is rapidly killed by an isolated cell population. Mackaness[7] clearly demonstrated using rabbit peritoneal macrophages and *Staphylococcus aureus* in a solid medium containing penicillin that the antibiotic did not contribute to the intracellular killing of the bacteria. On the other hand, Jenkin and Benacerraf[8], using mouse peritoneal macrophages and *Salmonella enteritidis*, observed a rapid drop in the number of bacteria at the end of a 3-h sampling period which was attributed to the presence of streptomycin in the tissue culture medium.

This article presents evidence that ingested *Staphylococcus aureus* is protected for as long as 24 h from the action of penicillin and streptomycin in the culture medium. This investigation was made possible by the availability of cells from a population of children in our clinic who suffer from a disease based on a defect of intracellular digestion of certain micro-organisms.

We have recently had the opportunity to investigate the phagocytosis and digestion of bacteria by the peripheral white blood cells of three boys with fatal granulomatous disease. This disease, described by Berendes, Bridges and Good[9,10], by Landing and Shirkey[11] and by Carson *et al.*[12], is characterized by chronic draining adenopathy, pulmonary disease, hepatosplenomegaly and dermatitis. The disease has been described only in boys and is familial, and presumably is based on a sex-linked recessive defect. Extensive investigations have failed to reveal the nature of the metabolic fault which underlies the chronic granulomatous infections in these children.

In recent investigations of all three cases, the patients' peripheral white blood cells phagocytize bacteria normally but, in a tissue culture medium containing antibiotics, do not digest the bacteria. This suggests that the metabolic abnormality underlying the tendency to granuloma formation in these children is indeed a failure to digest bacteria following phagocytosis. Perhaps of equal significance, the results provide evidence that the presence of antibiotics in the tissue culture medium does not significantly affect the viability of certain intra-cellular organisms.

Venous blood was collected in a heparinized syringe and placed in a siliconized tube containing a solution of dextran (6 per cent) in saline. After 1 h at room temperature, the leucocyte-rich plasma was withdrawn and the cells were sedimented at 800*g* for 8 min. The cells were washed once in heparinized saline (0·5 u./ml.) and resuspended in Hanks's balanced salt solution or gel-Hanks (0·1 per cent gelatine). An 18-h broth culture of coagulase-positive *Staphylococcus aureus* was centrifuged at 1,500*g* for 10 min and washed twice with normal saline. The deposit was resuspended in Hanks's balanced salt solution or gel-Hanks so that the final suspension had an optical density of 0·6 at 650 mμ, and the bacterial suspension was diluted 1 : 50 before addition to the white blood cell culture. A suspension of white blood cells (1×10^7 cells per ml.) was delivered into 15 × 120 mm siliconized screw-cap test-tubes. Pooled normal human serum and bacterial suspensions were then added to give a final serum concentration of 10 per cent and approximately equal numbers of cells and bacteria (5×10^6 per

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ml.). The mixtures were incubated in a reciprocating water bath shaker at 37° C for 20–30 min to allow phagocytosis to occur. The tubes were removed from the water bath and centrifuged at 800*g* for 8 min and resuspended in tissue culture medium containing 100 U. of penicillin per ml. and 100 U. of streptomycin per ml. The medium consisted of Hanks's balanced salt solution plus 50 per cent foetal bovine serum and 1 per cent chick embryo extract. Samples were removed at prescribed intervals, centrifuged at 800*g* for 5 min, and the cellular pellet washed twice in Hanks's balanced salt solution. The deposit was suspended in 1 ml. distilled water and vigorously pipetted for about 30 sec. Appropriate dilutions were made and equal portions removed for the determination of total number of viable bacteria by the pour plate method using nutrient agar. A simultaneous control patient assay was performed with each assay for patients with granulomatous disease.

In Fig. 1, counts of viable bacteria have been plotted from representative experiments showing the survival of *Staphylococcus aureus* in peripheral white blood cells of the three patients with the chronic granulomatous disease and of three control patients. In most instances, control cells were obtained from children of about the same age as the patients, although age does not seem to be a factor in this system. It can be seen that after 120 min the number of viable bacteria has decreased by less than 0·5 $\log_{10}$ in cultures using cells from the granulomatous patients. The control cultures consistently showed a decrease of 1 to 1·5 $\log_{10}$. When samples were taken at longer intervals (5 to 24 h) there was no significant decrease in numbers of viable bacteria in cultures from patients with granulomatous disease, while a continuing decrease was noted in the control cultures (Fig. 2). Air-dried smears were made at sampling intervals between 0 min and 120 min and stained with Wright's stain. In all smears, large numbers of intracellular bacteria were seen in polymorphonuclear leucocytes and macrophages and appeared to be morphologicallly intact (see Fig. 3).

Summarizing these findings: (1) the peripheral white blood cells of three patients with fatal granulomatous disease of childhood, when cultured in a medium containing antibiotics, fail to destroy intracellular *Staphylococcus aureus* at a normal rate; (2) the survival of intracellular bacteria is not affected when the fluid cultures are shaken for long periods of time in the presence of antibiotics.

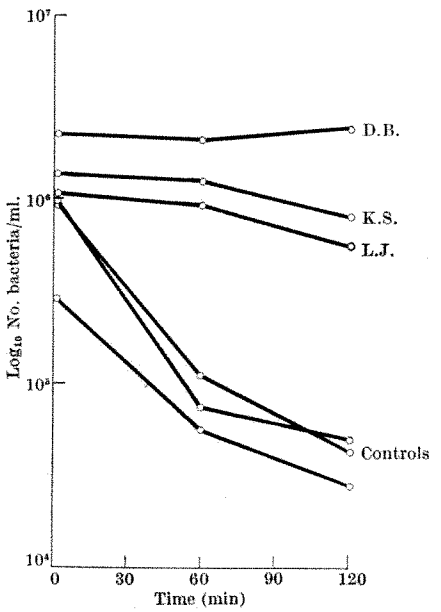


Fig. 1. A comparison of the destruction of *Staphylococcus aureus* by peripheral white blood cells of patients with fatal granulomatous disease (L.J., D.B., and K.S.) and of control patients

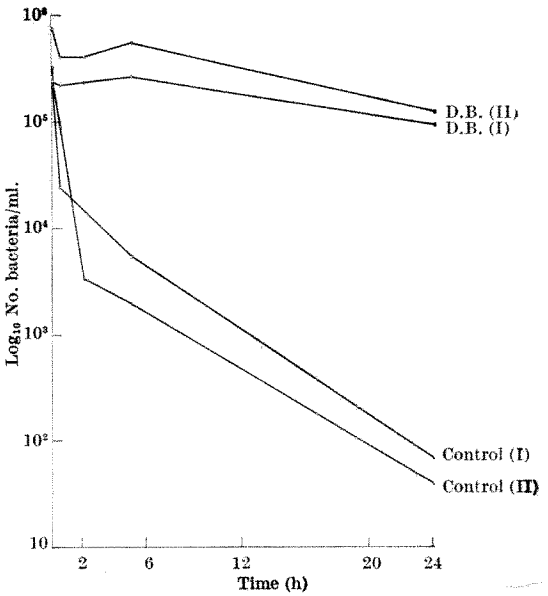


Fig. 2. Viable bacterial counts from two separate experiments (I and II) showing the fate of *Staphylococcus aureus* during prolonged incubation in the presence of peripheral white blood cells from a patient with chronic granulomatous disease (D.B.) and control patients

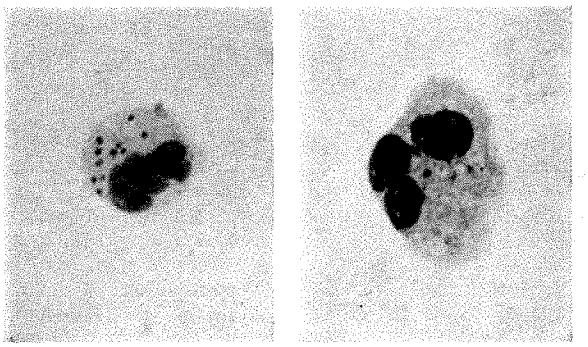


Fig. 3. *a*, A neutrophil from a patient with chronic granulomatous disease (K.S.) 30 min after infection *in vitro* with *Staphylococcus aureus*. *b*, A neutrophil from the control patient 30 min after infection *in vitro* with *Staphylococcus aureus*

Because the clinical disease of the patients investigated seemed to arise from faulty digestion of phagocytized micro-organisms, the latter finding indicates that phagocytized staphylococci are protected from bactericidal action of penicillin and streptomycin in the media. This suggests that when a search is made for abnormalities of intracellular digestion due, for example, to abnormalities of lysosomal content or structure, it may be useful to investigate the degradation of organisms located entirely within the cell while killing the extracellular micro-organisms with an appropriate antibiotic.

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POLYSOME PATTERNS AND PROTEIN SYNTHESIS DURING THE MITOTIC CYCLE OF *Physarum polycephalum*

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MITOSIS is synchronous in the plasmodium of *Physarum polycephalum* and RNA is synthesized in two distinct peaks, one premitotic and one postmitotic[1].

Since RNA is intimately associated with protein synthesis, it was of interest to determine the characteristics of protein synthesis and of polysome patterns during the mitotic cycle. A disappearance of polysomes during mitosis would suggest that certain cytoplasmic functions cease at the end of one cycle and start afresh with the beginning of each new cycle.

Sucrose was obtained from Baker and Adamson, and sodium deoxycholate (DOC) from Matheson, Coleman and Bell (Norwood, Cincinnati). L-lysine-^{3}H (specific activity 800 mc./mmole) was purchased from Schwarz Bioresearch Inc. (Orangeburg, New York). RNase (pancreatic ribonuclease, *EC* 2.7.7.16) came from Boehringer und Soehne (Mannheim, Germany). Medium *A* consisted of 0·25 M sucrose, 0·05 M potassium chloride and 0·005 M magnesium chloride, *p*H 5. Medium *B* consisted of 0·2 M sucrose, 0·05 M potassium chloride, 0·0015 M magnesium chloride and 0·1 M *tris* buffer, *p*H 7·2.

Growth and maintenance of the organism. Methods of cultivation of the organism have recently been reviewed by Daniel and Baldwin[2] and by Guttes and Guttes[3]. A detailed account of the growth and of the microscopic procedures has been given previously[4]. In this work the sub-line *M3C* was used.

Determination of total protein content in vivo. Plasmodia, each derived from 0·3 ml. of a shaken culture, were collected at different times of the cycle between mitoses 3 and 4 into 10 ml. of trichloroacetic acid–acetone (4 per cent trichloroacetic acid, 48 per cent acetone, 48 per cent water), and shaken. After centrifugation at about 1,000*g*, the pellet was resuspended and this procedure was repeated to remove all pigment. Three washes with 10 ml. 0·25 M perchloric acid (PCA) followed. The colourless precipitate was dissolved overnight in 3 ml. 0·4 N sodium hydroxide, and aliquots were taken to determine protein content by the Biuret methods[5].

Determination of the rate of amino-acid incorporation. Plasmodia at different stages of mitosis were incubated for 10 min on 13 ml. nutrient medium containing 20 μc./ml. lysine-^{3}H and processed as indicated earlier. The radioactivity of 0·5 ml. of the alkaline solution was determined in a liquid scintillation counter.

Preparation of ribosomes. Three plasmodia, taken about 2 h before mitosis 3, were homogenized in 30 ml. of either Medium *A* or Medium *B* with 15 strokes in a Potter–Elvehjem homogenizer with a 'Teflon' pestle. All steps were performed in the cold. To precipitate unbroken plasmodia and nuclei, the suspension was centrifuged for 10 min at 1,400*g*. The supernatant was then centrifuged at 7,500 r.p.m. in a 'Spinco' rotor 30, at an average of 5,000*g*. This pellet was designated 'mitochondria'. The 5,000*g* supernatant was divided into two halves: one was brought to a final concentration of 0·1 per cent DOC with 0·3 ml. aqueous DOC solution; the other received no additions. Both supernatants were then centrifuged for 1 h at 100,000*g* in a 'Spinco' centrifuge. The 100,000*g* pellet that had not been subjected to DOC was called 'microsomes', while the DOC-treated pellet was designated 'ribosomes'. The 100,000*g* supernatant was discarded. All three pellets (mitochondrial, microsomal and ribosomal) were washed three times with 10 ml. 0·25 M PCA, being carefully suspended and centrifuged each time as indicated above.

The RNA was hydrolysed with RNase in all pellets according to the method previously described[1], and the radioactivity and ribose contents were determined in the supernatant. A conversion factor of 2·1 was used to calculate μg RNA from μg ribose.

Preparation of polysomes without radioactive labelling. Plasmodia at about mitosis 3 were incubated for 1 h in fresh nutrient medium. At the desired times of the mitotic cycle they were placed in liquid nitrogen. This step had to be done rapidly, great care being taken to ensure that no cooling of the cultures took place in the air outside the 26° C incubator. Once collected, the samples could be kept for at least 5 h without destruction of polysomes. The next step included homogenization in a Potter–Elvehjem all-glass homogenizer (Kontes Glass Co., Vineland, New Jersey, type *B*). Homogenizer and pestle, as well as the vessel containing 2·5 ml. Medium *B*, were pre-cooled to 0° C, and the subsequent steps were performed in the cold room at 4° C. The frozen sample was placed in the homogenizer and immediately homogenized with ten strokes at 1,100 r.p.m. As soon as the frozen plasmodium was broken, five additional strokes followed, while the homogenizer was held in a cold mixture at −6° C. From then on the homogenate was kept in a glycerol–water–ice mixture at −6° C. The homogenate was centrifuged for 5 min at 3,500 r.p.m. in a 295 rotor of an International centrifuge at −6° C. The pellet was discarded, and the supernatant was brought to a final concentration of 0·4 per cent DOC with 0·2 ml. of a 5 per cent aqueous DOC solution. After being briefly shaken in the cold, 0·5–1·5 ml. of this supernatant was layered with pre-cooled pipettes over a sucrose density gradient. A 2-h run in an '*SW*25' bucket in a Spinco model *L* centrifuge at 25,000 r.p.m. followed, with the temperature in the centrifuge set at 10° F. After the centrifuge was stopped by braking, the tubes were punctured and the effluent passed through a flow-through cell (Beckman No. 96160) of cylindrical shape, 10 mm path length and 0·5 ml. volume. The whole flow-through assembly was filled with 0·5 M sucrose at the start. The ultra-violet absorption of the effluent was measured in a Beckman '*DB*' model at 260 mμ and registered continuously with a Sargent strip chart recorder. 0·5 M sucrose served as a blank.

Linear sucrose density gradients were prepared by mixing a 10 per cent sucrose solution with a 51 per cent sucrose solution, both containing 0·025 M potassium chloride, 0·005 M magnesium chloride, and 0·05 M *tris*, *p*H 7·3. The mixing procedure was performed in a gradient apparatus consisting of two 20-ml. syringes filled up to the 17-ml. mark, which were pushed with equal speed, resulting in a 28-ml. linear gradient.

Preparation of polysomes after radioactive labelling. 1 h before labelling, the cultures were transferred to a lysine-free, fully defined medium (*AV*-40)[2]. At the desired time in the mitotic cycle these cultures were transferred into a lysine-free *AV*-40 medium containing 20 μc./ml. lysine-^{3}H and were incubated for the specified times. Care was taken to bring the incubation medium

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to 26° C prior to use. After incubation the plasmodia were chilled rapidly in liquid nitrogen and processed as described above. Collection and precipitation of the radioactive samples were done as follows: twenty drops (about 2 ml.) were collected by a fraction collector into 0·5 ml. albumin solution (1 mg/ml.) and immediately precipitated in the cold with 1·5 ml. 0·5 M PCA or 0·6 ml. 100 per cent TCA (w/v). The samples were then filtered *in vacuo* through 'Millipore' filters, type *HA*, of 2·8 cm diameter with a pore size of 0·45μ. After being dried in air the radioactivity of these filters was measured in a liquid scintillation counter.

Determination of the load on the gradients. A direct and fast measurement of the RNA content of the sample loaded on the gradients was not possible, because of its contamination with non-ribosomal, ultra-violet-absorbing material. We therefore looked for a fast and accurate but indirect measurement of the RNA content of the load. This was found in the direct proportionality of pigment and RNA content of the postmitochondrial supernatant, as illustrated in Fig. 1. The routine procedure followed was to load a certain amount of postmitochondrial supernatant on the gradient, and to mix the same amount thoroughly with TCA-acetone in the cold and centrifuge this at 1,400*g* for 10 min. The optical density of the supernatant was measured at 415 μm, the spectral maximum of the yellow pigment. The sediment was washed three times with 10 ml. 0·25 M PCA and the RNA determined as described elsewhere[1]. When RNA was not determined in the sediment, its amount was calculated from the graph (Fig. 1).

Calculation of the amount of ribosomes and polysomes. Since the observed heights of the peaks are the result of overlapping of neighbouring peaks, all methods of comparing single ribosomes with polysomes are approximations[6]. The method we followed was to measure the areas below the single ribosome peak and the polysome peaks, having separated the two by a vertical line going through the trough between single ribosomes and dimer peaks. The ratio polysomes to single ribosomes was calculated from these figures.

Protein synthesis during the mitotic cycle. The total protein content of plasmodia during the mitotic cycle is shown in Fig. 2. The protein almost doubled between mitosis 3 and mitosis 4. In contrast to the more or less steadily increasing amount of total protein, the rate of incorporation of lysine-^{3}H into protein showed a biphasic pattern. The premitotic and postmitotic peaks parallel those of RNA synthesis. In contrast to RNA synthesis, however, the lysine-^{3}H incorporation exhibited a distinct lag phase of about 1 h after mitosis compared with the postmitotic rise in uridine incorporation.

Ribosomes. To determine optimal conditions for preparing ribosomes and polysomes, the influence of *p*H during the isolation procedure was investigated and

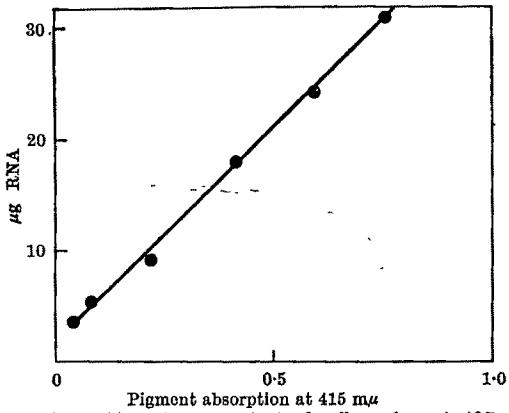


Fig. 1. Relationship between content of yellow pigment ($OD_{415\,m\mu}$) and μg RNA (orcinol-positive material) in a postmitochondrial supernatant containing 0·4 per cent DOC

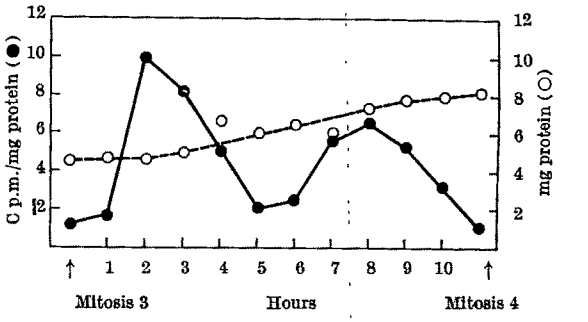


Fig. 2. Protein synthesis throughout the mitotic cycle: ○ - - - - - - ○, Total protein content determined with the Biuret method. ●——————●, *In vivo* incorporation of L-lysine-^{3}H into plasmodia between mitosis 3 and 4. Pulse labelling time, 10 min

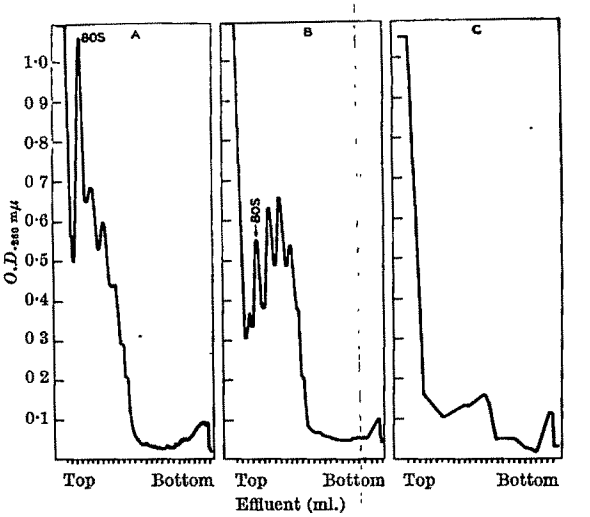


Fig. 3. Effect of temperature and RNase ion the polysome pattern. (*A*) The frozen plasmodium was homogenized at 0° C. Most of the polysomes were broken down. (*B*) The frozen plasmodium was homogenized with a rough-surfaced, all-glass homogenizer, and all steps were performed at −6° C. Polysomes prevail. (*C*) After homogenization the 16,000*g* supernatant was incubated at 37° C for 10 min with 2·5 μg RNase. This resulted in total disappearance of ribosomal structures. Load expressed in μg RNA. *A* = 45, *B* = 40, *C* = 42

the RNA contents of the ribosomes was compared with that of fractions containing mitochondria and microsomes. It will be noted from Table 1 that the amount of RNA in the microsomes obtained at *p*H 7·2 (145 μg) was greater than the amount obtained at a lower *p*H (84 μg). Fractionation at *p*H 7·2 in the presence of DOC resulted in further purification (168 μg RNA), yielding a fraction designated as 'ribosomes'. The mitochondrial pellet obtained at *p*H 5 contained more RNA (104 μg) than the fraction prepared at *p*H 7·2; this suggests that microsomes adhered to the mitochondria at the lower *p*H.

Polysomes. The critical phase in the isolation procedure of polysomes from the plasmodium is the prompt application and maintenance of low temperatures. As seen in Fig. 3*B*, only the plasmodia which were frozen in nitrogen and homogenized in this frozen state gave the characteristic pattern of polysomes. If the plasmodium was not converted from the solid frozen state directly into a homogenate, breakdown took place; melting or softening of the solidly frozen plasmodium in the homo-

Table 1. RNA CONTENT AND RNA/PROTEIN RATIO OF PLASMODIAL FRACTIONS PREPARED ABOUT 2 H BEFORE MITOSIS 3

Fractionation medium	Microsomes		Ribosomes		Mitochondria	
	μg RNA	RNA/protein	μg RNA	RNA/protein	μg RNA	RNA/protein
A, *p*H 5	84	0·58	—	—	104	0·41
B, *p*H 7·2	145	0·89	—	—	25	0·16
B, *p*H 7·2, + DOC	—	—	168	0·92	—	—

The figures given represent the averages of two independent experiments. See text for the preparation of the various fractions.

genizing medium caused breakdown. The selection of the proper homogenizer was also crucial. Only glass homogenizers with a rounded tip and rough inner surface were satisfactory. Homogenizing at 4° C resulted in the breakdown of polysomes (Fig. 3*A*). The breakdown of all ribosomal structures could be induced by adding 2·5 μg RNase to the postmitochondrial supernatant and incubating it for 10 min at 37° C (Fig. 3*C*). In the RNase-treated samples we regularly found a slight peak at about the position of the decamer. This peak did not disappear with higher RNase concentrations. The standard optical density pattern (Fig. 3*B*) showed a large amount of non-ribosomal material at the top. This was not surprising since no 'purification step' was used. The lightest particle to be recognized in the standard 2-h centrifugation was the 50*S* ribosomal subunit, the 30*S* subunit remaining in the highly absorbing material at the top of the sucrose gradient. Both subunits could be resolved with an 8-h centrifugation, and their amounts could be increased by omitting magnesium from the isolation medium. In the standard centrifugation, the second lightest peak was caused by 80*S* particles (single ribosomes); thereupon followed the polysomes, with the dimer, trimer, tetramer, pentamer, and hexamer peaks showing up at regular intervals (Fig. 3*B*).

In Fig. 4*A*, a better resolution was achieved owing to a smaller load; polysomes were separated in peaks up to heptamers. In other experiments with still smaller loads, nonamer peaks could be detected. However, the region comprising ten to twenty unit-aggregates is represented in *P. polycephalum* only by a low, broad peak. Fig. 4*B* shows the polysome pattern of a 48-h culture. This stage is characterized by movement of the plasmodial masses owing to lack of nutrients. The sedimentation pattern showed predominantly the single ribosome peak with a small polysome portion.

The effect of DOC at different concentrations on the polysome pattern is shown in Fig. 5. When no DOC was added to the postmitochondrial supernatant (Fig. 5*A*), single ribosomes prevailed. With increasing DOC concentrations (Fig. 5*B*, 0·1 per cent; Fig. 5*C*, 0·3 per cent) more polysomes were found. This indicates that polysomes were being liberated, on the assumption that the single ribosome population was not altered by DOC. A high concentration of DOC (1 per cent), as used by investigators with liver preparations[6–8], destroyed the entire patterns (Fig. 5*D*).

Polysome pattern during the mitotic cycle. Fig. 6 shows polysome preparations at different stages in the mitotic cycle. The optical density pattern is remarkably uniform at most times of the mitotic cycle. There was no rapid change during and immediately after mitosis (Fig. 6*A*). There was, however, a steady decline in the number of

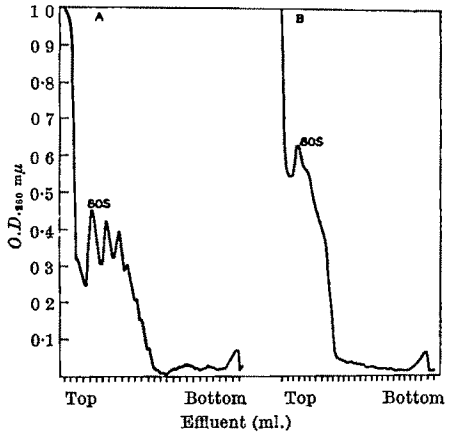


Fig. 4. Influence of age of plasmodium on the polysome patttern. (*A*) Standard polysome pattern of a plasmodium 23 h after inoculation of the culture. (*B*) Polysome pattern of a plasmodium 48 h after inoculation of the culture

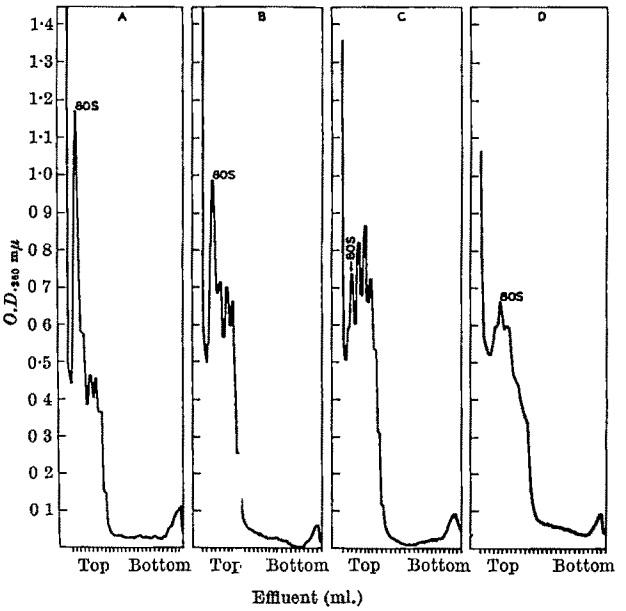


Fig. 5. Influence of different concentrations of DOC on the polysome pattern. (*A*) No DOC (32 μg RNA), (*B*) 0·1 per cent DOC (30 μg RNA), (*C*) 0·3 per cent DOC (33 μg RNA), (*D*) 1·0 per cent DOC (25 μg RNA). Freshly prepared DOC was added in aqueous solution to the postmitochondrial supernatant

polysomes relative to the number of single ribosomes after mitosis, becoming most pronounced 40 min after mitosis (Figs. 6*B* and 7). By 50 min after mitosis a polysome pattern comparable to that found in prophase was re-established (Fig. 6*C*). Similar determinations were made at various stages throughout the rest of interphase and resulted in patterns like those of Fig. 6*C*.

Labelling pattern of polysomes after pulses with lysine-^{3}*H.* Fig. 8 shows the radioactive labelling pattern as observed 1 h before mitosis 3. In Fig. 8*C* radioactive samples were precipitated with 1·5 ml. 0·5 M PCA. One peak can be observed, with its maximum at the position of the trimers. The single ribosome peak contained only slight radioactivity, indicating that there was little or no breakdown of heavy aggregates into single ribosomes. Precipitation of the radioactive samples with 0·6 ml. 100 per cent TCA (giving a final concentration of about 20 per cent) resulted in the curves shown in Figs. 8*A* and 8*B*. Two maxima of incorporation of radioactivity can be seen. The first peak paraleled roughly the optical density pattern and was similar to the radioactivity peak observed in Fig. 8*C*. The second peak was located in the area where aggregates of ten and twenty ribosomes would be expected. In contrast to the peak associated with the optical density pattern, this second peak increased with longer pulses of lysine-^{3}H (Fig. 8*A*).

Certain aspects of protein synthesis in the mitotic cycle of the plasmodium of *P. polycephalum* were investigated by measurement of isotope incorporation and by an investigation of polysome patterns. Such an investigation during different physiological states of a cell should reveal the presence of *m*RNA, which is at least potentially functional in protein synthesis.

The profiles of optical density show that the majority of polysomes obtained from *P. polycephalum* consist of aggregates of two to five ribosomes. Under specified experimental conditions, the patterns are very reproducible, but a slight change in the procedure may lead to complete breakdown of the polysomes. The most critical parameter appeared to be the temperature; it was necessary to carry out the extraction slightly below 0° C. A low concentration of deoxycholate was also essential. Although part of the action of deoxycholate was the release of polysomes from other cell structures (endoplasmic reticulum), it also helped to prevent the breakdown of polysomes, as did the

low temperature. Thus the polysomes of *P. polycephalum* are very labile to the action of nuclease; they appear to be intermediate in lability between those of bacteria[9] and those from rat liver[6–8]. Whether the optimal patterns obtained from *P. polycephalum* are the result of a partial breakdown of still larger polysomes cannot be decided with certainty[10].

Lysine incorporation into acid-insoluble material took place at two positions in the gradients. One coincided with the peak of the optical density pattern (fractions containing clusters of three to five ribosomes), the other at a position where polysomes of ten to twenty subunits should be found. If the latter peak also represents ribosomal clusters with nascent radioactive protein attached, it would appear that nascent protein protects polysomes from breakdown and that the optical density patterns found were caused by a partial breakdown of larger

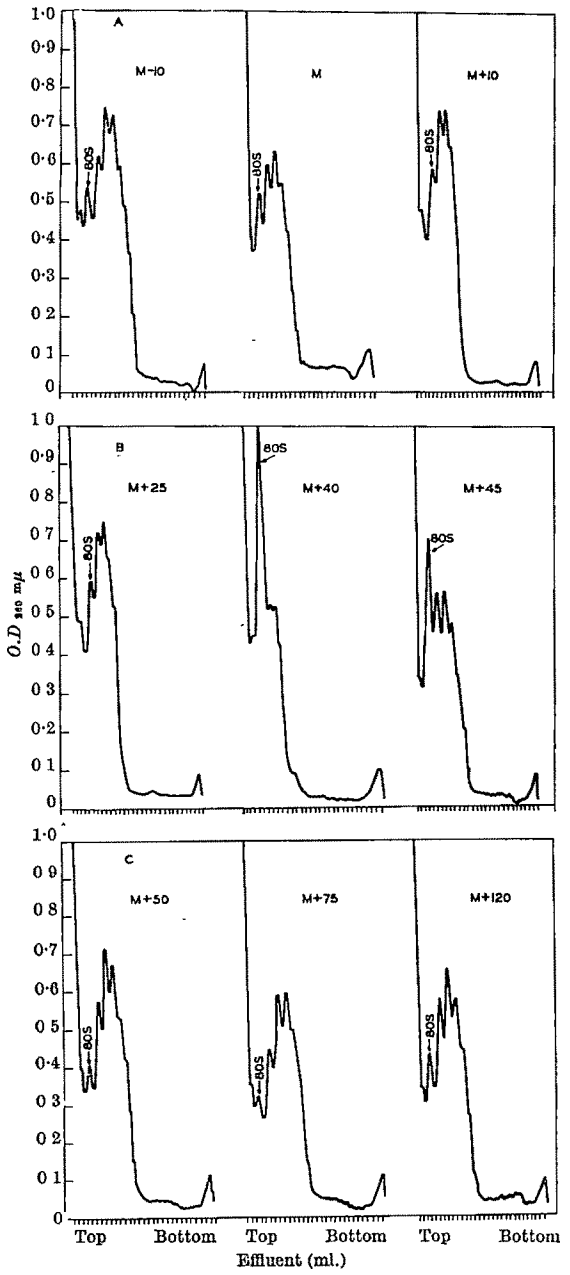


Fig. 6. Polysome patterns found at different stages of the mitotic cycle. (*A*) M − 10, 10 min before mitosis 3 (38 μg RNA load), M, Mitosis 3 (29 μg RNA load); M + 10, 10 min (33 μg RNA load); (*B*) M + 25, 25 min (29 μg RNA load); M + 40, 40 min (24 μg RNA load), M + 45, 45 min (32 μg RNA load), (*C*) M + 50, 50 min (35 μg RNA load), M + 75, 75 min (32 μg RNA load); M + 120, 120 min (33 μg RNA load), after mitosis 3

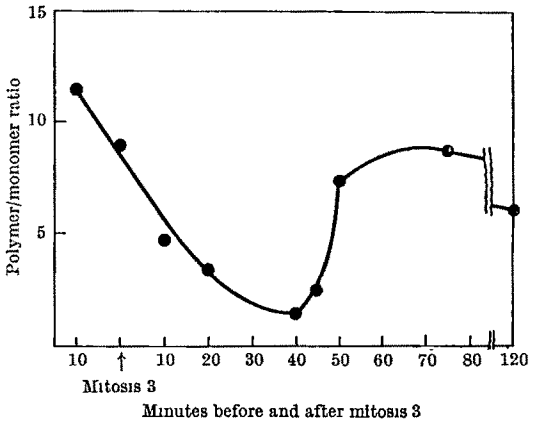


Fig 7 Ratio of polysomes to single ribosomes during the mitotic cycle. The areas occupied by the single ribosome peak and by the polysome peaks were taken from Fig. 6, and the ratio polysomes to single ribosomes (polymer/monomer) was calculated and plotted against the time

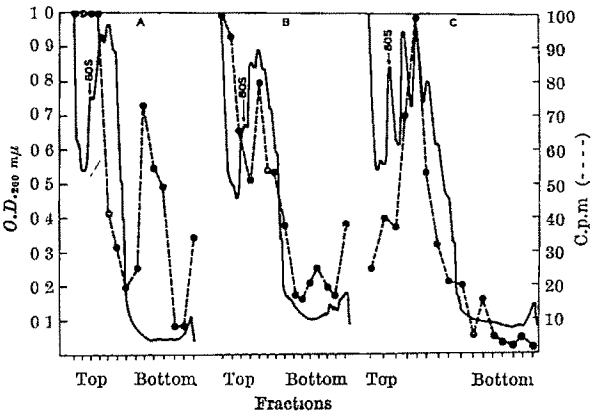


Fig. 8. Polysome pattern 1 h before mitosis 3. In *B* and *C* a 10-min pulse, in *A* a 20-min pulse with lysine-^{3}H was used. In *A* and *B* the samples were precipitated with 20 per cent TCA, in *C* with 0 25 M PCA. The radioactivity of the acid-precipitable material was measured on 'Millipore' membranes

polysomes. Even if this were the case, the optical density patterns obtained during the mitotic cycle show clearly whether or not the ribosomes are present singly or in clusters.

At all times of the mitotic cycle investigated, including mitosis, the majority of ribosomes were present in the form of polysomes. The structural revolution of mitosis in the nuclei therefore does not entail a radical and immediate reorganization of the cytoplasmic protein-synthesizing machinery: functional messenger RNA appears to be carried over from one mitotic cycle to the next. However, there was a partial shift from polysomes to single ribosomes during a period of 40 min immediately after mitosis, rapidly followed by the usual interphase pattern. This temporary shift from polysomes to single ribosomes may reflect the reduced synthesis of *m*RNA. The *m*RNA holding the polysomes together is constantly being degraded and may not be replenished as long as the chromosomes are condensed.

Lysine incorporation investigations show that protein synthesis takes place throughout the mitotic cycle, including mitosis, and corroborate the conclusion from the polysomes experiments. The extent of incorporation varies in the cycle. It is greatest 2 h after mitosis, drops off, and then increases again 2 h before the next mitosis. The pattern is thus reminiscent of that of uridine incorporation into RNA. Low lysine incorporation was observed shortly after mitosis, when the ratio of polysomes to single ribosomes was low.

The polysome pattern during the growth cycle of *P. polycephalum* differs somewhat from that observed with

HeLa cells, in which it is reported that a complete polyribosome disaggregation occurs during metaphase[11]. In *P. polycephalum* the disaggregation of the polyribosomes is less complete and occurs about 40 min after metaphase. Two factors may account for these differences: first, the period of metaphase–anaphase in HeLa cells lasts from 20 to 30 min, compared with a 5-min metaphase–anaphase in *P. polycephalum*; thus, the time of polysome breakdown in HeLa cells may include the comparable period in *P. polycephalum*, where the metaphase period is so much shorter. Secondly, the degree of polysome disaggregation may be correlated with the extent of cellular reorganization during cell division. One would expect such disaggregation to be more pronounced in uninucleate cells, such as HeLa cells, in which cytoplasmic division is concomitant with mitosis, as compared with the changes in a multinucleate plasmodium in which the nuclear wall remains intact during mitosis and cellular fission does not accompany nuclear division.

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GOITROGENIC ACTIVITY OF CASSAVA (A STAPLE NIGERIAN FOOD)

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A RECENT goitre survey[1,2] in the Nsukka Division of Eastern Nigeria revealed a widespread incidence of visible goitre which varied considerably from town to town (1·4–41 per cent). Several of the water supplies of these towns were tested[2], and all were found to be deficient in iodine (under 1·5 μg/l.) but the lowest levels were not found in the most goitrous towns. A search was therefore made for differences in dietary habits, and it emerged that those towns and villages with the highest incidence of goitre were those feeding mostly on dry unfermented cassava‡. Thus, dry unfermented cassava might contain goitrogens, the effect of which only emerges when the associated dietary components are also iodine-deficient.

Experiments were therefore planned to assess this hypothesis. Measurements of thyroid function were made on various groups of rats after they had been fed on either a standard diet, an iodine-deficient diet or on cassava as well as on a group which had received the cassava plus iodide supplements. The effects of the carefully standardized iodine-deficient diets differed from some previous findings and have been published elsewhere[3], while the cassava-feeding experiments, which are reported in this article, indicated that cassava contains a goitrogen with an action similar to those of the thionamide group of goitrogens (? thiaurelene).

Female albino rats weighing 170–198 g were investigated in groups of three, each after a different dietary for seven days, the dietaries having been chosen to allow the assessment of a possibly goitrogenic effect from cassava. Unfortunately, the feeding could only be continued for short periods and to small groups of rats, as only limited amounts of cassava had been brought to London from Nigeria for these experiments. However, some sufficiently definite effects emerged. At the end of the 7-day dietary and 24 h before killing, each rat was injected intraperitoneally with 20 μc. carrier-free iodine-131. Immediately after killing with ether, blood was withdrawn by cardiopuncture and the thyroid carefully dissected. The following measurements were made to assess thyroid function: (*a*) thyroid weight, finally expressed as mg/100 g body weight; (*b*) 24-h thyroid iodine-131 uptake (as percentage of dose); (*c*) 24-h serum protein-bound iodine-131 (as percentage dose/ml.); (*d*) from digests of thyroid tissue prepared as described elsewhere[4,5]: (i) total iodine concentration (μg iodine-127/mg tissue) from one aliquot, and (ii) from the second aliquot, after resin purification and chromatography, the distribution of iodo-amino-acids, measured by scanning the paper chromatograms for iodine-131; the parameters found most relevant being the monoiodotyrosine/diodotyrosine (MIT/DIT) ratio and the iodothyronines (thyroxine or triiodothyroxine) as a percentage of the total gland iodine-131.

The diets so assessed for their effect on thyroid function included: (1) the standard laboratory diet (41_B) detailed by Bruce and Parkes[6] for a control group; (2) an iodine-deficient but otherwise normal diet containing 100 μg iodine-127/kg (as detailed elsewhere[3]); (3) 100 per cent cassava; (4) 50 per cent cassava with 50 per cent standard 41_B laboratory diet; (5) 100 per cent cassava with added iodine in the drinking water (50 μg iodine-127/100 ml. which should provide 10 μg/rat/day).

Measured aliquots of each diet were offered daily (10 g/rat). The tap water of all the cassava-fed groups was supplemented with 'Compound Vitamin Drops' (1 ml./l. water, Paines and Byrne, Ltd.). The iodine-deficient group and the iodide-supplemented cassava group were included because the cassava diet proved to be iodine deficient; a portion being found to contain 90 μg iodine-127/kg, so that each rat on the 100 per cent cassava received less than 2 μg iodine-127/day. Conveniently, this diet approximated very closely in iodine content to the iodine-deficient diet; the former containing 90 μg iodine-127/kg and the latter 100 μg iodine-127/kg. The cassava meals were prepared fresh each day by the methods traditionally used in Nigeria.

As summarized in Table 1, after cassava, the radioiodine uptake by the thyroid is increased, probably because of the associated iodine deficiency of this diet; indeed the avidity of the thyroid for iodine after cassava was similar to that seen after the iodine-deficient diet, while the cassava diet with iodine supplements led to a normal thyroid avidity for iodine. However, the 24-h serum plasma-bound iodine-131 and the thyroid weight, while little increased after the iodine-deficient diet, were both increased after all 100 per cent cassava feeding—suggesting some additional effect from cassava other than iodine deficiency.

The thyroid digest findings corroborate these suggestions of some antithyroid factor in the cassava diet as well as

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‡ Cassava (*Manihot utilissima*) is sold in three forms at the markets in the Division: (i) fresh fermented, (ii) dry fermented; and (iii) dry unfermented. It is a staple food for most Nigerians, the daily diet of an average person consisting of pounded or cooked cassava morning, afternoon and night served with a stew.

Table 1. THYROID FUNCTION OF RATS AFTER CASSAVA FEEDING COMPARED WITH OTHER DIETS

Experimental groups (each 3 rats) (each given diet for 7 days)	Weight		Thyroid parameters (mean ± S.E.) ^{131}I-Uptake 24 h	Total ^{127}I	Thyroid digest findings ^{131}I (24 h) Iodothyronines (per cent gland ^{131}I)	MIT/DIT	Serum 24-h protein-bound ^{131}I (per cent dose/ml.)
	(mg)	mg/100 g rat	(per cent dose)	(μg/g)			
Cassava fed							
(1) 100 per cent	19·1 ± 0·8	11·1 ± 1·1*	18·2 ± 0‡	0·14	39·9 ± 1·5‡ (2)	1·44 ± 0·06‡	0 08
(2) 50 per cent cassava + 50 per cent standard diet	19 3 ± 1·5	10·2 ± 1·4	10 9 ± 0·9*	0 35	33·9 ± 1·0‡	1·4 ± 0·11‡	0·02
(3) 100 per cent cassava + iodide supplements (10 μg ^{127}I/day)	16·0 ± 1·3	9·1 ± 1	14·3 ± 0 7	0·36	53·2 ± 2·0‡ (1)	1·3 ± 0 02†	0·06
Controls							
(4) Standard diet (41 B)	13 0 ± 0·3	7·3 ± 0·06	14·4 ± 0·7	0 8	25·0 ± 0·8 (1)	0·6 ± 0·02	0·028
(5) Iodine-deficient diet§	(19·3)	8·3 ± 1·0	18·3 ± 1·0	0·68	48·4 ± 1·5	0·34	0 015

Differences from controls on standard diet: * Probably significant ($P < 0·05$). † Significant ($P < 0·02$). ‡ Clearly significant ($P < 0·001$). § Data from experiments reported more fully by Dimitriadou *et al.* (ref. 3) (iodine content of diet being 100 μg/kg compared with that of cassava 90 μg/kg). (1), Mostly thyroxine; (2), high proportion of triiodothyronine.

iodine deficiency. After cassava feeding the digest findings showed: (*a*) severe depletion of the gland's precursor and hormone iodine stores, that is, the total thyroid gland iodine was 0·14 μg iodine-127, or much less than the slight depletion seen after an equivalent duration of the equivalently iodine-deficient diet (0·68 μg/mg); (*b*) an impaired iodine-131 transfer from MIT to DIT, that is, a high MIT/DIT ratio (1·3–1·44), again contrasting with the low or normal ratio after the iodine-deficient diet (0·34); (*c*) high transfer of iodine-131 to iodothyronines, a feature also seen in iodine deficiency, but not eliminated when the cassava feeding is combined with adequate iodine supplements.

The diet of 100 per cent dry unfermented Nigerian cassava used for these experiments is deficient in iodine, approximately equivalent to the iodine-deficient diet with which we have compared it, as noted earlier. To this feature is attributable the increased thyroid uptake of iodine-131 seen after the feeding of cassava—for the thyroid uptake was equivalently increased after the iodine-deficient diet, while it was normal with the iodide-supplemented cassava diet. However, the cassava feeding led to several other changes not seen after iodine-deficient diets; the cassava diet is rapidly goitrogenic, it depletes the iodine stores of the gland more rapidly than does iodine deficiency, and it also impairs the transfer of iodine from MIT to DIT (high MIT/DIT ratio) in the gland. Furthermore, giving iodide supplements with the cassava does not remove these last two abnormalities, and merely lessens the first. Thus, many of the effects seen to follow cassava feeding suggest that it contains a weak goitrogen, acting like the thionamide group of antithyroid drugs, since its effects are not reversed by iodide supplements.

Low concentrations of thiouracil have been reported to cause increased thyroid weight with a variable or even high iodine-131 uptake, severely depleted thyroid iodine stores and a high MIT/DIT ratio[7,8], findings rather similar to these reported here after cassava feeding, which clearly might have a similar basis. The high proportion of thyroid iodine-131 found as iodothyronine along with the high serum plasma-bound iodine-131 are most probably due to the low hormone stores in the gland causing a rapid iodine turnover through the gland, once the block at the MIT/DIT transfer has been passed. Possibly another factor might be a diminished peripheral deiodination similar to that reported to occur with thiouracil[9,10].

We have no evidence about the nature of the goitrogen in the cassava. Cassava has long been known to contain a cyanogenetic glucoside[11,12] which the traditional method of fermentation hydrolyses. Recent workers[13,14], however, doubt whether fermentation and boiling before eating remove all this cyanogenetic glucoside. Thus, eating cassava, especially in the unfermented form, may tend to bring about continual ingestion of this cyanogenetic glucoside which could decompose in the body to a goitrogen. Furthermore, whatever the goitrogen in cassava may be, the higher incidence of goitre in iodine-deficient Nigerian villages, which also feed on much cassava, could be explained by the goitrogenic activity of cassava.

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TAXONOMIC IMPLICATIONS OF 'MYXOVIRUS'

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THE bases on which viruses can be grouped have changed fundamentally in the past twenty years or so[1]. Whereas it was once possible to evaluate a virus only from its effect on the host organism[2], and somewhat later on the susceptible cell, it is now possible to view viruses in a much more definitive way. In the light of this recent knowledge about viruses, many previously held concepts now appear inadequate. Groupings which had previously seemed meaningful have now been shown to have only superficial validity and, conversely, unsuspected relationships have come to light. This article sets out to consider the changed relationships that have emerged in the case of one particular group of viruses contained within the overall grouping of the compound (that is, enveloped) helical RNA viruses and, until now, termed myxoviruses.

Luria[3], in discussing the taxonomy of viruses, referred to this group when he observed that a "natural group may be that of the influenza-mumps-Newcastle disease viruses which have similar particles and common enzymic activity on some surface component of red blood cells and on other substrates". Two years later, Andrewes,

Bang and Burnet[4] formally suggested the very acceptable name of 'myxovirus' for this same group of viruses, which then comprised influenza *A*, *B* and *C*, fowl plague, mumps and Newcastle disease virus (NDV). The prefix 'myxo' means mucus and is derived from the Greek μυξα (nasal secretion). This name was chosen because all these viruses have two distinct interactions with mucoproteins, whether these are an integral part of a cell, as on an erythrocyte, or are free, as in serum. First there is attachment of virus to mucoprotein, and then elution occurs from it by means of the enzyme neuraminidase. This two-fold action is the truly distinctive property of the myxovirus group as originally designated. The effect on red cells had considerable practical importance, as adsorption of virus to them gives rise to the phenomenon of haemagglutination, while elution offered a means of obtaining relatively pure virus. In the light of the knowledge available at the time the segregation of these six viruses into a group was both natural and instructive. In addition to this effect on mucus, the members of the group had other properties in common, such as sensitivity to ether, adaptability to growth in the hen's egg, and maturation at the surface of the cell. However, it is the unique interaction with mucoproteins, which might be termed 'myxophily', that both unites them as a group and segregates them from other viruses.

As has already been stated, the decade since 1955 has seen the emergence of much more definitive information on viruses in general and on myxoviruses in particular. The members of the group have been shown to have similarities other than those originally demonstrated. For example, it is now known that they all have RNA as their nucleic acid and that this RNA is in the form of a ribonucleoprotein helix contained in a lipoprotein envelope partially derived from the host and partially from viral material. On the other hand, it has now become evident that some important inconsistencies arise when the term myxovirus is used at the present time.

The group now appears to be less homogeneous than was originally supposed. It no longer maintains its early unity, but has been shown to consist of two distinct sub-groups which can each be named after one of their well-characterized members. These sub-groups are the NDV sub-group of myxoviruses and the influenza sub-group of myxoviruses[5,6]. Although members of both sub-groups are compound helical RNA viruses they are of two distinct types and can readily be identified by the negative staining process in the electron microscope, and also by their reproductive cycle in the cell. The situation is further complicated because the refined techniques of investigating viruses, which have come into widespread use since 1955, have produced many new candidates for inclusion in the myxovirus group. These new candidates began to appear when the negative staining technique revealed the morphology of the myxoviruses in considerable detail, and showed that certain other viruses, of which the first was measles, had a virion indistinguishable from the classical NDV type of myxovirus. Subsequently a whole range of viruses morphologically identical with NDV have been described. These may be divided into (*a*) those which have the same effect on mucoproteins as the classical myxoviruses, and therefore are myxoviruses, (*b*) those which attach to mucoproteins, but do not elute from them because they lack neuraminidase (such as some bovine parainfluenza 3's), and (*c*) those which have no effect on mucoproteins, for example, the measles group.

It is this third group which has caused particular confusion, because their morphological similarity to NDV had led some to label them myxovirus[7] without realizing the implications of the word. The argument runs as follows: "NDV is a myxovirus, and its virion has a characteristic structure. Measles virus has an identical structure, and therefore it is a myxovirus." The fallacy here is that it is the property of myxophily, and not the accompanying morphology, which establishes NDV as a myxovirus. Measles does not share this property of myxophily in spite of its identity of form, and therefore has no valid claim to be in the myxovirus group. The case of the two related viruses, rinderpest and distemper, is the same as that of measles. Indeed, if the word myxovirus is used in the original sense, even those bovine parainfluenza 3's which lack neuraminidase have only a partial claim to be called myxoviruses.

In addition to these viruses which exhibit identical morphology to one sub-group of myxoviruses but are not myxophilous, there has emerged a second assemblage of viruses which have been reported to possess a certain morphological similarity to the myxoviruses (that is, they have a ribonucleoprotein helix enclosed in an envelope) but are not morphologically identical with either sub-group. Such viruses are avian erythromyeloblastosis virus[8] which has been reported to have an internal component of about 40 Å diameter, and rabies[7,9], which has a helix of about 90 Å but is quite different in appearance from the corresponding structure in the influenza group. Yet a third example is the group typified by vesicular stomatitis virus[10]. In spite of the fact that all these viruses possess compound helical structures, there are clearly marked differences both from the two sub-groups of myxoviruses and from each other. To sum up, we find that the term myxovirus, instead of being applied to a homogeneous population of viruses which exhibit myxophily, is actually being applied with varying degrees of inaccuracy to each of the following four groups:

(1) True myxoviruses, all of which have a distinct two-fold interaction with mucoprotein, and fall into one of two distinct sub-groups—the NDV sub-group or influenza sub-group.

(2) Viruses having a structure identical with the NDV group, but distinguishable in that the relation with mucoprotein is limited to attachment, that is, they do not elute, as they do not possess neuraminidase. These are some of the bovine parainfluenza 3's.

(3) The measles-rinderpest-distemper group, the members of which are also morphologically identical with the NDV sub-group, but have no interaction at all with mucoprotein.

(4) A number of viruses, which are compound helical RNA viruses, but are neither myxophilous nor structurally identical with either sub-group of myxovirus. Examples are rabies, vesicular stomatitis, and avian erythromyeloblastosis virus.

Table 1

	Compound helical RNA	Form like NDV	Neuraminidase-sensitive receptors on erythrocytes	Neuraminidase
Newcastle disease	+	+	+	+
Some bovine parainfluenza 3's	+	+	+	−
Measles	+	+	−	−
Vesicular stomatitis	+	−	−	−

Part of this information is summarized in Table 1, and it can be seen that instead of the aforementioned viruses falling into a number of distinct groups, they actually form a series without clearly demarcated boundaries. Since this is so, and if the term myxovirus is to be retained, it becomes necessary to decide to which viruses it is still applicable. If the word is used in its strictly limited sense, that is, for those viruses which are myxophilous, only the NDV and influenza sub-groups, which have the double interaction with mucoprotein, merit the term. However, this would mean that a large group of viruses all having identical morphology would be separated into different terminological categories—those which are myxophilous, such as NDV and mumps, and those which are not, that is, measles, rinderpest and distemper. In our opinion, this division would be artificial and we would like to put forward the following proposals (see Table 2):

Table 2. COMPOUND (ENVELOPED) RNA VIRUSES WITH HELICAL SYMMETRY

	Myxoviruses and pseudomyxoviruses	
	True myxoviruses	Pseudomyxoviruses
NDV sub-group	NDV Mumps Parainfluenza 1,2,3,4	Measles Rinderpest Distemper Some bovine parainfluenza 3's
Influenza sub-group	Influenza *A* (including human and animal influenza *A*'s) Influenza B Influenza C	? Bittner (ref. 11)
Other compound helical RNA viruses		
	Rabies Vesicular stomatitis Cocal Haemorrhagic disease of trout (ref. 12) Avian erythromyeloblastosis	

(1) All viruses having ribonucleoprotein in the form of a helix, and this helix enclosed in a partially cell-derived membrane (envelope), shall be included within the broad heading of the compound helical RNA group.

(2) This overall group would then be divided into two major categories. These would be (*a*) the myxoviruses and any virus having morphological identity with either of the two myxovirus sub-groups, and (*b*) the remainder of those included in (1).

(3) The first of the divisions propounded in (2), that is, the true myxoviruses and those morphologically identical with them, would be further sub-divided into those exhibiting, and those not exhibiting, myxophily. For the latter group we suggest the name pseudomyxovirus. There are, therefore, four categories:

(*a*) The influenza sub-group of myxoviruses.

(*b*) Pseudomyxoviruses, which have influenza morphology.

(*c*) The NDV sub-group of myxoviruses.

(*d*) Pseudomyxoviruses, which have NDV morphology.

The viruses contained within the division (2) (*b*) should, we suggest, be given merely the broad title of compound helical RNA viruses until further information becomes available.

We do not expect that the terminology we have suggested here will be generally adopted, but if workers in this field are more aware of the true implications of the word myxovirus and of the inter-relationships that exist between the myxoviruses and the broader compound helical RNA group, then this paper will have subtracted from, rather than contributed to, the present confusion about myxoviruses.

One of us (A. P. W.) is in receipt of a grant from the Medical Research Council.

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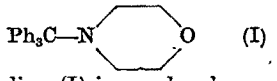
A NEW MOLLUSCICIDE, *N*-TRITYLMORPHOLINE

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BILHARZIASIS probably affects about 200 million people in the tropical and sub-tropical countries of the world, and in some areas is a very considerable public health problem. As a result of the introduction of new irrigation schemes the incidence of the disease is increasing.

The World Health Organization, in collaboration with the governments of the worst-affected countries, is now directing a major effort to break the transmission cycle of the parasite between man and the aquatic snails which act as intermediate hosts. If this is to meet with success comparable with that already achieved in the control of malaria, simple and effective means of breaking the transmission cycle must be developed. It has been shown[1,2] that it is possible to reduce the incidence of bilharziasis by controlling the intermediate host snails and it is generally considered that this method offers the greatest chance of immediate success.

This article reports the results of extensive field and laboratory trials with a new molluscicide, *N*-tritylmorpholine, which is not only highly effective against aquatic snails but also lends itself to simple methods of application.



(I)

N-tritylmorpholine (I) is a colourless, crystalline solid, m.p. 174°–6° C, which is stable to heat, sunlight and alkalis, but is hydrolysed under mildly acidic conditions to triphenylcarbinol which is not molluscicidal. It is almost insoluble in water (< 0·1 p.p.m.) but soluble in many organic solvents. It is conveniently formulated in tetrachloroethylene as an emulsifiable concentrate which gives satisfactory physical and molluscicidal performance and which is easy to handle in the field.

The concentration-mortality data which are given in Table 1 illustrate the activity of *N*-tritylmorpholine against some of the intermediate host snails of *Schistosoma mansoni* and *Schistosoma haematobium*, as well as of *Fasciola gigantica*. The median lethal concentrations (LC_{50}) for 1 h exposure periods are in the range of 0·1–0·5 p.p.m. ($3–15 \times 10^{-7}$ molar) while for 24-h exposures they are in the range 0·01–0·05 p.p.m. ($3–15 \times 10^{-8}$ molar). Similar molluscicidal activity has been observed in a number of field trials in several countries. For example, in Egypt no surviving *Bulinus truncatus* was found in a canal after the water had been treated continuously for 6 h to give a concentration of 0·25 p.p.m. of molluscicide. In Tanzania, *Biomphalaria pfeifferi* was killed in canals which had been treated for 6 h to give 0·1 p.p.m. of molluscicide, or for 15 min at a rate of 4 p.p.m. In Venezuela, *Australorbis glabratus* was killed in a canal after the water had been treated for 6 h to give a concentration of 0·25 p.p.m. of molluscicide.

However, despite the complete kill of snails in these trials, repopulation was relatively rapid because *N*-tritylmorpholine is not ovicidal and snail eggs were unaffected by the treatments. To control the whole of the snail population with this chemical we have developed a

Table 1. CONCENTRATIONS OF *N*-TRITYLMORPHOLINE, FOR VARIOUS PERIODS OF EXPOSURE, REQUIRED FOR 50 PER CENT AND 90 PER CENT KILLS OF SEVERAL SPECIES OF SNAIL (TEMPERATURE 21°–22° C AND A RECOVERY PERIOD OF 48 H IN FRESH WATER)

Snail species	Size and origin	Concentration of molluscicide (p.p.m.) 1 h LC_{50}	1 h LC_{90}	4 h LC_{50}	4 h LC_{90}	5 h LC_{50}	5 h LC_{90}	24 h LC_{50}	24 h LC_{90}
Australorbis glabatrus	12 mm diam. Laboratory strain	0·20	0·34	0·10	0·17			0·025	0·042
Bulinus truncatus	5 mm long Laboratory strain	0·45	1·0	0·11	0·25			0·053	0 10
Bulinus globosus	7·0–12·5 mm long. Wild	0 22	0·60			0·11	0·28	0·050	0 085
Biomphalaria pfeifferi	5·0–12·0 mm diam. Wild	0·13	0·25			0·034	0·062	0·014	0·023
Lymnaea natalensis	9·0–14·0 mm long. Wild					0 033	0 070		

simple method of treatment which shows great promise for use in irrigation systems. A simple, automatic device is used to apply the molluscicide continuously for several weeks to the intake of an irrigation system.

In initial small-scale trials at the Tanganyika Planting Co., Sugar Estate, near Moshi, Tanzania, no surviving *Biomphalaria pfeifferi* was found after canals had been continuously treated for 16 days to give a concentration of 0·02 p.p.m. of molluscicide in the water. In a later large-scale trial at the same estate, the treatment covered an area of 2,500 hectares which included five reservoirs, varying in capacity from 30,000 to 114,000 m^3, and an extensive system (about 200 km) of canals. The molluscicide was applied for 30 days at the main intake only of the irrigation system to give a concentration of 0·025 p.p.m. Chemical analysis of water samples taken throughout the system indicated that the molluscicide had penetrated most parts of the area within 5 days without a significant reduction in concentration. Snail mortality was assessed by sampling the natural population as well as by using caged snails. During the week before treatment 3,173 *Biomphalaria pfeifferi* were found in 426 samples. During the first week of treatment sixty-five snails were found in a similar series of samples, all in one of the reservoirs. In the following week only two snails were found, and in subsequent sampling none was found in the whole area. The method was thus efficient and eliminated many problems of water management. Furthermore, there was no interference with the irrigation of crops, and the labour force, which for conventional treatments with molluscicide would have involved about eighty people, including skilled staff, working full time for a week, was reduced to two unskilled people.

In another trial of the same technique in Rhodesia, the molluscicide was continuously introduced into a single concrete-lined irrigation canal, 22·5 km long, for 21 days to give a concentration of 0·02 p.p.m. Chemical analysis of water samples indicated that the molluscicide travelled the complete length of the canal without a significant reduction in concentration. Prior to the treatment the canal was populated by *Biomphalaria pfeifferi*, *Lymnaea natalensis* and large numbers of *Bulinus tropicus*. No living snails were found when the canal was re-examined 30 days after treatment, with the exception of a few *Bulinus tropicus*. In subsequent trials in Rhodesia and also in Egypt 0·04 p.p.m. of *N*-tritylmorpholine has proved to be fully effective against *Bulinus* spp.

An entirely different applicational problem is involved in the treatment of static water systems. *N*-tritylmorpholine, applied to give a concentration of 0·2–0·3 p.p.m., has been successfully used in six storage dams. These ranged in capacity from 500–10,000 m^3 and were typical of those which comprise a large proportion of the snail habitat in Rhodesia. In each case the snail population was eliminated or drastically reduced. A recent examination, 12 months after the initial treatment, has shown little evidence of repopulation.

Natural water systems frequently contain many intermittent pools, and drip-feed application in such places is usually impracticable. More effective treatment is generally possible by spraying the molluscicide from the banks. In Rhodesia a small branched water course, approximately 20 km long, was sprayed to achieve a final concentration of 0·5 p.p.m. of molluscicide in the water. The treatment was made during a period of limited breeding, and although a few surviving snails were found after 10 days none could be found one month after treatment.

A valuable feature of *N*-tritylmorpholine is its relatively high degree of selectivity. No effects on plants have been observed during any of the trials or in glasshouse tests. In one of the dams in Rhodesia, samples of the microfauna and microflora were taken before and after treatment with molluscicide. The results which are shown in Table 2 indicate that they were not significantly affected. The effect on fish populations in the trials described has been variable. Although *Barbus* and *Tilapia* spp. were killed in canals treated for 6 h to give 0·1 p.p.m. of molluscicide they survived continuous low-dosage treatments, for example, 0·025 p.p.m. for 30 days, which were fully effective against snails. It is therefore probable that an exploitable degree of selectivity between snails and fish exists and a field evaluation of this is in progress.

Table 2. ANALYSIS OF FAUNA SAMPLES

Fauna	Before treatment	After treatment
Turbellaria	10	6
Nematoda	43	19
Rotifera	3	78
Oligochaeta	7	6
Cladocera	145	103
Ostracoda	12	3
Copepoda	2,251	1,367
Ephemeroptera	302	206
Chironomidae	54	27
Acari	22	3

Nine composite samples each of three sweeps with a 12 cm diam. net were taken immediately before, and 1 week after, treatment with 0·2 p.p.m. of *N*-tritylmorpholine.

The acute oral toxicity of the technical material is partly dependent on the formulation. Concentrates in chlorinated solvents have an LD_{50} value in the range of 100–200 mg of active material per kg. However, when the compound is administered as a wettable powder or as a solution in dimethylsulphoxide the LD_{50} value is in the range of 1,200–2,000 mg/kg. The percutaneous LD_{50} to rats is greater than 1,000 mg/kg for all types of formulations tested. Rats were found to survive exposure for 90 days to 10,000 p.p.m. of *N*-tritylmorpholine in their diet. Histological changes such as bile duct proliferation were evident in the livers of rats receiving 3,000 p.p.m. or more, but no histopathological lesions have been seen in rats receiving 1,000 p.p.m. or less over a 90-day period[3]. It is therefore considered that the compound is safe for those using it and for those who may be exposed to water treated with it.

In view of these promising results, further trials are now being carried out on a larger scale to assess the value of this chemical as a weapon against bilharziasis.

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LETTERS TO THE EDITOR

ENGINEERING

Wind Forces and the Proximity of Cooling Towers to Each Other

RECENTLY a study has been completed in this Department of the effect of the geometry and spacing of particles on the forces experienced by them when fluid passes through the array. The results provide an interesting commentary on the recent destruction of three cooling towers at the Central Electricity Generating Board's Ferrybridge Power Station. The towers were destroyed in a severe gale on November 1, 1965.

In the experiments, spherical particles were supported in planes normal to the principal axis of flow, as shown in Fig. 1.

The spheres, 0·25 in. in diameter, were drilled centrally, threaded on to 0·01 in. diameter nylon line and fixed in position. The line was secured at either end to identical sheets of 'precision-perforated' plate, with the perforations aligned to give correct positioning of the spheres. The array of spheres, twenty planes deep with 225 spheres in each plane, was then mounted in a square, close-fitting 'Perspex' flow channel, liquid was passed through the array, and the drag forces on the spheres were obtained from measurements of pressure drop across the array.

The Reynolds number of flow was varied to span the range $0{\cdot}1–2 \times 10^3$. The spacing between centres of spheres in the plane normal to the main axis of flow, shown as x in Fig. 1, was varied from one to three diameters, and y, the spacing between planes, was varied from one to four diameters.

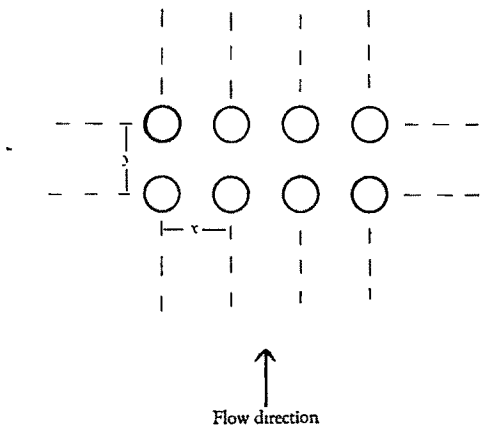


Fig. 1. Experimental layout

It was found that the drag forces on the individual spheres increased when y was increased, and decreased when x was increased. The forces measured were as much as 100 times as great as the forces on an isolated particle at the same Reynolds number of flow.

Fig. 2 shows the effect of a change in x at a constant interplanar separation of one diameter. The results are presented as the dependence of the drag coefficient (the ratio of force on unit projected area to twice the kinetic energy of the fluid) on Reynolds number. The relationship for x = one diameter is shown as line 1, and the coefficients for x = one and a half diameters, x = two diameters and the isolated single particle are shown as lines 2, 3 and 4. For Reynolds numbers greater than 100, the effect of bringing isolated particles into close proximity is to increase drag-forces by a factor of the order of 100.

The drag forces are less sensitive to variation in the interplanar distance y. The results for changing y when x remains constant at one diameter are shown in Fig. 3; for y = one diameter, the relationship is line 1, y = one and a half diameters—line 2, y = two diameters—line 3, y = four diameters—line 5, and the isolated particle—line 4. The individual drag coefficients are increased several-fold when the interplanar separation is increased.

Fig. 4 was published in *The Yorkshire Evening Post* on November 1, 1965 (reprinted by kind permission). The cooling towers were destroyed by winds of 75–90 m.p.h., the winds passing between the towers still standing and destroying the second row. Fig. 4 clearly shows that the tower separation between centres was slightly greater than one diameter—a position that suggests drag forces much higher than those experienced by an isolated tower at similar wind velocities.

The Reynolds number characterizing the interaction between the towers and winds of such high velocities would be in excess of 10^7, and as information on flow through groups of solids at these Reynolds numbers is very sparse indeed, we cannot be sure that the interaction between fluid and bodies will be similar to that observed at lower Reynolds numbers. However, the general reasons for the increase of drag coefficient due to proximity of

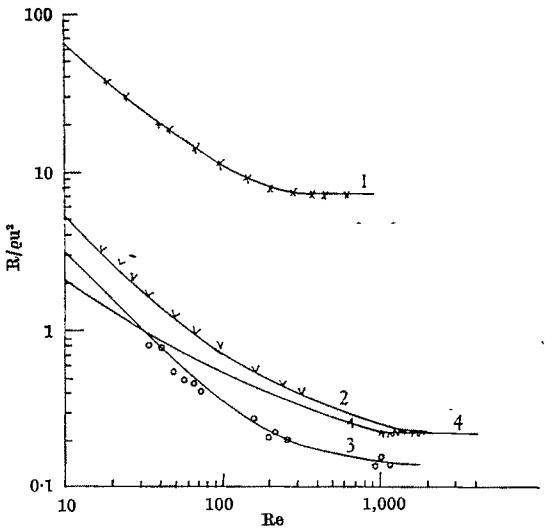


Fig. 2. (1) x = 0 25 in., y = 0·25 in.; (2) x = 0 375 in., y = 0·25 in.; (3) x = 0·5 in., y = 0·25 in.; (4) single sphere

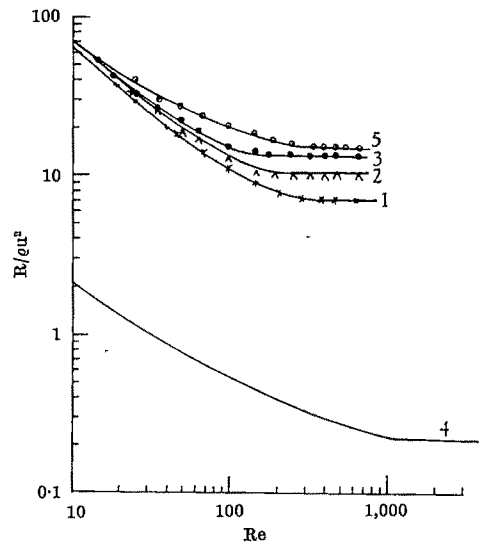


Fig 3. (1) x = 0·25 in., y = 0·25 in.; (2) x = 0·25 in., y = 0 375 in; (3) x = 0 25 in., y = 0 5 in.; (4) single sphere; (5) x = 0 25 in., y = 1 in.

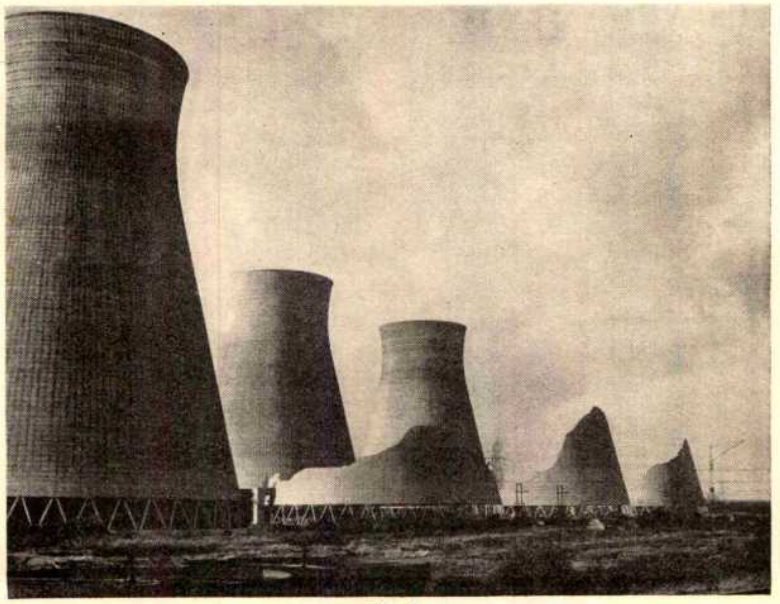


Fig. 4

bodies will be the same even at higher Reynolds numbers. The findings summarized here suggest that in view of uncertainty in wind direction, if the proximity of the cooling towers to neighbours is smaller than 2–2·5 diameters between centres, special provisions for the greater forces due to wind interaction should be made. The particular influence of geometry on drag forces for cooling towers will be somewhat different because of the shape, and because of the higher level of Reynolds numbers, but it is probable that the general nature of the pattern of interaction will be similar.

We thank the Science Research Council for financial support in these investigations.

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PHYSICS

Debris Particles from the Second Chinese Nuclear Bomb Test

ON May 14, 1965, the Chinese exploded their second nuclear bomb (*Hsinhua News Agency*, May 14, 1965). Debris first appeared over Sweden in an upper-air sample on May 24, 1965, which was also the strongest sample we collected from this test. Soon after, debris particles were also found in ground air (some of them stronger and larger than the strongest upper-air-borne ones).

The debris was examined in normal routine investigations in which autoradiographs were prepared of the air filters. Investigations were also undertaken of β-disintegration and γ-spectrography, using bulk samples as well as individual particles and particles grouped according to their class of radioactivity (in order to investigate fractionation phenomena)[1]. Finally, particles of all the classes of radioactivity available were prepared and treated according to reversal-autoradiographic methods in order that they could be individually measured and investigated microscopically[2–5].

Unlike the first Chinese test[2], the colour of the particles was remarkably uniform. They varied over a comparably narrow range from colourless to yellow to reddish yellow; sometimes they had a greyish, 'dirty' appearance, but they were always transparent. No optical activity was observed. The particles were for the main spherical (although seldom perfectly so); however, elongated ones were frequently observed, and these were sometimes more elongated than had been observed in earlier (mainly U.S.S.R.) tests (see refs. 2, 5 and 6).

The activity versus particle size of many samples at various ages between 70 and 110 days was investigated, and then recalculated to 100 days age with the aid of measured β-disintegration of some particles[1].

As can be seen in Fig. 1, the activity is strongly related to particle size. It should be noticed that, for the smaller particles, the activity increases faster than the volume. This phenomenon was observed earlier in a sample attributed to one of the first shots in the test series at Novaya Zemlya in the autumn of 1961 (ref. 5). (Even then the colour range of the particles was remarkably narrow, although all particles were red or reddish.) For purposes of comparison, the U.S.S.R. sample has been inserted schematically into Fig. 1. As can be seen, however, the specific activity of the Chinese particles is lower by a factor ~2. The average specific activity of the particles from the second Chinese test can be compared with similar investigations from other tests.

Specific activities of the U.S.S.R. tests from 1958 and 1961 range from about the same value as the second Chinese test, up to more than ten times greater[5]. A value obtained from a test carried out by the United States at Christmas Island in 1961 is about ten times higher[5], so also are those from the U.S.S.R. tests carried out in autumn 1962 (ref. 7). It is probable that these latter tests were air explosions[8].

The first Chinese test, which without doubt was a near-ground shot with considerable incorporation of ground material in the fire-ball, had specific activities almost a hundred times lower[2]. This value indicates an incorporation of ground material in the fire-ball in the order of magnitude of 1,000 tons which is in agreement with the statement that the test was a 20-kton explosion about 30–50 m above ground-level[8].

The high values from samples resulting from tests carried out by the U.S.S.R. and the United States, on the

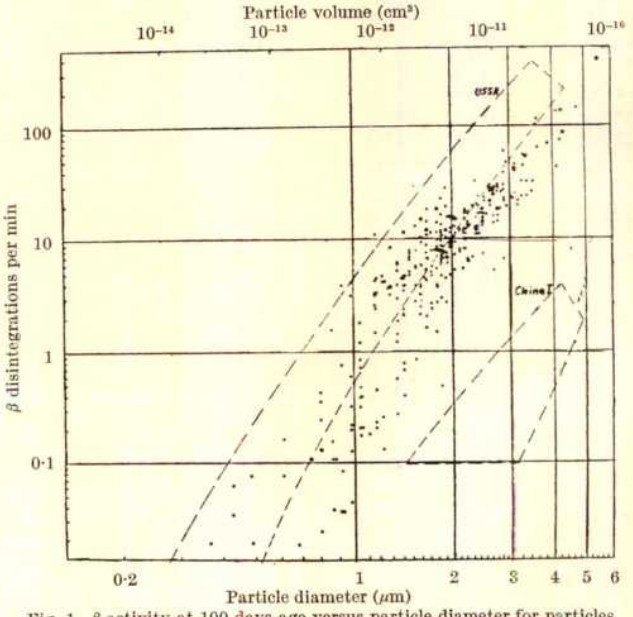


Fig. 1. β-activity at 100 days age versus particle diameter for particles from the second Chinese nuclear-weapon test. For purposes of comparison β-activities versus size for an U.S.S.R. sample (see text) and the first Chinese nuclear-weapon test (ref. 2) are shown schematically. The larger circles indicate particles which were measured in a β-counter. The others were indirectly measured with the help of the spot sizes on the autoradiograms (refs. 4 and 5)

other hand, seem to be remarkably low if it is supposed that only a minimum quantity of non-active material accompanied the fissile material in these explosions[9].

The values from the second Chinese test, however, indicate an intermediate inactive component of 10–100 tons. This finding would support the suggestion[8] that the bomb was a 20-kton device, exploded on a 100-m tower.

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Dielectric Properties of Freshly Cleaved Muscovite Mica

THE unique property of mica to split easily into thin lamina has long been exploited in the manufacture of mica condensers. Quantitative investigations of the electrification of mica crystal and the force necessary to split the material were undertaken by Metsik[1]. Ruthberg *et al.*[2] investigated the a.c. properties of the adsorbed film on the surface of mica cleaved in moist air. This communication reports the findings of some investigations of the dielectric properties of freshly cleaved muscovite mica.

Dissipation factors were determined by means of a Boonton *Q*-meter with the aid of a specially designed parallel plate sample-holder consisting of a micrometer (driven earthed) and a 'Teflon' insulated plate.

In the interblock layer of muscovite crystals molecules of partly bound water are present through which contact between individual blocks is established at a number of points[3]. On splitting the crystals along their cleavage planes, separation of heterogeneous particles occurs and the surfaces become electrified. Due to the symmetrical structure of mica crystal with respect to the interblock layer and the non-uniform distribution of water molecules in that layer, electrification of the splitting surfaces is in the form of mosaic with charges of alternating sign.

The freshly split mica specimen may thus be regarded as a composite dielectric consisting of the mica specimen and a charged layer of partly bound water molecules. The dielectric properties of such a split mica crystal would be expected to depend on the properties not only of the mica but also of the surface layer. Assuming that the thickness of the surface layer is the same as in the various cleavage layers of mica crystals, the dissipation factor of the freshly split mica seems to depend on the thickness of the mica. Because the surface layer is a layer of charged water molecules, both its conductivity and dielectric constant are much higher than those of mica. An increase in the dissipation factor with decrease of mica thickness (Fig. 1) is thus to be expected, because the total a.c. resistance of the specimen also decreases. The dipolar nature of the charged layer of water molecules on the surface of the freshly split mica would be expected to be responsible for the nature of the frequency dissipation factor curve (Fig. 2).

In Figs. 1 and 2 it can be seen that the charged layer on the surface of the freshly split mica has a greater effect on the dissipation factor for green than for ruby muscovite. It appears that the green muscovite mica contains more partly bound water between any two lamina.

Of all the possible mechanisms for the dispersal of the charge, leakage along the surface seems to be the most probable. Heating above 100° C evaporates the surface film of moisture, leaving the surface free from any layer.

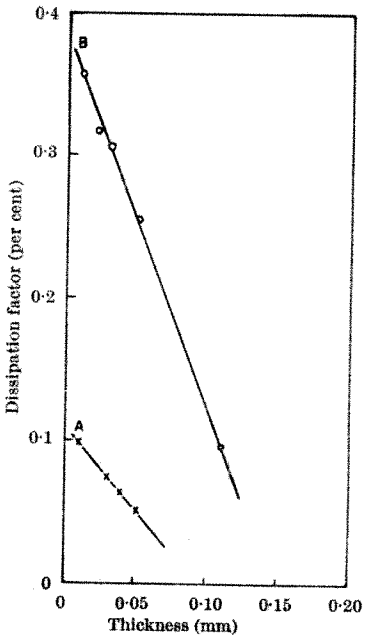


Fig. 1. Effect of thickness of mica on the dissipation factor of freshly split muscovite mica. *A*, Ruby muscovite; *B*, green muscovite

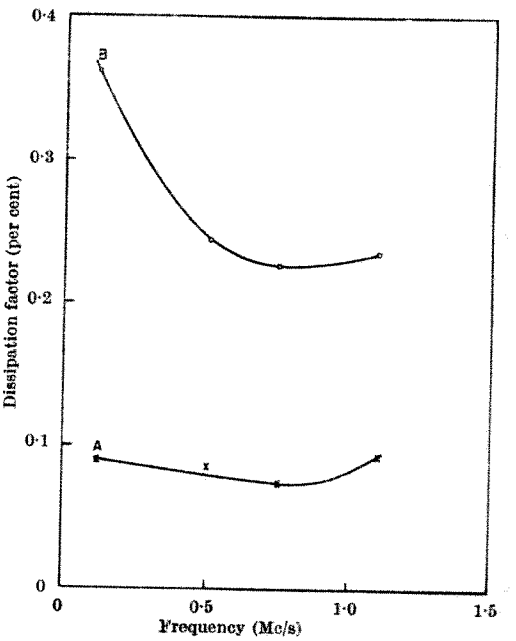


Fig. 2. Effect of frequency on the dissipation factor of freshly split muscovite mica. *A*, Ruby muscovite; *B*, green muscovite

Our findings concerning the improvement of dissipation factor with time and heating corroborated those of Ruthberg and Frenkel[2] and of Mahanti and Mandal[4] (Table 1).

To examine the effect of the charge on the properties of the freshly split mica, one specimen of mica was split

Table 1

(*a*) EFFECT OF TIME ON DISSIPATION FACTOR OF FRESHLY SPLIT MICA AT 500 KC/S

Thickness (mm)	Dissipation factor (per cent) Immediately after splitting	24 h after splitting
0·065	0·158	0·114
0·045	0·186	0·147
0·035	0·215	0·176
0·025	0·239	0·178

(*b*) EFFECT OF DRYING THE SPLIT SAMPLES AT 130° C FOR 2 H

Thickness (mm)	Dissipation factor (per cent) at 500 kc/s Before drying	After drying
0·325	0·148	—
0·080	0·238	0·151
0·060	0·140	0·115
0·010	0·228	0·171

Table 2. EFFECT OF FRESHLY SPLIT SURFACES TOUCHING EACH OTHER

Dissipation factor (per cent) at 500 kc/s							
Ruby Muscovite				Green Muscovite			
Unsplit sample	Sample *A*	Sample *B*	Samples *A* and *B*, freshly split surfaces touching each other	Unsplit sample	Sample *C*	Sample *D*	Samples *C* and *D*, freshly split surfaces touching each other
0 017	0 052	0 034	0 045	0 137	0 215	0 186	0 131
0 052	0 098	0 098	0·069	0 079	0 160	0 111	0 118
0 034	0 074	0 052	0 065	0 114	0 158	0 239	0 114

into two pieces, and the dissipation factor of each piece was determined. Thereafter the two pieces were recombined with the fresh surfaces facing each other and the dissipation factor was redetermined. It is observed from Table 2 that after recombination the dissipation factor of ruby muscovite changed from the initial value of the unsplit sample, but higher than the value of one of the split specimens, whereas the green muscovite almost regained the original value of the unsplit specimen. On recombination, it would be expected that the charges on the two faces would neutralize each other and that the properties exhibited could be those of an unsplit mica. (Consideration would have to be taken of the fact that during the process of recombining some air bubbles might stick between the layers, thereby increasing the dissipation factor[5]. Furthermore, leakage of charge as well as evaporation of surface moisture during the process of recombining might also affect the result.)

A few samples were split in alcohol to a thickness of about 0·025 mm. The dissipation factor at 500 kc/s was found to be much higher (0·270 per cent) than for samples split in atmosphere (0·073 per cent). During splitting in alcohol it is possible that some of the OH ions adhere to the charged layer of the freshly split mica, thereby increasing the dissipation factor.

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GEOLOGY

Age and Polarity of the Turkana Lavas, North-west Kenya

THE extension of the Trap Series of Ethiopia into north-west Kenya has previously been suggested on stratigraphical and petrological grounds[1]. This area has recently been mapped on the basis of a reconnaissance[2], and the following succession suggested:

Rhyolites and andesites	*Tvr, Tvd*
Olivine basalts	Tvb_3
Phonolites and nephelinites	*Tvp*
Augite and analcime basalts	Tvb_1
Turkana grits	*Tm*

The age of the lavas has hitherto been based on a sparse mammalian fauna found in grits intercalated in basalts (Tvb_1 ?) at Lothidok, 25 km north-east of Lodwar. Initially thought Burdigalian[3], recent work[4] has suggested that it is younger (Up. Miocene) in age. Some fossil wood found in the rhyolites 60 km north of Lokitaung (*Dryoxylon* sp.) hints at a Mio-Pliocene age.

Four lavas have been dated (R. L. G.) at the University of Cambridge; Table 1 gives the results obtained on whole rock samples.

Field mapping showed that the basalts and rhyolites interdigitated in two localities, but neither were observed to overlie phonolites or nephelinites. The *Tvp* group is thus younger than both basalts (but could be older than the rhyolites), and comparable in age with the Lothidok grits and the Kapitian phonolites. The basalts (Tvb_1 ?) at

Table 1

Sites	K_2O (per cent)	Vol. of $^{40}Ar/g$ (mm^3)	Atmos. contam. (per cent)	Age (m. yr.)	Polarity
260/1 (Tvb_1)	1 31	$1\,406 \times 10^{-3}$ $1{\cdot}375 \times 10^{-3}$	38·9 36·7	$32{\cdot}2 \pm 0\,5$ $31{\cdot}5 \pm 0{\cdot}7$	*R*
264/5 (Tvb_1)	0 61	$4\,21\square \times 10^{-4}$ $4\,683 \times 10^{-4}$	48·2 46·4	$20{\cdot}7 \pm 0{\cdot}4$ $23{\cdot}0 \pm 0{\cdot}7$	*R*
293/4 (Tvb_2)	0·90	$7{\cdot}04 \times 10^{-4}$ $6\,99 \times 10^{-4}$	78 0 51 7	$23{\cdot}5 \pm 1{\cdot}1$ $23\,3 \pm 0\,2$	*R*
243/50 (*Tvp*)	3·84	$1\,90 \times 10^{-3}$ $1{\cdot}56 \times 10^{-3}$	54·3 66 5	$14{\cdot}9 \pm 0{\cdot}5$ $12{\cdot}5 \pm 0\,3$	*N*

Lothidok are unconformably overlain by basalts which are considered, on petrological grounds, to be equivalent to the Tvb_2 basalts in the north. However, palaeontological evidence indicates that the Lothidok basalts are much younger, and this discrepancy remains to be resolved.

The disagreement between samples 260/61 and the apparently underlying sample 264/65 might be due to loss of argon in sample 264/65, which shows a high chlorite content, or to local repetition of the succession by faulting: a similar wide scatter has been observed in five dates[6] (30–69 m. yr.) for the Abbai basalts of Ethiopia. The age and character of the Turkana basalts are comparable with those of the (Upper) Magdala Group of the Ethiopian trap series (25 and 33 m.yr.).

The lava succession is best seen in the Lokitaung gorge, where 5,000 ft. of lavas, pyroclastics and sediments are exposed. The complex faulting in the vicinity of Lokitaung has also complicated the sequence of magnetic polarities previously described in the Lokitaung section[6]. The samples of the dated lavas appear to be magnetically stable. The results of thermal and alternating-field cleaning were found to agree closely (Fig. 1), and the shapes of the alternating-field demagnetization curves of the saturation remanence and of the natural remanence were similar (Fig. 2). High-field heating showed only single curie points—a value of 450°–500° C for the basalts, and 150°–300° C for the phonolites and nephelinites (*Tvp*). The last-mentioned, on heating, developed a new, strongly magnetic component with a curie point of about 500°–550° C. Preliminary examinations of the opaque minerals showed a low state of oxidation, with some maghemitization.

The polarities of the sites radiometrically dated are given in Table 1. A further site, 262/3, probably in the same flow as 260/1, had a polarity which became reversed after cleaning by either thermal or alternating-field demagnetization.

The existence of both polarities above the Lokitaung fault has been confirmed by 150 block measurements made in the field (1965) using an astatic magnetometer. No reversed polarities were observed below the fault. The

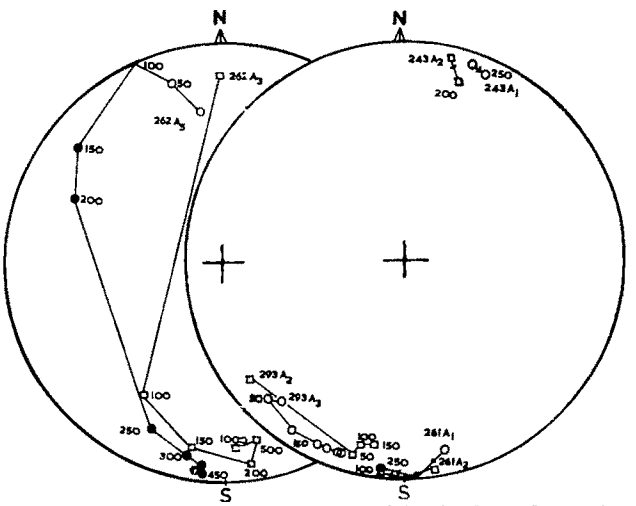


Fig. 1. Polar projection of typical changes of direction during demagnetization. ●, Dips down; ○, dips up: thermal demagnetization—numbers indicate temperatures in °C. ■, Dips down; □, dips up: alternating field demagnetization—numbers indicate peak field in oersteds

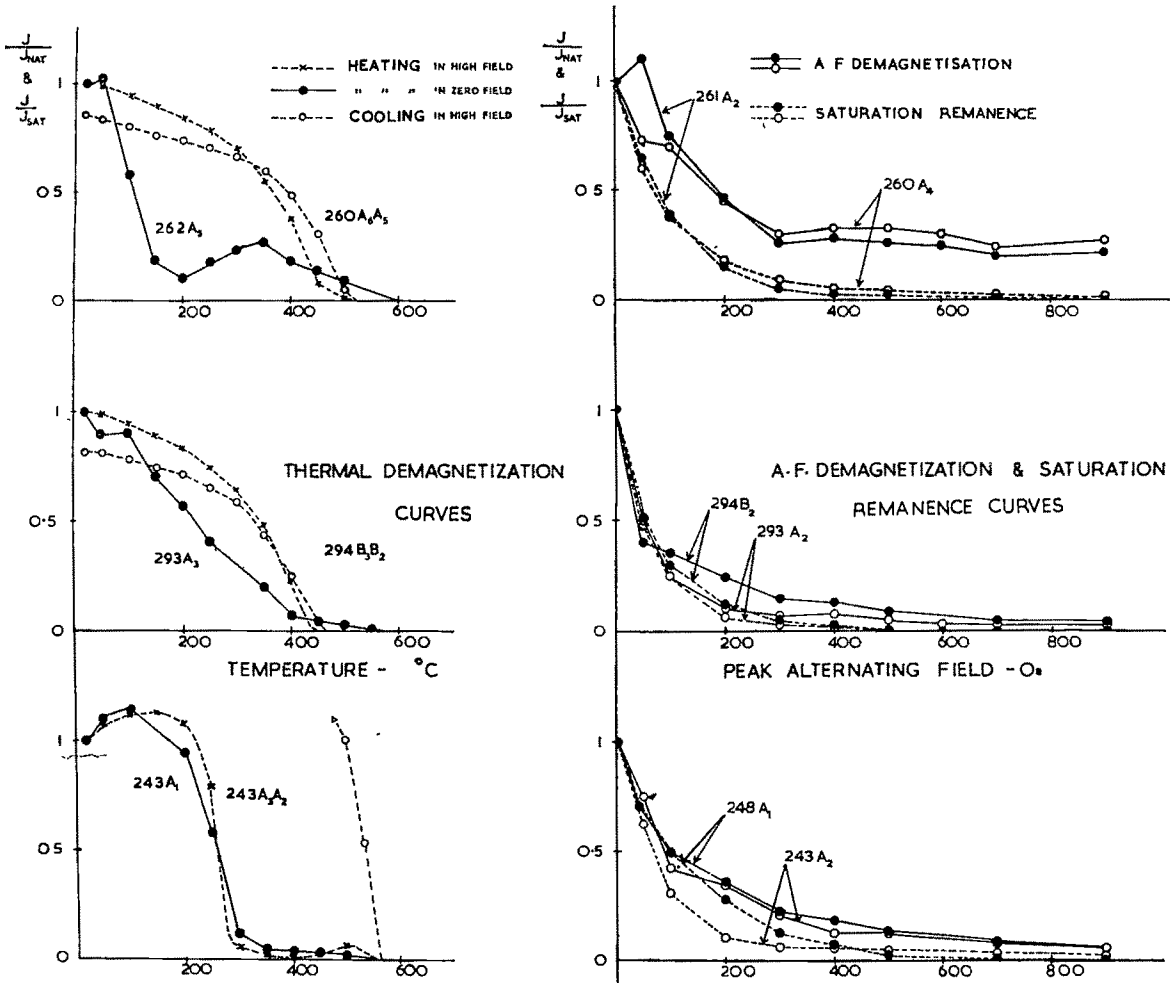


Fig. 2 Behaviour typical of dated sites during thermal and alternating-field demagnetization of natural remanent magnetism, alternating-field demagnetization of saturation remanence and high-field heating and cooling curves

reversed polarities occur in many rock types, an agglomerate horizon having a consistently reversed polarity, whereas highly mineralized fault breccias are magnetized normally.

Self-reversal and tectonic repetition are probably both present, but the foregoing observations support the previous suggestion of a field reversal in the Upper Oligocene preceded by a period of normal polarity.

We thank Prof. A. N. Hunter and Prof. P. M. S. Blackett for initiating the work, which has been carried out under a grant from the Rockefeller Foundation.

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Jebel Marra Volcano, Sudan

HAMMERTON'S suggestion[1] that the volcano of Jebel Marra is dormant rather than extinct is not only of considerable vulcanological interest, but also of considerable practical importance to the future development of the area at present being investigated by the United Nations.

These investigations, started for the Sudan government by Hunting Technical Services, Limited, in the winter of 1957–58, and subsequently participated in by an ecologist and pedologists from the company for the United Nations Special Fund Jebel Marra Project, have disclosed new information regarding the formation of the volcano.

The 1957–58 geological investigations led to the recognition of three phases of volcanic activity at Jebel Marra. The first phase resulted in the accumulation of a great thickness of basalt and trachyte lavas and tuffs which form the main mass of the volcano. A period of relative quiescence ensued during which the newly formed mountain was subjected to intense sub-aerial erosion resulting in the formation of extensive piedmont alluvial and colluvial deposits. Relatively small quantities of basaltic lavas, intercalated in, or overlying, the alluvial deposits, represent the second volcanic phase. The third and final phase was entirely explosive and resulted in the formation of extensive deposits of tuff.

No new evidence regarding the age of volcanic activity was obtained in 1957–58. Since then White[2] has made observations which suggest that the ash eruptions may have continued into recent, possibly early historical, times.

Leaf impressions were also discovered in an exposure in the piedmont during the course of the second phase studies and are still being examined. Sandford[3] suggested previously that the crater was of recent formation.

Clearly the volcano proper has been inactive for many centuries, and hot springs and fumaroles could be regarded, therefore, as the final manifestations of the earlier activity. The interesting point is, however, that fumaroles have not been reported previously from Jebel Marra although the mountain has been traversed a number of times by different observers[4,5]. During the traverse made by the Hunting team in 1957–58, warm springs were recorded at Jebel Idwa, a subsidiary cone 2 km north of the main crater[6]. Water temperatures were not recorded, but the description 'warm' suggests that they were considerably lower than the values recorded by Hammerton. No fumaroles were observed.

Lynes has commented on the bad 'sulphurous' smells within the crater. These can be interpreted as arising from the decay of organic matter in the saline lake. No odours were noticed during the 1957–58 studies and one of us (G.E.W.) also recorded the lack of sulphurous smells during a visit in February 1964. However, by mid-1964, other team members were reporting strong odours of sulphur dioxide.

Thus the possibility that the fumaroles reported by Hammerton have formed during the last few years, and that there has been a rise in the geoisotherms below Jebel Marra, presaging a renewal of volcanic activity, cannot be entirely discounted. Workers in the area should be on the watch for any further signs of increasing activity.

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GEOCHEMISTRY

Behaviour of Nickel during Magmatic Crystallization

THE enrichment of nickel in minerals such as olivine which appear at any early stage during fractional crystallization of magmas is well established[1,2]. This behaviour of nickel is contrary to all commonly accepted "rules" governing the distribution of minor elements during magmatic crystallization[3–6]. The behaviour is also anomalous when considered in relation to the binary system Mg_2SiO_4–Ni_2SiO_4 in which, on account of the higher melting-point of magnesium orthosilicate, early crystals are impoverished and late liquids enriched in nickel olivine[5]. Ringwood attributed the observed nickel enrichment in natural olivines to the preferential replacement of ferrous iron in the olivine structure, but this explanation is thermodynamically improbable. It was suggested recently by Ahrens[7] that the nickel–oxygen bond in olivine is stabilized by crystal-field effects, but no mechanism for the early uptake was given. An earlier interpretation[8] was apparently overlooked.

In order to account for the enrichment it is necessary to postulate that solid–liquid distribution coefficients may be inverted in systems of different chemical composition. Thus, in the binary system Mg_2SiO_4–Ni_2SiO_4 we have:

$$\left(\frac{Ni}{Mg}\right)_{olivine} < \left(\frac{Ni}{Mg}\right)_{liquid}$$

while in a basaltic system we find

$$\left(\frac{Ni}{Mg}\right)_{olivine} > \left(\frac{Ni}{Mg}\right)_{liquid}$$

A significant difference between these two systems is that, in the simple binary system, liquids are dominated by octahedral cation sites while both octahedral and tetrahedral sites are present in a basaltic liquid[8]. The crystal-field stabilization energy of divalent nickel ions in octahedral co-ordination in oxide structures (29·2 kcal) is larger than in tetrahedral co-ordination (8·6 kcal) and the resultant site preference energy (20·6 kcal) is the largest known for M^{2+} transition-metal ions[9,10]. The divalent nickel ion will occupy preferentially octahedral sites in a liquid and will enter tetrahedral sites only if thermal energy is comparable with site-preference energy or if the stability of the anionic framework of the liquid forces such entry. Magnesium may also favour octahedral sites through a comparable lattice energy effect, but the additional electronic stabilization to a transition-metal ion from crystal-field effects cannot be acquired by magnesium. These considerations applied to a 6 : 4 liquid will result in:

$$\Delta H_{fusion,\ Ni} > \Delta H_{fusion,\ Mg}$$

and

$$\Delta S_{fusion,\ Mg} > \Delta S_{fusion,\ Ni}$$

Both terms will lead to a higher melting-point or lower solubility for the nickel relative to the magnesium compound.

Table 1

	MgO	NiO	FeO
Melting-point (°C)	2,802	1,957	1,368
Heat of fusion (kcal/mole)	18·5	12·1	7·5
Entropy of fusion (cal/deg/mole)	5·8	5·4	4·6
Lattice energy (kcal/mole)	939	974	938

Previous attempts to explain the behaviour of nickel during magmatic crystallization illustrate the confusion that has developed in the geological literature over the use of melting-points as criteria of bond strengths[4–7,11]. Melting-point is related to the difference in bond energies and entropies of solid and liquid phases and not to the properties of either phase alone. At the melting-point (T), solid and liquid phases are in equilibrium. Therefore, $\Delta G_{fus} = 0$, and $\Delta H_{fus} = T\Delta S_{fus}$ or $(H_{liq} - H_{sol}) = T\ (S_{liq} - S_{sol})$, where H_{liq} H_{sol} and S_{liq} S_{sol} are the enthalpies and entropies of liquid and solid phases, respectively. Melting-points are determined by the relative values of heat of fusion, and entropy of fusion, high melting-points being favoured by a large ΔH_{fus} and small ΔS_{fus}. Generally, heats of fusion of binary compounds are one or two orders of magnitude smaller than lattice energies, which indicates that bond energies are comparable in the solid and liquid phases. Consider, for example, the oxides magnesium oxide, nickel monoxide and ferrous oxide, thermodynamic data for which are summarized in Table 1[12,13]. The lattice energies indicate that the nickel–oxygen bond energy is larger than magnesium–oxygen and iron–oxygen in the solid phase. There is a correlation between melting-point and heat of fusion; magnesium oxide has the largest heat of fusion and highest melting-point.

Similar correlations exist for most pairs of diadochic elements. Generally, compounds of B-sub-group elements or transition-metals have lower melting-points, smaller heats of fusion, and comparable or higher lattice energies than the A-sub-group compounds with which they are

matched[14]. These data indicate that melting-points cannot be used as criteria of bond energy in the solid state.

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MINERALOGY

A Green Variety of Yoderite

THE spectacular purple magnesium–iron–aluminosilicate, yoderite[1,2], was described from Mautia Hill, Kongwa, Tanganyika (now Tanzania), by McKie in 1959. Since that date, no other occurrence of the mineral has been recorded and unpublished analyses of yoderite from variant associations at Mautia Hill have been found to be not significantly divergent.

During the field season of 1961 a party of geologists engaged on training traverses across Mautia Hill sent a parcel of specimens, including a small specimen of a quartz talc schist containing an emerald-green mineral, to the laboratories of the Geological Survey of Tanganyika, where it was discovered that the optical properties (α, 1·691; β, 1·693; γ, 1·712 (all $\pm 0{\cdot}003$); $2V_y$, 30°; with pleochroic scheme α, green; β, pale yellow; γ, yellow) and X-ray-powder diffraction pattern of the green mineral were closely similar to those of yoderite. The locality on Mautia Hill from which the specimen came was not recorded at the time of finding and subsequent visits to the hill have failed to rediscover it. In due course, single crystal X-ray studies served to confirm the identification of the green mineral as yoderite.

The green mineral has the same space group, its unit-cell dimensions are identical (within the limits of accuracy quoted by McKie[1]), and its diffraction pattern exhibits the same distinctive array of subsidiary (non-Bragg) reflexions as yoderite. The chemical composition of the green yoderite has been determined by Mr. J. H. Scoon as: SiO_2, 36·05; TiO_2, 0·10; Al_2O_3, 43·46; Fe_2O_3, 3·85; FeO, 1·72; MnO, $<0{\cdot}01$; MgO, 12·25; CaO, $<0{\cdot}01$; H_2O+ 3·13 (Σ100·56) corresponding to an average unit-cell content of $(Fe^{II}_{0\cdot16}Ti_{0\cdot01}Mg_{1\cdot99}Fe^{III}_{0\cdot32}Al_{3\cdot51})^{[5,6]}Al^{[5]}_{2\cdot00}(Al_{0\cdot07}Si_{3\cdot93})^{[4]}O_{17\cdot72}(OH)_{2\cdot28}$.

The estimation of Fe^{II} in purple yoderite[1] was never regarded as completely satisfactory, and its titanium and calcium contents have been shown by electron-probe analysis, kindly performed by Dr. J. V. P. Long, to have been significantly over-estimated; a new analysis (shortly to be published elsewhere) leads to an average unit-cell content of $(Ca_{<0\cdot01}Mn^{II}_{0\cdot03}Ti_{0\cdot01}Mg_{1\cdot98}Mn^{III}_{0\cdot06}Fe^{II}_{0\cdot41}Al_{3\cdot55})^{[5,6]}Al^{[5]}_{2\cdot00}(Al_{0\cdot08}Si_{3\cdot92})^{[4]}O_{18\cdot06}(OH)_{1\cdot94}$. Fe^{II} in green yoderite and excess oxygen in purple yoderite have been determined by the method of Ingamells[3] and both values are now thought to be reliable. The new analysis suggests that the colour of purple yoderite is to be attributed to the presence of the chromophore group Mn^{3+}—O—Mn^{2+} rather than Fe^{3+}—O—Fe^{2+} as was suggested earlier[1]; the green colour of manganese-deficient yoderite bears out this conclusion in that in the absence of the manganese chromophore the mineral exhibits much the same colour as a wide range of aluminosilicates of similar ferrous and ferric iron content.

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CRYSTALLOGRAPHY

Crystallographic Data on New Rare Earth–Germanium and Silicon Compounds

DURING an investigation of samarium–germanium compounds of composition near Sm_5Ge_3, X-ray powder diffraction photographs indicated additional lines which could not be indexed on the basis of the hexagonal unit cell established for Sm_5Ge_3 by Gladyshevskii[1]. Investigations of single crystals showed the phase to be orthorhombic and of a new structure-type. An analysis of the crystal structure[2] showed the stoichiometry to be Sm_5Ge_4.

Five other lanthanide metals in combination with both germanium and silicon have now been examined for the occurrence of this phase. The preparations were made by arc-melting the component metals in an argon atmosphere. Lattice constants were derived from oscillation and Weissenberg photographs with an estimated accuracy of about 0·2–0·3 per cent.

The results are as follows: when germanium was used as the combining element, all the rare earths studied exhibited the orthorhombic 5 : 4 phase. The lattice constants, summarized in Table 1, follow a uniform lanthanide contraction so that the axial ratio, $a : b : c$, remains rather constant. It seems likely that most, if not all, of the rare-earth elements will form a germanide of this type.

Table 1. CRYSTALLOGRAPHIC DATA. SPACE GROUP. $Pn2_1a$ (FROM STRUCTURE DETERMINATION ON Sm_5Ge_4). UNIT CELL CONTAINS FOUR FORMULA UNITS

Compound	a (Å)	b (Å)	c (Å)	Volume (Å^3)
Nd_5Ge_4	7·86	15·06	7·93	939
Sm_5Ge_4	7·75	14·94	7·84	908
Gd_5Ge_4	7 69	14·75	7 76	880
Tb_5Ge_4	7·62	14·66	7 72	862
Er_5Ge_4	7·51	14·41	7·59	821
Y_5Ge_4	7·63	14·68	7 68	860
Tb_5Si_4	7·41	14·58	7 69	831
Er_5Si_4	7·27	14·32	7·58	789
Y_5Si_4	7·39	14 52	7·64	819

For the analogous silicon compounds, the situation is more complex. Three of the lanthanide elements, terbium, erbium and yttrium, formed Sm_5Ge_4-type compounds. It is probable that gadolinium also forms a similar compound. However, this preparation did not yield sufficiently large crystals for investigations to be carried out on individual ones. X-ray powder diffraction patterns, using chromium $K\alpha$ radiation and a 57·3-mm radius camera, indicated that gadolinium monosilicide[3] (FeB-type) was the major phase. The remaining lines fit a pattern expected for the orthorhombic 5 : 4 phase. Additional work is necessary to establish the occurrence of Gd_5Si_4 with absolute certainty. The results indicate that the orthorhombic R_5Si_4 compounds will occur most readily for the heavier lanthanides, that is those having smaller atomic radii.

In support of this, the silicide preparation using neodymium yielded crystals with a primitive tetragonal cell in which $a=7{\cdot}87$ Å and $c=14{\cdot}79$ Å. The unit cell volume corresponds with the orthorhombic 5 : 4 series;

however, substantial differences in the reflexion intensities indicate that this material has a different structure.

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Three Different Isomers in the Same Crystal

WE wish to report the discovery of a crystal in which three different isomers occur in the proportion 1 : 1 : 1.

In connexion with the problem of the identity of two different forms of diborinotetramethylbiphosphine[1], $BH_3(CH_3)_2P\text{-}P(CH_3)_2BH_3$, we have examined by X-ray diffraction the lump form having the lower vapour pressure. The crystals were found to be monoclinic, with $a = 6{\cdot}78$ Å, $b = 11{\cdot}64$ Å, $c = 20{\cdot}41$ Å, and $\beta = 95°$. The space group is $P2_1/c$, with six molecules per unit cell, a result which indicates that at least two of the six molecules must have centres of symmetry. It therefore follows that these two molecules are in the *trans* conformation. (This situation is reminiscent of the case of biphenylene, where Waser and Lu[2] found that crystals of the presumed biphenylene synthesized by Lothrop[3] were in space group $P2_1/c$ with six molecules per unit cell, a result which eliminated the non-centrosymmetric structures for the molecule of $C_{12}H_8$ favoured by Baker[4] and Coulson[5], and confirmed that Lothrop had, in fact, synthesized biphenylene.)

The structure was solved by means of a Patterson function calculated with three-dimensional Weissenberg data. The identification of the carbon and boron atoms was then carried out by three different and independent approaches.

(1) Least-squares refinement of the three positional and one thermal parameter per atom was carried out in which the form factor curve for carbon was used for all nine of the crystallographically distinct terminal ligands to phosphorus. The resulting values of the thermal parameters, B, are: (i) on $P(1)$: 6·2, 4·9 and 8·6; (ii) on $P(2)$: 5·3, 5 6 and 9·7; (iii) on $P(3)$ (in the crystallographically centric molecule): 4·1, 6·4 and 8·8. The higher B values for the last member of each of the three groups represent an attempt by the least-squares procedure to lower the form factor, and indicate that these three atoms are boron rather than carbon.

(2) Least-squares refinement was then continued, with the boron form factor curve used for the three atoms identified as such above. The following bond lengths to phosphorus were obtained: (i) to $P(1)$: 1·84, 1·85 and 1·93 Å; (ii) to $P(2)$: 1·87, 1·83 and 1·96 Å; (iii) to $P(3)$: 1·81, 1·82 and 1·97 Å. The shorter bond lengths correspond to *P–C*, the longer to *P–B*, and the three longer bonds involve the same three atoms already identified as boron. (The nine thermal parameters now range from 6·1–6·8 (boron) and 4·9–6·4 (carbon).)

(3) A three-dimensional electron density function, phased with the results of the least-squares calculation, gave the following relative peak heights, in electrons per Å^3: (i) $P(1)$, 23·1, ligands to $P(1)$, 4·62, 4·82 and 3·88; (ii) $P(2)$, 22·2, ligands to $P(2)$, 5·35, 5·65 and 3·66; (iii) $P(3)$, 24·0, ligands to $P(3)$, 5·63, 5·05 and 4·25. The three lowest peak heights correspond to the same three atoms previously identified as boron.

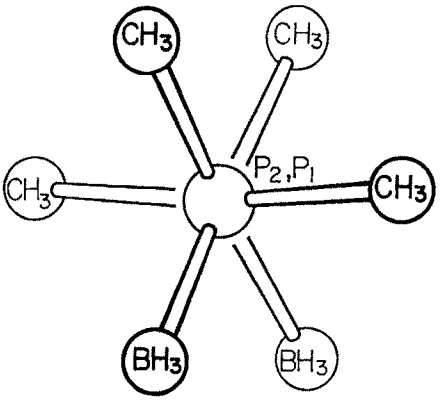


Fig. 1. View of the molecule as projected along the $P(1)$–$P(2)$ bond

There is thus little doubt concerning the identification of the boron and carbon atoms.

The molecule containing $P(3)$ and its centric counterpart is, of course, *trans*, as already pointed out. The molecule in the four-fold general position which contains $P(1)$ and $P(2)$ is not *trans*, but *gauche*, as may be seen in Fig. 1, which shows a projection of that molecule down the $P(1)$–$P(2)$ bond. The operations of the space group require that two of these asymmetric molecules in the unit cell have chirality opposite to the other two. The unit cell thus contains two *trans* molecules, two D-*gauche* molecules, and two L-*gauche* molecules.

The refinement of this structure is being continued. Meanwhile, we feel that the discovery of a crystal which consists of three different isomers is of more general interest.

This work was supported by the National Science Foundation. Fig. 1 was kindly prepared by Mrs. Maryellin Reinecke. We thank Prof. A. B. Burg for providing us with crystals suitable for X-ray analysis.

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CHEMISTRY

A Non-seeded Electrically Augmented Flame System

THE augmentation of flames by the addition of electrical energy has recently received considerable attention. Most of the systems reported use a.c. augmenting power in one form or another and require the electrical conductivity of the flame to be enhanced by the addition of easily ionized seed material. By using d.c. augmentation, we have found it possible to operate a suitable augmented flame with no seed addition once the discharge has been established.

Briefly, the addition of distributed electrical energy to a turbulent flame increases the enthalpy of the flame gases by a form of ohmic heating. Karlovitz[1–3] has proposed that the dispersion of such a discharge depends on a high level of turbulence, such that the turbulence exchange time is less than the time for a discharge to break down into an arc filament. It is also necessary to increase the electrical conductivity of the flame by adding an easily ionized seed

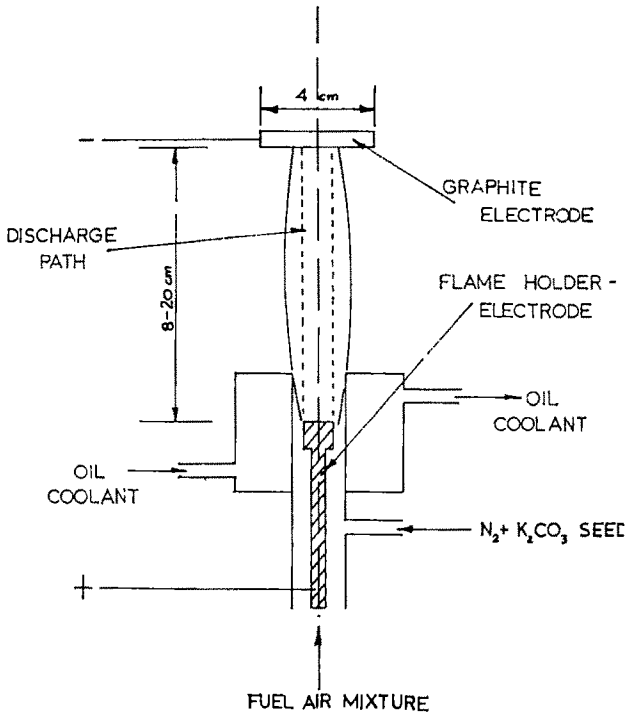


Fig. 1. Burner arrangement

material. Using a.c. augmenting power, Karlovitz found that once the discharge had been initiated, it was possible to reduce the concentration of potassium chloride seed material in the flame gases, but in order to maintain a steady discharge it was essential to continue seeding the flame.

The necessity for continuous seeding is a drawback. Seed materials, usually potassium salts, are expensive and, worse, corrosive. One of the aims of our work has been to reduce the seeding to a minimum and, if possible, to eliminate it altogether.

By using the configuration shown in Fig. 1, and a d.c. rather than an a.c. discharge, steady operation of the augmented flame has been found possible over a wide range of operating conditions, without any seed material being added, except when initiating the discharge. Once it has been established, the seed supply can be cut off.

Table 1. OPERATING CONDITIONS OF UNSEEDED D.C.-AUGMENTED HYDROCARBON GAS-AIR FLAMES

Burner	
Burner nozzle diameter, 1·875 cm	
Diameter of central electrode (flame holder), 1·25 cm	
Distance between electrodes, 8–20 cm	
Flow rates	
Propane, 2–9 l./min	
Air, 20–160 l./min	
Nitrogen (seed carrier), 0–1 l./min	For initiation only
K_2CO_3 seed (max. rate), 0·6 g/h	For initiation only
Electrical power	
Voltage, 300–800 V d.c.	
Current, 5–8 amp d.c.	
Concentration of free electrons for a 1 1 boost ratio: 10^{13}–10^{14}/cm³ (no seed flow)	

The characteristics of the discharge and operating conditions are given in Table 1.

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Non-enzymatic Formation of Nitrogen Gas

SINCE comprehensive treatment of the biochemical reduction of nitrate of Gayon and Dupetit[1] it has been generally assumed in denitrification studies that nitric oxide, and particularly nitrogen gas, evolved either from cell preparations or soil systems under moderate conditions of acidity (*p*H 5·0–7·0) and temperature (28°–30° C), are dissimilatory products of 'nitrate or nitrite respiration'[2–4]. Recent evidence has shown, however, that nitric oxide can also be formed from nitrite in sterile, moderately acid systems when certain transition-like metals are present[5]. Subsequent experiments to be presented in this communication further indicate that nitrogen gas can be formed as a result of reacting potassium nitrite and ferrous sulphate.

All experiments were carried out in electrolytic respirometer flasks with gas analyses being made with a Beckman '*GC*-2' gas chromatograph[6]. *p*H determinations were made using a Beckman 'model *G*' *p*H meter. The following reagents were used: aqueous potassium nitrite equivalent to 10,000 p.p.m. nitrogen; aqueous ferrous sulphate mixtures equivalent to 5,000 p.p.m. of iron, and brought to the appropriate *p*H values with the addition of potassium hydroxide; sulphuric acid adjusted to *p*H 3·0. Where potassium hydroxide was added, *p*H determinations were recorded only after equilibria were established.

Flask preparations were obtained by pipetting 20 ml. of the ferrous sulphate or sulphuric acid directly into the open flasks and then inserting an appropriate vial into which 20 ml. of the potassium nitrite was pipetted. After sealing the flasks in the usual manner[6], and flushing with purified helium for 30 min, the vials were tipped, allowing the observed reactions to proceed. All experiments were conducted at 29° ± 1° C. Values given are averages of duplicate determinations.

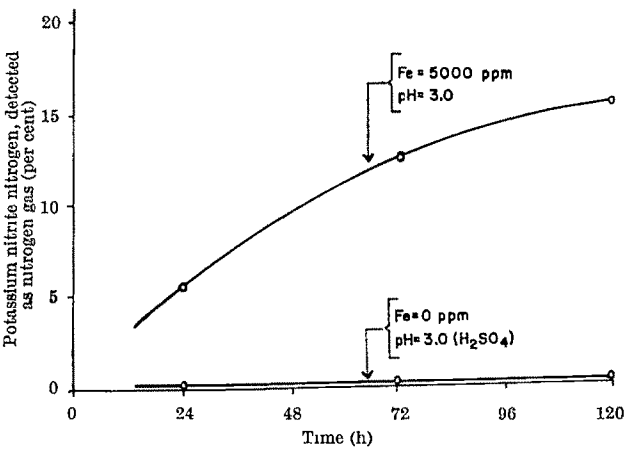


Fig. 1. Formation of nitrogen from potassium nitrite and ferrous sulphate

The formation of nitrogen by reacting nitrite ions with iron or other closely related metals is not well defined. (We were unable to locate specific experimental evidence attesting to the observed reactions.) However, it is apparent from Fig. 1 that nitrogen was evolved in the presence of the iron reagent. It is noteworthy that no nitrogen was detected at 0 p.p.m. iron when we attempted to react the nitrite ions with sulphuric acid. Clearly the observed reaction was iron-dependent. But it is not clear whether the reaction involved any intermediates, such as nitrogen oxides and/or metal complexes[7]. It will be remembered that nitric oxide is formed under similar conditions and was, indeed, detected in these experiments except where iron was omitted. At no time was nitrous oxide detected.

In order to determine what effect *p*H might have, NO_2^- was reacted with ferrous sulphate adjusted to *p*H 6·4. The results are shown in Table 1.

Table 1

Time	Fe p.p.m.	pH	Percentage nitrogen in KNO_2 recovered as nitrogen gas
72 h	5,000	3·0	13·0
72 h	5,000	6·4	15·0

Little or no effect was observed, that is to say, with decreased acidity there was little change in the quantity of nitrogen detected. Nevertheless the possibility existed that nitrogen formation might relate to *p*H, as it has been established that acidic conditions generally favour the reduced state of transition-like metals which is more active than the oxidized form with respect to nitric oxide formation[8]. The effect of *p*H, however, on oxidation-reduction processes is exceedingly complex and may vary with different ranges of *p*H. And, as emphasized by Hewitt[9], even though the effect is complicated it is not necessarily great.

The implications of the data presented here are far-reaching and may be related to denitrifying systems since intermediate fermentation products formed under anaerobic conditions may participate directly or indirectly in the reduction of such metals as iron[10]. Thus the very conditions which favour classical denitrification may be expected to promote metal-nitrite reactions. This possibility places in question the status of nitrogen as a strictly biochemical product. It appears that the key intermediate between the gaseous and non-gaseous states is the nitrite ion whether it arises via denitrification, nitrification, nitrate reduction, direct addition or by some anomalous route. The speculative scheme given in Fig. 2 seems appropriate at this time. As indicated, the formation of nitrogen may be a direct effect or may occur through one or more intermediates. The nitrite ion and nitric oxide are reactive with metal solutions and commonly form complexes with them such as $(FeNO(H_2O)_5)^{2+}$ formed from nitric oxide and aqueous ferrous sulphate[11]. These complexes may be stable or reactive depending on the conditions of the total system. It is conceivable, therefore, that the complexes could play an important part irrespective of whether they are products of nitrite or nitric oxide reactivity. Another possibility is that the metals may react directly with nitrite or nitric oxide without forming easily recognizable intermediate or complexes. Whatever the actual mechanism(s) in operation, it is reasonable to theorize that both nitrite and nitric oxide may be reduced to nitrogen by active metals. Of course a more thorough understanding of metal-nitrite reactions is needed to test the validity of the pathways proposed.

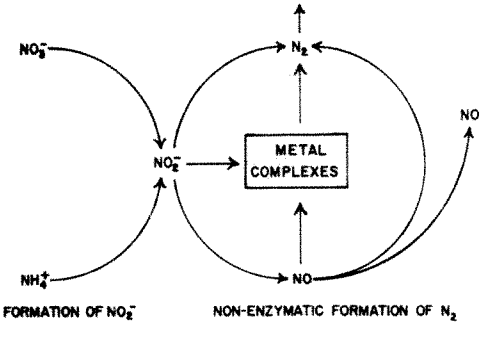


Fig. 2. Schematic diagram of hypothetical non-enzymatic pathways of metal-nitrite reactions leading to nitrogen production

Non-enzymatic formation of nitrogen mediated by transition-like metals may help to clarify heretofore inexplicable losses of mineral nitrogen from both soil and cell preparations. In addition these findings may partially explain certain data which have been interpreted as 'aerobic denitrification'[12]. Additional parameters which may affect the reaction reported here are being investigated.

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Reaction of 2,2′-Diphenyl-1-picrylhydrazyl with Hydroperoxides

It has been reported that the stable free-radical 2,2′-diphenyl-1-picrylhydrazyl (DPPH) is entirely unsatisfactory for most rate measurements of free-radical production[1,2]. These findings have been confirmed and extended during our investigations on the use of DPPH for the quantitative determination of hydroperoxides of unsaturated fatty acids[3]. We have found that the rate of the reaction of DPPH with different hydroperoxides depends on the concentration of both reactants. Furthermore, we have discovered that while the rate of the heat-induced decomposition of, for example, methyl oleate hydroperoxides is 0·01 per cent/h, at 70° C, the rate increases a thousandfold in the presence of DPPH[4]. It was, therefore, concluded that the function of DPPH is not that of a reliable ·OH counting device, which may reflect the primary O-O bond cleavage of long-chain, unsaturated fatty hydroperoxides. For the same reasons, it was thought that the actual chemical reaction between DPPH and hydroperoxides should be different from that previously reported[5–8].

This communication reports an investigation of the reaction between DPPH and hydroperoxides, as well as the identity of the predominant reaction products.

250 ml. of chromatographically purified *n*-heptane containing 1×10^{-4} M concentrations of DPPH and pure methyl oleate or linoleate or linolenate hydroperoxides were heated under purified nitrogen at 70° C until all measurable absorbance at 517 mμ had disappeared. Similar blank experiments showed a negligible change in the absorbance of DPPH.

The solvent was removed in a rotating evaporator heated at 30° C in a water bath, under a vacuum of 15 mm of mercury. The powders thus recovered were dissolved in a small amount of chloroform and the solutions were spotted on thin-layer plates prepared from 'Silicagel-*G*', 0·3 mm thick. Chromatographically purified benzene was used as mobile phase.

Several spots became visible when the plates were sprayed with molybdophosphoric acid, while the powder from the blank gave one spot only (DPPH). Similar chromatograms did not give any colour reaction with potassium iodide or 2,4-dinitrophenylhydrazine.

After developing fresh plates by the aforementioned method, all spots were scraped off, eluted with chloroform or benzene, combined and filtered. Most of the solutions were respotted and the plates were developed with a

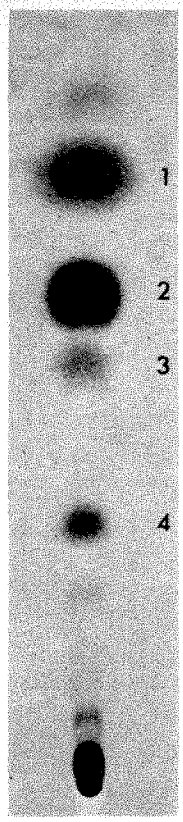


Fig. 1. Thin-layer chromatography of DPPH-hydroperoxides reaction mixture, developed by spraying with molybdophosphoric acid

variety of solvents to ascertain the purity of the eluted compounds.

The above experiments were carried out both under diffused daylight and in the dark.

The ultra-violet, visible, and mass spectra of all fractions, as well as of model compounds, were recorded, using a 'Unicam' *SP* 800 or 200 recording spectrophotometer or an *MS* 2 *H* and *MS* 9 'Vickers' mass spectrometer.

The decomposition of DPPH after reaction with hydroperoxides is illustrated in Fig. 1, while the compounds identified as corresponding to the numbered spots and representing approximately 85 per cent of the total are shown in Table 1. Under the conditions of the experiments and the solvents used, all decomposition products of the hydroperoxides have moved together with the solvent front.

Table 1. COMPARISON OF ABSORPTION MAXIMA AND MASS OF VARIOUS SPOTS ISOLATED BY THIN-LAYER CHROMATOGRAPHY AND THOSE OF MODEL COMPOUNDS

Spot*	% of total	Compound	Absorption maxima (mμ) Known	Found	Mass Known	Found
1	30–35	Diphenylamine	286	285	169·2	169
		Hydroxyphenyl-phenylamine†			185·2	185
		Diphenylhydrazine†			184·2	183
		2-hydroxyphenyl-2′-phenyl-1-hydroxyhydrazine†			216·2	216
2	30–35	2,2′-diphenyl-1-picrylhydrazine	250, 315	250, 315	395·3	395
		2-hydroxyphenyl-2′-phenyl-1-picrylhydrazine	294, 375	294, 375	411·3	412
3	5	2,2′-diphenyl-1-picrylhydrazyl	332, 517	332, 517	394·3	394
4	15	Picramide	312, 396	310, 395	227·1	227
		Trinitrobenzene	303, 370	301, 369	213·1	214
		Picric acid	352	352	229·1	229

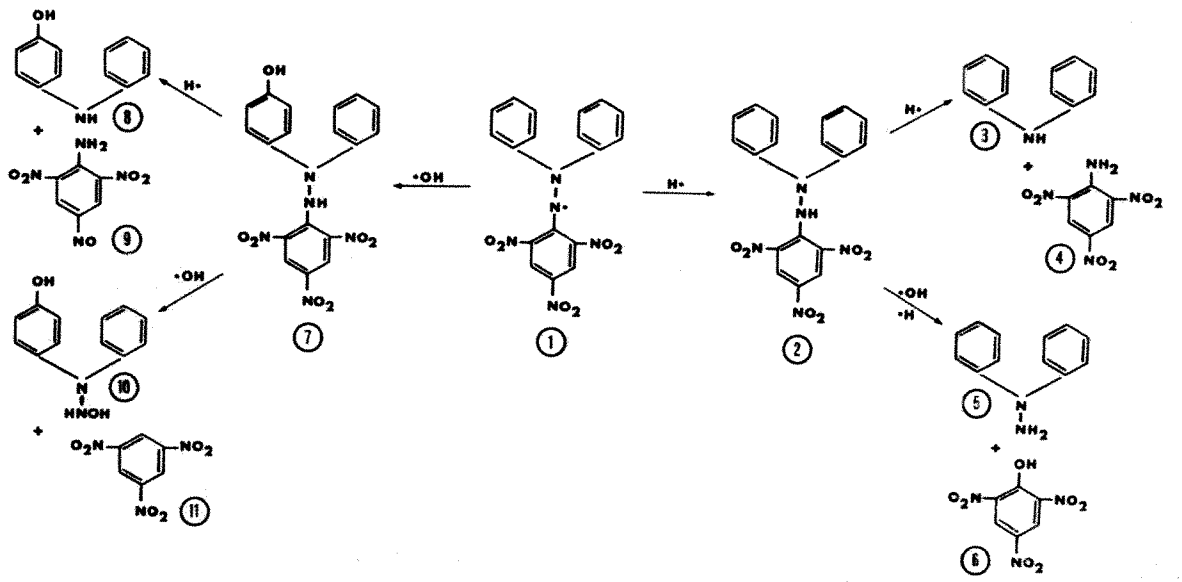
* As in Fig. 1.

† The absorption maxima are not known.

The formation of all products identified cannot be explained as taking place only by a direct and/or secondary ·OH attack on the DPPH molecule. Only compounds 7, 10 and 11 may be predicted as resulting from destruction of DPPH by the trapping of ·OH radicals, yet it was found that compounds 7, 10 and 11 actually consist of less than 15 per cent of the total amount of identified products. Since identical results were obtained when the reaction of DPPH with hydroperoxides was carried out in diffused daylight or in the dark, there is no question of photoreduction of DPPH[9], nor of photo-oxidation of the solvent. No effect on the DPPH has been observed when blank experiments were carried out. In addition, the absence of water from the reaction mixture has been strictly assured, thus excluding the possibility of hydrolysis.

It is, therefore, reasonable to assume that most of the decomposition products of DPPH are formed by a combined ·OH and ·H attack. The latter may be visualized as being abstracted from any of the known labile sites of the long-chain unsaturated fatty hydroperoxides.

The results also give evidence that the addition of the hydroperoxide decomposition products to the intact DPPH takes place on the phenyl group and not at the —N free-radical site, as formerly suggested[5–8]. Further-



more, since only (DPPH)OH and (DPPH)H addition products have been identified, and not even one product isolated in measurable amounts gave mass higher than 412 in the mass spectrum, any reaction between RO· or ROO· and DPPH may be considered as negligible.

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Reliability of the Thiobarbituric Acid Test in the Presence of Inorganic Iron

IN recent years, the validity of the 2-thiobarbituric acid (TBA) test for the determination of oxidative deterioration in animal and plant tissues, or their food products, has been challenged. However, since most authors prefer to use their own modification of a TBA test, comparison of results obtained at different laboratories is virtually impossible. Considerable confusion is therefore created by these claims, rather than clarification of the subject.

It has been demonstrated time and again that the TBA test is one of the most reliable tests for measuring the extent of oxidative deterioration in substrates containing unsaturated fatty acids. Only in the case of measurements involving either the initial or the very late stages of oxidation is the TBA test not very valid.

The TBA test measures the amount of malonaldehyde which is formed during the oxidation of the unsaturated fatty acids[1]. Malonaldehyde is primarily produced from the oxidative cleavage of 2-enals and 2,4-di-enals[2], which in turn are products of the breakdown of hydroperoxides[3]. Therefore no malonaldehyde is expected to be found at the early stages of oxidation, during which the rate of decomposition of hydroperoxides is negligible.

On the other hand, it has been shown conclusively that malonaldehyde does not accumulate as a stable end product during the oxidation of unsaturated fatty acids, but that it is destroyed at the very late stages of oxidation[4]. It is not, therefore, surprising that malonaldehyde cannot be found after an 18 h oxidation of methyl linolenate under ultra-violet radiation at 45° C (ref. 5).

It has been reported recently that inorganic iron present in the medium during the performance of the TBA test gives much higher results, in comparison with tests during which no iron is present[6]. Because of the extremely high concentration of free iron used in this investigation (10,000–100,000 p.p.m.), which is scarcely to be expected in any animal or plant, we have re-investigated the effects of iron on the TBA test.

The TBA methods used for assay were those described in previous publications[7,8].

100–5,000 p.p.m. of ferric ions were added before or after distillation to 0·1–1 g of methyl linolenate, rape seed, soyabean or groundnut oil, which were oxidized to a peroxide value (P.V.) from 1 to 40 mmoles/kg[9]. After the TBA test was carried out on a portion of the distillate[7,8] and under a variety of conditions, including heating at 20° C or 100° C, in water or 90 per cent acetic acid solution, the absorbance of all samples was measured at 530 mμ in a 'Unicam' *SP* 800 recording spectrophotometer. In no case was any significant difference in absorbance observed between the samples containing iron and the appropriate blanks. However, when 10,000–100,000 p.p.m. of ferric ions were added to the same oils and the experiments were repeated, significant increase in absorbance was observed in the samples containing iron before distillation, for example, the absorbance was increased from 0·9 to 2·8 by the presence of ferric ions. This increase was directly proportional to the P.V. of the substrate before the test, and it was much lower when the test was performed in water at 20° C than in acid at 100° C.

The effects of iron on TBA and the TBA-malonaldehyde complex[10] were investigated because of suggestions that the increased absorbance may be due to formation of metal chelates.

Ferric ions were added to 1×10^{-2} M TBA or 5×10^{-5} M TBA-malonaldehyde solutions in water or acetic acid, which were then heated to 20° C or 100° C for 15 h or 40 min respectively. In all cases, even when the concentration of iron was as high as 300,000 p.p.m., no difference in absorbance at 530 mμ was observed from that of the blanks. It is, therefore, concluded that inorganic iron does not form complexes with the above compounds, or if it does, the change in absorbance at 530 mμ is negligible and it does not alter the results of the TBA test.

The effects of iron on malonaldehyde have been investigated previously[11]. It has been shown that although malonaldehyde chelates iron, the recovery by distillation is not affected by iron chelation even with 10 : 1 ratios of iron to malonaldehyde.

The results of the experiments show that ferric ions present in a medium up to a concentration of 5,000 p.p.m. do not alter the results of the TBA test. Higher concentrations of iron induce changes which may be attributed to an acceleration of the decomposition of malonaldehyde precursors by the iron[12] rather than to formation of iron chelates. Since the changes induced by iron take place almost exclusively when the test is performed in acid medium and at high temperatures, the use of milder conditions during the performance of the TBA test[8] is recommended.

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Exchange Reactions in the Radiolysis of Adsorbed Hydrocarbons

THE radiolysis of hydrocarbons adsorbed on silica gel was first investigated by Caffrey and Allen[1] and later by Sutherland and Allen[2], who found that the hydrogen yield was several times larger than that expected from the amount of energy absorbed directly by the hydrocarbon itself. In an attempt to investigate more thoroughly the role of the silica gel, we have studied the radiolysis of *cyclo*hexane-d_{12} adsorbed at low coverages on silica gel.

Silica gel (50–60 mesh) was obtained from Analabs, Inc., Hamden, Connecticut, and had a surface area of

710 ± 60 m²/g, as calculated by the Brunauer–Emmett–Teller (B.E.T.) method using nitrogen as the adsorbate. The gel was dried by heating at 500° C in air for 72 h or more, and was then evacuated at 400° C to 10^{-6} mm mercury for 18 h. This procedure should give a silica gel surface about one-half covered with hydroxyl groups[3]. The *cyclo*hexane-d_{12} (Merck, Sharp and Dohme of Canada, Ltd.) was shaken with concentrated sulphuric acid and distilled. Samples were irradiated at room temperature with a cobalt-60 γ-ray source at a dose rate of about 9×10^{17} eV/g min to doses of between 3 and 90×10^{18} eV/g. All experiments reported here were carried out at a surface coverage of *cyclo*hexane of 0·51 per cent of a monolayer, taking the area of the *cyclo*hexane molecule[4] to be 39 Å².

The yields of hydrogen as a function of dose are shown in Fig. 1, the G values being the number of molecules of hydrogen produced for every 100 eV absorbed in the entire sample. The yields of hydrogen and deuterium hydride are much larger than that of deuterium, showing that chemical reactions involving the hydrogen atoms of the gel are at least as important as the decomposition of the hydrocarbon itself. No hydrogen whatsoever was obtained by irradiation of the gel alone.

At low doses, the primary liquid products are those observed in liquid-phase radiolysis[5], that is, bi*cyclo*hexyl and *cyclo*hexene; these can be recovered in amounts equivalent to the hydrogen yield (Fig. 2). The yields of these two products are extremely dose-dependent.

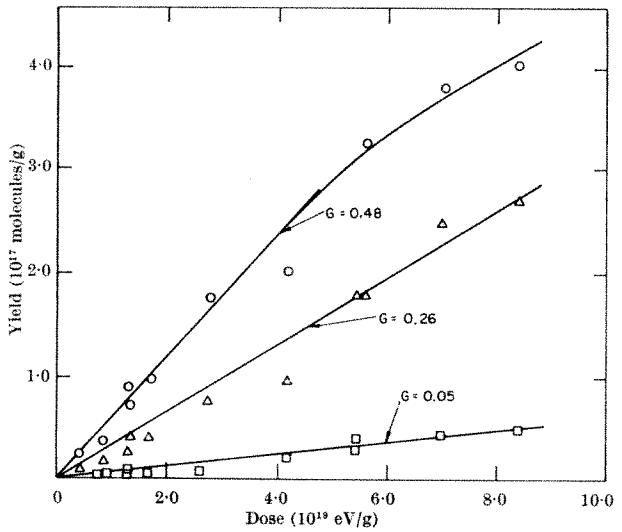


Fig. 1. Hydrogen yields for the irradiation of *cyclo*hexane-d_{12} adsorbed on silica gel. ○, Hydrogen; △, deuterium hydride; □, deuterium

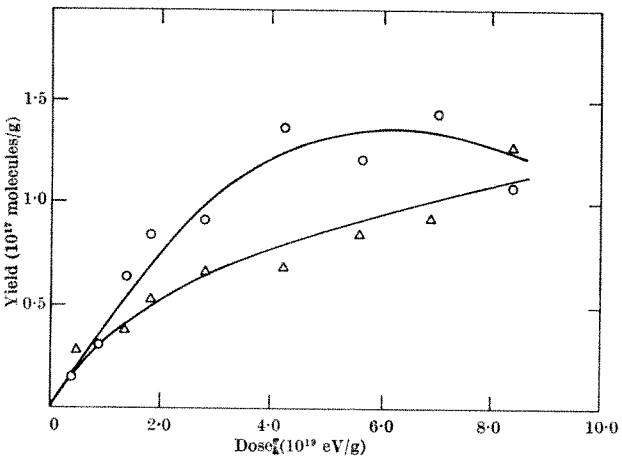


Fig. 2. Yields of the major liquid products of the radiolysis of *cyclo*hexane-d_{12} adsorbed on silica gel. ○, Bi*cyclo*hexyl; △, *cyclo*hexene

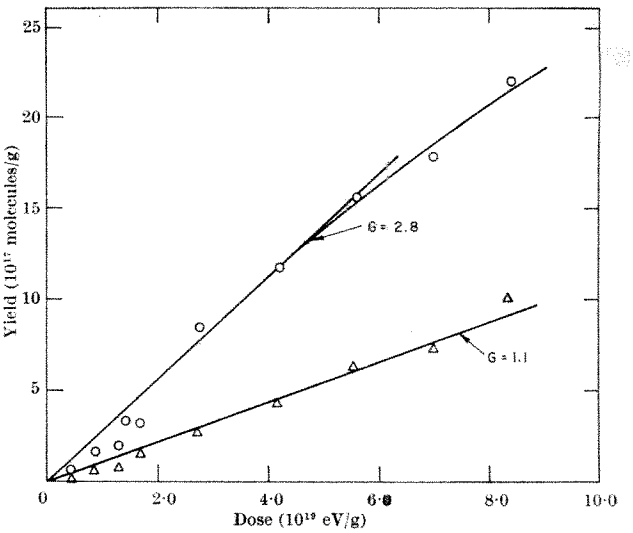


Fig. 3. Radiation-induced exchange of *cyclo*hexane-d_{12} adsorbed on silica gel. ○, $C_6D_{11}H$; △, $C_6D_{10}H_2$

This dose-dependence explains the very different products obtained by Zhabrova *et al.*[6], who irradiated *cyclo*hexane on silica gel to the much higher dose of $5{\cdot}4 \times 10^{21}$ eV/g.

Our most important observation is that the bi*cyclo*hexyl was highly exchanged. Mass spectral analysis of this product showed parent-ion peaks with masses covering the range 188 ($C_{12}D_{22}$) to 182 ($C_{12}D_{16}H_6$); masses 188 and 187 were about equal, with smaller amounts of the more highly exchanged bi*cyclo*hexyls.

The recovered *cyclo*hexane was examined for exchange and the yields of $C_6D_{11}H$ and $C_6D_{10}H_2$ are shown in Fig. 3. G(H), the total number of hydrogen atoms found in the *cyclo*hexane-d_{12} per 100 eV absorbed in the system, was 8 ± 2, which shows the existence of chemical processes which would not be detected by simple measurements of decomposition products. Although a radiation-induced exchange between light methane and deuterated silica gel has been observed by Kohn[7], this is the first comparison of exchange rate and decomposition rate in a system in which the hydrocarbon is strongly adsorbed.

In the present system (*cyclo*hexane on silica gel) radiolysis in the adsorbed state involves much more than an energy transfer from the solid to the adsorbate. Many efficient exchange reactions occur with even greater yield than the decomposition.

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Pulse Radiolysis of Aqueous Eosin

WE have been investigating the radiolysis of aqueous eosin induced by 1 μsec pulses of 33-MeV electrons from a Linac accelerator at approximately 1,200 rads per pulse. The flash spectra were taken on a Hilger *E*498 quartz prism spectrograph by synchronizing a 15 μsec (overall) flash from an E.G. and G. type *FX*-33 flash-tube to give

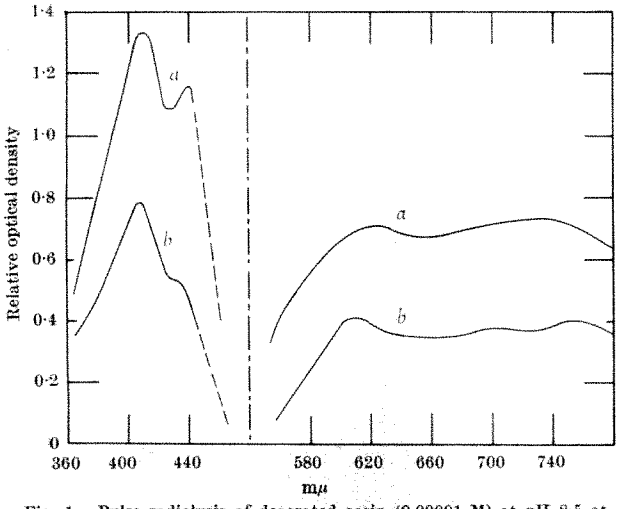


Fig. 1. Pulse radiolysis of deaerated eosin (0·00001 M) at *p*H 8·5 at 5 μsec (*a*) and 500 μsec (*b*) time delay. The spectra were taken on a Kodak '*I–N*' spectroscopic plate and are not corrected for emulsion sensitivity

a minimum 5 μsec peak-to-peak time delay. The rate data were obtained by measuring the transmission transients in narrow wave-length bands by means of photoelectric detection and oscilloscope display, with an Osram *XBO*-450 xenon arc as the monitoring lamp. The irradiations were performed in a 5-cm long fused-silica cell, located in a two-pass optical system, which could be evacuated to 2×10^{-6} mm mercury. The solutions were prepared with chromatographically purified eosin–sodium in triply distilled water and C.P. grade chemicals.

Fig. 1 shows flash spectra taken of 1×10^{-5} M eosin in 0·001 M sodium tetraborate buffer (*p*H 8·5). The transient at 5 μsec delay consists of a strong band at 405 mμ, a band to longer wave-lengths superimposed on the transmission increase due to dye bleaching, and a diffuse absorption in the red region. The 405 mμ band was identified in flash photolysis studies as semi-reduced eosin di-anion (*R*) (refs. 1 and 2). The band shifted to 369 mμ when eosin was radiolysed at *p*H 5·5, which corresponds to the absorption of the mono-anion[2]. The superimposed band is attributed to semi-oxidized eosin (*X*) (λ_{max} = 462 mμ; ref. 3), where the true maximum is shifted by the "negative absorption" caused by eosin bleaching (λ_{max} = 516 mμ). Kinetic measurements at 700 mμ showed that the red absorption has a fast component (~ 2 μsec) due to the hydrated electron (e^-_{aq}) and a slow component (~ 2 msec) attributed to triplet eosin (*T*). The e^-_{aq} life-time agrees with measurements in deaerated water at high dose rate[4] and is independent of eosin concentration up to 1×10^{-5} M, which sets an upper limit of ~ 4×10^{10} M^{-1} sec^{-1} on the reaction of e^-_{aq} with eosin. The e^-_{aq} absorption was obscured by the triplet above 2×10^{-5} M eosin. The slow decay at 700 mμ is of second order ($k/\varepsilon = 1{\cdot}1 \times 10^5$ cm sec^{-1}) and independent of eosin concentration from 2×10^{-5} M to 1×10^{-3} M. Taking $\varepsilon \sim 1 \times 10^4$ M^{-1} cm^{-1} (ref. 2) gives $k \sim 1 \times 10^9$ M^{-1} sec^{-1}, which is in agreement with the flash photolysis value of $1{\cdot}1 \times 10^9$ M^{-1} sec^{-1} for bimolecular triplet decay[3]. The disappearance of semi-reduced eosin is of second order, where the rate constant decreases with increasing eosin concentration (Table 1).

Table 1. Rate Constants for the Disappearance of Semi-reduced Eosin

Eosin concentration (M)	Rate constant* (M^{-1} sec^{-1})
0·00006	$0{\cdot}62 \times 10^8$
0·00004	$0{\cdot}75 \times 10^8$
0·00003	$2{\cdot}4 \times 10^8$
0·00002	$3{\cdot}2 \times 10^8$
0·00001	$3{\cdot}9 \times 10^8$

* ε (*R*) at 405 mμ, $3{\cdot}8 \times 10^4$ M^{-1} cm^{-1} from ref. 2.

Although it has been found that radiolysis produces aromatic triplets from a number of molecules in organic solvents[5,6], this would seem to be the first case reported of substantial triplet formation in aqueous solution. Water is not known to have a low-lying triplet state available for energy transfer and electron exchange is postulated as the mechanism of triplet production[5]:

$$S(\uparrow\downarrow) + e^-(\uparrow) \rightarrow T(\uparrow\uparrow) + e^-(\downarrow) \qquad (1)$$

Eosin reduction cannot be explained by the triplet electron-transfer reactions[3]: $T + T \rightarrow R + X$ and $T + S \rightarrow R + X$, because the rate constants of ~ 1×10^8 M^{-1} sec^{-1} are too slow to account for the "prompt" semi-reduced dye. The oxidation of impurities by OH which might later reduce eosin can be discounted also, because *R* was not suppressed by the addition of formate to scavenge OH. The remaining possibility is the capture of hydrated electrons:

$$S + e^-_{aq} \rightarrow R \qquad (2)$$

A comparison of Fig. 1 with the e^-_{aq} yield in deaerated water gives $G(R) \sim 1{\cdot}6$ and requires $k_2 \geqslant 1 \times 10^{10}$ M^{-1} sec^{-1}, which is not inconsistent with the upper limit deduced from the scavenging observations. The origin of *X* is explained by a fast reaction of OH with eosin:

$$S + OH \rightarrow X + OH^- \qquad (3)$$

The rate was estimated by measuring the competition with carbonate ion in the reaction:

$$OH + CO_3^{2-} \rightarrow CO_3^{-}\cdot + OH^- \qquad (4)$$

Taking $k_4 = 2 \times 10^8$ M^{-1} sec^{-1} (ref. 7) leads to $k_3 \sim 1 \times 10^{10}$ M^{-1} sec^{-1}. Thus, at low dye concentrations the decay of *R* is predominantly:

$$R + OH \rightarrow S + OH^- \qquad (5)$$

while at high dye concentrations hydroxyl is scavenged by way of reaction (3) and *R* disappears by the presumed slower dismutation:

$$R + R \rightarrow S + L \qquad (6)$$

where *L* is leuco-eosin (see Table 1).

The formation of triplet eosin raises the question as to whether dye-sensitized radiolysis can occur. Preliminary results with *p*-cresol indicate that this may be the case. Pulse radiolysis of deaerated *p*-cresol (0·005 M, *p*H 11) gives a spectrum at 5 μsec delay showing the e^-_{aq} absorption, the narrow bands of *p*-methylphenoxyl at 405, 385 and 369 mμ (ref. 8), and a broad absorption below 340 mμ. The addition of 0·1 M sodium formate suppresses all but e^-_{aq}, indicating that hydroxyl is the predominant oxidizing species:

$$OH + CH_3C_6H_4OH \begin{cases} \nearrow CH_3C_6H_4O\cdot \\ \searrow CH_3C_6H_4(OH)_2\cdot \end{cases} \qquad (7)$$

where the short wave-length band is attributed to the addition product. Fig. 2 shows the results obtained with the eosin/formate/*p*-cresol system. The shoulders on the *R* absorption located at the maxima of the *p*-methylphenoxyl bands (the arrows) prove that the latter species is present even though OH was scavenged by formate. (The absence of the semi-oxidized eosin band confirms that OH was not available to react with eosin and *p*-cresol.) The result is explained by the triplet reaction:

$$T + CH_3C_6H_4OH \rightarrow R + CH_3C_6H_4O\cdot \qquad (8)$$

which has been studied in detail with flash photolysis[2]. Although our preliminary work does not make a full comparison possible, it is interesting to note that eosin parallels methylene blue (MeB) by its high reactivity with hydroxyl and hydrated electrons, but differs markedly in its high triplet yield by radiolysis: $k(e^-_{aq}$ + MeB) = $2{\cdot}5 \times 10^{10}$ M^{-1} sec^{-1} and *G* (triplet MeB) $\leqslant$ 0·1 (ref. 9).

We thank Dr. J. Ovadia for his advice and for his contributions during the initial stages of this work, which

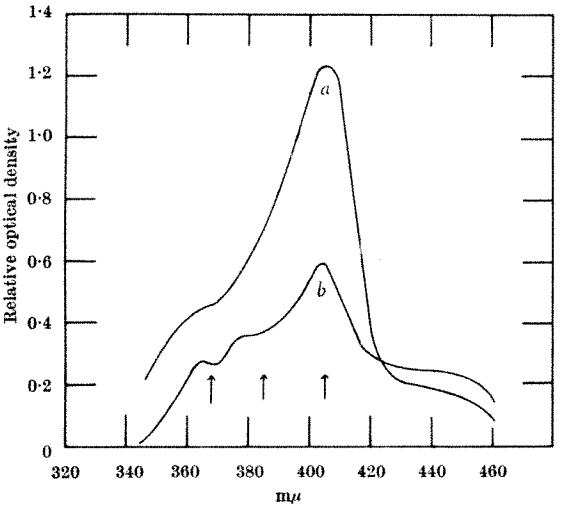


Fig. 2. Pulse radiolysis of deaerated eosin (0·00001 M) and *p*-cresol (0·005 M) in the presence of 0·1 M formate (*p*H 8·7) at 5 μsec (*a*) and 50 μsec (*b*) time delay. The arrows indicate the absorption maxima of *p*-methylphenoxyl (ref. 8). The spectra were taken on a Kodak '103–0' spectroscopic plate and are not corrected for emulsion sensitivity

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BIOCHEMISTRY

Structural Aspects of Polyribosomes and Endoplasmic Reticulum Fragments isolated from Rat Liver

THE isolation of polyribosomes from rat liver has been described in previous communications[1–4]. In addition to the polyribosomes found in the 75,000*g* pellet, three coloured layers localized in the interface between 1·5 M and 2 M sucrose can be isolated. The lower layer consists mainly of rough endoplasmic reticulum (membranes with attached ribosomes)[2]. In the upper layers the membranes are almost devoid of ribosomes. In both these layers polyribosomes are frequently encountered which are connected to the membrane surface only by way of one single terminal monomer (compare Fig. 1*E*).

In the negative stained preparations the attachment of these monomers to the membranes seems to be either a tight junction or a short stem (Fig. 1*D*). Furthermore, it appears that the membranes are built up by small globular sub-units with a diameter of about 40 Å. This substructure can be detected as well in the smooth membranes as in the rough ones.

Since membrane-attached polyribosomes are observed in the upper layers, the question arises whether part of the unattached polyribosomes in the pellet may have been detached during homogenization and centrifugation. However, when the layers are re-submitted to the whole isolation procedure, no second pellet is found. This evidence favours the assumption of a substantial number of attached ribosomes.

Webb *et al.*[5] arrived at the conclusion (based on a comparison of the pellet obtained from untreated and deoxycholate-treated 15,000*g* supernatant) that at least 50 per cent of the polyribosomes in normal rat liver are tightly bound to membranes.

Our results confirm these observations in that the amount of RNA recovered from the three coloured layers is approximately the same as that recovered from the pellet and the 2 M sucrose layer. Moreover, after treatment of the combined coloured layers with 1 per cent deoxycholate after centrifugation at 100,000*g* for 30 min, a pellet is obtained which contains about the same amount of RNA as the pellet obtained from deoxycholate-treated 2 M sucrose layer combined with the polysomal pellet. One might expect that a tight binding would originate from attachment of polyribosomes to membranes by way of a great number of monomers, but the electron microscopic evidence suggests that the link

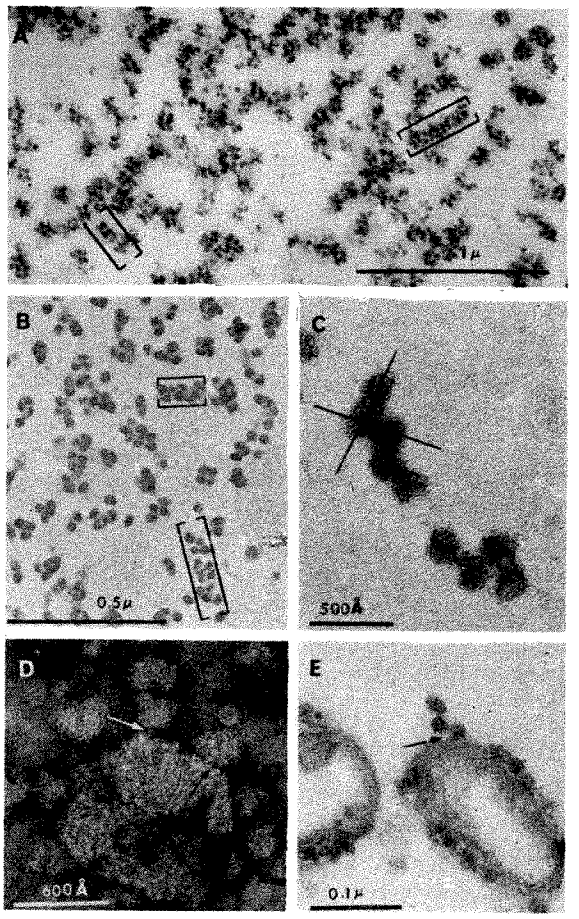


Fig. 1. (*A*) Pellet of polyribosomes, thin section stained with 1 per cent uranyl acetate. Some ribosomal aggregates show a helical configuration. (*B*) Pellet of polyribosomes, spread preparation shadowed with carbon–platinum while rotating. Polyribosomes formed by rows of paired units. (*C*) Pellet of polyribosomes, spread preparation positive staining with 1 per cent uranyl acetate. These ribosomal aggregates show clearly a helical configuration, with an angle of approximately 78°. (*D*) Lower layer, spread preparation negatively stained with 1 per cent uranyl acetate. The white arrow points to a stem-like attachment, the black arrow to a tight junction. Note also the globular structure of the membraneous sheet. (*E*) Lower layer, thin section stained with uranyl acetate. The arrow points to a small chain of ribosomes. The connexion with the membrane element is made by a monomer

between the membranes and the chain of polyribosomes often consists only of a single ribosome.

Further observations on the fine structure of the polyribosomes localized in the pellet after centrifugation of the postmitochondrial supernatant reveal that many ribosomal aggregates appear as helices with angles which vary in the range 50°–80° C (Fig. 1*A*, *B* and *C*). This finding is in agreement with a recent report by Pfuderer *et al.*[6]. The results of their physico-chemical investigation of polyribosomes in solution favour a helical model. This configuration may well result from a transition during isolation of clusters of ribosomes which are arranged *in vivo* as spirals or rosettes. The latter configurations have been related to free and membrane-bound ribosomal aggregates[7].

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Separation and Recombination of Ribosomal Ribonuclease from Ribosomes

Ribosomes obtained from *Escherichia coli* contain ribonuclease (RNase) which is latent if the ribosome is intact[1]. It has been reported that this enzyme originates in the cell wall and adds to the particle only during preparation[2]. This finding is in contradiction to earlier work[3,4] and the work of Anraku[5] which suggest that the enzyme is an intrinsic part of the ribosome. The experiments of Neu *et al.*[2] showed addition of RNase to ribosomes but no inactivation had been mentioned. If ribosomes are treated with 0·5 M ammonium chloride solution the RNase is released[6,7] and subsequently separated from the ribosome by 'Sephadex' filtration[8].

It has been found that in intact ribosomes the RNase becomes released and activated at 62° C, whereas sensitivity to the action of this enzyme occurs already at 54° C, in both cases leading to the breakdown of the whole ribosome. Thus, the temperature of thermal decomposition is an indication of the activation of the RNase[9]. Using this technique we have investigated to what extent release and activation of ribosomal RNase by 0·3–0·5 M sodium chloride can be reversed by removing the salt.

Ribosomes have been prepared as described by Matthaei *et al.*[10]. Thermal degradation has been carried out in a silica cell by raising the temperature in 3°–5° C gradients every 15 min. The degradation, resulting in formation of a precipitate, was measured photometrically; since this was carried out at 260 mμ any hypochromicity could also have been noted. A typical degradation pattern resulting from this procedure is shown in Fig. 1*a*. Enzyme activity was tested as described before[9] using poly-*C* (Miles) as the substrate. Ribosomes suspended in 50 per cent caesium chloride still form distinct particles of Svedberg constants 23*s* and 42*s*[11]. After removal of caesium chloride by dialysis, these particles still contain RNase

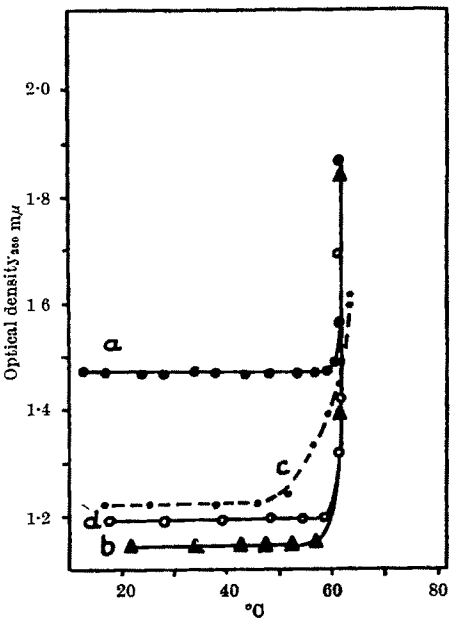


Fig. 1. Light absorption of ribosome solutions at various temperatures: (*a*) ●—●—●—●—● Untreated (*b*) ▲—▲—▲—▲—, Treated with 50 per cent caesium chloride followed by dialysis. (*c*) ● – – ● – – ● –, In 0·3–0·5 M sodium chloride solution. (*d*) ○—○—○—○—, 0·3–0·5 M sodium chloride, treatment followed by dialysis. In the temperature region of the flat parts of *a*, *b*, and *c* increase of light absorption at prolonged heating was negligible

in latent form; this has been shown by comparison with untreated ribosomes in the following two experiments:

(1) Untreated and caesium chloride-treated ribosomes in 0·01 M *tris*-hydrochloric acid (*p*H 7) and 0·01 M magnesium acetate were heated up as described. Degradation of both took place at nearly identical temperatures (62° C) (Fig. 1*a*, *b*). In both cases, addition of 0·5 mg/ml. pancreatic RNase caused this degradation to occur below 54° C.

(2) Untreated and caesium chloride-treated ribosomes in 0·01 M magnesium acetate, 0·01 M *tris*-hydrochloric acid (*p*H 7·4), and 0·005 M β-mercaptoethanol (1 mg/ml.) were heated for 20 min to various temperatures and then cooled down to 4° C. A schlieren sedimentation analysis was performed with each sample. The bands ascribed to the ribosomes and their sub-units (traces) were only slightly changed at temperatures up to 59° C but were absent in samples heated up even higher (65° C). This temperature step was also marked by the appearance of RNase activity.

Ribosomal RNase can be liberated by treatment with 0·3–0·5 M sodium chloride. The effect of this treatment can be seen from Fig. 1. Degradation begins at a lower temperature and occurs over a much wider temperature range (*c*) than the degradation of untreated ribosomes (*a*). When the salt is removed by dialysis the degradation pattern of (*a*) is almost restored (*d*).

We note that centrifugation of such a sodium chloride-containing ribosome solution at 39,000 r.p.m. in an '*SW* 39' Spinco rotor for 3 h is not sufficient to remove the RNase completely from the particle. Those ribosomes have also been found to be a little more labile than untreated particles (Fig. 2*e*). When 0·5 M sodium chloride enzyme supernatant solution and pellet ribosomes are remixed in their original proportions, the degradation pattern (*f*) in Fig. 2 is obtained. Removal of salt from this mixture by dialysis again results in almost complete restoration (Fig. 2*f*). Under those conditions no enzyme activity can be demonstrated in the supernatant when this recombined sample is centrifuged at 39,000 r.p.m. in the Spinco '*SW* 39' rotor.

Ribosomal particles obtained by treatment with 50 per cent caesium chloride and subsequent dialysis do

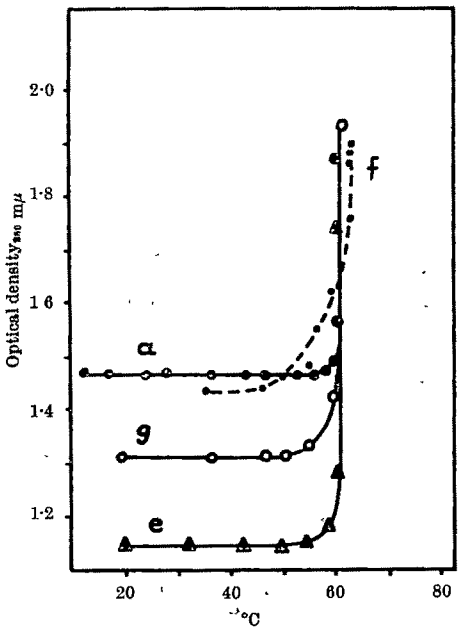


Fig. 2. Light absorption of ribosome solutions at various temperatures. (*a*) ●—●—●—, Untreated, identical to (*a*) in Fig. 1. (*e*) ▲—▲—▲—, Pelleted in buffer containing 0·35–0·5 M sodium chloride and subsequently heated in 0·01 M *tris*-hydrochloric acid, *p*H 7, and 0·01 M magnesium acetate. (*f*) ● – – ● – – ● – –, Pellet and supernatant remixed. (*g*) ○—○—○—, Pellet and supernatant mixture dialysed

not differ in their thermal degradation behaviour from untreated ones. This effect can be explained by recombination of the enzyme with the particle during dialysis. This interpretation is supported by results obtained from ribosome solutions containing 0·5 M sodium chloride. In such solutions the enzyme is released and activated. Removal of the salt, however, restores the original shape of the thermal degradation diagram. Thus, the conditions under which the enzyme is thermally released approximate to those conditions found with ribosomes which were not treated with sodium chloride. Within the limits allowed by the procedure, we conclude, therefore, that the enzyme can recombine with the ribosome, and become latent on gradual removal of the 0·5 M sodium chloride from the solution.

Separation of the enzyme activity from the ribosome itself, by centrifuging 0·5 M sodium chloride ribosome solutions, is not complete. We still found RNase activity using poly-*C* as a substrate. Moreover, this centrifugation procedure renders the ribosome a little more labile. This might interest workers who include washing with such salt concentrations in their ribosome purification.

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Isolation of a Pre-viral Deoxyribonucleic Acid

IN 1943, we found that hydroxylamine can induce viral polyhedrosis in silkworms[1]. Pursuing this finding, one of us has established a metabolic cycle producing virogens and mutagens[2]. It has further been demonstrated that the polyhedral pre-viral genome is located in the chromosome of the host cell[3]. We have now succeeded in isolating the pre-viral deoxyribonucleic acid from the larval nucleus and have confirmed that hydroxylamine is able to accelerate its separation from the other nucleic acid portion.

Cellular deoxyribonucleic acid was prepared with sodium dodecylsulphate according to our method[4] from nuclei which had been collected from digestive organ-free tissues of healthy silkworms using the procedure described before[5]. Viral deoxyribonucleic acid was also prepared with the same reagent in a similar way[4] from polyhedra which had been obtained from larvae inoculated with the virus[6].

The results of analysis showed that the cellular and viral deoxyribonucleic acid have almost the same base composition. The data of sedimentation with an analytical ultracentrifuge indicated, however, that the average molecular weight of the former is 46×10^6 and that of the latter 24×10^6. Therefore, we tried to fractionate the cellular nucleic acid, to find a component which has the same molecular weight as the viral nucleic acid. For this, a gradient centrifugation was first applied to the viral deoxyribonucleic acid. The freshly prepared nucleic acid was dissolved in a mixture of 0·2 N sodium chloride plus 0·01 M sodium citrate to a concentration of 40 μg/ml. and the solution obtained was put into a tube containing 5–20 per cent sucrose solution in 0·1 N sodium chloride and 0·05 M potassium phosphate buffer, *p*H 6·7. The tube was centrifuged at 30,000 r.p.m. for 4 h in a 'Spinco *L*' ultracentrifuge at 10° C. The centrifugation pattern showed that the viral deoxyribonucleic acid is represented by the 35 *S* (sedimentation coefficient) component as a linear form. The cellular nucleic acid was then dissolved in the same way in a chloride–citrate mixture. To facilitate the fractionation, the solution was stirred at 2,000 r.p.m. for 30 min in a blender under cooling. Immediately after that, the sucrose gradient centrifugation was applied to the solution as in the case of the viral nucleic acid. The obtained pattern indicated that the cellular deoxyribonucleic acid consists of 62 *S*, 55 *S*, 44 *S*, 35 *S* and 24 *S* components. The amount of 35 *S* was about 4 per cent of the total acid.

It seemed reasonable to assume that the 35 *S* component in the cellular deoxyribonucleic acid was the polyhedral pre-virus. To confirm this, the 35 *S* fraction was collected, dialysed and freeze-dried. The dried component was dissolved in 0·01 M sodium carbonate and 0·02 ml. of the solution was injected into each larva on the first day of fourth instar. At the same time, the injection was also carried out with the carbonate solution of viral and cellular deoxyribonucleic acid. In addition, the cellular nucleic acid was treated with 0·01 M hydroxylamine at 35° C for 2 h at *p*H 7·0, and after dialysing against 0·01 M sodium carbonate the solution was injected into larvae. Some of the results are given in Table 1.

Table 1. VIROGENIC ACTION OF DEOXYRIBONUCLEIC ACID (DNA) PREPARATIONS

Kind of preparation	Viral DNA	Pre-viral DNA	Cellular DNA	Cellular DNA + hydroxylamine
Concentration of preparation	10 μg/ml.	25 μg/ml.	450 μg/ml.	450 μg/ml.
Number of test larvae	15	15	15	15
Number of virozed larvae	5	4	2	6

In the control, it was verified that the injection of 24 *S* (35 μg/ml.) or hydroxylamine (0·01 M) in 0·01 M sodium carbonate causes no polyhedral disease. From these observations, one can conclude that the 35 *S* component in the host cell must be a pre-viral deoxyribonucleic acid. There was also the possibility that the larger components

still involve the pre-viral nucleic acid molecule and the data of hydroxylamine-treatment appeared to suggest that the action of this amine is to break appropriately chromosomal nucleic acid to liberate the pre-virus. The cellular deoxyribonucleic acid was therefore mixed with 0·005 M hydroxylamine and after 2 h at 35° C the mixture was centrifuged with a 'Spinco *L*' ultracentrifuge according to the procedure mentioned above. It was corroborated that the yield of 35 *S* amounts to 6 per cent of the cellular nucleic acid employed. The centrifugation of the intact cellular deoxyribonucleic acid which had not been treated with a blender or hydroxylamine gave only about 2 per cent 35 *S* component. It can thus be confirmed that the pre-virus is pre-existent in the larval cell and that its separation is promoted by suitable means.

Recently, Northrop[7] demonstrated that a new phage can be produced by a long treatment of *Escherichia coli* with triethylene melamine. Ikuta and Zimmerman[8] also proved that a new virus is produced in the precancerous state of mice implanted with carcinogenic hydrocarbons. These results may be explained by the appropriate cleavage of cellular nucleic acids by chemicals. It is further assumed that duplicants proposed by Butenandt[9], Druckrey[10] and Euler[11] for precursors of cancers may correspond to pre-viral nucleic acids.

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Pyridoxal and Pyridoxamine Phosphate Breakdown by an Acid Phosphatase Preparation

DURING investigations of the controlled inactivation of pyridoxal phosphate-dependent enzymes, an acid phosphatase preparation has been used to effect co-enzyme destruction. Alkaline phosphatases of mammalian origin are known to hydrolyse both pyridoxal and pyridoxamine phosphates[1,2] and an alkaline phosphatase preparation from *E. coli* has been used for the resolution of tryptophanase[3]. A preliminary report on the inactivation of L-α-alanine : phenylglyoxal transaminase by treatment with alkaline phosphatase has also appeared[4]. The breakdown of vitamin B_6 phosphates by acid phosphatases does not appear to have previously been reported.

In the present work a commercial preparation of wheat germ acid phosphatase (B.D.H., Ltd.) was used. Activities with a variety of substrates were measured by the colorimetric assay of orthophosphate production according to the method of Gomori[5], readings being made at 715 mμ. Reaction mixtures contained buffer (0·2 M), 1·0 ml.; enzyme preparation in water 0·4 ml., substrate (7·0 mM), 0·5 ml.; and additions up to 3·5 ml. Incubations were carried out at 37° C and terminated by the addition of 0·5 ml. trichloroacetic acid (50 per cent).

With pyridoxal phosphate as substrate, preliminary experiments showed that optimum activity occurred at *p*H 5·5. Acetate buffers were used in this region, maleate and veronal buffers being used to cover a wider range of *p*H. Pyridoxal phosphatase activity rapidly declined between *p*H 5·5 and 7·0, and no evidence of a second peak at the alkaline side of neutrality was obtained, even at high enzyme concentrations. Pyridoxamine phosphate was hydrolysed optimally at the same *p*H but at a much lower rate. The respective specific activities were 88·0 and 10·8 mU. per mg protein, compared with 340·0 mU. per mg for disodium phenylphosphate used as a reference substrate.

Magnesium ions, as magnesium sulphate, had no effect on pyridoxal phosphatase activity at a concentration of 1 mM, and EDTA had no inhibitory effect at concentrations below 0·1 mM although 30 per cent inhibition was observed at 1 mM. Sodium fluoride caused marked inhibition at concentrations as low as 10·0 μM (10 per cent) and inhibited 70 per cent at 1 mM. Iodoacetate, β-mercaptoethanol, or cysteine had no effect on phosphatase activity when tested over the same concentration range, after 30 min preincubation with enzyme. Preincubation of pyridoxal phosphate in buffer with cysteine or histidine, which are known to form respectively thiozolidine and imidazo-tetrahydro-pyridine derivatives with the coenzyme[6], had no detectable effect on the rate of enzymatic hydrolysis.

The effect of substrate concentration on the initial rates of enzyme activity, using either pyridoxal or pyridoxamine phosphates, was investigated. Michaelis constants for these substrates were determined from double-reciprocal plots by the method of Lineweaver and Burk[7], and found to be 0·52 and 1·30 mM, respectively, when measured at *p*H 5·5.

The foregoing results show that wheat germ acid phosphatase readily hydrolysed pyridoxal phosphate. Pyridoxamine phosphatase activity was lower, however, and less favoured by the Michaelis constant. This result is in contrast to the results obtained using mammalian preparations[1,2]. It is interesting to note that an alkaline phosphatase from human brain hydrolysed pyridoxal phosphate more rapidly than all other substrates tested[2], and that whereas a bacterial acid phosphatase preparation had no activity with vitamin B_6 phosphates[3], a preparation of prostatic acid phosphatase was also more effective with pyridoxal phosphate than any other substrate[8]. The possible role of phosphatases in the regulation of pyridoxal phosphate requiring enzymes has been discussed elsewhere[9,10].

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Loss of Adenine during the Hydrazine Degradation of Deoxyribonucleic Acid

THE treatment of deoxyribonucleic acid (DNA) with anhydrous hydrazine causes the degradation and removal of the pyrimidine bases but leaves the rest of the polynucleotide chain more or less intact[1,2]. This degradation of the pyrimidine ring occurs irrespective of whether the pyrimidines occur as part of a nucleic acid or as free base, nucleoside or nucleotide[1–5]. This reaction has recently been considered in detail by Temperli *et al.*[5]. The pyrimidine-free DNA (apyrimidinic acid) can be hydrolysed by potassium hydroxide[4] to tracts of oligopurine nucleotides having the general formula, $(\text{purine nucleoside})_n$ $(\text{phosphate})_{n+1}$, where *n* has been observed to vary from 1 to 13

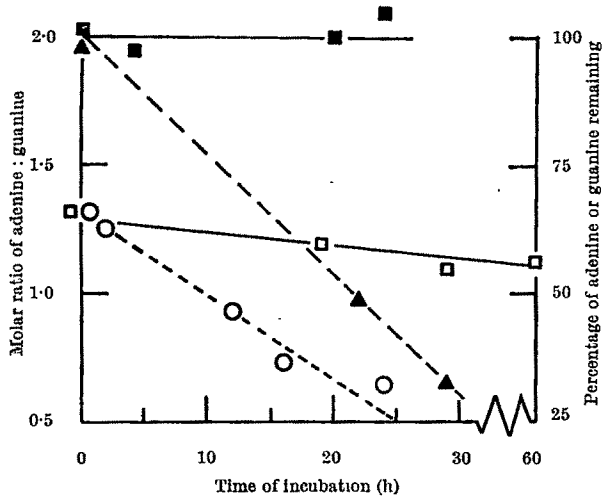


Fig. 1. Effect of anhydrous hydrazine on the loss of purines from calf thymus DNA and from deoxymononucleotides. Dried calf thymus DNA was incubated with anhydrous hydrazine (Fluka, water-free) at 37° C □—□ and 60° C ○ - - - ○. At the end of the incubation, the hydrazine was removed by co-evaporation with ethanol using a rotary evaporator. The product was heated in 0·3 M potassium hydroxide at 100° C for 1 h, desalted by adsorption on and elution from DEAE-cellulose (bicarbonate) and hydrolysed with 72 per cent perchloric acid at 100° C for 1 h. 5′-*d*AMP ▲ - - - ▲ and 5′-*d*GMP ■——■ were incubated with anhydrous hydrazine at 60° C, dried and hydrolysed with perchloric acid. In all cases, the bases were separated and estimated by quantitative paper chromatography. Data in duplicate experiments agreed with the results shown

(refs. 4, 6, 7). For any one value of n, these tracts are known as isostichs[8].

It has been claimed[1,2,6] that the treatment of DNA with anhydrous hydrazine at 60° C causes a rapid and complete degradation of the pyrimidine bases while leaving the purine residues unaffected. Although we have confirmed that the pyrimidines are rapidly removed from DNA under these conditions, we have found that there is also a loss of adenine as indicated by the decreasing molar ratio of adenine to guanine with time of incubation at 60° C (Fig. 1). This ratio fell almost linearly with time and had decreased to 90 per cent of its original value (1·32) after 4 h incubation, 85 per cent after 6 h and 51 per cent after 20 h. As expected, the rate of loss of adenine was much lower with an incubation temperature of 37° C; at this temperature there was a negligible loss after 4 h and a 7 per cent loss after 20 h incubation. In agreement with the results of other workers[1–6], the degradation of pyrimidines is rapid and results obtained for the loss of cytosine and thymine from DNA at 37° and 60° C are given in Table 1.

Table 1. DEGRADATION OF PYRIMIDINES ON INCUBATION OF CALF THYMUS DNA WITH ANHYDROUS HYDRAZINE*

Time of incubation (h)	Cytosine remaining (per cent) 37° C	60° C	Thymine remaining (per cent) 37° C	60° C
0	100	100	100	100
2	22	0	72	15
4	15	0	35	0
8	2	0	10	0
16	0	0	0	0

* Experimental conditions as for those described in Fig. 1.

This effect of hydrazine on adenine was confirmed by following the breakdown of 5′-*d*AMP in anhydrous hydrazine at 60° C (Fig. 1). It was found that the rate of loss of adenine was very similar to that observed with DNA with a 50 per cent loss after 20 h (Fig. 1). On the other hand, under similar conditions, there was no loss of guanine from 5′*d*GMP (Fig. 1).

It is difficult to explain the discrepancy between the claims that anhydrous hydrazine at 60° C causes negligible loss of purines[1,2,4–6] and the results reported here. Where an incubation time of 4 h has been used[2,4–6] a 10 per cent loss of adenine, as found here, could well go undetected. However, Sedat and Sinsheimer[7], using an incubation time of 18–20 h at 60° C, reported that there was little loss of adenine compared with the nearly 50 per cent found under our conditions. Whatever may be the explanation for these discordant results, it appears that prolonged incubation of DNA with anhydrous hydrazine at 60° C can cause extensive loss of adenine. The results in Fig. 1 and Table 1 indicate that incubation of DNA and anhydrous hydrazine at 37° C for 16 h or at 60° C for 4 h are the optimum conditions of incubation, ensuring maximum degradation of pyrimidines commensurate with minimum degradation of adenine.

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Inactivation of Rennin by Dansyl

RENNIN (*EC* 3.4.4.3) is a proteolytic enzyme[1] that is of considerable industrial importance because of its ability to clot milk. Evidence has been presented that its clotting action on milk depends on the splitting of an ester bond[2]. If this is so, the active centre of rennin could be expected to be similar to those of other enzymes with esterase activity such as chymotrypsin (*EC* 3.4.4.5) in which catalytic activity is associated with reactive serine[3] and histidine[4] side-chains. We have shown that rennin may be inactivated by photo-oxidation with methylene blue and that the loss of activity is probably due to destruction of histidine as there is no loss of activity when photo-oxidizable side-chains other than histidine are modified[5].

Cheeseman has reported[6] that rennin is not inactivated by di-isopropylfluorophosphate (DFP), which may inactivate esterases by reacting with a single serine side-chain[7]. We have confirmed this, and have found in addition that rennin does not react with toluenesulphonyl chloride. Like DFP, this reagent has been shown to react with active-centre serine[8] and the failure of both these reagents to inactivate rennin suggests that this enzyme may not possess a catalytic serine residue.

In a search for a means of labelling the active centre we have used dimethylamino-naphthalene sulphonyl chloride (dansyl), a fluorescent reagent recently introduced for determining the end groups of proteins[9]. We have found that, at *p*H 8 and 3° C, rennin is inactivated by adding to the solution sufficient dansyl to react with 1·5–2 residues of the rennin. In a typical experiment, 50 μl. of a solution of dansyl in acetone (6 mg/ml.) was added to 12 ml. of rennin solution (2 mg/ml.) at *p*H 8 and 3° C in phosphate buffer (I = 0·2), and the solution stirred continuously. 50 μl. of acetone was added to a control solution kept in the same conditions. At intervals of about 30 min, 0·1 ml. of the solution was pipetted into 10 ml. of phosphate buffer at *p*H 6·5, and used for activity tests with skimmed milk reconstituted in 0·01 M calcium chloride[10]. After 30 min the activity of the dansylated rennin had fallen to about 50 per cent, and after 1 h to about 40 per cent, of that of the control, which was almost unaffected. Addition of a further 20 μl. of dansyl was required at this stage and in these experiments caused a drop in activity to a minimum of 15 per cent of that

of the control. The rennin was then separated from free and hydrolysed dansyl by gel filtration on 'Sephadex G25'. The amount of dansyl bound to the protein was estimated from ultra-violet difference spectra, assuming that the absorption of bound dansyl was the same as that of dansyl in isopropanol. At maximum (85 per cent) inactivation, the bound dansyl was estimated at 1·4 moles dansyl per mole rennin, and at lower levels of inactivation (such as 70 per cent) less dansyl (0·7 residues) was bound. When the dansyl was added at *p*H 6·5 instead of at *p*H 8, there was no inactivation.

Our results suggest rather strongly that the rennin is inactivated by reaction of the dansyl with a single residue. Work aimed at identifying the site of the reaction is in progress and will be reported later. However, the present results encourage the hope that this reagent may be useful for labelling the active centre of rennin and suggest its use in similar investigations of other enzymes.

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Mechanistic Interpretation of Empirical Enzyme Rate Equations

THE kinetic behaviour of most enzymes is more complicated than that described by Michaelis and Menten[1] for the relatively simple mechanism: $E+S = ES \rightarrow E+P$. Many enzymes have more than one substrate or product; others are inhibited by product and/or substrate, and their rate equations are correspondingly complex. For such enzymes, Michaelis–Menten kinetics are sometimes applicable at limiting conditions (such as velocities measured in the absence of product—that is, in zero time—using a large excess of all but one substrate), but this normally precludes any rigorous interpretation of the results. Oversimplifications of this kind have resulted in conflicting interpretations of the same data, leading to unnecessary controversy.

Recently, Cleland[2] has discussed rate equations derived from hypothetical mechanisms for enzymes having two or more substrates or products. Such techniques are extremely useful. Proposed mechanisms can be excluded if they do not predict the observed kinetic behaviour of the enzyme. Some enzymes, however, have very complicated mechanisms, consisting of perhaps ten steps. Many possibilities must then be considered, each resulting in a rate equation which may contain many hundreds of terms. Often the experimental evidence required to select between the possibilities is unobtainable by the available techniques. It then becomes necessary to reason directly from the experimental kinetic results, and to decide what generalizations about the mechanism can be inferred from these. It is intended here to suggest what deductions can be made about mechanisms from experimental rate equations. For the sake of simplicity, only non-random mechanisms will be considered.

A convenient technique for deriving steady state rate equations from proposed mechanisms is the schematic method of King and Altman[3]. This method gives rate as a quotient. The numerator consists of a positive and a negative term, containing respectively the product of the concentrations of all the substrates consumed, and the product of the concentrations of all the products formed. The denominator is a sum of terms containing most possible combinations of the concentrations of the various substrates and products. If an experimental rate equation is arranged in this form, an interpretation of the terms can be made from the significance of analogous terms in theoretically derived rate equations.

Numerator terms are related to the overall rate of production of substrates from product. The positive term is proportional to the overall rate of the combined forward reactions, and the negative term to that of the reverse reactions. Their ratio is the equilibrium constant for the reaction catalysed, and can be used to calculate the free energy change. If the numerator contains more than the two terms mentioned above, it follows that there is more than one way of producing products from substrates, that is to say, the reaction is non-ordered, and the treatment discussed here does not apply. If the numerator does not contain additional terms, it can be concluded that the rate of alternative pathways is negligible within experimental error. If the numerator contains only a positive term, the reaction can be considered to be irreversible, at least until more accurate measurements reveal the existence of a small negative term.

The denominator as a whole is proportional to the total enzyme concentration. It consists of combinations of terms relating to the concentrations of the various (free and combined) forms of the enzyme. The coefficient of any term is proportional to the fraction of the total concentration of the enzyme which consists of forms which can arise from sequences containing all the reactants in that term. In a reaction of n steps, then, any coefficient is the sum of a number of products of rate constants: one product for each possible combination of $n-1$ reactions which (*a*) is directional—that is, does not contain a cyclic process or both directions of a reversible reaction; and (*b*) consumes all and only the reactants governed by the coefficient.

Although many of the denominator terms are mechanistically unintelligible, two generalizations may usefully be made. First, terms which contain the concentration of a single reactant as the only variable represent the total binding power of the enzyme system (in the steady state) toward that reactant. The binding power of the enzyme system toward the various substrates and products can therefore be compared if there are terms containing these as the only variable. Second, it is sometimes informative to consider the denominator to be two sets of terms, one related to the relative concentration of free enzyme, and the other to that of bound enzyme, that is, the magnitude of one of the sets of terms represents the tendency of the enzyme to become free, the magnitude of the other its tendency to become bound. The two sets can be distinguished as follows. If the reaction is (as assumed) non-random, only one of the substrates and only one of the products will be found to react with free enzyme in the absence of all the others. Terms related to the tendency of the enzyme to become bound must contain the concentration of one or the other of these reactants, since a molecule of one of them must be consumed in any reaction in which free enzyme becomes bound. Terms related to the tendency of the enzyme to become free can contain neither of them, since a process involving either must result in the net consumption of free enzyme.

The mechanism by which factors such as temperature, *p*H, ionic strength, inhibitors or activators alter the coefficients of experimental rate equations can in this way be interpreted rigorously in terms of increased or decreased affinity of enzyme for substrate or product, increased or decreased tendency of the enzyme to become bound, or

an increased or decreased rate of the forward or reverse reactions.

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Lipoperoxidation of Rat Liver Microsomal Lipids induced by Carbon Tetrachloride

IT has been known for 17 years[1] that *d*-α-tocopherol can protect rats from the lethal effects of carbon tetrachloride. Anti-oxidant protection implies that the reaction of the liver to the toxin involves destructive lipoperoxidation. Under certain circumstances, lipoperoxidation can be detected by following the evolution of malonaldehyde by means of the thiobarbituric acid (TBA) reaction. We have monitored malonaldehyde evolution in *in vitro* experiments utilizing the microsome-supernatant fraction of rat liver, and have found that carbon tetrachloride can act as a pro-oxidant in this system. Diethyl ether and heptane were inert. These *in vitro* findings support the hypothesis that the hepatotoxic chloroalkanes have as one of their vectors of toxicity the initiation of destructive lipoperoxidation. If carbon tetrachloride acts similarly *in vivo*, it should be possible to find supportive evidence. We have found that malonaldehyde is readily metabolized by rat liver mitochondria, hence the TBA reaction cannot be used as a tool in the search for evidence of lipoperoxidation *in vivo*. However, the appearance of malonaldehyde is only one index of destructive lipoperoxidation. It has been known for 20 years, from the work of Bolland and Koch[2], that during the course of autoxidation of ethyl linoleate there occurs a rearrangement of double bonds into diene conjugation. The spectra of normal conjugated dienes, that is, molecules such as butadiene, are characterized by a very intense absorption, the so-called *K*-band[3], at 215 to 250 mμ (ref. 4). The spectra of peroxidized lipids are characterized by an intense *K*-band at 233 mμ, with a shoulder, due to ketone dienes, in the region from 260 to 280 mμ (ref. 2). The appearance of diene conjugation in peroxidized, unsaturated fatty acids, or their derivatives, is due to resonance following free radical attack on methylene bridges separating the double bonds in these compounds[5]. It should be borne in mind that microsomal lipid is largely phospholipid.

It appeared to us not unreasonable to imagine that if there were a free-radical attack on the methylene bridges of unsaturated fatty acid side-chains of microsomal lipids, resonance could occur, at least in a certain number of instances, with displacement of the organic free-radical electron toward the methyl end of the fatty acid. Subsequent formation of an organic peroxide, and cleavage of the carbon chain would result in degenerate complex lipids with diene conjugation in the fatty acid residues still tied to the glycerol moiety by way of an ester bond. Accordingly, lipids extracted from peroxidized rat liver microsomes should exhibit typical diene conjugation absorption, whereas lipids extracted from control, non-peroxidized rat liver microsomes should not. The expectation was realized. The left panel of Fig. 1, taken from Bolland and Koch[2], shows the typical diene conjugation absorption of a peroxidized unsaturated fatty acid (upper trace). The non-peroxidized lipid (lower trace) shows only simple end-absorption. The right panel of Fig. 1, upper trace, shows the absorption spectrum of the lipid extracted from rat liver microsomes peroxidized *in vitro* at 38° C, in air, in the presence of ascorbic acid, at *p*H 5·6. Typical diene conjugation absorption is present. The lower trace of the right panel of Fig. 1 shows only simple end-absorption for lipids extracted from an equivalent amount of microsomes which were also incubated in air at 38° C in presence of ascorbic acid, but with peroxidation prevented by addition of ethylenediamine tetraacetic acid (EDTA). The middle trace is the difference spectrum. There can be no doubt that peroxidation *in vitro* is accompanied by the appearance of typical diene conjugation absorption in the lipids extractable from the peroxidized microsomes. If the microsomes are protected from peroxidation by EDTA, the extracted microsomal lipid is completely devoid of diene conjugation absorption. If carbon tetrachloride acts *in vivo* by initiating autocatalytic, destructive, lipoperoxidation, it should be possible to extract from the liver microsomes degenerate complex lipids exhibiting the characteristic 233 mμ absorption of diene conjugation. Six rats were fasted overnight, following which three were given 250 μl. of carbon tetrachloride, in mineral oil, intragastrically, per 100 g body-weight. The remaining three rats received mineral oil alone. After 6 h the rats were killed and the liver microsomes recovered. Microsomal lipid was extracted in chloroform–methanol (2 : 1). The chloroform–methanol extract was forced into a two-phase system by addition of water. The recovered chloroform phase was dried down under oxygen-free nitrogen. The recovered lipids were redissolved in methanol and examined in the ultra-violet spectrophotometer. The mean difference spectrum, carbon tetrachloride-poisoned minus controls, is shown in Fig. 2. There is no doubt that the expected diene conjugation absorption is there. A similar experiment was carried out in which the rats were killed 90 min after carbon tetrachloride poisoning Typical diene conjugation absorption was also found in this experiment.

It has been suggested by Butler[6] and by Wirtschafter and Cronyn[7] that carbon tetrachloride is cleaved homolytically in the liver to yield trichlormethyl free-radical and monatomic chlorine. Rubinstein and Kanics[8] showed that hepatic metabolism of carbon tetrachloride takes place in the microsome fraction, in presence of the supplemented supernatant fraction. The work reported here supports the hypothesis that carbon tetrachloride is cleaved homolytically in or near the lipoidal components of the endoplasmic reticulum of the hepatic parenchymal cells. We envisage that free radicals, arising from carbon tetrachloride cleavage, then attack the methylene bridges

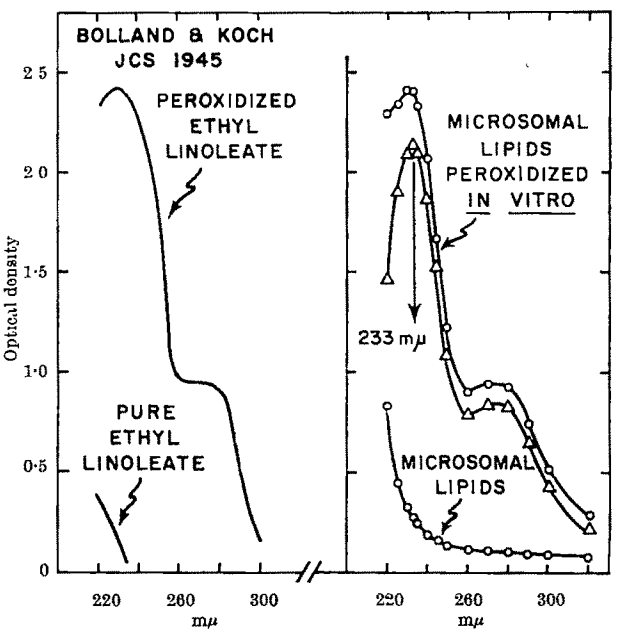


Fig. 1. Diene conjugation in lipids of rat liver microsomes peroxidized *in vitro*

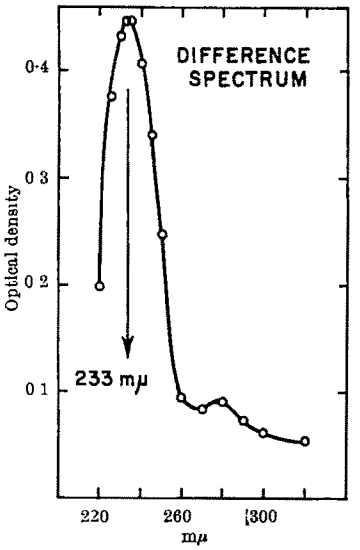


Fig 2. Diene conjugation absorption of rat liver microsomal lipids 6 h after carbon tetrachloride poisoning

of the unsaturated fatty acid side-chains of complex structural lipids of the endoplasmic reticulum, setting off autocatalytic chains of peroxidative lipid decomposition. Loss of physiological and biochemical properties of the endoplasmic reticulum would then follow, leading eventually to the well-known pathological consequences of carbon tetrachloride poisoning.

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Alkaloids as Possible Intermediaries in Plant Metabolism

SINCE their discovery 150 years ago, alkaloids have been the subject of innumerable research papers, practically all of which have been concerned with the occurrence, production in the plant and properties of the final molecular species. Even when the plant produces a series of closely related alkaloids, it has normally been considered sufficient to show their order of formation and which was the ultimate molecule. In all this work the tacit assumption has been that alkaloids are the end-products of interesting biosynthetic sequences and that functionally they represent slowly accumulating heaps of metabolic sludge. Only rarely is there any evidence of return to metabolic circulation and the quantities involved are extremely small[1]. However, some workers have shown that nicotine, which itself is derived partly from nicotinic acid[2], may not be an end-product substance but suffers extensive metabolic change, acting as a methyl donor to choline[3] and is also incorporated into insoluble organic substances[4], related alkaloids, amino-acids, sugars and other common metabolites[5]. Another approach has been to study the variation in alkaloidal pattern during plant growth; Hemberg and Flück[6] found some variation in total alkaloids in *Datura* species at 4-h intervals, and Heydenreich and Pfeifer[7] have demonstrated marked variation in the alkaloidal pattern in *Papaver somniferum* fruits at 6-h intervals. These variations suggest a dynamic role for the alkaloids, and our own work on developing fruits of *Conium maculatum* and *Papaver somniferum* has shown that even more marked variations occur at 3-, 2- and 1-h intervals[8–10]. Furthermore, our results indicate that the major alkaloids, conine and morphine, respectively, disappear rapidly soon after formation, again indicating that they are not 'dead-end' substances but may be the precursors of non-alkaloidal substances of metabolic significance. In order to confirm this and gain further information on the problem, we carried out a preliminary experiment by feeding radioactive morphine (the major alkaloid) to poppy plants (*Papaver somniferum* L.) in 1963. Examination of the fruits a few days later showed that the morphine had been converted into non-alkaloidal substances; accordingly, we decided to repeat the experiment more carefully the following year.

Radioactive morphine was prepared by feeding carbon-14 labelled tyrosine hydrochloride (universally labelled) to poppy plants and separating the now active morphine from the latex collected about a week after feeding. The purity of the radioactive morphine was checked by R_F values, melting point and reverse isotope dilution. In the first experiment, 5 mg, as the hydrochloride dissolved in water at *p*H 7·2, was fed to four poppy plants just below the developing capsules by the method already described[10], 18 days after petal fall. Three days after feeding, the capsules were gathered and the seeds separated and extracted with methanol. In the second experiment, 23 mg of another batch of radioactive morphine was fed to four plants and the seeds collected and treated as before, 5 days after feeding. The methanol extracts were examined for radioactivity, and the results recorded in Table 1 show that about 3–6 per cent of the fed activity was present in these extracts. This was in marked contrast to the alkaloidal fractions which were separated from the capsules and other parts of the plant (stem, leaf, root) which showed practically no radioactivity apart from the phenolic alkaloids of the capsule which contained about 0·01 per cent of the fed radioactivity.

Table 1

^{14}C-morphine fed	Seeds collected after feeding (days)	Total radioactivity in methanol extract of the seeds (d.p.s.)	Radioactivity incorporated (per cent)
5 mg containing 680 d.p.s.	3	20	3·0
23 mg containing 2,040 d.p.s.	5	116	5·7

The methanol extracts were examined by paper chromatography using *n*-butanol–water–acetic acid (5 : 2 : 1) as running solvent and spraying with Dragendorff and ninhydrin reagents. Appropriate spots were also cut out and their radioactivity measured in a scintillation counter. As was to be expected, no alkaloids were detectable in the seeds and only very slight radioactivity was present in the position on the chromatogram where morphine would occur. About 90 per cent of the total radioactivity was present in three diffuse ninhydrin spots of low R_F value (distinctly lower than that of morphine in the same system). The radioactivity of these ninhydrin positive substances must be derived from the body of the morphine molecule and not from the labile *N*-methyl group since the latter would be non-radioactive in the morphine we used. It is interesting to note that methanol extracts of seeds from plants fed with carbon-14 labelled tyrosine also showed similar radioactive ninhydrin spots which contained about 75 per cent of the total activity of the extracts. As we had more active material to work with, we examined seeds collected 3, 4 and 5 weeks after feeding and found that the radioactivity in the methanol soluble fraction decreased with age but that in the methanol insoluble fraction increased. It may well be that these ninhydrin substances are themselves gradually built into larger molecules as the seed develops. We are now preparing larger quantities of specifically labelled

morphine for further investigation of these interesting changes.

Our results are obviously of only a preliminary nature and leave unanswered the question of the fate of the 94–97 per cent of the morphine activity not accounted for. Clearly there are many ways in which the newly formed substances could be translocated, such as within the ramifying latex system or by transfer to the phloem and xylem vessels of the vascular bundles. Dispersal by these means to other parts of the plant might account for some of the missing activity. Some of the newly formed substances might be broken down into $^{14}CO_2$, for which, unfortunately, we were not able to test under the conditions of the experiment. In further experiments we intend examining the newly formed compounds as soon as possible after their formation in the latex.

Interest in the seeds stems from our earlier observation that there is no anatomical connexion between the latex vessels and the seeds[11]. Latex vessels occur in all parts of the plant except the stamens, ovules and seeds; alkaloids are also absent from these organs. The presence of derivatives of morphine in the seeds is therefore of some interest.

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Significance and Regulation of Methaemoglobinaemia

Beutler and Mathai[1] have rather simplified my thesis[2]. A central theme, that the methaemoglobin–oxyhaemoglobin and oxidized-reduced glutathione (GSSG–GSH) systems are loosely linked in a buffering system against the selective and destructive effects of different types of oxidizing compound, has already been discussed[3]. On the basis of published[4–10] and otherwise unpublished[2] observations, it was also proposed that the pentose phosphate pathway governs the equilibrium between methaemoglobin formation and GSH depletion, and thus generally between the degrees of methaemoglobinaemia and haemolysis in erythrocytes exposed to catalytic oxidants. The suggestion was made that, by providing a source of reductant energy which varies in degree and direction with the oxidant stress, the pentose phosphate pathway might well have played an important part in the evolution of the anuclear mammalian erythrocyte. These concepts, relating to the adaptive role of the pentose phosphate pathway, seemed essentially in accord with previous and concurrent observations concerning the role and control of the pentose phosphate pathway in other mammalian tissues (see refs. 11–22, and refs. 70–74 cited by Jandl *et al.*[23]).

Of more immediate relevance were the differences observed *in vitro*, which appeared sufficiently striking to warrant the following tentative classification of oxidant haemolysins[2]: (1) 'simple' oxidants such as nitrite, which cause methaemoglobin formation, with GSH depletion only if the pentose phosphate pathway is inadequate, and irreversible damage in normal cells only after prolonged exposure; (2) catalytic oxidants such as quinone, *p*-aminophenol and the phenylhydrazines, which cause in succession GSH depletion, methaemoglobin formation, oxidative destruction of haemoglobin, and osmotic hyperfragility, and which are relatively ineffective stimulants of NADPH-dependent methaemoglobin reduction; (3) catalytic oxidants such as naphthoquinones, which catalyse methaemoglobin formation and GSH depletion in normal cells potently and to a similar relative degree, cause irreversible damage only after gross GSH depletion, and are powerful catalysts of NADPH-dependent methaemoglobin reduction; (4) powerful catalysts of NADPH-dependent methaemoglobin reduction, such as methylene blue, which cause gross GSH depletion (and frank haemolysis *in vivo*[24]) only with an inadequate pentose phosphate pathway of GSSG reduction; (5) catalytic oxidants such as 2-naphthol and 'Primaquine', which selectively increase osmotic fragility relative to other changes; (6) aromatic compounds such as phenacetin and acetylsalicylic acid, which presumably oxidize erythrocytes only after conversion *in vivo* to an active derivative; (7) aromatic compounds such as 'Primaquine', which are active *in vitro*, but appear likely to exert an additional effect after degradation *in vivo*[24].

Because acetylphenylhydrazine causes complete loss of GSH before achieving methaemoglobin formation, prior formation of methaemoglobin by nitrite would not be expected to favour GSH depletion by this aromatic compound. The experiments of Beutler and Mathai[1] thus seem in agreement, rather than in disagreement, with my thesis. Clearly, their findings neither confirm nor refute the buffering effect of the oxyhaemoglobin-methaemoglobin system on the GSH–GSSG system in erythrocytes exposed to more potent methaemoglobin-forming catalysts, such as naphthoquinones, or to 'simple' oxidants such as nitrite. However, our observation that methaemoglobin formation usually precedes both haemoglobin destruction and osmotic hyperfragility suggests that the production of methaemoglobin may buffer the cell against irreversible oxidative changes, even by drugs which have first depleted GSH. The very conversion of oxyhaemoglobin to a reconvertible derivative, incapable of catalysing the generation of erythrotoxic hydrogen peroxide by oxidants[25,26], might well be regarded as a cellular adaptive mechanism.

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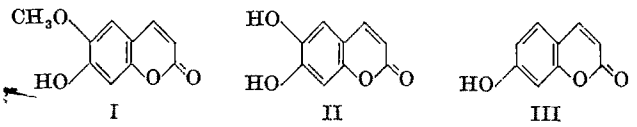
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Production of Coumarins by *Phytophthora infestans*

THE causal organism of potato blight, *Phytophthora infestans* (Mont) de Bary, has been subjected to very close investigation since it was first described just over a century ago. It is only in recent years, however, that it has been possible to culture this organism *in vitro* on a chemically defined medium, and very little work has been done on its secondary metabolism. Such investigations are of interest because they may provide some insight into the basis of the host–parasite relationship.

The tissue surrounding blight lesions in potato tubers of several varieties fluoresces strongly in ultra-violet light and at least part of this fluorescence is due to an accumulation of oxygenated coumarins and their glucosides. Scopoletin (I) and scopolin accumulate to the greatest extent[1], but increases in aesculetin (II) and umbelliferone (III) also occur[2] (Fig. 1).

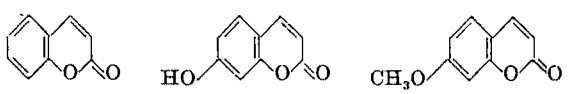


I II III

Fig. 1. Coumarins from blight lesions

Although the potato tuber is capable of producing these compounds[3], and in fact coumarin itself[2], since they are present in uninfected tubers, the possibility exists that *P. infestans* can also produce them. This has now been shown to be the case.

When cultured on an extract of French beans, prepared by blending 250 g of beans in 1 l. of distilled water and filtered through celite, an isolate of race 4 of *P. infestans* grew well, but no coumarins were detected either in the mycelium or in the culture filtrate. However, when grown on a synthetic minimal medium[4], small amounts (about 0·1 mg/l.) of three coumarins were produced. Although *P. infestans* grows well on this synthetic medium, better growth occurs on the French bean extract, and it may be that the formation of these compounds is stimulated by stress conditions as in certain higher plants[5–7]. The coumarins were isolated and shown to be coumarin (IV), umbelliferone (V) and herniarin (VI) by paper and thin-layer chromatography, characteristic fluorescence and reactions with base, R_t values in gas–liquid chromatographic analysis, ultra-violet spectra and, not least, odour (for coumarin and herniarin). The properties of the isolated compounds corresponded with those of authentic samples.



IV V VI

Fig 2 Coumarins produced by *P. infestans*

Recent work with higher plants has clarified the biosynthetic routes from phenylalanine to the coumarins[8]. Accordingly, radio-tracer experiments were carried out with *P. infestans* to determine whether similar or different pathways are operative. They involved the administration of 150 μmole/l. of generally ^{14}C-labelled L-phenylalanine and L-tyrosine and specifically 2-^{14}C-labelled *trans-p*-coumaric acid to one-day-old surface cultures growing on the minimal medium. Ten Roux bottles, each containing 200 ml. of medium, were used for both amino-acid experiments, and six were used for the *p*-coumaric acid experiment. Hyphal fragments, obtained by lightly blending the mycelium of seed cultures in a Waring blender, were used for the inoculum. After eight days growth, the mycelium was extracted with acetone and the culture filtrate with ethyl acetate. The incorporations of activity into coumarin and umbelliferone were measured by inverse dilution analysis[9]. Thus, known weights (about 12 mg) of unlabelled coumarin and umbelliferone were added to each organic extract and completely dissolved. The coumarins were then isolated from the extracts by repeated thin-layer chromatography on Kieselgel *G* using solvents (9 : 1 chloroform-methanol; chloroform) that effected their complete separation. Each was then eluted from the Kieselgel, fractionally sublimed, and crystallized to constant activity from water. The incorporation levels into coumarin and umbelliferone are shown in Tables 1 and 2. The herniarin present was not quantitatively measured, but the pattern of its activity was identical with that of the umbelliferone. It should be recognized that the low incorporation levels are due in part to the very small quantities of coumarins formed in the experiments.

Table 1. INCORPORATIONS INTO COUMARIN

Precursor	C p.m. fed	Percentage incorporation ($\times 10^{-2}$) Mycelium	Broth	Total
L-Phenylalanine	$3\cdot502 \times 10^6$	3 93	5 67	9 60
L-Tyrosine	$3\cdot362 \times 10^6$	0 170	0 316	0 486
p-Coumaric acid	$1\cdot081 \times 10^5$	0·264	0 315	0·579

Table 2. INCORPORATIONS INTO UMBELLIFERONE

Precursor	C p.m. fed	Percentage incorporation ($\times 10^{-2}$) Mycelium	Broth	Total
L-Phenylalanine	$3\cdot502 \times 10^6$	6 16	6 31	12 47
L-Tyrosine	$3\cdot362 \times 10^6$	0·298	0 060	0 358
p-Coumaric acid	$1\cdot081 \times 10^5$	2 30	5 83	8·13

The results show that phenylalanine is a good precursor of coumarin and umbelliferone. They further indicate that *p*-coumaric acid is a good precursor of umbelliferone, but not coumarin, and that tyrosine is not significantly used for either. The pattern of incorporation into herniarin matches that into umbelliferone.

The involvement of *trans-p*-coumaric acid in the biosynthesis of both umbelliferone and herniarin indicates that deamination of an aromatic amino-acid is part of the operating sequence. The negligibly small incorporation of tyrosine is good evidence that the pathway lies from phenylalanine to *trans*-cinnamic acid, which may then give rise to coumarin. The specific *para*-hydroxylation of cinnamic acid will result in *trans-p*-coumaric acid[10], the precursor of umbelliferone and herniarin. Clearly, these compounds may be ultimately derived from shikimic acid, as they are in higher plants[11].

The direct 7-hydroxylation of coumarin to give umbelliferone cannot be excluded, but the good incorporation of *p*-coumaric acid into umbelliferone suggests that the hydroxylation pattern is established at the cinnamic acid stage The methylation of umbelliferone to give herniarin may occur, and this would constitute a variant of the apparent pathway to herniarin in lavender[12]. The steps from the cinnamic acid stage to the lactone ring system may involve hydroxylation *ortho* to the side chain, direct attack by the acid group, or spirolactone rearrangement[8]. These alternatives are under investigation.

These preliminary observations permit a definition of the overall pathways that may give rise to the coumarins in *P. infestans* cultures (Fig. 3), since control experiments have demonstrated that their presence is a result of the metabolic activities of the fungus, rather than a spontaneous cyclization of cinnamic acids[13].

Thus, *P. infestans*, using glucose as its main carbon source, is capable of total syntheses hitherto observed solely in higher plants[14]. Its observed secondary metabolism bears striking resemblances to that of certain higher plants since similar intermediate compounds are involved in similar biosynthetic pathways.

The results suggest that *P. infestans* may contribute to the accumulation of coumarins which occurs in infected potato tubers. The apparent relationships of the synthetic mechanisms used by both host and parasite

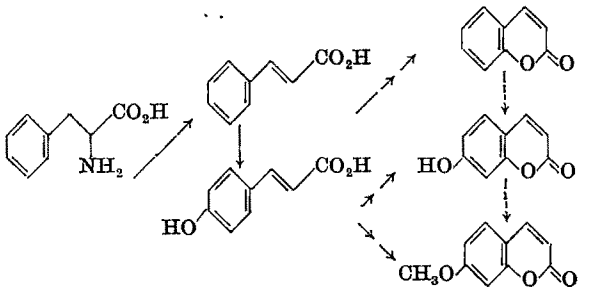


Fig. 3. Possible routes to coumarins in *P. infestans*

offer the possibility of an interaction between the two in which intermediates synthesized by one are further metabolized by the other. Although coumarin and certain of its oxygenated derivatives possess well-documented physiological activity[15,16], there is no evidence as yet to implicate them as primary factors in the control of the development of the host–parasite relationship.

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BIOLOGY

Phosphorous Metabolism and Auxin Action

SEVERAL workers have proposed that an increase either in utilization or synthesis of adenosine triphosphate (ATP) accompanies growth induced by applied auxin, and they imply that the increased rate of metabolism of ATP is concerned directly in the mechanism of auxin action. Sen Gupta and Sen[1] reported that treating coleoptile sections of *Avena* with indolylacetic acid (IAA) increased the amount of ^{32}P incorporated from orthophosphate into adenosine pyrophosphate and hexose phosphate. Marrè and Forti[2] reported a transitory increase in concentration of ATP 30 min after treating sections of etiolated pea stem with IAA. In the work reported here the effect of IAA on metabolism of phosphorylated compounds in sections of pea stems grown in light was investigated by measuring the amount of ^{32}P incorporated into nucleotides in short 'pulse-chase' experiments.

The stem sections were similar to those used by Galston and Kaur[3]. After cutting, they were suspended in phosphate buffer (10 mM, *p*H 6·1) or buffer plus 0·1 mM IAA (Sigma Chemical Co.) as required. Replicate batches of sections held in small stainless-steel baskets were treated with ^{32}P-orthophosphate by lowering into 4 ml. of the appropriate solution containing 0·15–0·2 mc./ml. After the time required, baskets and sections were removed, drained on paper tissue and washed in unlabelled medium.

Preliminary experiments demonstrated that ^{32}P-orthophosphate was washed from the 'free space' of the tissue in approximately 4 min and that treating with IAA did not alter the rate of uptake of ^{32}P-orthophosphate into the tissue. Treating sections with IAA for 30 min at 25° C induced elongation significantly greater than in controls. To examine the effect of treating sections with IAA on incorporation of ^{32}P into organic phosphate, replicate batches of fifteen sections were treated with ^{32}P-orthophosphate with or without IAA (0·1 mM). After 1 or 2 min, the sections were removed, drained and washed in unlabelled medium for 0·5, 1, 2 or 4 min. Phosphates were extracted and prepared for chromatography by the method of Bieleski and Laties[4]. The extracts were analysed by chromatography in two dimensions on Whatman No. 3 *MM* paper washed in 1 N acetic acid, using *n*-propyl acetate—90 per cent (v/v) formic acid–water (11 : 5 : 3, by volume)[5] followed by *iso*-butyric acid–ammonia (specific gravity 0·91)–water (66 : 1 : 33, by volume)[6]. Organic phosphates were located in preliminary experiments by co-chromatography. Autoradiographs were prepared by leaving in contact with X-ray film (Kodak, 'Blue brand') for 10–12 days. Circles of paper containing radioactive spots were placed in large planchets and radioactivity was measured using a Nuclear Chicago '*D* 47' gas-flow counter. When required, phosphates were eluted from the paper and from appropriate blank areas of equal size by soaking in sulphuric acid (10 mM) and total phosphorus estimated[7]. Specific activities were calculated using a mean value of total phosphorus from all four batches of tissue receiving the same treatment.

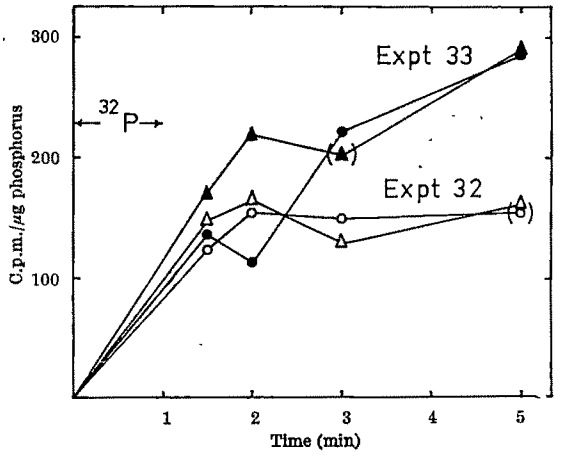


Fig. 1. Change in specific activity of ATP in sections of light-grown pea stem after treating for 1 min with ^{32}P-orthophosphate. Before and after treatment, the sections were washed in ^{32}P-orthophosphate buffer (10 mM, *p*H 6·1) with (△ and ▲) or without (○ and ●) IAA (0·1 mM). Treatment with IAA was not significant ($P = 0·05$) when tested by split-plot analysis. Symbols in brackets are calculated missing values

Fig. 1 shows that the change in specific activity of ATP during the chase in unlabelled medium was not altered by treating with IAA. In treated tissue, the concentration of ATP decreased and the ratio ADP/ATP appeared higher (Table 1) though a reliable estimate of ADP concentration was made only in experiment 33.

Label could not be detected in glucose phosphate on autoradiographs of all experiments. The label recovered in this fraction from tissue washed for 4 min is shown in Table 2.

Table 1. Mean Concentration of Nucleotides and the Ratio ADP/ATP in Tissue Incubated with and without IAA

Expt.	Treatment	Nucleotides: μmoles per 15 sections ADP	ATP	ADP/ATP
32	Control	0·0355*	0·0229	1·55
	IAA	0·0266	0·0150	1·77
33	Control	0·0331	0·0237	1·40
	IAA	0·0349	0·0116	3·00

* Single observation only.

Table 2. Amount of ^{32}P Recovered in Glucose Phosphate from Tissue Washed for 4 min in Unlabelled Medium

Expt.	Duration of pulse of ^{32}P (min)	^{32}P recovered in glucose phosphate (percentage of total ^{32}P esterified) Treatment IAA	Control	IAA/Control
28	2	28·2	17·4	1·62
30	2	9·4	3·8	2·47
31	1	7·1	4·5	1·58
33	1	8·9	2·7	3·30

If we represent the synthesis and utilization of ATP as

$$P_i \xrightarrow{k_1} ATP \xrightarrow{k_2} XP$$

and assume first order kinetics for these reactions,

$$(k_1 + k_2) \cdot t_{\frac{1}{2}} = \log_n 2$$

where $t_{\frac{1}{2}}$ is the half-time of maximum labelling of ATP (ref. 8). As treating with IAA does not alter $t_{\frac{1}{2}}$,

$$k_{1(\text{control})} + k_{2(\text{control})} = k_{1(\text{IAA})} + k_{2(\text{IAA})}$$

If we assume that the concentration of ATP is at a steady state and that the concentration and specific activity of orthophosphate at the phosphorylation sites are equal in both treated and control tissue:

$$\frac{k_{2(\text{control})}}{k_{1(\text{control})}} \cdot AT^{32}P_{(\text{control})} = \frac{k_{2(\text{IAA})}}{k_{1(\text{IAA})}} \cdot AT^{32}P_{(\text{IAA})}$$

As the concentration of $AT^{32}P$ is greater in control than in treated tissue,

$$\frac{k_{2(\text{IAA})}}{k_{1(\text{IAA})}} > \frac{k_{2(\text{control})}}{k_{1(\text{control})}}$$

We cannot tell directly from these results if treating the tissue with IAA increases k_2 or lowers k_1. The greater amount of ^{32}P-glucose monophosphate recovered from treated tissue suggests that IAA increases k_2, although decreasing utilization of glucose monophosphate could account for this effect also. However, glucose 1-phosphate is a precursor of cell-wall components, and increasing the rate of synthesis of glucose monophosphate could be an important part of the mechanism of action of IAA. Increase in label recovered in hexose monophosphate is the only change common both to light-grown (Table 2) and dark-grown[1] tissue treated with IAA, as the increased concentration of ATP (ref. 2) and increased recovery of $AT^{32}P$ (ref. 1) does not occur in the light-grown tissue (Fig. 1 and Table 1). In these experiments we cannot distinguish if label enters glucose monophosphate by way of photosynthesis or by the reactions catalysed by amylose phosphorylase or hexokinase. The investigation is continuing using a starch-free tissue grown in darkness.

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Bispecific Cell Aggregation in Sponges

Recently, Curtis[1] has pointed out that cell aggregation in sponges is not species specific as was generally thought[2] but that bispecific aggregates can be obtained by controlling the time of addition of the cell suspension of one species to that of the other.

In the case of a mixture of *Hymeniacidon perleve* and *Suberites* (= *Ficulina*) *ficus*, Curtis also obtained simultaneous intermingling of the cells in the aggregates. Previously, de Laubenfels[3], using two *Pachychalina*, and Sarà[4], with two related species of *Leucosolenia*, have also observed intermingling of cells of two different species.

To test the capacity to intermingle of cells from taxonomically distant sponges, we have used the following species of various orders of the class *Demospongiae: Tethya aurantium* (Pallas), *Polymastia mammillaris* (Bow.), *Hymeniacidon sanguinea* (Grant), *Petrosia ficiformis* (Poiret), *Haliclona elegans* (Bow.), *Ircinia variabilis* (O. Schmidt) and one species of the class *Calcispongiae*, *Clathrina coriacea* (Montagu).

In order to follow the intermingling, one of the pair of species was previously vitally stained over a period of 48 h with neutral red diluted 1 : 50,000 in sea water. By staining with neutral red it is possible to control the behaviour even of single cells adhering to mixed aggregates. Cell suspensions were obtained by squeezing 1 g of sponge through bolting cloth '*n. XXXOO*'. 100 mg of the resulting cellular suspension were diluted with 1 ml. of filtered sea water. Suspensions of $0{\cdot}7–1 \times 10^6$ cells per ml. of each species were obtained. For the experiments on mixed aggregations, 0·5 ml. of this suspension of each of the pair of species was used and the cells were allowed to settle on coverslips in Petri dishes, 5 cm in diameter, containing 5 ml. of filtered sea water.

Bispecific aggregations of the following pairs of *Demospongiae: Tethya aurantium* and *Hymeniacidon sanguinea*; *T. aurantium* and *Haliclona elegans*; *T. aurantium* and *Petrosia ficiformis*; *H. sanguinea* and *Polymastia mammillaris* were observed at different time intervals for 24 h at 18°–20° C. A large number of aggregates were formed with intermingling of cells of the two associated species (Fig. 1 *a*, *b*). Monospecific aggregates were present in smaller numbers.

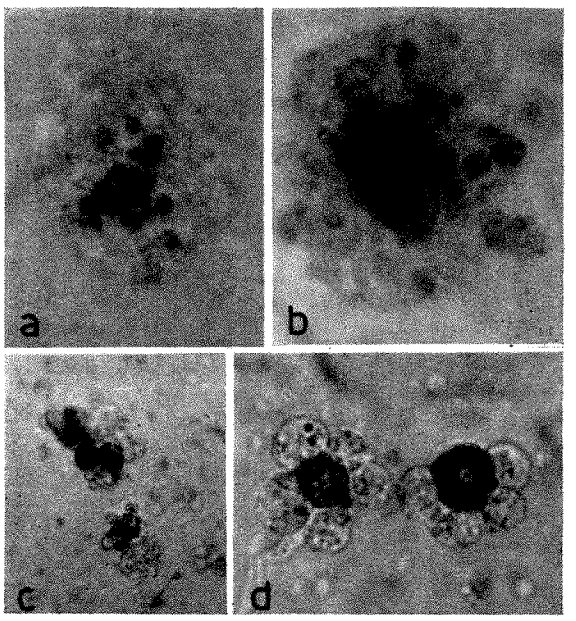


Fig. 1. (*a* and *b*) Bispecific aggregates of *Hymeniacidon sanguinea* (stained) and *Tethya aurantium* (after 3 h, ×850); (*c*) bispecific aggregates of *Clathrina coriacea* (stained) and *Tethya aurantium* (after 3 h, ×240); (*d*) cells of *Haliclona elegans* adhering to a cell of *Clathrina coriacea* (stained) (after 3 h, ×875)

A certain number of aggregates with intermingling of cells was also obtained in aggregations of *Clathrina coriacea* and *Hymeniacidon sanguinea*, *C. coriacea* and *Haliclona elegans* (Fig. 1 *d*), *C. coriacea* and *Tethya aurantium* (Fig. 1 *c*), consisting of mixed cell suspensions of a calcareous and a siliceous sponge. Monospecific aggregates were present in larger number. However, one or more cells of the other species were often incorporated in these monospecific aggregates of *Clathrina* or of the siliceous sponge.

In the aggregation of *Tethya aurantium* and *Ircinia variabilis*, a few small aggregates were formed and sometimes patches of cells of the two species loosely adhered. Only in this type of reaggregation was a significant intermingling of cells not observed.

In conclusion, bispecific aggregates of sponge cells may easily be obtained by mixing simultaneously cell suspensions of two species, even if they belong to different classes, *Calcispongiae* and *Demospongiae*.

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Mixed Cell Aggregation between Sponges and the Anthozoan *Anemonia sulcata*

OUR observations[1] show that sponge species of distant taxonomic position, even if they belong to different classes such as *Calcispongiae* and *Demospongiae*, may form bispecific cell aggregates after dissociation.

To test the capacity of sponge cells to form bispecific aggregates with cells of other animal phyla we have carried out experiments on mixed aggregation between the sponges *Clathrina coriacea* (Montagu) (*Calcispongiae*), *Tethya aurantium* (Pallas) Gray, *Haliclona elegans* (Bow.) and the anthozoan *Anemonia sulcata* (Pennant).

Sponges were vitally stained in advance for 48 h with neutral red diluted in sea water 1 : 50,000. With this method a cell marking in living aggregates was obtained that was more satisfactory than the difference in appearance between sponge cells and sea-anemone cells. Marking with fluorochrome-acridine-orange was also utilized.

Cell suspensions were obtained by squeezing the sponge tissue or sea-anemone tentacles through bolting cloth without chemical treatment or after treatment for 2 h with 0·004 ethylenediamine tetraacetate (EDTA) in 0·55 M sodium chloride buffered with 0·004 M hydroxymethylaminomethane (*tris*). Sponge dissociates were diluted with filtered sea water to give suspensions of $0{\cdot}7$–1×10^6 cells per ml. More concentrated sea-anemone suspensions (6–8×10^6 cells $+ 1$–$1{\cdot}5 \times 10^6$ *Zooxanthellae* per ml.) were used.

For the experiments on bispecific aggregation (*Clathrina-Anemonia*; *Tethya-Anemonia* and *Haliclona-Anemonia*) 0·5 ml. of these sponge and sea-anemone suspensions were used. Cells of both species were allowed to settle on coverslips in Petri dishes of 5 cm diameter containing 5 ml. of filtered sea water.

Controls were performed on monospecific aggregates of sponges and *Anemonia*. Cell aggregation after dissociation in Anthozoa is known in the sea feather *Leptogorgia*[2]. In this case aggregates are formed without a further development. In *Anemonia* the aggregation process is very slow and small aggregates can be observed only after some hours, while much of the cell material is not aggregated. *Zooxanthellae* and nematocysts are present in large numbers.

In all bispecific experiments mixed aggregates (Fig. 1) were formed: they are viable at 18°–20° C even 48 h after the beginning of the culture. Moreover, as compared to the monospecific controls, aggregation appears to be stimulated by the coexistence of sponge and sea-anemone cell suspensions.

The appearance of these aggregates is variable, depending in part on the different species of sponge utilized in the experiments. In some cases, adhesion of sponge and cnidarian aggregates leads to the formation of chains of aggregates; in these cases, the single aggregates are totally or nearly totally monospecific, whereas in other cases aggregates show an intimate mixture of sponge and sea-anemone cells.

Aggregates of *Tethya aurantium–Anemonia sulcata* obtained by mixing suspensions respectively of $1{\cdot}5$–2×10^6 cells (*Tethya*) and 6–8×10^6 cells $+ 1$–$1{\cdot}5 \times 10^6$ *Zooxanthellae* (*Anemonia*) per ml. after 24 h in synthetic sea water[3] and attachment on wooden substrates were transferred to natural sea water in aerated aquaria.

At 72 h a mosaic pattern of tissue patches of *Tethya* and *Anemonia* was observed (Fig. 2) after fixation in Bouin's liquid and staining in Carazzi's haemalum-eosin. Due to detachment of aggregates from their substrate we have so far been unable to follow their further development.

In conclusion, viable cell aggregates may be formed from mixtures of cell suspensions of species of two different phyla, Porifera and Cnidaria. A selective organization of tissue of both species takes place.

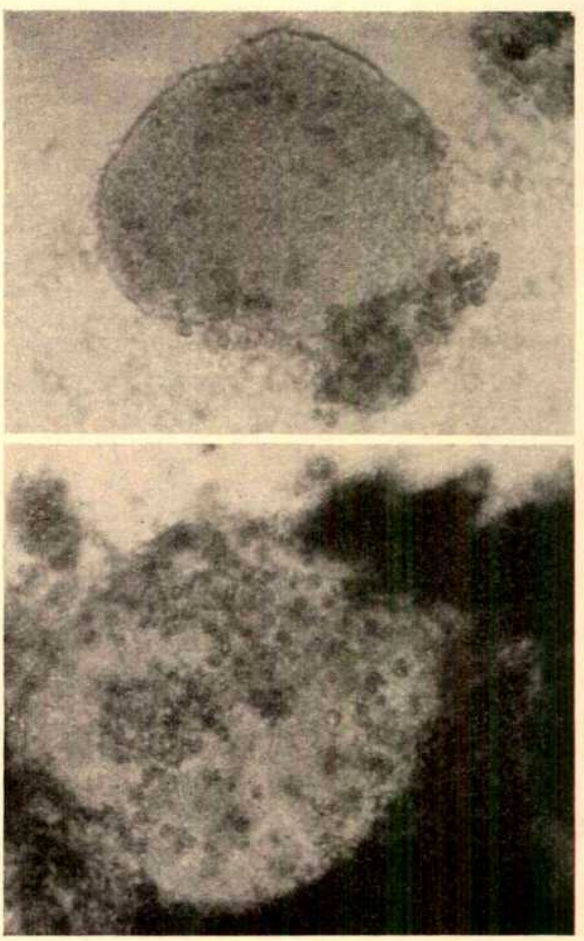


Fig. 1. Interspecific living aggregates of *Haliclona elegans* (dark, vitally stained) and *Anemonia sulcata* (light, unstained). After 11 h. ×225

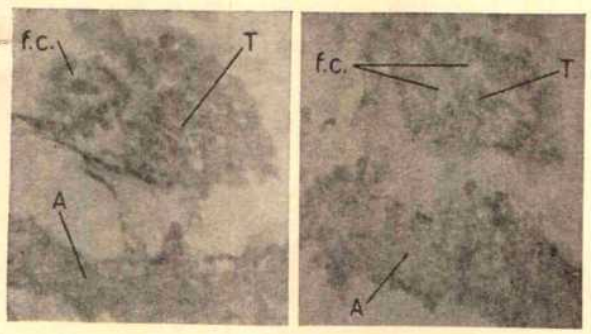


Fig. 2. Mosaic pattern from a *Tethya–Anemonia* aggregate (*T*, *Tethya* tissue, *A*, *Anemonia* tissue, *f.c.*, flagellate chambers). After 72 h. ×650

Our results agree with recent work on mixed cell aggregation[4,5] or heterokaryon formation[6,7] in emphasizing that compatibility may exist between cells from different species.

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Temporary Alteration of the Behavioural Repertoire of a Hermit Crab

DURING intraspecific interactions, hermit crabs rarely do physical damage to one another, but communicate by means of displays[1,2]. The aggressive displays executed by a crab during an interaction are determined by a number of factors. Each species has an aggressive repertoire, in which some behaviour patterns occur frequently and others are rare. Every individual has gone through physiological and psychological developmental sequences which affect that crab in any interaction[3]. Characteristics of the environment will also determine, in part, what will happen in an aggressive interaction.

When specimens of *Calcinus tibicen* Herbst, a common intertidal hermit crab of the Caribbean area, interact, several types of aggressive displays may be executed. The two most common displays have been termed the cheliped extension and the ambulatory raise[1]. They involve stereotyped upward-outward movements of the respective appendages. The frequencies of occurrence of these behaviour patterns during aggressive interactions have been determined[4]. Also it has been found that individuals of *C. tibicen* react to the presentation of aggressive models fashioned from conspecific specimens[1]. A series of presentation experiments were carried out to determine how a crab's immediate past experience might affect its behaviour in aggressive interactions.

Two models were fashioned from dried specimens of *Calcinus tibicen* and attached with Duco cement to glass rods, 6 mm in diameter. The ambulatory legs of one model were in a neutral position and its chelipeds in the raised position of a double cheliped extension. The chelipeds of the second model were in a neutral position but the first ambulatory legs in the fully extended position of a double ambulatory raise. Twenty individuals of *C. tibicen* were removed from a maintenance tank and placed in individual culture dishes, 8·5 cm in diameter, which contained a layer of sand. Three hours after placing in the dishes, presentations were started. Ten animals were presented with the cheliped extension model and ten with the ambulatory raise model. Each presentation consisted of slowly moving the model toward the animal, holding it about 5 mm from the animal for 3 sec, and then removing the model. The animal's reaction to the presentation was recorded (execution of a cheliped display, ambulatory display, retreat, ducking back into shell, move toward the model or no observable reaction) and the model presented to the next animal.

Thirty presentations were made to each animal the first day, forty each day for the next four days and ten the last day for a total of 200 presentations to each animal of four groups (Groups 3–6), while two groups (Groups 1 and 2) were given only 100 presentations (10, 40, 40, 10 on four consecutive days). Presentations were made in series of ten and at least 1 h elapsed between the end of one series and the start of the next. Three hours after the final series of presentations, the twenty animals of a group were placed together in an aquarium and the displays executed by each animal during aggressive interactions were recorded for 1–2 h. Each animal had been marked on the back of the gastropod shell it inhabited so that individual recognition was possible. No shell exchanges occurred during the observation periods. The animals to which the cheliped model had been presented will be referred to as 'cheliped-pre' animals and those presented with the ambulatory model the 'amb-pre' animals.

The responses of one group of animals to the presentations of the cheliped model were compared with the responses of the other animals to the ambulatory model by a two-by-two contingency test. The number of cheliped and ambulatory displays executed by the cheliped-pre animals during aggressive interactions was compared with the number of displays executed by the amb-pre animals by a chi-square contingency test. In addition, the frequency of execution of the two types of displays by the members of each class of animals was compared with the frequencies of these two types observed in the general aggressive interactions of untreated groups of *Calcinus tibicen* (ambulatory displays = 0·55 of aggressive displays executed, cheliped displays = 0·45) (ref. 4). Finally, the individuals were ranked within their group according to the number of displays, of the same type as the model, executed in response to the model presentations and according to the number of displays, of the same type as the model, executed during the general interaction period. These two rankings were compared by a Spearman *r* rank order correlation test.

The results for the six groups of animals are presented in Table 1. In all groups, the responses of the animals to the presentations of the two types of models were very

Table 1. RESPONSES OF SPECIMENS OF *Calcinus tibicen* TO PRESENTATIONS OF AGGRESSIVE MODELS AND THEIR BEHAVIOUR WHEN PLACED TOGETHER AFTER A NUMBER OF PRESENTATIONS

Grp.	No. of presentations		Responses to models Ch	Am	Displays during interactions Ch	Am	χ^{2}*	χ^{2}†
1	100	Cheliped-pre animals	116	24	60	43	3·4	
		Amb-pre animals	92	65	63	73	0·5	2·0
2	100	Cheliped-pre animals	37	13	48	36	2·4	0·7
		Amb-pre animals	5	37	44	43	0·1	
3	200	Cheliped-pre animals	246	135	49	22	7·0‡	94·1§
		Amb-pre animals	21	184	43	52	0·1	
4	200	Cheliped-pre animals	251	73	79	32	12·8‡	23·7§
		Amb-pre animals	31	171	54	87	1·2	
5	200	Cheliped-pre animals	255	30	79	50	7·3‡	
		Amb-pre animals	12	110	43	74	1·8	15·0§
6	200	Cheliped-pre animals	140	33	131	74	30·0§	20·8§
		Amb-pre animals	24	81	51	82	1·3	

* χ^{2} obtained by comparison with frequencies of displays observed in 1,000 interactions of untreated animals.

† χ^{2} obtained by comparison of cheliped-pre animals' behaviour with ambulatory-pre animals' behaviour.

‡ $P < 0.05$.

§ $P < 0.01$.

significantly different ($P < 0.01$). In no group was the rank order correlation significant. The behaviour of the two classes of animals during the second hour of interaction was not significantly different in any group ($P \geqslant 0.05$).

It is clear that the presentation of the different models elicits different responses. Cheliped displays were much more frequently executed at the cheliped extension model and ambulatory raises more frequently executed at the ambulatory raise model. This occurs during natural aggressive interactions between crabs[4] and appears to be an example of social facilitation[5]. Retreat from the models and no observable response to the presentation occurred frequently with both models and no significant difference in the elicitation of these acts by the two models occurred. One hundred presentations was not sufficient to effect a significant change in subsequent behaviour whereas 200 presentations did effect a change. However, the change was very temporary since the behaviour patterns of the two classes of animals during the second hour of interaction were not statistically different by the tests used. Individuals were exposed to both kinds of displays during the first hour of general interaction and this may have counteracted the effects of the model presentations.

In groups 3 to 6, the behaviour of the cheliped-pre animals was very significantly different when compared with that of the amb-pre animals. However, when the frequencies of execution of displays was compared with the frequencies observed in untreated groups of *Calcinus tibicen*, only the cheliped-pre animals appeared to show a statistically significant change in behaviour. These frequencies were obtained for individuals of populations collected and observed in Curaçao, N.A., and it is conceivable that the frequencies of displays are different in the Miami populations of this species. If this were so, the behaviour of both classes of animals could show a change when compared with the behaviour of untreated groups. That is, the behaviour of a crab in any given situation is dependent, in part, on its past experience insofar as the particular display that a crab executes is affected by the kind of displays recently executed towards it.

The data give little information as to the basis of action of these presentations, that is, how they effected a change. The effect of the presentations cannot be explained by simple conditioning. Removal of the aggressive model probably is a re-enforcing stimulus, but that re-enforcement was given whether the animal did nothing, retreated from the model, or executed a display at it. The lack of correlation between an animal's responses to the model presentation and its behaviour when placed with the other members of the group indicates that it was not the repeated execution of a given display by a crab that tended to increase the frequency of execution of that display. It appears that seeing a display temporarily changes the probability of execution of that display.

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Movements and Co-ordination of the Latero-frontal Cilia of the Gill Filaments of *Mytilus*

THE observations described here were made on very young mussels (*Mytilus edulis*) in which the valves are sufficiently transparent to allow direct observation of the gill cilia in the intact animal. In young animals, the structure of the gill filaments and their ciliature is essentially similar to that of adult mussels; some evidence was obtained that the same holds for the movements of the cilia. Our observations concerned the latero-frontal cilia, which are responsible for the filtration of suspended food particles from the sea water. With this material the frontal gill surface may be viewed. The latero-frontal cilia move to and from the observer and, consequently, are only momentarily in focus. This disadvantage was overcome by the use of Gregory's solid image microscope[1], which offers a greatly increased depth of focus when compared with the conventional microscope. With this instrument it was possible to observe the entire latero-frontal cilium during all stages of its beat.

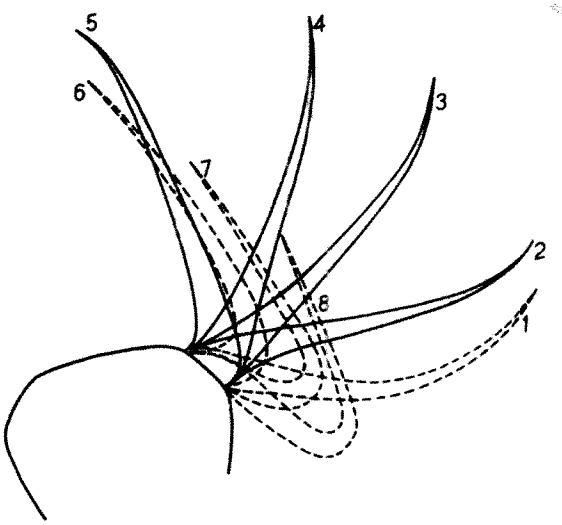


Fig. 1

Fig. 1 is a schema of the beat which is usually performed by the latero-frontal cilia of the intact mussel spat. During the effective phase of the beat (stages 2–5 in Fig. 1) only the base of the cilium bends, usually until the cilium has reached a position roughly perpendicular to the gill surface. In many cases, however, the amplitude of the beat is reduced and the movement is already reversed when the cilium has reached stage 4 or even somewhat earlier. The recovery beat (stages 6–8) starts at the base of the cilium while the distal part goes on moving in the effective direction. During recovery, the basal flexure moves distally along the cilium.

The velocity of the movement decreases after the recovery beat is completed. The movement from stage 1 to stage 2 takes at least as much time as the performance of both effective and recovery beat together. From stage 2 the movement suddenly increases in speed and reverses at stage 5 without perceptible delay.

The velocities of the effective and recovery beat differ with circumstances. When the lateral cilia are active, producing a current of water which supports the recovery beat, this phase of the beat lasts about as long as the effective one. When the lateral cilia are inactive, however, the recovery beat takes more time than the effect beat, presumably because of the increased water resistance. As a result, the duration of the whole cycle of the beat is somewhat prolonged in the latter case.

When the mussel is offered a dense suspension of food particles, one may assume that food retention by the animal diminishes[2]. In these circumstances the type of movement of the latero-frontal cilia appeared to remain unaltered, except that the trajectory of beat is shifted more or less in a frontal direction. The effective beat is now started from a position conforming to a more advanced stage, such as stage 3 in Fig. 1. In consequence of the shift the tips of the latero-frontal cilia of both sides of a filament can sometimes be seen to cross above the frontal filament surface. In most cases, however, the amplitude of beat is reduced simultaneously, in the same way as has already been described. Combination of these changes results in the effective beat again ending when the cilium is in a position perpendicular to the gill surface.

Under these circumstances the latero-frontal cilia sometimes execute quite another type of movement. While the base of the cilium stays at rest, a flexure travels along the cilium from the tip towards the base during the effective beat and in the opposite direction during the recovery beat. This type of movement, which has been described by Gray[3], is more frequently observable in excised gill fragments.

The frequency of beat of the latero-frontal cilia shows slight temporal and spatial variations, in the same way as described for the lateral cilia[4], but to a much lesser extent. On the average, a frequency of 7–8 beats/sec was most commonly found; extremes amounted to about 4 and 9 beats/sec. The prolonged duration of the beat during periods of rest of the lateral cilia mentioned earlier gives rise to a small (of the order of 0·2 beats/sec) acute decrease of beat frequency of the latero-frontal cilia when such a period sets in. The initial frequency of beat is restored as soon as the lateral cilia start their activity again.

The movements of the latero-frontal cilia are co-ordinated in such a way that cilia of one row beat alternately in phase, and each cilium differs from its immediate neighbours by half a phase. The wave-length, measured as usual between synchronously beating cilia, is equal to twice the interciliary distance, which in *Mytilus* amounts to 5–6μ. It is obvious that in a case such as this, where each 'wave' comprises two cilia only, waves as such cannot be evident. Nevertheless, a progressing movement, resembling a metachronic wave through the row of cilia, is quite often discernible. Unlike that in the lateral cilia, the 'wave' in the latero-frontal cilia moves on both sides of the filaments in a ventral direction. In many cases this direction reverses on the dorsal parts of the filaments. In between, there is a short zone, from which the 'waves' seem to originate. We are inclined to explain these phenomena by the fact that the gill filaments follow the curvature of the overlying valve, with the result that synchronously beating cilia will pass through the plane of focus in a metachronic sequence. The 'waves' originate in the part of the filament nearest to the microscope objective. Thus a metachronic movement is suggested which does not really exist. This explanation is supported by the possibility of transferring the 'origin' by moving the mussel in such a way that another part of the filament is brought nearer the objective.

The regularity of the co-ordinated latero-frontal ciliary beat appeared to be easily disturbed by external factors, such as lack of oxygen and decrease of light intensity, indicating that the automatism of the ciliary movement does not go so far as has generally been supposed. It may be for this reason that the regular movement of the latero-frontal cilia has not been observed by earlier investigators, who made their observations mainly on gill fragments taken from adult Lamellibranchs. In gill fragments we did not see the type of movement of the latero-frontal cilia, which, on evidence from our work with undamaged individuals, is considered to be normal; co-ordinated (flexural) movement is only occasionally seen in excised material. The observations above, which will be described in greater detail elsewhere[5], may illustrate the importance of the use of undamaged animals in the study of normal ciliary behaviour.

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Transport of Sediment by Crustaceans and Fish

Erosion and transportation of sediment by organic agents is a well-known phenomenon, and sediment transport is known to occur over large areas in many parts of the world[1]. However, the best-known examples of organic transportation are confined almost exclusively to coarse-grained sediments. We know of no well-substantiated examples of transport of sediment finer than coarse sand over distances greater than a few centimetres.

While working on the feeding habits of the pink shrimp (*Pandalus montagui*) from the River Crouch, Essex, we noticed small but significant amounts of fine sand (grain diameter 30–200μ) in the stomachs of a considerable proportion of the specimens examined. This material is taken incidentally with food, and does not appear to serve any digestive function (cf., for example, gastroliths). When shrimps were kept without food in the laboratory the stomachs were emptied of food and sand within a period of 24–36 h. We were able, by using a Coulter counter, to determine accurately the grain size distribution of these small quantities of sand. Samples of one hundred stomachs gave ample material for accurate counts to be obtained.

In some instances, the size frequency distribution of the sediment from the stomachs was identical to that of the bottom on which the shrimps were caught. In other cases there were small but significant differences.

It was possible, after making a sediment survey over the area in which the shrimps were found, to follow small-scale migrations by comparing the size-frequency distributions of material from the stomach with that of the substrate. This work will be published in detail elsewhere.

The weight of sand carried varied from 0·00027 g to 0·0038 g per individual. Large shrimps carried more material than small shrimps. There was no difference between the distributions of grain size in separate samples of large and small shrimps.

Similar observations were made on several fish species commonly found in the River Crouch and its associated offshore channels. Significant quantities of sand were removed from the digestive tracts of each species examined, but several whiting (*Merlangius merlangus*) and one sole (*Solea vulgaris*) were found to be empty of both food and sand.

Soles (*Solea vulgaris*), plaice (*Pleuronectes platessa*), whiting (*Merlangius merlangus*) and pouting (*Gadus luscus*) all carried sediment identical in grain size characteristics to the substrate in the sampling area (see Fig. 1).

One large skate (*Raia batis*) carried sediment with a grain size distribution matching that of sediment from an area 12 nautical miles (21 km) to seaward of the sampling area (see Fig. 1). It is perhaps significant that commercial catches of skate were taken by a local fisherman from this offshore area 24 h before the arrival of several skate in our daily trawl samples.

Shore crabs (*Carcinus maenas*) carried sediment with grain size characteristics similar to that from the lower intertidal mud flats adjacent to the sampling area. Mid-

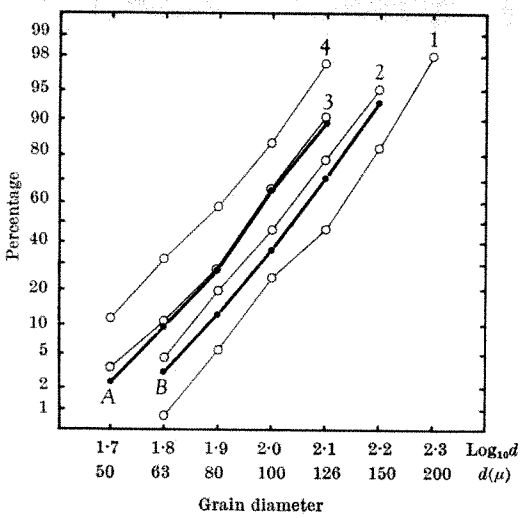


Fig. 1. Grain size distributions from stomachs and bottom sediments. Bottom sediments: ○——○, (1) East Swin Channel. 18 miles seaward of sampling area. (2) South Buxey. 9 miles seaward of sampling area. (3) Sampling area. (4) River Crouch. 8 miles upstream of sampling area. Stomach contents: ●——●, (*A*) Sole. (*B*) Skate

river sediment with similar grain size characteristics is found in the River Crouch about 3 miles upstream; however, it is reasonable to assume that the shore crabs had carried sediment from the adjacent intertidal area.

We found that for individuals with significant amounts of sediment in their digestive tracts, the weight of sediment was proportional to the body weight. In the species examined, 0·1 per cent of the total body weight was found to be sediment (grain size 30–200μ). It would seem that at any one time a significant proportion of the total mass of benthonic fish and crustacea is made up of sedimentary material and in some instances it is possible that this material is moved over a distance of several miles in a few hours.

It is doubtful whether organic transportation of sediment is of great significance in the River Crouch as normal sedimentation is relatively rapid (about 2·5 mm/yr.)[2], but in areas where sedimentation is relatively slow fish and crustaceans could well be the major agents of sediment transport.

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Colonization of Fresh Waters in the Republic of South Africa by *Lymnaea columella* Say (Mollusca : Gastropoda)

Lymnaea (*Pseudosuccinea*) *columella* Say 1817, described from specimens probably obtained in the vicinity of Philadelphia[1], is widely distributed in still and slow-flowing waters in the eastern United States of America, Mexico, Central America, Cuba and the northern and central regions of South America[1,2]. Fossil specimens are known from the Pleistocene of North America, and another species of the sub-genus has been described from

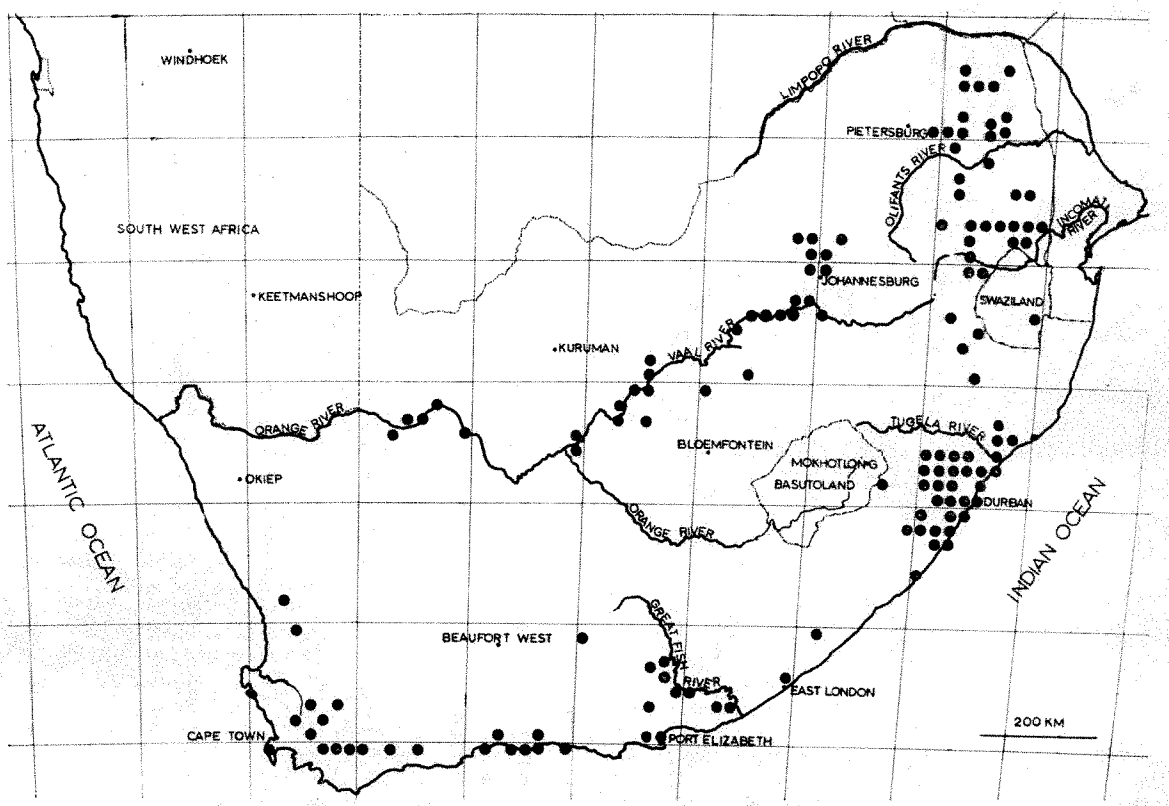


Fig. 1. Distribution of *L. columella* Say in the Republic of South Africa

e Pliocene of the same region[3]. *L. columella* is believed have been introduced by human agency, probably with aquatic plants, into Puerto Rico, Tenerife, South Africa and many European countries[4].

The earliest recorded collection of *L. columella* in Africa was made in 1944 at Jonkershoek Fish Hatchery, western Cape, South Africa[5]. While Harrison and Elsworth[6] record its presence in the Berg River, van Eeden[7] reports on its recovery from the Vaal River near Vereniging, and populations were later found in several other areas remote from the western Cape Province[8–10] and in Rhodesia[11].

During a survey, begun in 1956 and at present continuing, of the distribution of freshwater molluscs in the Republic of South Africa, many populations of *L. columella* have been found. These localities are plotted in Fig. 1, from which the impression is gained that, although first discovered in the western Cape Province, this snail could have entered the country at all the major ports, having been found at Cape Town, Mossel Bay, Port Elizabeth, East London and Durban. Although we have no records to support this theory, it seems probable that it could have spread to the eastern Transvaal via Lourenço Marques and might have been transported to Johannesburg from Durban with supplies for the aquarium trade. Once in the Vaal River catchment it could have spread down-stream in this river and into the Orange River, where it has already been located west of Upington.

It is reasonably certain that *L. columella* was either absent from South Africa or very rare before 1944 as this species was not known to Connolly[5], who thoroughly reviewed the freshwater Mollusca of the region on the basis of extensive material collected by himself and many other collectors; there are no specimens of *L. columella* in material collected during the first half of the present century in the collection of the Natal Museum. It thus appears likely that the greater part of the present distribution of *L. columella* in South Africa has been established within approximately 20 years. The rapid dispersal achieved by this snail may be related to its well-developed capacity for self-fertilization[12], and in view of proposed developments of irrigation schemes in the Orange River, whereby the ecology of large areas, at present arid, will considerably changed, it is probable that this range will be considerably extended in future.

In Europe *L. columella* has been reported only in botanical hothouses, but in South Africa this snail has in many areas become one of the most abundant freshwater molluscs, occurring in a variety of natural and artificial habitats. One factor contributing to this success may be the ability of the snails to exist on moist mud often several centimetres away from free water, thereby inhabiting a niche not occupied by any of the indigenous freshwater molluscs. On the other hand, *L. columella* also leads a submerged existence and has frequently been found in company with indigenous species, including *Bulinus* (*Physopsis*) *africanus* (Krauss) and *Biomphalaria pfeifferi* (Krauss), the snails concerned in the transmission of human bilharziasis, and *Lymnaea natalensis* Krauss. Competition might be expected to occur in this situation, but at present no evidence can be offered regarding the effect on these snails of the introduced species.

The successful colonization of a new area by a freshwater snail may have important ecological consequences that depend on the trematode parasites for which the snail is capable of providing an intermediate host. *L. columella* from Durban has already been successfully infected with South African strains of both *Fasciola hepatica* Linn. and *F. gigantica* Cobbold[13] and cercariae recovered, so that its widespread occurrence in South Africa may be expected to have serious economic consequences.

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PHYSIOLOGY

Formation of Acid Mucopolysaccharides by Fibroblasts and Serosal Cells

THE colloidal iron method modified for electron microscopy[1,2] has been used to investigate the distribution of acid mucopolysaccharides in the granulomatous reaction that takes place on the surface of implants of egg-white placed in the peritoneal cavity of the rat[3]. This reaction leads to the formation within 4 or 5 days of a thin layer (25–50μ) of avascular fibrous tissue which is covered 1 or 2 days afterwards by a layer of serosal cells. The fibroblasts and the serosal cells both possess a very prominent Golgi apparatus and a well-developed endoplasmic reticulum. When granulomata are reacted in bulk with colloidal iron the Golgi apparatus of both types of cell gives a positive colloidal iron reaction; this is confined to the round, more superficial vesicles (Fig. 1) and the central flattened sacs do not react. In contrast the endoplasmic reticulum never takes up iron. Isolated vesicles scattered at random throughout the cytoplasm also take up iron.

These results suggest that acid mucopolysaccharide is formed, or at least that its synthesis is completed, within the Golgi apparatus of fibroblasts and serosal cells. In the case of the fibroblasts it may be assumed that this acid mucopolysaccharide is released into and becomes a major component of the ground substance of the collagenous tissue that surrounds these cells ; and in the same way the positively-reacting Golgi apparatus of the serosal cells may be linked with a fairly thick layer of acid muco-

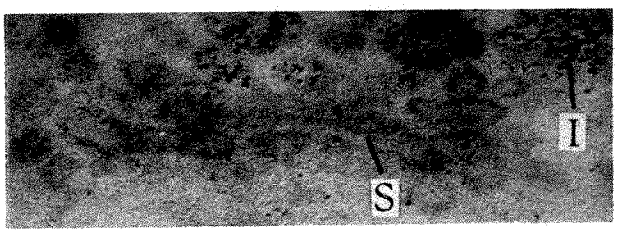


Fig. 1. Electron micrograph of part of Golgi apparatus of a fibroblast. Numerous iron particles (*I*) have collected within several of the larger vesicles, but none are present within the central flattened sacs (*S*). (× 120,000)

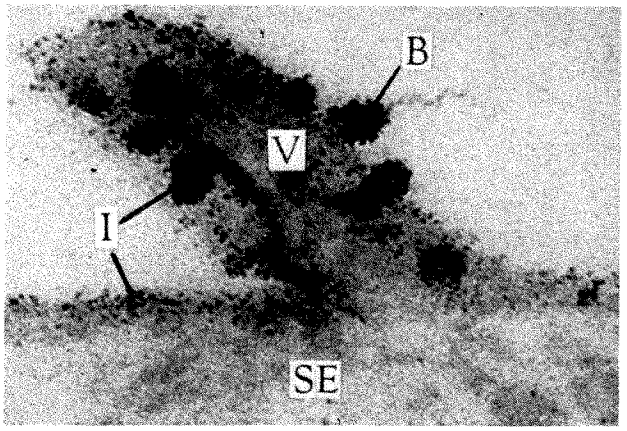


Fig. 2. Electron micrograph of the outer surface of a serosal cell (*SE*) on the surface of the implant. Iron particles (*I*) adhere to the plasma membrane of the cell and its villous process (*V*) and large numbers of them coat the surface of spherical bodies (*B*), approximately 300 Å diameter, on the surface of the villous process. (× 174,000)

polysaccharide that covers the external surface of these cells. This layer also shows focal concentrations which, when they take up colloidal iron, appear as rounded bodies 300 Å in diameter (Fig. 2). Incidentally, it is the appearance of this layer and particularly of these bodies that provides the first sign of differentiation of the serosal cells, around 5 days, from cells that also appear to act as precursors of the fibroblasts.

The colloidal iron method has good specificity, and further evidence for believing that the sites taking up iron do contain acid mucopolysaccharide is provided by the fact that when the tissues are treated with testicular hyaluronidase, after fixation and before embedding in 'Epon 812', no uptake of iron occurs.

Evidence already exists linking the Golgi apparatus with the formation of acid mucopolysaccharides. Thus in chondrocytes radioactive sulphate becomes localized within this organelle[4] and in goblet cells glucose-6-^{3}H does likewise[5]; and the Golgi zone of several varieties of normal and tumour cells gives a positive colloidal iron reaction in the light microscope[6].

The findings presented here are in accordance with this earlier work.

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Plasticity of Reactions to Light in Frogs and a Possible Role for the Pineal Eye

THE reaction of frogs to light varies considerably. Negative phototaxis as high as 80 per cent has been reported with tests in a two-choice jumping stand or Y maze[1]; in another Y maze the distribution between positive and negative responses was about even[2]. In yet other tests a strong positive reaction to light was seen[3], sometimes reaching the 90 per cent level[4]. The importance of the exact behavioural situation used to assess the level of positive or negative responses to light can be demonstrated with a sequential choice apparatus (Fig. 1). In this, different batches of *Rana temporaria* showed some variability in the initial choice between a black or white surrounded water compartment. But, once in the water, there was a 5–1 probability, at the least, that at the second choice point the dark area would be selected, even if the first response had been to the lighter side. Much higher negative phototaxis scores are therefore obtained if second choice is taken into account. The first response appears to sample only part of a behavioural sequence.

What physiological mechanisms underlie this behavioural plasticity in the response to light? Muntz[4,5] has suggested that positive phototaxis is mediated by activation of a system of 'on' fibres projecting to the diencephalon, because the electrophysiological response of these fibres was maximal to blue light, and blue light was also the most effective in producing positive reaction to light. It was also proposed[2] that the red rods might act as an inhibitory mechanism and that the development of the rods in ontogeny was responsible both for the increasing relative effectiveness of blue light and the higher level of negative phototaxis in older animals. In this theory the same mechanisms control the wave-length preferences and the level of positive or negative response. However, a number of considerations suggest that there may be separate mechanisms for determining what wave-length the frog selects and those regulating the sign of the photic response.

To begin with, subsequent analysis[1] of Muntz's[4] data showed that the bulk of positive scores were not due to phototactic responses because the animals had been forced. Since the areas from which the frogs made the choice was dark the animals were, in a sense, showing a negative reaction to light, until forced, when the blue was maximally selected. But there was nothing to show that the same mechanisms controlled whether the animal moved out of the dark area or not and the selection of the wave-length. In fact, behavioural preference for blue has been seen both in situations where there are positive responses to light, and in those giving mainly negative responses. In the experiments of Boycott *et al.*[1] the level of negative phototaxis was manipulated by changing the experimental milieu of the frog, but there was a preference for blue over green in nearly every condition.

An alternative to the red rods for controlling the level of positive or negative phototaxis is the pineal eye. Boycott *et al.*[1] found that placing an opaque cover over the starting area in a black–white choice situation had two effects: the frogs stayed in the starting area longer and they showed a decrease in their preference for the black over the white stimulus (the same effects can be seen here in Table 1, cols. 3 and 4). Measurements showed that covering the starting area reduced the amount of light coming down into the frog from above much more than that reflected off the stimulus panels from in front.

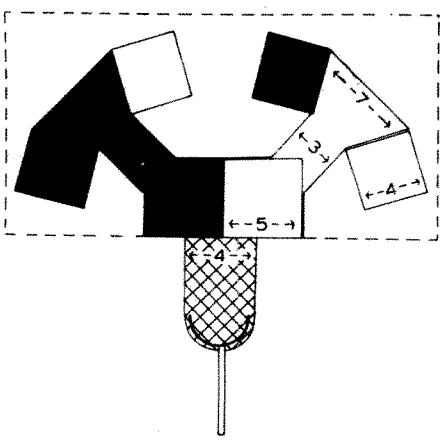


Fig. 1. Plan of sequential choice apparatus for frogs. Measurements in inches. Area within dotted line is under water, except for the walls of the apparatus not shown in this figure. Cross-hatched area is starting area about 2 in. above the water level

Table 1

Batch. Date. Experimenter	Starting area illuminated by cold cathode tube light: Opaque hats	Trans-parent hats	Differences between opaque and transparent hats, one-tailed test	Unop-erated controls	Starting area dark: Unop-erated controls
Batch 6 Aug. R.K.	21*W*(0) 7*B*(0) 14·5 $n=7$	7*W*(2) 15*B*(0) 21·7 $n=6$	$P<0.05$ $z=1.65$		
Batch 8 Oct. N.M.	22*W*(3) 13*B*(2) 12·9 $n=10$	11*W*(2) 26*B*(1) 20·2 $n=10$	$P<0.01$ $z=2.53$		
Batch 9 Oct. N.M.	24*W*(3) 19*B*(2) 24·7 $n=12$	24*W*(7) 15*B*(2) 20·2 $n=12$		17*W*(0) 29*B*(2) 23·0 $n=12$	15*W*(22) 2*B*(17) 34·8 $n=14$
Totals	67*W*(6) 39*B*(4) $n=29$	42*W*(11) 56*B*(3) $n=28$		17*W*(0) 29*B*(2) $n=12$	15*W*(22) 2*B*(17) $n=14$
Percentage of all unforced responses made to black	36·8	57·1		63·0	11·8

W, *B*: shows unforced responses to white and black sides. In brackets are the number of forced responses to black and white sides. n = No. of animals per group. The other figures show mean latencies in sec for the unforced responses.

(For a diagram of this apparatus see Mrosovsky[6]). The pineal eye, lying on top of the head, is a structure well situated to mediate behavioural differences such as those produced by reducing the light coming down from above the animal. Moreover, the pineal nerve runs to the diencephalon and light inhibits the electrical activity of the pineal nerve[7]. Therefore it is suggested here that the pineal system interacts with other diencephalic mechanisms in controlling phototaxis. To test this hypothesis, since placing the animals in darkness will alter the contribution of the red rods (due to Purkinje shift) as well as the activity in the pineal nerve, some experiments were made with frogs in which the pineal eye was selectively covered.

A simple method for covering the pineal eye is shown in Fig. 2. Frogs, adult *R. temporaria*, were lightly anaesthetized (placed in a jar with ether for 25–75 sec). A pliable opaque plastic hat was fixed between the lateral eyes with two stitches. The position of the hat was such that it should also screen the photosensitive area in the brain itself[8] from overhead illumination. Translucent hats of similar weight were fitted on to other groups of frogs. The animals were then light adapted for at least 12 h in boxes described previously[1]. Each frog was given four trials in a two-choice apparatus. A diagram of this has been given elsewhere[1]. The starting area was illuminated from above by a cold cathode tube. The black and white stimulus panels were illuminated by a 150-watt bulb about 5·5 ft. above, and by daylight from a window behind. The starting area was painted matt black inside to restrict the light reaching the frogs from the walls as much as possible when the cold cathode tube was on. If no response was made within a minute the animals were forced by means of a rod and an attached plaque at the back of the apparatus. Animals in each batch were tested on the same day by the same experimenter, except batch 9 which was run in two replications separated by a week.

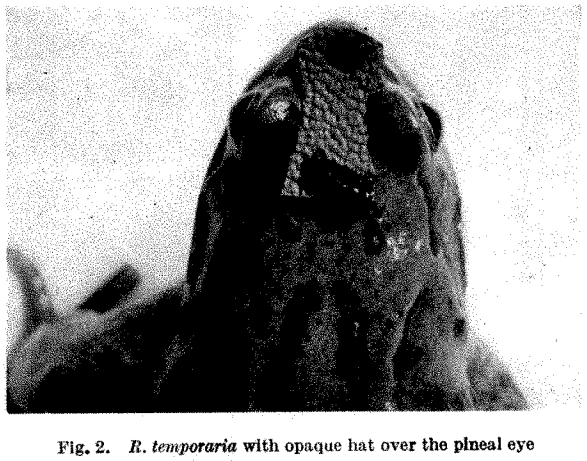


Fig. 2. *R. temporaria* with opaque hat over the pineal eye

Table 1 gives the results of these experiments. The significance of the differences between opaque and translucent hatted groups was assessed as follows: each animal was given a score for black–white choice on the basis of its unforced responses. *S* was then computed and the *z* value obtained was calculated. The test was in effect a Mann–Whitney with ties. For two batches the opaque-hatted animals showed a significantly greater choice for the white stimulus panel than the translucent-hatted ones. Somewhat similar behavioural effects have been seen in lizards: animals with the 'third eye' removed appeared easier to catch and emerged from cover more than controls[9].

However, in the third batch tested here the opaque hats did not increase the choice of white stimulus above the level for translucent-hatted frogs. A number of possibilities might account for this. The pineal system may degenerate in some animals: Dodt and Heerd[7] found that the electrophysiological response to light in the pineal nerve could not be found in about 30 per cent of the animals they tested. More probably the failure to get high scores for the white stimulus in these opaque-hatted frogs is connected with the already relatively high scores for this in other animals in this batch. It was previously suggested[1] that some of the variation in the phototaxis between different batches of frogs is associated with the latency of the response; but the relationship has not been fully worked out. These differences between different batches of frogs make it necessary to manipulate variables within given batches and test groups at the same time.

The fact that the significant differences between the opaque and translucent hatted animals were found in some batches tested on the same day, and in the same conditions, suggests that the pineal system may make some contribution in controlling phototaxis. But these effects are probably closely dependent on the details of the apparatus used. In other experiments, with an open jumping stand with grey walls of the type used by Mrosovsky[6], covering the pineal eye had slight or no effect. Moreover, in experiments shown in Table 1, covering the pineal eye with the present method did not increase the latency of the moving out of the starting area, whereas covering the starting area with an opaque lid[1] or turning off the cold cathode tube light has this effect (Table 1). Other systems must also, therefore, be important in controlling the level of the positive or negative reaction to light. If the richness and diversity of the behavioural repertoire are examined and analysed in different situations a more complete idea of the controlling systems may be found. To explain the behaviour of the frog from physiological abstractions may give too simple an idea of the mechanisms at work.

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Failure of Thymectomy in New-born Guinea-pigs to influence Contact Sensitization

RECENT evidence indicates that thymectomy in new-born laboratory animals of some species may result in immunological deficiencies. These may become manifest as compromised capacity to form circulating antibodies[1], as delayed homograft rejection[2,3], as diminished capacity to develop tuberculin-type sensitivities[1], and as lowered resistance to infection[1]. These deficiencies may be related to a reduction in the number of circulating lymphocytes and a failure of germinal centre development, as observed in neonatally thymectomized mice[2].

There is evidence in favour of an important part played by mononuclear cells in allergic contact sensitivity. The dermal infiltrate and blister fluid generally contain significant numbers of these cells[4]. Allergic contact sensitivity has been passively transferred in guinea-pigs by means of leucocytes[5]. However, the results of attempts at such transfer in man have been conflicting[6,7]. The importance of the regional lymph nodes in the sensitization process also has been demonstrated[8].

Decreased sensitizability to dinitrofluorobenzene has been shown in some cases of ataxia telangiectasia associated with thymic aplasia[9], and in a case of lymphopenic ('Swiss') agammaglobulinaemia associated with thymic aplasia[10]. On the other hand, agammaglobulinaemias without lymphopenia or decreased thymic tissue showed normal responses to dinitrofluorobenzene[10].

The purpose of the present experiment was to test the effect of thymectomy in new-born guinea-pigs on the subsequent development of contact sensitivity to 2,4-dinitrochlorobenzene (DNCB) and to ascertain whether any observed changes correlated with any changes in blood leucocyte counts.

New-born offspring of Hartley strain albino guinea-pigs were divided into a thymectomized group, a sham-operated control group and a non-operated control group. Operations were performed under 'Nembutal' anaesthesia usually within 32 h and no later than 56 h after birth. Thymectomy, with certain minor modifications, was carried out by the method described by Park[11]. Sham-operated animals were treated similarly but without excision of the thymus. The non-operated controls were anaesthetized with 'Nembutal' but were otherwise not manipulated.

Sensitization was attempted in all animals when one month old by dropping 0·05 ml. of 2 per cent DNCB in alcohol on the clipped nuchal areas and gently spreading with a glass rod on five successive days. The degree of induced sensitivity was assayed one month later by contact skin-testing with 0·2 per cent, 0·09 per cent and 0·05 per cent dilutions of dinitrochlorobenzene in olive oil and with olive oil alone. Reactions were rated from 0 to 4, depending on the degree of erythema.

The majority of animals were killed with an overdose of 'Nembutal' when 4 months old. White blood cell counts and differential counts were carried out on blood specimens, usually obtained by cardiac puncture just before death.

No consistent differences were noted in the susceptibility of neonatally thymectomized guinea-pigs to become sensitized to dinitrochlorobenzene as compared with sham-operated or non-operated control guinea-pigs (Table 1). The total mononuclear and polymorphonuclear cell counts tended to be lower in the thymectomized animals compared with the controls. However, the differences were not always statistically significant. The wasting described by other authors in neonatally thymectomized animals of other species was not observed in the guinea-pigs.

Table 1. DEGREE OF SENSITIVITY TO DNCB ONE MONTH AFTER SENSITIZATION IN FIFTY-SEVEN NEONATALLY SENSITIZED GUINEA-PIGS*

Type of treatment	Thymectomized (23 animals)			Sham-operated (18 animals)			Non-operated (16 animals)		
Percentage of DNCB in olive oil	0·2	0·09	0·05	0·2	0·09	0·05	0·2	0·09	0·05
Average sensitivity ratings in arbitrary units	2·3	1·1	0·5	2·0	1·1	0·6	2·0	1·4	0·7

* In 7 thymectomized, 5 sham-operated and 5 non-operated animals a second course of sensitizing exposures was given after they failed to become sensitized after the first course.

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Respiratory Changes during Electrical Stimulation of Rhinencephalic Structures in the Foetal Guinea-pig

THE rhinencephalon regulates a number of vegetative and somatic functions such as the respiration, blood pressure, reflexes and tonus of musculature, and movements of the intestine[1–3]. The most important role of the rhinencephalon seems to lie in the higher organization and integration of somatic and vegetative regulative functions.

As regards the different parts of the rhinencephalon, those which have the most distinct influence on the respiration are the gyrus cinguli and insulo-orbito-temporal cortex. During electrical stimulation of these areas in adult animals, there have been observed both activation and inhibition of the respiration, dependent on the parameters and localization of the stimulus, and the condition of the animal[2].

No attention has been focused on the functional role of these rhinencephalic areas during foetal life. The present work is concerned with the influence of electrical stimulation of the rhinencephalon on the respiration pattern in foetal, young and adult guinea-pigs.

Sixteen guinea-pig litters were used with an average of two to three foetuses, four young (weight 120–370 g) and five adult (weight 640–1,000 g) guinea-pigs. The foetuses were measured and their weights were ascertained for estimation of their age in accordance with Draper's length and weight curve[4]. Foetuses were aged between 28 and 64 days (the full term is 65 days). The dams were narcotized with 'Thiogenal' (methyl-thiogenyl-2[1]-penthylthiobarbituric acid sodium, Merck, 100 mg/kg). Only light narcosis was applied. After sectioning of the abdomen and the uterus, the foetuses were exposed without the umbilical cord being touched. The temperature of foetuses was maintained at a constant height (37° C) by covering the foetus with warm paraffin oil. The animals were tracheostomized, and the brains exposed for electrical stimulation. Monopolar stimulation with monophasic square wave pulses was used. The stimulus parameters were: frequency 1–150 c/s, duration 1–10 msec, amplitude 1–8 V, average current 0·002–24 m.amp, with the stimulation lasting 3–5 sec. The structures corresponding to the precallosal area, the Sylvian fissure and orbito-temporal cortex of the rhinencephalon, were selected for investigation in pursuance of the results obtained by Kaada, who,

by electrical stimulation of these areas in the cat, recorded extremely distinct responses. The precallosal area was exposed by pulling the opposite hemisphere to the side or by removing a part of it. The orbito-temporal cortex was reached stereotaxically, or by lifting the brain. The electrodes were left in position with a view to localizing the stimulation point. The respiratory volume was measured by floating chamber technique, using a photocell, and registered together with the stimulus on a two-channel graph. In the smallest of the foetuses, the respiratory movements were observed only visually. The condition of the foetus was estimated from the electrocardiogram.

In the group of adult guinea-pigs serving as controls, it was possible to reproduce only partially the results obtained earlier in the cat, rabbit and monkey. Even on application of a strong stimulus (200 c/s, 10 msec, 10 V), no changes in the respiration were obtainable from the precallosal area of the cingulate gyrus, or from the area of Sylvian fissure. When we applied the same method to cats serving as controls, we obtained a distinct inhibitory effect from the cingulate gyrus on the respiration. The orbito-temporal cortex of the guinea-pig was sensitive to the electrical stimulation (Fig. 1). Both inhibition and facilitation of the respiration appeared, depending on the localization of the electrodes. However, during stimulation the respiratory rate did on occasion become irregular, and short arrests of respiration were noticed. At times, on the other hand, the respiratory rate rose distinctly (Fig. 2, III*A*). The clearest inhibitory and facilitatory effects were obtained with stimulus parameters of 50 c/s, 10 msec and 5–8 V, although the changes mentioned above were still apparent when a pulse frequency as low as 1 c/s was applied.

In the group of young animals (2–11 weeks old), the findings were similar to those obtained with the adult animals. Both diminished and raised respiratory rates were observed. In an animal three weeks old there occurred a respiratory arrest which lasted the whole time of stimulation. Immediately after the electrical current had been switched off, the spontaneous respiration returned to normal (Fig. 2, II*A–B*).

As regards the group of foetuses, electrical stimulation of the surface of the dura mater at different points of the convexity of the brain triggered 'gasps' in those foetuses in which spontaneous gasps had previously occurred. By stimulation of the orbito-temporal cortex, the gasps became stronger and more regular from the beginning of application of the stimulus. Electrical stimulation of these areas also induced breathing in those foetuses which had not as yet breathed at all (Fig. 2, I*A–B*). All the foetuses exceeding an age of 45 days displayed respiratory movements on electrical stimulation. The youngest one which could be made to breathe was 39 days old, although no foetus showed respiratory movements in another litter of the same age. For the sake of comparison, some older foetuses were made to breathe by compressing the umbilical cord and by pinching the skin. The respiratory movements obtained in this way were similar to those resulting from electrical stimulation (Fig. 2, IV).

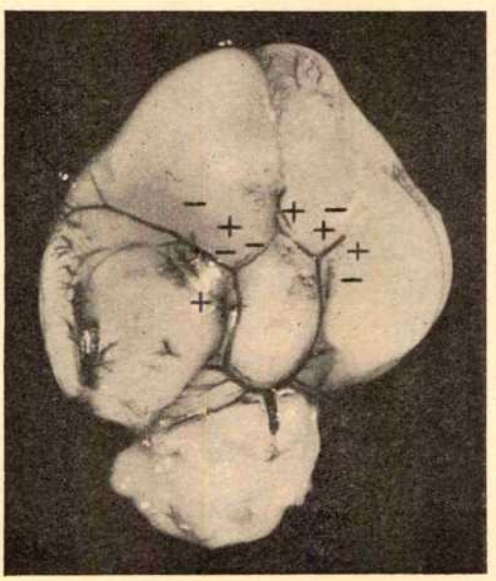


Fig. 1. The areas in the orbito-temporal cortex of the guinea-pig foetus which gave the facilitatory (+) and inhibitory (−) respiratory responses when stimulated electrically

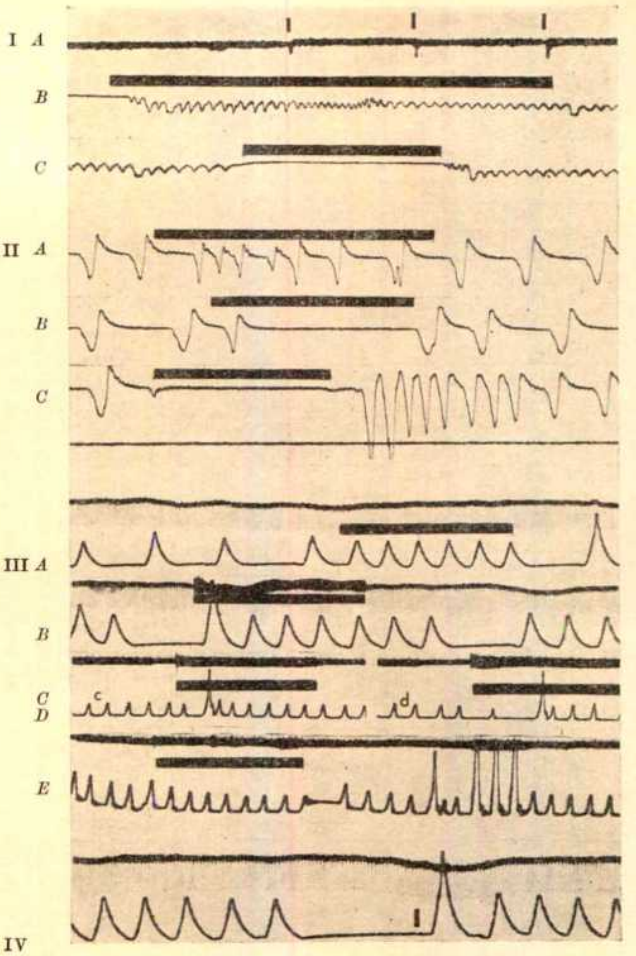


Fig. 2. The effect of electrical stimulation of rhinencephalic structures on the respiration. The stimulus is indicated with a black bar above the tracing indicating the respiration. I, *A–C* are from foetuses. *A*, A foetus, 60 days, gasping in the step of the stimulus; *B*, a foetus, 62 days, started to breathe when the stimulus was operated; *C*, the same foetus as in *B*, but another stimulation point of the cortex. A clear inhibition of respiration during the stimulus. The respiration started weakly again after the current was switched off. II, *A–C* are from a young guinea-pig, two weeks old. *A*, Acceleration of the respiratory rate; *B*, inhibition of respiration; *C*, an almost complete inhibition and a post-inhibitory acceleration of the rate and increase of the volume after the stimulus was switched off. III, *A–E* are from adult guinea-pigs. *A*, Acceleration of the respiratory rate; *B*, acceleration of the respiratory rate and increase of the first breath; *C* and *D*, two degrees of inhibition. In both cases a post-inhibitory deep breath; *E*, a weak inhibition during the stimulus, but after the stimulus a complete cessation of respiration. IV, An increase of the respiratory volume when the nose was pinched. The effect is quite similar to the effect of the electrical stimulus

The present results indicate that the higher centres of the rhinencephalon have already developed into functioning structures during the foetal life of the guinea-pig. The appearance of the different respiratory responses to electrical stimulation indicates an early differentiation of the vegetative regulatory functions in the rhinencephalon. At about the same time (41 days), electrical activity begins in the parietal cortex, indicating the functional readiness of these areas[5].

The rhinencephalic system in the adult guinea-pig apparently does not attain such a versatile development as that of the cat. Otherwise, it would be difficult to understand why the cingulate gyrus in the adult guinea-pig did not respond to stimulation, although this has been indicated as an important area for regulation of the vegetative functions in the higher mammals. The cingulate gyrus is phylogenetically younger than the orbitotemporal cortex, so it is possible that in lower animals all the functions of the cingulate gyrus have not yet developed.

The anaesthesia used in such experiments always exercises some influence on the respiratory functions; in this work, accordingly, an endeavour was made to use as light a barbiturate narcosis as possible. Such a form of anaesthesia does not affect the respiratory functions of the brain cortex[6]. To bring about muscular relaxation of the dam, curare was used; this cannot exert any effect on the musculature of the foetus, as curare in low doses does not pass the placental barrier[7]. During the experiments the reflexes of the foetuses were tested and found to be vigorous, so that it can be presumed that the respiratory changes obtained in this work are equivalent to reality.

The significance of the rhinencephalon for the functions of the human foetus and new-born infant is not accurately known. From the findings in the experiments described here, it is possible that it plays a part in initiating respiration during delivery. It has been stated that neuronal factors influence the respiration on parturition, but the exact way in which these events are regulated is not understood.

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Reciprocal Influences of the Ventromedial and Lateral Hypothalamic Nuclei on Blood Glucose Level and Liver Glycogen Content

SINCE the discovery by Bernard[1] that puncture in the floor of the fourth ventricle results in hyperglycaemia and glycosuria, many investigations have been carried out on the rise in blood glucose level and the appearance of glycosuria after stimulation of the various areas of the diencephalon.

In this laboratory, Shimizu[2] has investigated the relationship between changes of blood glucose level and the nuclei of the hypothalamus by selective stimulation of each nucleus in rabbits, and has shown that blood glucose increases after stimulation of the hypothalamic nuclei belonging to the medial hypothalamic area (sympathetic area) while it decreases after stimulation of the lateral hypothalamic nucleus (parasympathetic area). Similar, but transient, changes of blood glucose following hypothalamic stimulation have also been observed in adrenalectomized, thyroidectomized and hypophysectomized rabbits[8]. The increase in blood glucose level after electrical stimulation of the hypothalamus might be due to promoted glycogenolysis or gluconeogenesis in liver, or the fall of blood glucose utilization, but the exact metabolic events involved in these changes of blood glucose are still obscure.

In the present investigations the previous observations on the changes of blood glucose following hypothalamic stimulation were extended, and the results are presented here showing that stimulation of the ventromedial hypothalamic nucleus (VMH) caused a rapid increase in blood glucose level followed by a pronounced decrease in liver glycogen content, whereas stimulation of the lateral hypothalamic nucleus (LH) resulted in a decrease in blood glucose level with an insignificant change of liver glycogen content.

Male rabbits weighing about 2,500 g were used. The VMH and the LH were stimulated electrically as previously described[4] except that 20 sec stimulations were repeated every 4 min for a 12- to 20-h period. At various times indicated during the stimulation, about 1 ml. of blood was taken from the auricular vein with a heparinized syringe. Serum was immediately separated by centrifugation and the concentration of blood glucose was determined by the *o*-aminobiphenyl method of Shibata[9]. At the end of the electrical stimulation, the animals were killed under ether anaesthesia. Liver was perfused, *in situ*, with isotonic potassium chloride solution and then immediately homogenized in a glass homogenizer with nine volumes of the ice-cold potassium chloride solution. To 2 ml. of the homogenate was added 2 ml. of cold 10 per cent trichloroacetic acid (TCA) and the precipitate of proteins was separated by centrifugation. The precipitate was washed seven times with 2 ml. of 5 per cent TCA until no detectable glycogen was extracted. The resulting supernatants were combined and diluted to 20 ml. with 5 per cent TCA. 2 ml. of this solution was analysed for glycogen according to van der Vies's anthrone method[6]. The position of the stimulating electrode in the hypothalamus was checked histologically in serial sections after the experiment.

Fig. 1 shows the changes in blood glucose level during hypothalamic stimulation. Shortly after the onset of stimulation of the VMH, blood glucose level was increased about 60 per cent, and increased level of blood glucose was maintained during 5–8-h stimulation, but the level returned gradually to normal when the repeated stimulation was continued for a longer period. On the contrary, stimulation of the LH caused a decrease of about 20 per cent in blood glucose level during 8-h stimulation, and it returned to normal level by the end of a 20-h stimulation period. In the course of a long stimulation period, such as for 20 h, hormonal balance would be shifted so as to restore the level of blood glucose to normal.

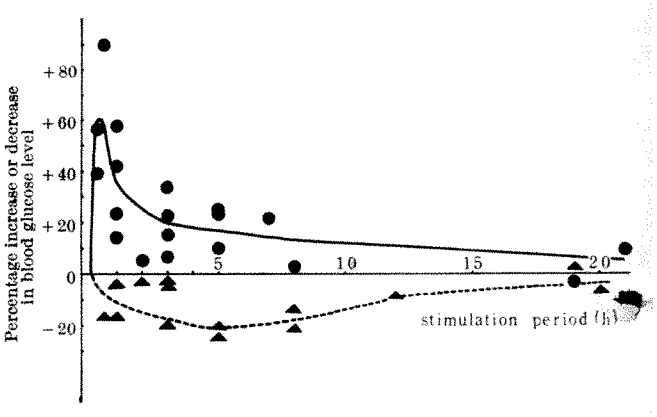


Fig. 1. Changes in blood glucose level during hypothalamic stimulation of rabbits. —●—, Stimulation of the ventromedial hypothalamic nucleus; - - -▲- - -, stimulation of the lateral hypothalamic nucleus

Table 1. Liver Glycogen Content after Hypothalamic Stimulation of Rabbits

	Control	VMH*	LH*
	(mg of glycogen/g liver)		
Summer season	20·9	0·8	19·7
	27·9	1·1	20·4
	25·7		
	21·4		
Mean value and standard deviation	24·0 ± 2·9	1·0	20·1
Winter season	56·0	28·9	56·0
	65·0	20·8	52·2
	49·3	32·0	76·5
	75·0	36·7	77·5
	67·5	19·6	62·0
	68·8		50·0
Mean value and standard deviation	63·6 ± 8·5	27·6 ± 6·6	62·4 ± 11·0

* Liver glycogen was measured in each rabbit after stimulation of the ventromedial hypothalamic nucleus (VMH) or the lateral hypothalamic nucleus (LH) for 12–20 h as described in the text.

Liver glycogen content after stimulation of the hypothalamus for a 12–20-h period is shown in Table 1. A marked decrease in liver glycogen content was observed when the VMH was stimulated. As the liver glycogen content was greatly affected by dietary composition and seasonal factors, all the animals were subjected to fasting during the day before killing and the data of two different seasons are included in Table 1. The liver glycogen content of control animals was much lower in summer than in winter. Almost all glycogen of the liver was depleted in summer, and about half the liver glycogen was depleted in winter, by stimulation of the VMH. Thus, the depletion rate of liver glycogen (ratio of level in stimulated animals to level in control animals) differed considerably between two seasons, but the absolute value depleted during stimulation of the VMH was approximately the same in both seasons (20–30 mg of glycogen per g liver was depleted). In both seasons, an insignificant effect on glycogen content was observed when the LH was stimulated.

These results lead us to assume that stimulation of the VMH promotes the degradation of liver glycogen to cause an increase in blood glucose. The VMH is one of the nuclei in the sympathetic area of the hypothalamus (and the LH is in the parasympathetic area), according to previous investigations on the hypothalamus[7]. Therefore, these results suggest that excitement of the sympathetic area would result in an acceleration of glycogenolysis in liver. This assumption is further fortified by the findings[8], recently obtained in this laboratory, that the activities of glycogen phosphorylase and glucose-6-phosphatase in the liver were rapidly and pronouncedly increased after stimulation of the splanchnic nerve of rabbits.

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Hypo-osmotic Re-absorption due to Active Salt Transport in Perfused Collecting Ducts of the Rat Renal Medulla

Urine reaches its maximal osmolality in the collecting ducts of the renal medulla[1], but the mechanisms causing the transformation from isotonic tubular fluid are in dispute. Most modern attempts to explain the concentrating mechanism are based on the hypothesis of Wirz, Hargitay and Kuhn[2], that the loops of Henle function as counter-current multipliers to raise the medullary interstitial osmolality so that water can be extracted from the collecting duct urine by simple osmosis. Experimental evidence[3,4] supports the validity of this hypothesis only in the outer medulla; the major increase of osmolality occurs in the inner medulla where the loops of Henle are not capable of active salt transport[5] and so cannot be countercurrent multipliers. In this communication I shall propose an alternative hypothesis for the inner medulla and present the results of experimental tests of its more unusual features.

The collecting ducts of the inner medulla reabsorb salt actively[1]. As will be discussed later, the osmotic work provided by this active transport can concentrate the urine provided that a hypo-osmotic reabsorbate is generated. Stationary microperfusion techniques were used to explore this possibility. Male Wistar rats, 250–300 g body-weight, had free access to food and water until the time of the experiment, when they were anaesthetized with ether. The left kidney was excised and from it the entire inner medulla was removed and submerged in a bath maintained at 37° C and bubbled gently with 100 per cent oxygen. The piece of tissue was fixed in place by suction applied from below, and was allowed to incubate for 30 min before the perfusion experiments were begun. A polyethylene catheter (*O.D.* 70–100μ) filled with coloured 'Ricinus' oil was then inserted into the orifice of a collecting duct at the tip of the papilla and advanced about 1 mm up the tubule so that oil injected from the catheter filled the entire tree of collecting ducts draining into the catheterized duct. A sharpened micropipette containing a test solution was next placed in the lumen of one of these collecting ducts lying on the surface of the medulla. With this arrangement the test solution could be injected and isolated between oil columns in segments of collecting duct located 2–4 mm from the tip of the papilla. The perfusate was collected into a second oil-filled pipette after the desired perfusion period had elapsed. The osmolality of the specimens and of the bath fluid was measured in a thermoelectric microfreezing point apparatus[6]. The composition of the various solutions is given in Table 1.

The behaviour of collecting ducts perfused in this manner is qualitatively similar to that of proximal and distal convoluted tubules studied *in vivo*[7–9]: the collecting ducts reabsorb sodium against an electrochemical potential gradient, transmural water flow accompanies net solute flow, and active salt transport is inhibited by cardiac glycosides. These results will be presented in detail elsewhere, while this report is restricted to experiments dealing with the osmolality of the reabsorbate.

The purpose of these experiments was to see whether active salt transport by the collecting ducts could lead directly to reabsorption of hypo-osmotic fluid, in circumstances close to those found *in vivo*. For the first group of experiments concentration values of the bath were selected to correspond to the medullary interstitium near the junction of the inner and outer medulla. The perfusion fluid had exactly the same composition, except that it contained no vasopressin. Although there were no concentration gradients across the epithelium, the volume of the perfusate decreased with time, because of active salt reabsorption. The osmolality of the fluid remaining in the tubule increased progressively, as can be seen from Fig. 1; this could have happened only if the fluid absorbed from the tubule was hypo-osmotic to both the initial perfusion fluid and the bath.

Table 1. Composition of Bath and Perfusion Solutions

Expt.	Na	K	Ca	Mg	Cl	HCO_3	H_2PO_4	Urea	Glucose
(1) Bath	250	8·0	3·6	1·05	251	11·9	0·33	343	7·7
Perfusion	250	8·0	3·6	1·05	251	11·9	0·33	343	7·7
(2) Bath	423	16·1	3·6	1·05	432	11·9	0·33	425	7·7
Perfusion	237	49·9	3·6	1·05	280	11·9	0·33	684	0

All ionic concentrations are in m.equiv./l.; urea and glucose given as mmoles/l. Both bath solutions contained vasopressin, 20 mμ/ml.

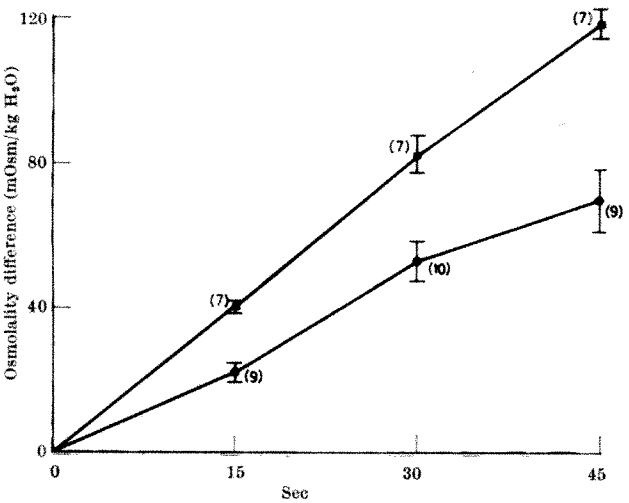


Fig. 1. Changes in perfusate osmolality as a function of time, expressed as the difference between the perfusate and a specimen taken from the bath. Bars indicate ± one standard error of the mean; figures in parentheses are the number of perfusions. Closed circles depict the results from experiment (1), while the squares are data from experiment (2)

The luminal sodium concentration *in vivo* may never be as high as in the adjacent interstitium, and in fact is known to decrease progressively toward the tip of the papilla even while the interstitial salt concentration is increasing[1]. Thus, a second set of experiments was necessary to determine whether or not hypo-osmotic reabsorption continues when bath and perfusion concentrations reflect these dissimilarities. The relative proportion of sodium chloride to urea used in the first series was retained for the bath solution of the second series, but the perfusion solution had higher urea and potassium concentrations than the bath. The osmolalities of bath and perfusion solutions were identical, but greater than those in the first series. These relationships mimic those found *in vivo* in antidiuresis at a point about midway toward the papilla. The volume of the perfusate again decreased with time while the osmolality of the perfusate increased to levels higher than the bath and the initial perfusion solution. These experiments show that active salt reabsorption by the collecting ducts leads directly to the formation of a hypo-osmotic reabsorbate under conditions close to those found *in vivo*.

At least two different models have been published that predict hypo-osmotic reabsorption coupled to active salt transport[10,11]. Whether either of these is applicable to the collecting duct cannot be decided yet, but the observations reported here provide a sufficient basis for formulating a new hypothesis of urine concentration.

Any acceptable model of the inner medulla in antidiuresis must explain certain observations: the osmolality of the interstitium increases along the corticomedullary axis to a maximum at the tip of the papilla[2]; this increased osmolality is due almost entirely to increased concentrations of sodium chloride and urea[12]; the reabsorption of a hypo-osmotic fluid causes the osmolality of collecting duct urine to increase toward the tip of the papilla[1]; the osmolality increase in the collecting ducts closely parallels that in the interstitium[4,13]. These facts cannot be explained without some source of osmotic work in the inner medulla[14]. The collecting ducts are the only structures capable of this work, and it is relevant to examine their role in the concentrating mechanism.

Consider a small element of volume in the collecting duct lumen from which salt is actively reabsorbed. Since the reabsorbate generated by salt transport is hypo-osmotic to the original tubular fluid, the fluid remaining becomes more concentrated. This element of volume then moves axially down the collecting duct lumen to the next level; again active salt transport generates a reabsorbate hypo-osmotic to the tubular fluid. Note, however, that the tubular fluid at the second level is already more concentrated than at the first level. If the absorbate osmolality is governed by concentrations in the tubular fluid, the reabsorbate at the second level will be more concentrated than the reabsorbate at the first level. Repetition of these steps along the length of the inner medullary collecting ducts would produce parallel increases of the osmolalities of collecting duct urine, of the collecting duct reabsorbate, and thus also of the interstitial fluid, all with maxima at the tip of the papilla. Because the osmolalities of all three fluids are so closely interrelated, the actual differences among them need not be large or even detectable. In this view, the collecting duct reabsorbate is the primary determinant of interstitial osmolality. The countercurrent exchange properties of the loops of Henle and vasa recta maintain the osmotic stratification achieved by hypo-osmotic reabsorption along the collecting ducts, and sodium chloride and urea accumulate in the interstitium because these are the only two solutes that leave the collecting duct lumen in appreciable quantities—the former by active transport, the latter by passive process. The high osmolality of the medullary interstitium is not only a consequence of hypo-osmotic reabsorption, but is also its necessary concomitant. If the interstitial osmolality did not increase *pari passu* with the urinary osmolality, transmural osmotic gradients would result. These gradients would cause osmotic flow of water back into the lumen of the collecting ducts and limit the maximal osmolality that could be achieved. Thus, the collecting ducts can concentrate the urine only because they penetrate a region the function of which assures the maintenance of high interstitial osmolality.

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PHARMACOLOGY

Inhibition of Haemagglutination by Benzylpenicillin adsorbed on Erythrocytes

INVESTIGATIONS of the inhibition of haemagglutination have demonstrated the role of the penicilloyl group as the major antigenic determinant[1]. Coating of erythrocytes either with benzylpenicillin or with penicillenic acid leads to covalent bonding of penicilloyl haptene groups either by irreversible reaction of penicillenic acid with lysine ε-amine groups or by coupling involving the direct addition of the β-lactam carbonyl to lysine amino residues of membranous proteins. According to Levine[2] the former mechanism is more likely. Combination of penicillenic acid through disulphide linkages, as reported by De Weck and Eisen[3], has been attributed by Levine and Ovary[4] to

small numbers of penicilloyl groups in the penicillenate conjugates. However, such groups were not detected by De Weck[1] and no cross-reactions were observed with anti-penicilloyl antisera. Occasional human sera are encountered in which inhibition is more effective with other degradation compounds of benzylpenicillin, namely, penamaldate, penilloate, penicillenate and penicilloate. Consequently, it is probable that other haptene specificities may be involved in the haemagglutination system, but their role is so far unestablished. In general, unaltered benzylpenicillin is a less effective inhibitor at equimolar concentrations. Because of this and the failure of benzylpenicillin to form stable covalent bonds with proteins it has been considered unlikely that the unaltered compound can function as an effective haptene.

A characteristic feature of the agglutination of benzylpenicillin-coated cells is the occurrence of spontaneous reversal of agglutinates, the rate of which is increased by benzylpenicillin and other haptenes[5]. The effect of penicillinase on agglutination reversal was therefore investigated as a means of determining the presence of biologically active benzylpenicillin adsorbed to erythrocyte membranes and its possible role in the haemagglutination system.

Erythrocytes were coated with benzylpenicillin as described previously[6]. After storage at 4° C for variable periods up to 3 weeks, aliquot volumes were washed exhaustively in repeated changes of sterile 0·85 per cent saline. Cells were then lysed in sterile distilled water and the deposited membranes were washed in three changes of fresh distilled water. Membranes were then placed on the surface of nutrient agar plates seeded with a lawn of a 6-h culture of a penicillin-sensitive strain of *Staphylococcus pyogenes*. Similar membrane preparations treated with penicillinase and membranes from normal cells were included. Fig. 1 shows wide zones of inhibition and the effect of penicillinase in destroying the biologically active penicillin. Only slight differences were noted in zone sizes after storage of cells for 24 h or 3 weeks at 4° C.



Fig. 1. Top, zones of inhibition caused by washed membranes after storage of cells for 21 days. Bottom left, same membrane preparation after treatment with penicillinase. Bottom right, membranes from uncoated erythrocytes

Agglutinates were prepared by incubating coated group O, rhesus negative cells with 1/4 dilutions of positive human antisera (titres > 1/128). At this low dilution the agglutinates resuspend as a single mass of clumped cells. After removal of the supernatant sera, the agglutinates were resuspended in three sets of four replicate tubes containing, (*a*) penicillinase, (*b*) penicillinase solution heated to 75° C for 1 h, and (*c*) 0·85 per cent saline, respectively. Times required for complete disappearance of haemagglutination were then noted (Table 1).

Table 1. HAEMAGGLUTINATION REVERSAL BY PENICILLINASE

Serum	Haemagglutination reversal (time) Penicillinase (min)	Penicillinase (inactivated) (h)	Saline (h)
1	< 20	24	24
2	< 20	24	24
3	< 20	24	24
4	< 20	36	36
5	60	48	48
6	90	72	72

These findings indicate that biologically active penicill adsorbs to the erythrocyte surface and its destruction l penicillinase leads to the rapid reversal of agglutinatio Agglutination is not, however, due to antibody wi specificity against the unaltered benzylpenicillin hapter since we have shown previously that treating coated ce with penicillinase, followed by its removal by washin does not affect the ability of the cells to be agglutinat by positive antisera[6]. Additionally, each of the s sera was inhibited to a markedly greater extent l the monovalent haptene penicilloyl-ε-amino-caproa than by benzylpenicillin. Further experiments we carried out by incubating aliquot volumes of coat cells with penicillinase and saline respectively for 2 h 37° C, after which they were washed five times in lar volumes of 0·85 per cent saline. Cells were then aggluti ated in replicate tubes with 1/4 dilutions of the six positi antisera. Agglutinates formed were then resuspended penicillinase. Table 2 shows the effect on the tir required for haemagglutination reversal.

Table 2. EFFECT OF PRIOR TREATMENT WITH PENICILLINASE ON REVERS TIMES

Serum	Haemagglutination reversal (time) *A* (h)	*B* (min)
1	24	< 20
2	24	< 20
3	24	< 20
4	36	< 20
5	48	60
6	72	90

A, Coated cells treated with penicillinase, then agglutinated and agg tinates resuspended in penicillinase.

B, Coated cells treated with saline, then agglutinated and agglutina resuspended in penicillinase.

In this instance, prior treatment with penicillina prevented the rapid reversal of agglutination caused penicillinase added later and was again associat with loss of biologically active antibiotic from the c surface.

Haemagglutination reversal caused by penicillinase explained most readily by conversion of adsorbed benz penicillin to penicilloic acid. This compound is unable adsorb effectively to the cell surface because its fr α-carboxyl group does not combine readily with ami groups of cell protein, an observation in agreement wi the findings of Josephson[7] that penicilloic acid is r antigenic in rabbits. However, penicilloic acid is effective inhibitor of haemagglutination in virtue of close structural similarity to the penicilloyl haptene. seems probable that the spontaneous reversal of haem glutination referred to results from conversion of so fraction of the adsorbed benzylpenicillin to penicill acid.

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HAEMATOLOGY

Nature of Haemoglobin $M_{Oldenburg}$

In 1962, Tönz, Simon and Hasselfeld[1] described a German family with forty-six cyanotic members. This cyanosis was found to be due to an abnormal haemoglobin. This abnormal haemoglobin was characterized as an Hb M type on the basis of electrophoresis on starch block at *p*H 7·0 and on the absorption spectrum of the haemolysate. The abnormal haemoglobin was called Hb $M_{Oldenburg}$.

Cyanosis was present from birth, so an α-chain abnormality was postulated. The purpose of this communication is to describe the more detailed chemical investigation to find the amino-acid replacement in this Hb $M_{Oldenburg}$.

Haemolysate was prepared in the usual manner from the blood of one of the Hb $M_{Oldenburg}$ carriers. The Hb M fraction was separated on starch block electrophoresis at *p*H 7·0 (0·025 M $Na_2HPO_4.2H_2O$ + 0·025 M $NaH_2PO_4.2H_2O$) after converting the haemoglobin into the oxidized form[2]. The purified Hb M was digested with trypsin and the peptides were separated with high-voltage electrophoresis at *p*H 6·4 (water–pyridine 9 : 1, acidified with acetic acid). In Fig. 1 the pattern is given in comparison with that of a Hb A_1 digest. In the case of Hb M, peptide αT 8·9 is missing. No new peptide could be detected. Because peptide αT 9 has the same amino-acid composition as αT 8·9, with the exception of the N-terminal lysine, which is split off, differences in the amino-acid composition must occur in both peptides. We therefore compared the Hb M peptide αT 9 with the normal Hb A_1 αT 9 peptide. αT 9 usually occurs in the neutral zone after electrophoresis at *p*H 6·4. To separate this peptide, the neutral zone was eluted, dried and again electrophoresed at *p*H 3·6 (water–pyridine–acetic acid–*n*-butanol 7·6 : 1 : 7 : 2). Instead of the normal pattern, a new peptide appeared in the case of Hb $M_{Oldenburg}$ with sulphur, histidine and tyrosine staining reactions. A second sulphur-positive peptide could also be detected almost on the site of the normal peptide αT 9 (see Fig. 2). This suggests an amino-acid replacement, which acidified the peptides αT 9 and αT 8·9. On account of this more acid character, peptide αT 8·9 shifts to the neutral zone at *p*H 6·4 on electrophoresis, and both peptides αT 8·9 and αT 9 will run less to the negative side after electrophoresis at *p*H 3·6.

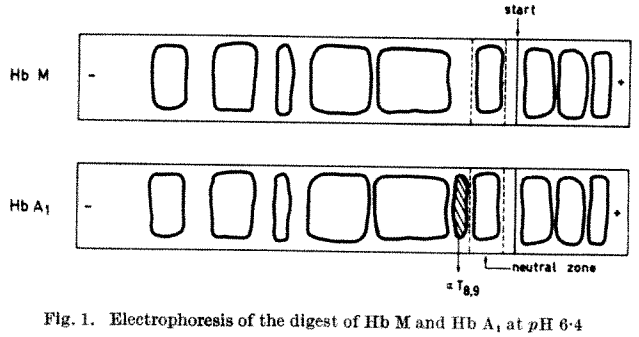


Fig. 1. Electrophoresis of the digest of Hb M and Hb A_1 at *p*H 6·4

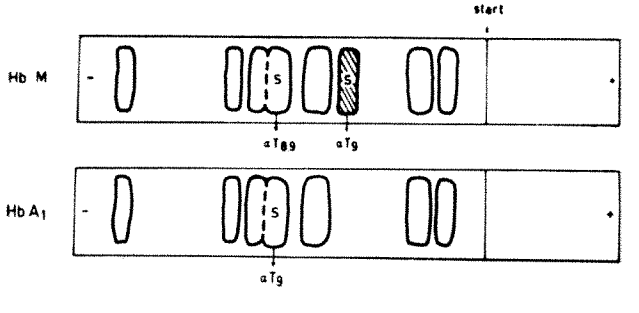


Fig. 2. Electrophoresis of the neutral zone at *p*H 3·6

The new peptide was extracted, hydrolysed and analysed. The amino-acid composition was that of the normal αT 9 peptide, except that one histidine unit was missing and a half-unit of tyrosine appeared in addition. Because of the positive tyrosine staining of the peptide and the fact that tyrosine breaks down on acid hydrolysis, it was assumed that a histidine group had been replaced by tyrosine. Which of the three histidines was replaced was not clear. It is reasonable to assume that it is histidine 87, because this histidine is connected to the iron atom of the haem group. This would explain the abnormal absorption spectrum of the haemolysate and the biochemical behaviour (lack of reduction of the methaemoglobin with sodium sulphate[1]). Such a replacement has already been described for Hb M_{Iwata} and Hb $M_{Kankakee}$ by Shibata *et al.*[3] and Jones *et al.*[4]. For this reason, Hb $M_{Oldenburg}$ is probably the same as these abnormal haemoglobins.

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IMMUNOLOGY

Heterogeneity of Heavy (γ) Chain Preparations from Human γG-Immunoglobulins

In recent publications, Cohen[1] and Cohen and Porter[2] have presented evidence that light chain preparations from partially reduced and iodoacetamide-alkylated γ-globulins of several species are heterogeneous. In glycine-urea starch-gel electrophoresis, *p*H 7–8, some eight to ten electrophoretically distinct bands were separated. Under similar electrophoretic conditions the heavy (γ) chains migrated as a diffuse band showing approximately the same spread as the original γ-globulin. In contrast to the light chains, the heavy chains did not give a band pattern.

We have prepared heavy and light chains of γ-globulins essentially according to the method of Fleischman, Pain and Porter[3]. The experimental conditions for reduction of the γ-globulin in neutral aqueous solution were similar to those described, but the chains were dissociated by dialysis against 0·1 M formic acid immediately after alkylation. Iodoacetic acid or iodoacetamide were used as alkylating agents. The chains from either reduced and dissociated products or reduced, alkylated and dissociated products were separated on a 'Sephadex *G*-200, *A* 50' (Pharmacia *AB*, Uppsala, Sweden) column in 0·1 M formic acid[4]. The separation of γ- and light chains from reduced but non-alkylated material was carried out in 0·1 M formic acid containing 1 ml. mercaptoethanol/l.

Urea starch-gel electrophoresis was performed in horizontal trays in formate buffer[5] and in glycine buffer[2]. When non-alkylated material was subjected to electrophoresis mercaptoethanol was incorporated into the gel.

The normal human γG globulins (7*S*) used in our experiments were either (1) Cohn-fraction II (supplied by Kabi *AB*, Stockholm, Sweden), further purified by chromatography on DEAE-'Sephadex', or (2) preparations

obtained by chromatography of normal human serum on DEAE-'Sephadex' in 0·0175 M phosphate buffer, *p*H 6·5. Myeloma globulins were prepared by the latter procedure from sera of patients diagnosed as suffering from multiple myelomatosis.

The purity of the γ-globulin preparations was checked by immunoelectrophoresis against rabbit or horse anti-human sera.

The results of the glycine-urea starch-gel electrophoresis of iodoacetic acid-alkylated γ- and light chains from a normal γG preparation and from two individual myeloma γG preparations are shown in Fig. 1. The band pattern of normal light (κ and λ) chains is consistent with that published by Cohen and Porter[2]. That is, about ten distinctly separated bands were obtained (Fig. 1*A*). In contrast to Cohen and Porter's finding of only one or sometimes two bands in light chain preparations from myeloma γG material[2] several myeloma light chain fractions showed more than two distinct bands (Fig. 1*B*). However, these were fewer than those from normal γ-globulin and the number varied from one myeloma protein to another.

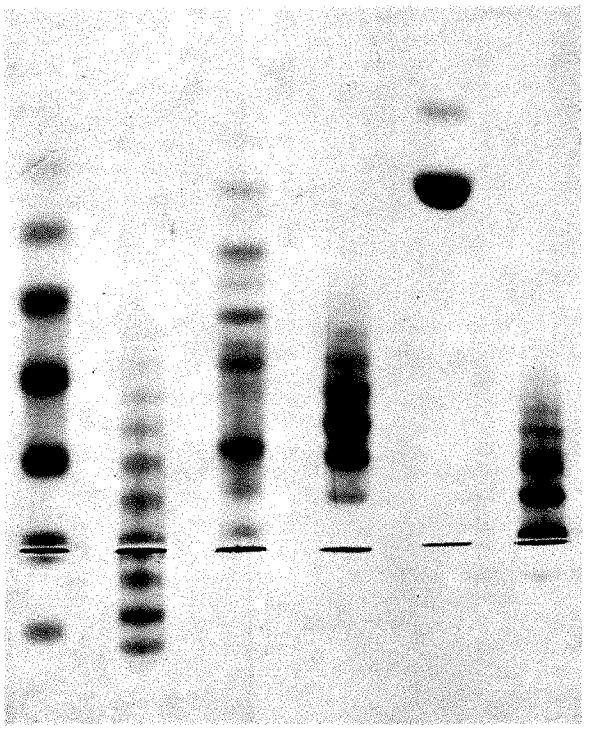


Fig. 1. Starch-gel electrophoresis in urea[2] of heavy (*H*) and light (*L*) chain preparations from (*A*) a normal γG preparation and (*B*) and (*C*) two individual myeloma γG preparations

Urea-glycine starch-gel electrophoresis of the γ-chain preparations from normal and abnormal γ-globulins also showed distinct band patterns as shown in Fig. 1. These preparations showed different band patterns, analogous to the patterns given by normal and abnormal light chains. In a γ-chain preparation from normal γG (Fig. 1*A*) some fifteen bands could be recognized. The γ-chains from myeloma proteins always gave fewer bands (Fig. 1*B* and *C*) and, in addition, the distribution of bands varied among different abnormal γ-chain preparations.

All our γ-chain preparations from normal γG-globulins gave similar band patterns. The most striking effect was obtained either with γ-chain material alkylated with iodoacetic acid or γ-chains prepared from material that had been reduced in mercaptoethanol only. In the latter case the γ-chains gave a pattern similar to that of iodoacetic acid-alkylated material except that the mobilities of the bands were somewhat less. In contrast, the material obtained by alkylation with iodoacetamide gave only a smear, although a few bands could be recognized within the smear. Obviously, the carboxymethyl groups attached to the molecules after alkylation with iodoacetic acid increase the net negative charge and thereby enhance the electrophoretic mobility of the material.

If we accept that the banding of light chain material is an expression of light chain heterogeneity[2], then it is reasonable to assume that the band pattern of γ-chain material also represents heterogeneity. Were banding a result of aggregation it would be difficult to explain the differences in band pattern between normal γ-chains and γ-chains from myeloma proteins and also the differences among γ-chain preparations from individual myeloma proteins.

The results suggest that the banding of γ-chain preparations is due to charge differences between molecules; that is, differences in primary structure. Furthermore, the observation that preparations of myeloma γG immunoglobulins are more homogeneous than those of normal γG preparations is consistent with earlier results[2]. However, in some myeloma proteins in which only one or two light chain bands could be recognized as many as five to seven γ-chain bands were detected. These results seem to indicate that a great heterogeneity also exists within individual human myeloma γG preparations and that the γ-chains of these proteins contribute most to this heterogeneity.

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GENETICS

Phosphoglucomutase Polymorphism: an Unusual Type in Negroes

PHOSPHOGLUCOMUTASE is present in human erythrocytes in different forms. Spencer *et al.*[1] showed that polymorphism results from the inheritance of two autosomal-allelic genes, PGM^1 and PGM^2. The homozygous state for PGM^1 results in pattern 1, for PGM^2 in pattern 2, and the doubly heterozygous state results in pattern 2-1. In the English population the gene frequencies for PGM^1 and PGM^2 are respectively 0·76 and 0·24.

At the University of California Medical Center, San Francisco, I examined the haemolysate of 555 persons —271 Caucasians and 284 Negroes—63 of whom were healthy students and hospital employees and 492 were patients attending the out-patient clinic or admitted to the hospital wards. Results are presented in Table 1. In addition to the usual patterns 1, 2-1 and 2, an entirely different pattern was observed in two healthy female Negroes, whose strong *g* zone pattern differed from the usual very faint one (see Fig. 1). Moreover, their pattern had another similar strong, more anodic zone and a faint, even more anodic spot was also observed, especially when the haemolysate became older. The *e* and *f* zones, however, were very faint in these two individuals. The cathodic portion of the unusual pattern resembled that of pattern 1, but the anodic portion did not resemble patterns

1 or 2. This suggested the existence of a third allele or a second structural locus that determined the *PGM* patterns. This rare pattern was found constant, even when the person was re-examined several months later. To find out whether this unusual pattern was genetically determined, available relatives of one subject with the unusual pattern were examined. The mother and sister had exactly the same pattern as the propositus; the father was not available for investigation.

The calculated gene frequencies for PGM^1 and PGM^2 in the Caucasian group were respectively 0·777 and 0·223, essentially the same as those found in the English population examined by Spencer *et al.* In the Negro group the gene frequencies were PGM^1–0·801, PGM^2–0·195 and the unusual type 0·004. The gene frequencies for PGM^1 and PGM^2 in the Negro group differ little from those of the Caucasian group and no significant difference was observed between male and female in either group.

Since the completion of this study, I have read the recent publication of Hopkinson and Harris[2] on the role of a second structural locus in determining human phosphoglucomutase. The Atkinson type they described would seem to be the same as the unusual type found in the Negro group in this investigation.

Table 1. FREQUENCIES OF DIFFERENT *PGM* PHENOTYPES IN 555 UNRELATED PERSONS (271 CAUCASIANS AND 284 NEGROES)

PGM phenotypes	Caucasians	Frequencies	Negroes	Frequencies
1	169	0·624	188	0·662
2–1	83	0·306	77	0·271
2	19	0·070	17	0·060
1-unusual	—	—	2	0·007
Total	271	1·000	284	1·000

During my investigation of the electrophoretic pattern of phosphoglucomutase, several of the group of patients with different diseases produced a very dark pattern even when the haemolysates applied were adjusted to the same concentration as those of normal controls, indicating a high increase of phosphoglucomutase-level in the erythrocytes in those patients. Some variation was also apparent in the healthy group, but it was not so striking. In addition to the variation in quantity several had an abnormal distribution of quantities among the electrophoretically normal migrating spots. These findings may indicate that an abnormal variation in quantity or in relative distribution of quantities among the zones may be clinically significant, just as has been shown for the lactodehydrogenase isozymes. In one patient with chronic congenital non-spherocytic haemolytic anaemia due to severe glucose-6-phosphate dehydrogenase deficiency of the erythrocytes, a definite abnormal distribution was found that was not demonstrated in the parents or siblings (see Fig. 2).

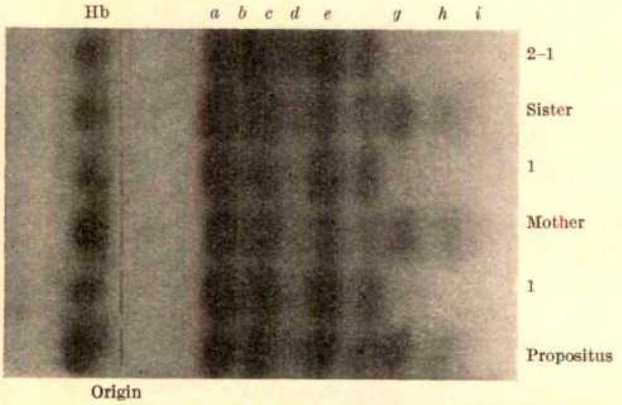


Fig. 1. Starch-gel electrophoresis with *tris*-EDTA-maleic acid and magnesium chloride buffer *p*H 7·8, stained for phosphoglucomutase (ref. 1). Note unusual pattern of the haemolysate of propositus, her mother and her sister, compared with controls 1 and 2–1

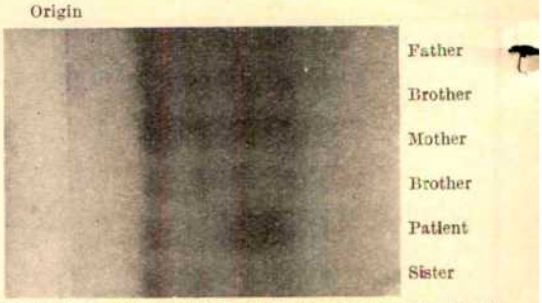


Fig. 2. Phosphoglucomutase patterns after starch-gel electrophoresis of a patient with congenital non-spherocytic haemolytic anaemia and his family. Note abnormal pattern in patient

Studies on other racial groups are in progress. Although the number in each is too small to be significant, the unusual pattern 3–1 described by Hopkinson and Harris was observed in one of fifty Malayan Aborigines examined, but the other forty-nine had the usual 1, 2–1 and 2 patterns.

I thank Miss Fermor Lloyd and her staff at the Outpatient Clinic, Department of Medicine, and Mrs. Gladys Spaulding of the Employee Health Service for collecting the blood samples. I also thank Mrs. Marlene Scham for her technical assistance.

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Effect of Centrifugation of the Eggs on the Sex of *Carausius morosus* Br.

THE stick insect *Carausius morosus* Br. reproduces parthenogenetically. However, non-functional males appear spontaneously by loss of a sex-chromosome. This occurs with a frequency of about 0·5 per cent at 16°–20° C. Sexually aberrant individuals showing gynandromorphism and/or intersexuality are also found at frequencies of up to about 2 per cent. Higher percentages of 'non-females' are obtained when eggs are treated with high temperatures or X-rays[1–3]. The following experiment shows that centrifugation of the eggs may also influence the sex of *C. morosus*.

Eggs were collected daily and divided into two samples during September 2–11, 1964. One series was centrifuged at 2,520*g* (4,600 r.p.m.) for 2 h on September 11, while the other series was kept as control. During centrifugation the temperature of the eggs rose to ± 26° C, and so the eggs of the control series were also similarly heated. The nymphs were sexed just after hatching, using the differences in the external genitalia as the criterion. The results are summarized in Table 1 and Fig. 1.

The sensitivity to centrifugation can be expressed either as the fraction of individuals showing some further development after treatment or as the fraction hatching successfully. This function is at its peak during meiosis, decreases during segmentation and blastoderm development, and increases again during formation of the germ anlage. The period of embryonic development is lengthened by the treatment, particularly when the meiosis and the early cleavage stages are treated, while the developmental phases which follow are influenced progressively less. The

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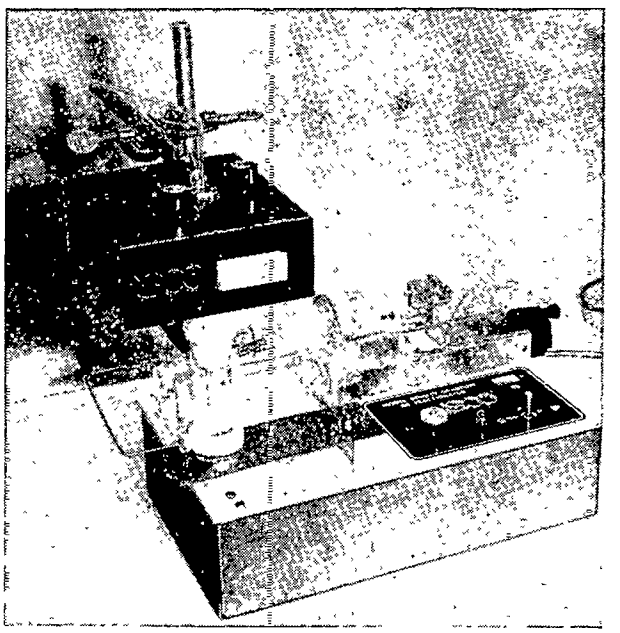
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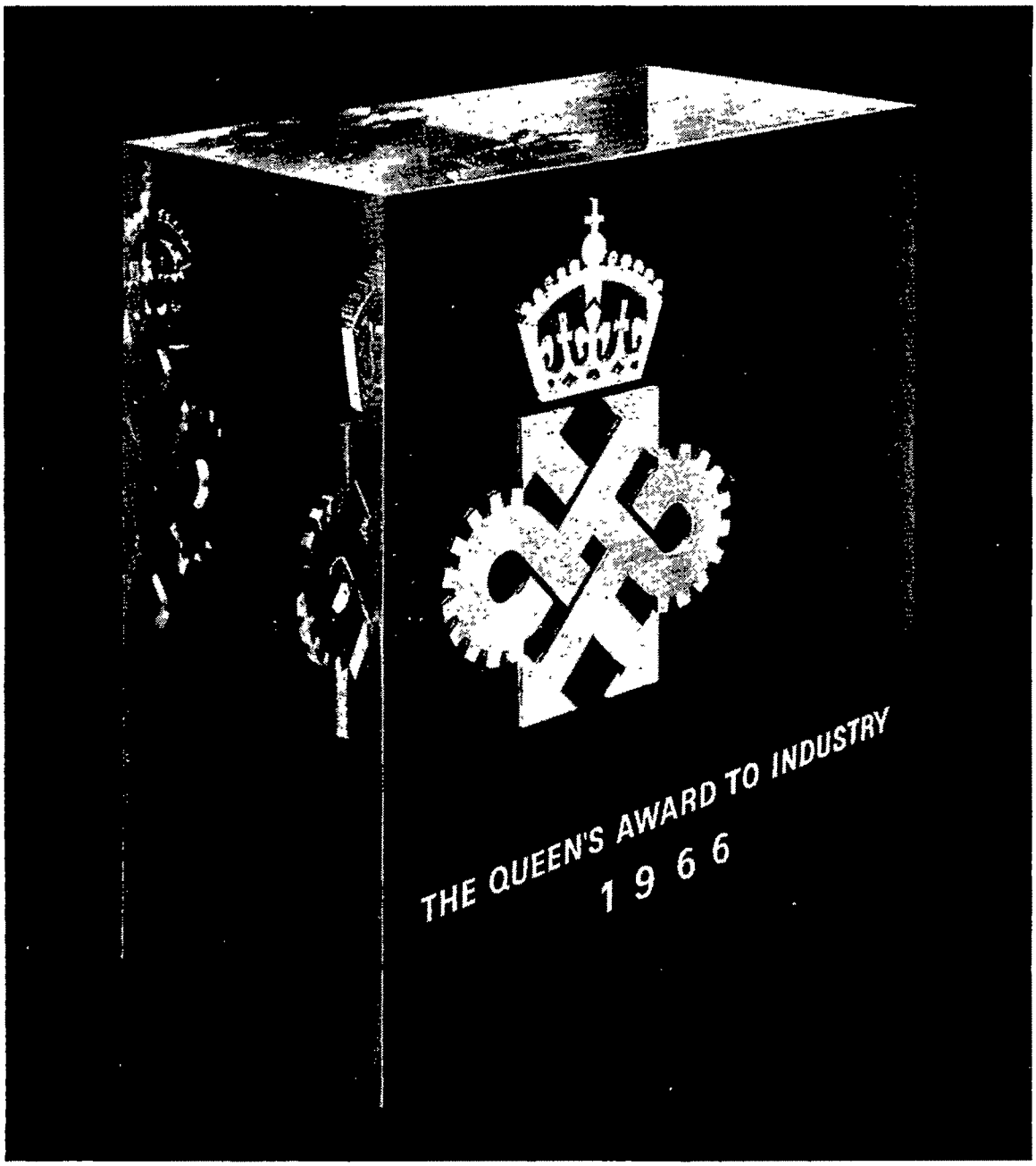
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Table 1. EFFECT OF CENTRIFUGATION OF THE EGGS ON THE SEX OF *Carausius morosus* BR.*

	Age of eggs at treatment (days)†	Number of eggs	Percentage of eggs with some development after treatment	Percentage of eggs hatched	Mean embryonic development period (days)	Percentage of nymphs sexed as 'non-females'	Percentage of nymphs sexed as males
Treated	0–1	148	12·8	2·7	129 5 ± 5 3	25 00	0·00
	1–2	143	32·9	7 0	133·3 ± 7·3	40·00	20 00
	2–3	161	60 3	17·4	133·2 ± 10 9	46·43	7·14
	3–4	161	72·1	31·1	133 6 ± 8 2	58·00	10 00
	4–5	138	84 8	42·8	120 3 ± 9 2	44·07	8·47
	5–6	134	88·8	48·5	118·2 ± 6·6	23·08	1 54
	6–7	139	87·1	50·4	116 3 ± 6·8	15·71	1 43
	7–8	131	88·6	55·0	113 0 ± 8·7	18·06	1·39
	8–9	135	89·6	60 7	113·6 ± 8 3	12·20	0·00
	9–10	136	75·0	38·2	111·9 ± 9 7	11·54	0·00
Control‡	0–1 to 9–10	±180 per sample	88 9 ± 1·4	81 9 ± 1·7	98·9 ± 1·7 6·4 ± 1·2	2·32 ± 1·28	0·15 ± 0·47

* All samples received a treatment of 2,520*g* for 2 h.
† 0–1, non-reductional meiosis; 1–2, 1–4 cleavage nuclei; 2–3, 20–50 cleavage nuclei; 1–5, segmentation; 5–9, blastoderm; 9–13, germ anlage.
‡ For the control series only the means with standard deviations of the sample means are given.

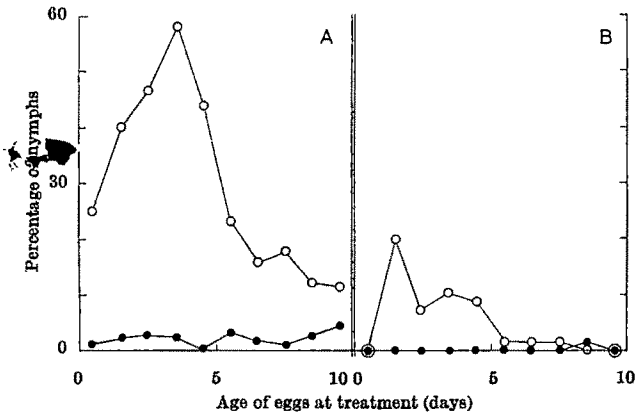


Fig. 1. The production of 'non-females' (*A*) and males (*B*) by centrifugation of eggs of *Carausius morosus* Br. at different stages of development. ●, Control; ○, treated

increase in the average time needed for development in the treated series cannot be attributed to an increase in the number of non-female individuals despite the fact that these have a longer development period than is normal. In the treated series, both normal and abnormal individuals show an appreciably longer period of development. The total numbers of 'non-females', gynandromorphs and/or intersexes, and males are increased significantly (χ^2_1's $\gg$ 10, P's $\ll$ 0·001) by centrifugation. As centrifugation may cause chromosome elimination[4] and can induce males, it seems likely that gynandromorphs rather than intersexes arise. Whether the sex chromosome elimination arises through direct or through indirect effects of centrifugation cannot be inferred from this experiment (due to the paucity of data on the effect on meiotic stages). Most of the gynandromorphs and males are induced when cleavage stages during segmentation are treated. The effect of treatment at later stages of development decreases progressively. The decrease in the number of induced gynandromorphs may be explained by a decrease in the expressivity of the gynandromorphism. This arises because proportionately fewer nuclei mutate at the more advanced stages of embryonic development. The fact that males can also arise through treatment of cleavage stages implies either that all the treated nuclei must mutate, or that there is chance mutation of those blastoderm nuclei which ultimately give rise to the germ anlage. This explains the observation of a decrease in the frequency of induction of males with the stage of development at treatment.

I thank Prof. J. A. Beardmore for his help and advice.

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MICROBIOLOGY

Reciprocal Genetic Changes in Mycobacterial Host–Virus Systems: Effect of Lysogeny on the Phage

Mycobacteriophage phlei B2, one of several bacteriophages, isolated in Borstel in 1963 (ref. 1) was used in this investigation.

Phage *phlei B2* was obtained from dung and the slightly polyvalent original filtrate could be rendered specific for *Mycobacterium phlei* in two transfers on *M. phlei* strain *F89*. The host range of phage *B2* proved to be stable in more than thirty passages. Phage *B2* showed a drastic change in host range only on establishment of lysogeny and subsequent re-isolation from lysogenic complexes.

In the course of screening for lysogenic mycobacteria, organisms were picked from the residual growth in centres of massive (phage-) lysis and were purified by serial single-colony transfers on solid media. Phages re-isolated from lysogenic complexes, on the other hand, were purified by single-plaque transfers. The experiments were carried out with the agar overlay method of Gratia as modified by Froman *et al.*[2]. Phage stocks were maintained in 'Difco' heart infusion broth.

The first series of lysogenization experiments led to the formation of *SN*109(*B2*) complexes, the second to the formation of *F*89(*B2*) complexes.

On re-isolation from several *SN*109(*B2*) complexes, phage *B2* showed a genotype which differed in several respects from wild *B2*. Table 1 shows the changes which were in host range (extension of host range to *M. smegmatis SN2*), immunity and, to some extent, in plaque morphology. Concurrently with these changes in phages, respective lysogenic hosts showed an altered colony morphology and a decrease in growth rate (lysogenic conversion).

Table 1. HOST RANGE, IMMUNITY AND PLAQUE MORPHOLOGY CHARACTERISTICS OF PHAGE *B2* AND ITS *B2h* . . . DERIVATIVES

	Host range*	Immunity†	Plaque morphology‡
Wild *B2*	h^+	imm^+	c^+
*B2h.SN*109			
*B2'h.SN*109	h^-	imm^-	$c^{(-)}$
*B2"h.SN*109			

* h^+: propagates only on *M. phlei*; h^-: host range extension to *M. smegmatis*.
† imm^+: *B2* immunity; imm^-: insensitive to *B2* immunity.
‡ c^+: hazy plaque; c^-: mutation from hazy to clear plaque; $c^{(-)}$ intermediate between hazy and clear plaque.

It was clear that modification could not account for the emergence of the *B2h.SN*109 series of phages in view of the stable hereditary character of changes and to the absence of any previous contact with *M. smegmatis*.

A mutational hypothesis for the origin of the phages *B2h.SN*109 could not be discarded so easily as was the case with modification. Nevertheless, in view of the multiplicity of changes, it appeared to be somewhat less probable than recombination. The question of origin of the *B2h* genotype, however, could not be resolved at this stage and had to remain unanswered until lysogenic

complexes between *M. phlei* *F*89 and phage *B*2 had been established.

The variant phages emerging from *F*89(*B*2) strains appeared on re-isolation to be different from wild *B*2 as well as from each other. Some showed host range extension similar to that of phages *B2h.SN*109 (examples of this are *B2h.F*89 and *B2′′′′h.F*89). Others, such as *B2′.F*89, kept the wild-type host range. While all host-range variants proved to be immune variants as well, the immunity variant *B2′.F*89 had the wild *B*2 host range.

By grouping the newly obtained *B*2-variants according to the extent of the change in their immunity and subgrouping them according to a change in host range, a definite pattern could be demonstrated: a gradient of reciprocal changes in phages and respective lysogenic organisms.

Table 2. CO-IMMUNITY PATTERN AND HOST RANGE OF PHAGE *B*2 AND ITS DERIVATIVES EMERGING FROM *F*89(*B*2) . . . LYSOGENIC SERIES

Mycobacterium strain	Phage* Wild *B*2	*B2″.F*89	*B2‴h.F*89	*B2′F*89	*B2h F*89
Wild *F*89	+	+	+	+	+
*F*89(*B*2)″	0	0	+	+	+
*F*89(*B*2)‴	0	0	0†	+	+
*F*89(*B*2)′	0	0	0	0	+
*F*89(*B*2)	0	0	0	0	0
Wild *SN*2	0	0	+	0‡	+

* +, Lysis; 0, no lysis: protection due to immunity or species barrier.
† "Dismune" mutants of *B2‴h* can lyse *F*89(*B*2)‴.
‡ Recent segregation into *B2h* type phage changed this picture. *B2h* segregants of *B2′.F*89 can lyse strain *SN*2.

Table 2 shows that *F*89(*B*2)″, which differs from wild *M. phlei* *F*89 only with respect to lysogeny, releases a phage (*B2″*). This phage behaves as wild *B*2. At the other extreme, in *F*89(*B*2) phage *B*2 underwent the most drastic changes in the whole series. The resulting phage (*B2h.F*89) has a distinctly different plaque morphology—it forms clear plaques—and in contrast to *B*2, it does not respect either species or immunity barriers. It attacks *M. smegmatis* *SN*2 as well as related complexes of *F*89 lysogenic for phage *B*2. The change in its host seems as drastic as that in the phage. *F*89(*B*2) shows a two- to three-fold decrease in growth rate in conjunction with definite changes in colony appearance. *B2′F*89 and *B2′′′′h.F*89 represent intermediates between these two extremes.

The hypothesis of recombination as the mode of origin of the *B2h* series of phages gained powerful support by the establishment of a gradient of reciprocal changes in host *F*89 and phage *B*2. Subsequent serological, ultra-violet, and heat inactivation experiments confirmed the results of previous findings and contributed valuable additional support to this view.

The analysis of serological data revealed that (1) *B2h.F*89 and *B2′′′′h.F*89 are very closely related or identical phages; (2) the former group can be sharply delineated from the *B*2 group which comprises *B*2, B2″ (and *B2‴*) by the latter's very high sensitivity to inhibition by normal serum; and (3) *B2′F*89 shares antigens with both *B*2 and *B2h.F*89. (This, in view of a *B*2-host range and a *B2h.F*89 immunity, and the segregation of phage *B2′*F89 into parental *B2h.F*89, established this phage as a hybrid between *B*2 and *B2h.F*89.)

On the basis of heat and ultra-violet inactivation studies, an even sharper line could be drawn between members of group *B*2 and that of *B2h*, and in addition to this, the ultra-violet and heat sensitivity of *B2h.F*89 (or *B2′′′′h.F*89) has been shown to be intermediate between that of phage *B*2 and *M. phlei* strain *F*89.

A more detailed account of serological and physical characteristics of phages belonging to the *B*2- and the *B2h*-group will be presented at the International Symposium on Mycobacterial Variation (Borstel, W. Germany).

To sum up, we have presented evidence to show that phages of the *B2h*-type do not emerge from wild *B*2 due to modification or mutation. Observations supporting their recombination origin and nature are as follows: (1) they were invariably isolated from lysogenic complexes; (2) reproducible changes in the phage were accompanied by reproducible changes in the bacterium; (3) in heat and ultra-violet inactivation studies, the *B2h*-group behaved as would be expected if they were hybrids formed between phage *B*2 and *M. phlei* *F*89. The number of *B*2-variants was not sufficient to construct a chromosome map of phage *B*2; nevertheless, immunity appeared to be closely linked to host range and we would not be surprised to find later that immunity, virulence and 'clear' mutation lie within the same region somewhat similarly to phage λ (ref. 3).

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Comparative Gel-electrophoretic Investigation of the Protein Patterns of *Septoria* Species

THE genera *Septoria* in the class Fungi Imperfecti comprises some 1,200 species, each parasitic on one or several closely related genera of host plants. Little is known regarding their phylogenetic relationships since only a few perfect stages have been found. The host range and spore morphology have therefore been the main bases for the determination of species. However, the value of these characteristics for delimiting *Septoria* species has been questioned[1].

Recently, protein patterns obtained by electrophoresis have also been used to demonstrate taxonomic relationships. Clare[2] suggested that they may be helpful in classifying *Pythium* species; he found that isolates from different locations of any one species were similar in their protein patterns. Chang, Srb and Steward[3] demonstrated differences among patterns from species of *Neurospora*. They also showed that a mutant strain of *N. crassa* possessed a number of slow-moving bands not present in the pattern of the wild-type strain which they used. The last finding suggests that the protein patterns of fungi, such as *S. avenae*, the wild-type isolates of which are known to be highly variable in cultural type and pathogenicity[4,5] may also vary significantly. This communication describes the results obtained when the soluble proteins from several species of *Septoria*, parasitizing plants in the family Graminae, were analysed using disc electrophoresis.

Twelve isolates of *S. avenae*, varying in pathogenicity and cultural characteristics, were collected from the North Central oat-growing area of the United States. A similar series of isolates of *S. nodorum* and *S. tritici* were obtained. They were grown in Czapek's 2 per cent sucrose medium for 10–14 days on a reciprocating shaker at 24° C. The colonies were collected by filtration, and washed with distilled water. The soluble proteins were extracted in a

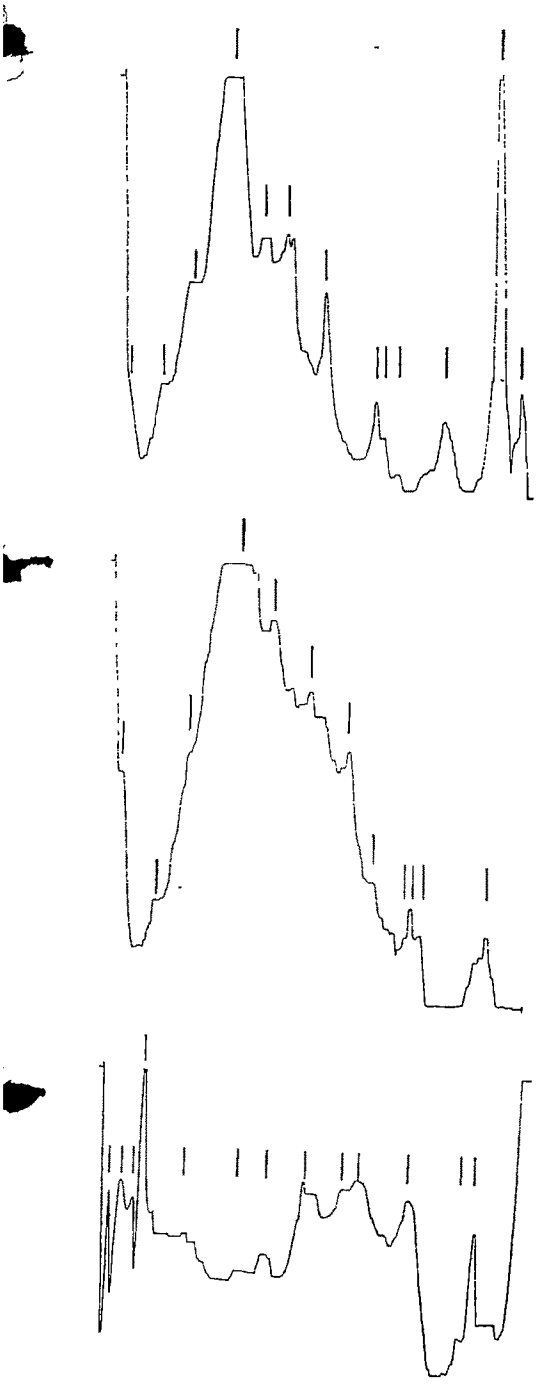


Fig. 1. Densitometric traces of amidoschwarz 10*B*-stained protein patterns from three isolates of *S. avenae* showing the variability among isolates. Tracings were made with a Joyce 'Chromatoscan' and the position of each band was marked with a vertical line

nitrogen atmosphere at 2° C with a Pirie press[6] using 0·1 M *tris* buffer, *p*H 7·8, containing 0·1 per cent ascorbic acid, 0·1 per cent cysteine-hydrochloride and 17 per cent sucrose. The extract was centrifuged, and a portion of the supernatant containing 200γ protein layered on top of the spacer gel just before electrophoresis. The electrophoretic procedure of Ornstein and Davis[7] was followed.

The protein pattern of any single isolate of *S. avenae* was not altered even though colonies of different ages were used or the fungus was grown on Czapek's, Fries's or Richard's medium. The majority of the patterns were either identical or quite similar, there being in some cases small variations in band position. However, there were significant differences between this 'type' pattern and those of the remainder of the isolates. The differences, although occasionally quantitative, were usually qualitative (Fig. 1). Similar results were obtained with the other two species investigated.

These findings indicate that the use of protein patterns for identifying *Septoria* species could become a valuable adjunct to other techniques for investigating *Septorias* parasitic on grasses, where the problem is relatively restricted in scope. However, it should be viewed with caution until the limits of natural variability within each species are established and the various species compared. Variability within *S. avenae* is considered, but has not conclusively been proved, to arise as "a process of continuing mutation within a heterocaryotic system"[5]. The probable occurrence of these processes in other fungi does suggest that here, too, variants may arise which differ in protein pattern much as they do in morphology and pathogenicity. As yet, the extent to which these findings can be applied to other fungi is not known.

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SOIL SCIENCE

Stimulation of Nitrification following Temporary Acidification of Soil

THE use of acidifying materials for reducing soil *p*H and the concentration of soluble salts in saline and alkali soils in order to improve structure and mineral nutrient relations is commonly practised. Sulphur and the sulphates of aluminium or iron are often used for this purpose[1,2]. During an investigation of the effects of varying degrees of acidification of soils on nitrogen metabolism during subsequent incubation some interesting results were obtained on nitrification rates when the *p*H of the acidified soils was increased before incubation. These results are presented here, since a search of the literature revealed that no similar effects have been reported.

Two soils (a saline soil, *p*H 7·2, and a degraded saline–sodic soil, *p*H 7·6, from Colombia, South America) of poor fertility were mixed with varying amounts of the three acidifying materials at similar sulphur-equivalent levels (0–0·5 per cent) and kept moist with intermittent leaching for 20 weeks. The soils were then air-dried and ground to pass a 2-mm sieve. *p*H values of the acidified degraded saline–sodic soil in relation to level of acidifying material are shown in Fig. 1. *p*H values for both soils were very similar irrespective of the type of acidifying material used. 10-g portions were incubated (30° C) under aerobic conditions (33 per cent of maximum water-holding capacity) with and without addition of 1 per cent calcium carbonate before incubation. This amount of calcium carbonate was sufficient to neutralize the acidity of even the most acid samples. After 6 weeks incubation ammonia and nitrate were extracted with N sodium chloride and determined by a microdiffusion method[3].

In the absence of added calcium carbonate, nitrate accumulation during incubation was negligible in all

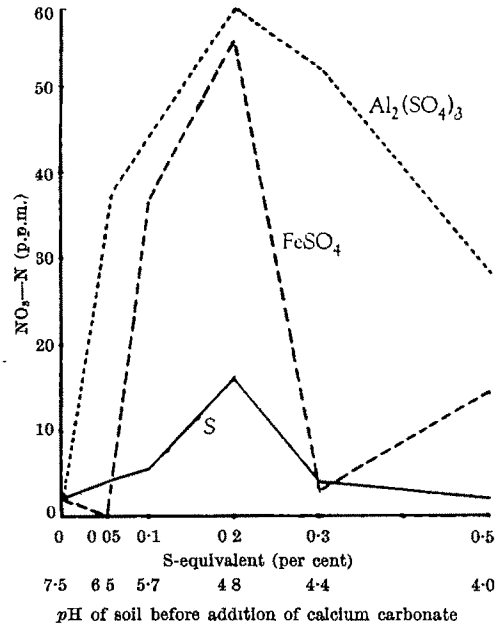


Fig. 1. Nitrate production during incubation of acidified soils treated with 1 per cent calcium carbonate

samples irrespective of *p*H. This would be expected in the soils of low *p*H because of the negligible activity of nitrifying organisms under these conditions[4,5]. At the higher *p*H values, low nitrate production was probably due to the absence of easily ammonifiable, and thus nitrifiable, organic nitrogenous compounds.

Of more interest, however, are the results obtained with added calcium carbonate. These are shown (for the degraded saline–sodic soil) in Fig. 1. Nitrate production increased with decreasing original *p*H (that is, *p*H before calcium carbonate addition) up to a maximum at original *p*H of 4·8 and then decreased with further increasing original *p*H. The same trend was noted irrespective of the type of acidifying material which had been used. The effects were, however, greater where aluminium or iron sulphate than where sulphur had been used. Similar results were obtained with the other soil investigated, namely, maximum nitrate production occurred where an intermediate level of acidifying material had been used. The original *p*H corresponding to this optimum nitrate level was also 4·8, as with the first soil.

Many reasons may be put forward to explain the results, but the most probable explanation lies in the changes occurring when the soils were being acidified. During this period stable organic compounds were probably being degraded to simple organic nitrogenous compounds. On increasing the *p*H before incubation these simple organic nitrogenous compounds were then easily ammonified and then nitrified. The extent of degradation of the complex to the simpler organic nitrogenous compounds appeared to increase with intensity of acidity during the acidifying period, being at a maximum in the region of *p*H 4·8. The low nitrate production in samples which had been brought to *p*H values less than 4·8 before incubation with added calcium carbonate may have been due to much reduced degradation of organic compounds during acidification. Alternatively, at the very low *p*H values the population of nitrifying bacteria may have been reduced to such low levels[6] that even after neutralization of the soils they could not multiply at a sufficiently high rate to nitrify all the ammonia produced. This is confirmed by the fact that ammonia accumulated during incubation of neutralized soils only where *p*H had been reduced below 4·8. The absence of ammonia accumulation where soils of *p*H 4·8 or higher were neutralized indicates that the rate of nitrification exceeded the rate of ammonification so that the rate-limiting factor was organic nitrogen which could be mineralized.

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Weathered Biotite from Strathdon, Aberdeenshire

WALKER[1] showed that biotite weathered to vermiculite in the soil. This transformation involved the complete replacement of interlayer potassium ions by water molecules and exchangeable magnesium ions together with various changes in the silicate layers such as oxidation of ferrous iron and the substitution of octahedral iron by magnesium. MacEwan[2], however, found that in poorly drained soils biotite broke down into a macroscopic trioctahedral montmorillonite. Recent work, summarized by Jackson[3], reveals that the interlayer positions of expanded 2 : 1 layer silicates in soil clays may be incompletely filled with positively charged hydroxy aluminium, such as $Al_8(OH)_{12}(OH_2)_{12}^{4+}$. Thus the expanded 2 : 1 layer silicates are effectively converted to a 2 : 2 intergrade in which the interlayer positions are partially occupied by hydroxy aluminium islands. These intergrades which usually show a 14 Å *c*-spacing have been referred to as 'dioctahedral vermiculite' or 'chlorite-like' and possess properties intermediate between homogeneous 2 : 1 or 2 : 2 layer silicates.

Experiments at this Institute appear to indicate that biotite may weather in a similar way to expanded 2 : 1 soil clays. Samples of weathered biotite were collected from the fine and coarse sand fractions of two freely drained soils and also from a weathered biotite-rich gabbro near Strathdon, Aberdeenshire. X-ray diffraction measurements of both powdered material and single flakes almost invariably showed a strong line at approximately 14 Å and a weaker, more diffuse, line at about 7·3 Å. At first it was thought that the 14 Å line indicated vermiculite, but the behaviour of the mineral on heating and on potassium saturation was inconsistent with this interpretation. Heating at 400° C for several hours followed by sealing in a glass capillary tube to prevent rehydration caused a shift in spacing to between 11·0 Å and 11·4 Å. Had true vermiculite been present as a component, this treatment should have caused a collapse to 10 Å (ref. 4). The weathered material was also treated with 1 N potassium chloride, potassium hydroxide and potassium oxalate for periods up to a week, but this failed to produce any significant change in the *c* dimension. These results pointed to the presence of interlayer contaminants, suggesting in particular the occurrence of non-exchangeable aluminium in interlayer positions which is known to inhibit lattice contraction of vermiculite[5]. Using the method of Follett *et al.*[6] for the dissolution of amorphous alumina and silica, the weathered biotite flakes were repeatedly treated with cold and then hot 5 per cent sodium carbonate in an attempt to remove any less well ordered alumina and silica present. The weight percentage total of alumina extracted by this means varied from 2·5 to 5·4 per cent; the extracted silica ranged from 2·9 to 4·6 per cent. After this treatment, the weathered biotite flakes were re-examined by X-ray diffraction. It became clear that the behaviour of the mineral on heating to 400° C and on potassium saturation had been considerably modified. Heating to 400° C now caused a

collapse to between 10·0 and 10·6 Å, and potassium saturation contracted the lattice to between 11·3 and 11·8 Å. These results indicate that much of the dissolved material must have come from inter-layer positions and appear to confirm the existence of non-exchangeable alumina, possibly associated with silica, in this part of the biotite structure.

The line at about 7·3 Å referred to already is observed in powders and in single flakes and is invariably associated with a (060) dioctahedral line at 1·49 Å. When the flakes are heated to 600° C, both the 7·3 and the 1·49 Å lines disappear, while the trioctahedral line at 1·53 Å associated with the biotite remains unchanged. These results strongly suggest the presence of a kaolin zone within the weathered biotite, possibly possessing a definite crystallographic orientation with the host mineral. Using the potassium acetate method of Wada[7], it was shown that the kaolin mineral behaved like kaolinite and not halloysite.

It may be seen, therefore, that in this particular instance the weathered biotite consists of a 14 Å intergrade alumino vermiculite–chlorite, together with zones of kaolinite. This transformation seems to take place fairly rapidly, since biotite weathered in such a manner has been found in slightly disintegrated rock, where the visual appearance of the mineral is fairly fresh. Normal 10 Å biotite was found only in the completely unweathered rock. This suggests that the alumination of the interlayer positions takes place at the same time as the expansion of the lattice in the *c* direction. It is of interest to note that Kato[8] has found that the weathering product of biotite of a granodiorite at Shinshiro City, Japan, is somewhat similar to that described here and that it is characteristic of an open, freely drained environment. Walker[1] and MacEwan[2] found that the transformations of biotite to vermiculite and biotite to trioctahedral montmorillonite occurred under intermediate and poor drainage conditions respectively. It is clear, therefore, that the nature of the weathering product of biotite in the soil depends on several environmental factors, one of which is drainage. A more detailed report of the weathered biotites described above will be published elsewhere.

I thank Mr. W. A. Mitchell, Mr. B. D. Mitchell and Mr. A. Thomson for encouraging discussions and for helpful advice on techniques. I also thank Mr. B. F. L. Smith, who did the alumina/silica determinations, and Mr. E. L. Birse for considerable help in the field.

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PSYCHOLOGY

Control of Time Estimation by a Chemical Clock

THE hypothesis of a temperature-dependent internal chemical clock as a basis for man's estimation of time intervals of short duration was made more than 30 years ago.

Francois[1] had found that the speed of tapping a Morse key at a rate estimated to be 3 taps/sec increased in a total of sixteen experiments on three subjects when their axilla temperature was raised by diathermy by between 0·3° C and 1·0° C. He found, also, that the frequency of a metronome beat which was perceived as 4 beats/sec increased in a total of five experiments on three subjects when their axilla temperature was raised by diathermy by between 0·3° C and 0·8° C.

Six years later Hoagland[2] had observed that his wife's speed of counting at an estimated rate of 1 digit/sec increased as her oral temperature rose due to influenzal fever. He obtained average counting speeds over three to six judgments at five levels of oral temperature between 36·33° C and 39·44° C. In 1880, Arrhenius had described an empirical equation relating chemical velocity to temperature, and directly from the slope of the line it was possible to calculate a constant of much theoretical importance to the physical chemist known as the energy of activation (μ) (refs. 2 and 4) from the formulae:

$$\log_e \frac{k_1}{k_2} = \frac{\mu}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right) \text{ or } \log_{10} \frac{k_1}{k_2} = \frac{\mu}{2{\cdot}30258R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)$$

where k_1 and k_2 are proportional to the velocity constants of an irreversible chemical reaction (log speed of counting, tapping etc.) at the respective absolute temperature (°K) T_1 and T_2, R is the gas constant with a value of 1·987 calories/degree/mole, $\log_e x = 2{\cdot}30258 \log_{10} x$, and μ is the energy of activation or temperature characteristic in calories. Hoagland, therefore, analysed the time estimation data from his wife and obtained an energy of activation value of μ = 24,000 calories (to the nearest 1,000 calories) which he suggested "falls within one of the major modal groupings of temperature characteristics found for a variety of rhythmic processes investigated in poikilothermic animals"[2]. Data from a similar experiment on a colleague with influenzal fever revealed an oral temperature increase from 36·57° C to 38·15° C and an increased speed of counting with an energy of activation value, μ, of 18,500 calories. Data from a student heated by diathermy revealed an increase in counting speed over three occasions at oral temperatures between 38·15° C and 38·74° C and a μ value of 24,000 calories (to the nearest 1,000 calories).

Hoagland[2] then plotted the full results (three subjects, sixteen experiments, of Francois's study of Morse key tapping at an estimated rate of 3 taps/sec together with his own data from three subjects engaged in estimating a counting rate of 1 digit/sec, and found a composite plot with a value of μ = 24,000 calories. "These results definitely imply the existence of a unitary chemical process serving as a basis for the subjective time scale, a process quite possibly irreversible in nature and perhaps catalysed in a specific way, since the critical increment of 24,000 calories corresponds to one of the modal groups characteristic of kinetics in living organisms . . . (and) . . . indicate that our sense of time depends on the speed of a chemical step in some group of cells, presumably in the brain, that acts as a chemical clock"[2,3].

In order to subject this hypothesis to further experimental testing, two series of experiments have been performed in which the subjects' speed of counting at a rate estimated to be 1 digit/sec and speed of tapping at an estimated rate of 3 taps/sec was compared with increase in body (oral) temperature[5,6]. In both experiments, body temperature was raised significantly by immersion of the legs below the knee in a bath of warm water. Eight subjects estimated the 1 digit/sec counting rate over an estimated 60 sec interval. Their body temperature rises during the experiments ranged from 0·4° C to 1·2° C. A '*t*' test assessment of the significance of the difference between the counting speeds at the lowest and highest temperatures recorded revealed a significant increase in counting speed with the increase in body temperature. There was, however, no significant association as assessed by the calculation of a product moment correlation coefficient between the extent of rise in oral temperature and the magnitude of the change in time estimation performance. One subject failed to demonstrate a change in his counting speed in the predicted direction. When the energy of

activation constant was calculated, for the data from each of the seven subjects whose counting speed had increased, in order to assess the constancy of the μ value on which the biochemical clock hypothesis is based, it was found that μ had a mean value of 74,000 calories within a range over the seven subjects of 9,000 to 139,000 calories.

The eight subjects examined on the 3 taps/sec task had increases in oral temperature of 0·3° C to 1·2° C. A product moment correlation coefficient of $r = +0{\cdot}84$ ($P < 0{\cdot}05$) revealed a significant association between the extent of rise in oral temperatures and the magnitude of the changes on the tapping task. However, three subjects failed to show an increase in tapping speed with raised body temperatures, and for the five subjects whose performance changed in the predicted direction energy of activation constants ranged from 20,000 calories to 138,000 calories about a mean of $\mu = 83{,}000$ calories.

In view of the very great discrepancies between these findings and those of the original research, Arrhenius equation analyses were performed on Francois's[1] data. It was found that with increases in axilla temperature of between 0·3° C and 1·0° C, the energy of activation constants ranged between 14,000 and 34,000 calories in sixteen experiments on three subjects.

In view, therefore, of this variability in the Arrhenius equation energy of activation value, the constancy of which is essential to the hypothesis of a potent biochemical influence on time estimation performance in humans, and because Hoagland[2] has pointed out that "a temperature range of 3·2° C is too short to enable one to say with certainty that the Arrhenius equation adequately describes the data", the position that man has demonstrated a control of his time estimation of short intervals by a chemical clock would seem to be untenable.

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MISCELLANEOUS

'Significant' and 'Highly Significant'

Frequently in scientific papers one finds the terms 'insignificant', 'significant' and 'highly significant' used in discussing the difference between control and experimental, with their ranges usually established by $P = 0{\cdot}05$ and $P = 0{\cdot}01$. Statistics text-books provide little or no guidance as to their proper usage because statisticians apparently disapprove of the convention. Yet the universality of the convention attests to the need for clearly defined, commonly accepted terms which properly weight the results of the experiment. The present convention is commonly accepted among non-statisticians, but, as will be shown here, is not clearly defined. Therefore an improved convention is proposed for the consideration of those who would benefit from it—the non-statisticians in research. Perhaps by improving the convention, it can be made more palatable to statisticians, as well as more useful to non-statisticians.

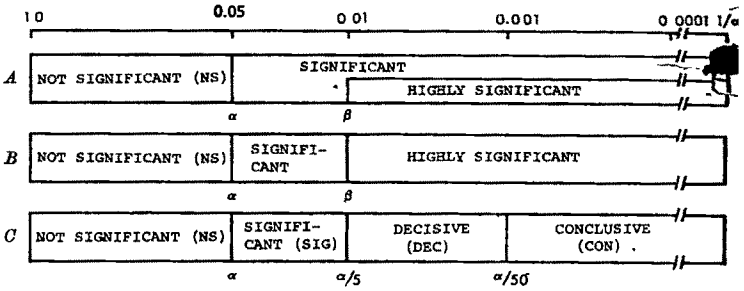


Fig. 1. Range of probabilities covered by present and proposed terms. *A*, Present convention, by definition. *B*, Present convention, as commonly used. $\alpha = 0{\cdot}05$ and $\beta = 0{\cdot}01$ unless otherwise specified (no set relation between α and β). *C*, Proposed convention: $\alpha = 0{\cdot}05$ unless otherwise specified, other values always $\alpha/5$ and $\alpha/50$

The proposed convention is as follows: The significance level, α, for rejection of the null hypothesis will be 0·05 unless otherwise specified. The significance zones will be established by the points 0·05, 0·01 and 0·001; or, in general, α, $\alpha/5$ and $\alpha/50$. When a statistical test is not designed to distinguish, for example, a probability between $\alpha/5$ and $\alpha/50$ from one which is less than $\alpha/50$, any P less than $\alpha/5$ will be designated by the term for P between $\alpha/5$ and $\alpha/50$. The terms that follow are capitalized to indicate that the idiomatic definition is intended.

The abbreviations may be used in conjunction with the symbols greater than, less than and different from, as follows: $A \overset{\text{DEC}}{>} B$; $B \overset{\text{NS}}{\neq} C$, meaning A is decisively greater than B, and B is not significantly different from C.

The present convention has several defects which the proposed modification will remedy. 'Significant' at present is defined as $P < 0{\cdot}05$, and 'highly significant' as $P < 0{\cdot}01$, unless otherwise specified. Since the latter is included within the former, either term could be applied to values less than 0·01. This ambiguity is avoided in practice because, when using both terms, one never refers to values less than 0·01 as merely 'significant'. In practice, the terms are mutually exclusive, with significant restricted to P between 0·05 and 0·01. In the proposed convention, there is no overlap of definitions, and usage agrees with definition (see Fig. 1).

Flexibility in designating the chosen significance level, α, for rejection of the null hypothesis is retained, but in the proposed system the three levels of significance bear a constant relationship to each other. Since the same criteria would be used to establish each significance level these should have a constant relationship. Only in the proposed convention is this found. In addition, the present scale of probabilities is too compressed, for the two extreme terms are separated by only 5 per cent. The proposed terms, as one may see in Table 1, allow a more precise discussion of probabilities.

Finally, there are semantic difficulties with the old convention. 'Significant' and 'highly significant' sound so much alike that clarity is lost when both must be used several times. Also, the adverbial form, 'highly significantly greater than' is awkward construction. The proposed terms, by contrast, are single, easily distinguished, easily used words with meanings which suggest their idiomatic use.

The foregoing is proposed in the hope of clarifying and simplifying the use of statistics in research, and thus encouraging investigators to make use of statistics in evaluating their data.

Table 1. Definitions of Proposed Terms for Statistical Significance

Term	Abbreviation	Conventional meaning	General meaning
Not significant (ly)	NS	$P \geq 0{\cdot}05$	$P \geq \alpha$
Significant (ly)	SIG	$0{\cdot}05 > P \geq 0{\cdot}01$	$\alpha > P \geq \alpha/5$
Decisive (ly)	DEC	$0{\cdot}01 > P \geq 0{\cdot}001$	$\alpha/5 > P \geq \alpha/50$
Conclusive (ly)	CON	$P < 0{\cdot}001$	$P < \alpha/50$

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, June 13

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 3 p.m.—Annual General Meeting.

Tuesday, June 14

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

PARLIAMENTARY AND SCIENTIFIC COMMITTEE, GENERAL COMMITTEE (in Committee Room 11, House of Commons, Westminster, London, S.W.1), at 5.30 p.m.—Discussion on "Air Pollution—Progress since the Passage of the Clean Air Act and Future Developments in Connexion with Air Pollution".

Wednesday, June 15

GEOLOGICAL SOCIETY OF LONDON, ENGINEERING GROUP (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. C. S. Hitchen: "Materials Problems Connected with the Kainji Multi-Purpose Project"; Mr. D. B. Morris. "The Application of Ground and Marine Geophysical Methods to Material Surveys"; Mr. A. A. Archer: "Economic Minerals Survey".

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Mr. F. Pasquill: "Lagrangian Similarity and Vertical Diffusion from a Source at Ground Level"; Mr. A. J. Dyer: "The Flux-Gradient Relation for Turbulent Heat Transfer in the Lower Atmosphere"; Mr. K. L. Calder: "Concerning the Similarity Theory of Monin/Obukhov".

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—132nd Annual General Meeting. 6 p.m.—Mr. L. H. C. Tippett "Statistics in Manufacturing Industry" (Presidential Address).

Thursday, June 16

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Prof. R. G. W. Norrish, F.R.S.: "The Progress of Photochemistry Exemplified by Reactions of the Halogens" (Bakerian Lecture).

INSTITUTION OF MINING AND METALLURGY (at the Geological Society of London, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. Murrogh V. O'Brien: "Review of Mining Activities in the Republic of Ireland".

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. G. W. Whitehead: "Stable Homotopy Theory".

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at Manson House, 26 Portland Place, London, W.1), at 7.30 p.m.—59th Annual General Meeting, followed by Prof. A. W. Woodruff. "Medicine in Burma".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER or ASSISTANT LECTURER (with an interest in electrochemistry or thermodynamics) IN CHEMISTRY—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (June 13).

SECOND TUTORIAL FELLOW IN PHILOSOPHY—The Warden, Merton College, Oxford (June 14).

SENIOR DEMONSTRATOR IN THE DEPARTMENT OF CHEMISTRY to help with the practical teaching of physical chemistry and to carry out research in the general field of mechanisms of reactions in solution—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (June 14).

ASSISTANT LECTURER IN ARCHAEOLOGY (including Roman Britain)—The Registrar, University of Bristol, Senate House, Bristol, 2 (June 17).

ASSISTANT LECTURER or LECTURER (preferably with experience in the application of quantitative techniques in human geography) IN THE DEPARTMENT OF GEOGRAPHY—The Registrar, The University, Liverpool, quoting Ref. CV/76/N (June 18).

LECTURER (with an honours degree) IN MATHEMATICS; and a GRADE B ASSISTANT LECTURER (with an honours degree) IN MATHEMATICS IN THE DEPARTMENT OF MATHEMATICS AND PHYSICS, College of Engineering and Science—The Director of Education, The Polytechnic, 309 Regent Street, London, W.1 (June 18).

ASSISTANT LECTURER (preferably with interests in reaction kinetics, surface chemistry, or in thermochemistry and of doctoral standing) IN PHYSICAL CHEMISTRY—The Registrar, The University of Manchester Institute of Science and Technology, Sackville Street, Manchester, 1 (June 20).

LECTURER (preferably with experience or interest in neurophysiology) IN PHYSIOLOGY—The Secretary, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (June 20).

LECTURER IN PSYCHOLOGY in the Institute of Education—The Registrar, The University, Hull (June 23).

LECTURER IN ENGINEERING MATHEMATICS (Computation)—The Secretary, The Queen's University, Belfast, Northern Ireland (June 24).

LECTURER (with special qualifications in petrology (igneous or metamorphic) or in economic geology) IN GEOLOGY—The Registrar, The University, Sheffield (June 27).

CHAIR OF BIOLOGY—The Secretary, University of Stirling, Stirling (June 30).

FOOD TECHNOLOGIST, SCIENTIFIC OFFICER GRADE (with a university degree, preferably with honours) IN THE DEPARTMENT OF AGRICULTURE AND HORTICULTURE, Long Ashton Research Station—The Registrar, University of Bristol, Senate House, Bristol, 2 (June 30).

LECTURER (with a distinguished academic record, and preferably with interests in some field of invertebrate zoology) IN THE DEPARTMENT OF ZOOLOGY, School of Biological Sciences, University of New South Wales—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, June 30).

LECTURERS IN STATISTICS IN THE DEPARTMENT OF MATHEMATICS—The Deputy Secretary, The University, Southampton (June 30).

ASSISTANT LECTURER IN THE DEPARTMENT OF MATHEMATICAL PHYSICS—The Assistant Registrar (Science and Engineering), The University of Birmingham, Birmingham, 15 (July 1).

ASSISTANT LECTURER or LECTURER IN THE DEPARTMENT OF VETERINARY ANATOMY, Royal (Dick) School of Veterinary Studies—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (July 1).

CHAIR OF THEORETICAL PHYSICS at Westfield College—The Academic Registrar, University of London, Senate House, London, W.C.1 (July 1).

LECTURER or ASSISTANT LECTURER (with research or industrial experience in one of the following fields: theoretical and experimental fluid dynamics, advanced propulsion systems, aircraft structures, aircraft control systems or automatic control) IN THE DEPARTMENT OF AERONAUTICS AND ASTRONAUTICS—The Deputy Secretary, The University, Southampton (July 1).

READER IN EXPERIMENTAL PHYSICS at Queen Mary College—The Academic Registrar, University of London, Senate House, London, W.C.1 (July 1).

SENIOR EXPERIMENTAL OFFICER IN THE DEPARTMENT OF MICROBIOLOGY whose main duties will be the operation and maintenance of an electron microscope, examination and preparation of specimens of micro-organisms and infected tissues, and to develop or improve techniques for the examination of such specimens—Secretary to the Academic Council, University of Belfast, Belfast, Northern Ireland (July 1).

CHAIR AND HEAD OF THE DEPARTMENT OF BIOCHEMISTRY—The Registrar, The University, Leeds, 2 (July 4).

LECTURER IN HAEMATOLOGY AND BLOOD TRANSFUSION IN THE DEPARTMENT OF PATHOLOGY, University of Ibadan, Nigeria—The Secretary, Senate Committee on Higher Education Overseas, University of London, Senate House, London, W.C.1 (July 11).

LIBRARIAN at the University of Ife, Nigeria—The Inter-University Council, 33 Bedford Place, London, W.C.1 (July 11).

ASSOCIATE PROFESSOR IN BIOLOGY (in the field of cellular biology) at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, July 15).

LECTURER (Member of the College of Pathologists, or with an equivalent higher qualification, and experience of teaching students) IN MORBID ANATOMY IN THE DEPARTMENT OF PATHOLOGY, University of Hong Kong—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, July 15).

READER IN MICROBIOLOGY at King's College Hospital Medical School—The Academic Registrar, University of London, Senate House, London, W.C.1 (July 15).

CHAIR OF PSYCHOLOGY at the Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, July 18).

READER (with at least ten years experience in the petroleum industry and preferably interests and experience in petroleum reservoir engineering) IN PETROLEUM ENGINEERING at Imperial College of Science and Technology—The Academic Registrar, University of London, Senate House, London, W.C.1 (July 20).

CHAIR OF MINING AND METALLURGICAL ENGINEERING at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, July 29).

SENIOR LECTURER IN GEOPHYSICS at the University of Tasmania, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, July 29).

SENIOR LECTURER/LECTURER (with an honours degree in an appropriate branch of science, applied science or engineering, a number of years of research and/or industrial experience, and preferably with experience in the use of modern analytical techniques and their application in the process industries) IN APPLIED CHEMISTRY IN THE DEPARTMENT OF INDUSTRIAL SCIENCE, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office) Marlborough House, Pall Mall, London, S.W.1 (August 8).

LECTURER/SENIOR LECTURER IN AGRONOMY at Massey University of Manawatu, Palmerston North, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, August 15).

BIOCHEMIST (basic grade) in the Biochemistry Laboratory of the Endocrine Unit—The Secretary, New End Hospital, London, N.W.3.

CHAIR OF PHYSIOLOGY in the Institute of Advanced Studies, Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1.

CHAIR OF THEORETICAL SOLID STATE PHYSICS—The Registrar and Secretary, The University of Sussex, Stanmer House, Stanmer, Brighton, Sussex, marking envelope "Confidential".

CHEMISTS (with a good honours B.Sc. degree and preferably of Ph.D. standing) to work in the fields such as relation between structure and properties in polymeric materials, modification of polymer properties by cross-linking and grafting, the application of reverse osmosis to the desalination of water and to non-aqueous systems and biomedical applications of new membranes—The Director, Arthur D. Little Research Institute, Inveresk, Musselburgh, Midlothian, Scotland.

LECTURER and an ASSISTANT LECTURER (with a good honours degree in electrical engineering or an honours diploma in technology) IN THE DEPARTMENT OF ELECTRICAL AND ELECTRONIC ENGINEERING—The Academic Registrar, Northampton College of Advanced Technology, St. John Street, London, E.C.1, quoting Ref. EE/N.

PROFESSOR (with high academic qualifications and substantial industrial and/or research experience in some aspect of the broad field of automotive engineering, preferably in vehicle dynamics) IN AUTOMOTIVE ENGINEERING WITHIN THE DEPARTMENT OF AERONAUTICAL AND AUTOMOBILE ENGINEERING—The Academic Registrar, Loughborough University of Technology, Loughborough, Leicestershire.

RESEARCH FELLOW (theoretical physicist or mathematician, preferably of post-doctoral status) IN THE DEPARTMENT OF PURE AND APPLIED CHEMISTRY, to work under Dr. Pugh on the theory of electronic states near dislocations in iron crystals—Prof. M. Gordon, Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, C.1.

RESEARCH OFFICER (with a background in forestry and a sound knowledge of the structure of wood in relation to its use) IN THE DEPARTMENT OF ORGANIC CHEMISTRY (at present the research programme centres on the quality of Eastern Canadian softwoods in relation to their use in the pulp and paper industry)—Personnel Officer, Ontario Research Foundation, 43 Queen's Park Crescent East, Toronto, 5, Ontario, Canada.

SENIOR GRADE PHYSICISTS (2) (with previous experience in radiation protection, and preferably experience with radio-isotopes) IN THE PHYSICS DEPARTMENT—The House Governor, The London Hospital, Whitechapel, London, E.1.

SENIOR LECTURER IN ZOOLOGY/PHYSIOLOGY, a SENIOR LECTURER IN PLANT PHYSIOLOGY; LECTURERS IN CHEMISTRY (2); and a LECTURER IN MICROBIOLOGY—The Principal, Barking Regional College of Technology, Longbridge Road, Dagenham, Essex.

SENIOR SCIENTIFIC OFFICER (veterinary graduate, preferably with experience in physiological research, and an interest in, and aptitude for, experimental surgery) IN THE PHYSIOLOGY DEPARTMENT, to collaborate with members of the Physiology and other Departments in experiments involving surgical procedures and to carry out physiological research in some field related to experimental surgery—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, Berkshire, quoting Ref. 66/19.

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BOMBS AWAY

THE common interest that there should be a limit to the spread of nuclear weapons is uncommonly difficult to ensure by technical means. It is now more than three years since the international agreement was made between most countries except China and France not to test nuclear weapons in the atmosphere. For much of that time the Disarmament Sub-Committee of the United Nations Assembly has been talking about other steps that might be taken. Governments and groups of governments have proposed various devices for limiting the spread of nuclear weapons including an extension of the test ban to underground tests and nuclear-free zones, as they are called, in various parts of the world. Unofficial groups have zealously pursued the same objectives. Most often, frustration has won the day.

By now the reasons why these problems are difficult should not be a surprise. The spread of the technology of nuclear weapons is not a process of unrestrained contagion, like the spread of plague. Refrigerators and motor-cars may be diffused to the corners of the Earth, but nuclear weapons need not follow. So far, five nations have embarked on the manufacture of nuclear weapons. Nuclear powers appear to have been created at a rate of one every five years. Yet for every nation which manufactures nuclear weapons, there is at least one other which has deliberately decided not to do so. The causes of this self-restraint are only occasionally technical, and concerned with such things as the cost of making nuclear weapons in meaningful numbers, or the difficulty and cost of delivering them in anger. Political restraints have usually been more telling. Some nations have been discouraged from making nuclear weapons, or otherwise obtaining them, by the fear of political and even military repercussions. Others have sought to preserve an international reputation for pacific ways of conducting international business. The influence of the Soviet Union and the United States has frequently been decisive in preventing the spread of nuclear weapons.

The political disincentives to the independent manufacture of nuclear weapons are so many and so powerful that it is even possible to argue that there are natural limits to the extent to which nuclear weaponry will spread throughout the world. If nuclear weapons are like an infection, only some nations are susceptible. The trouble with this line of argument is that, even if it should be sound, nobody can risk relying on it. The same is true of the beguiling, but less plausible, analogue of Le Chatelier's principle that the spread of nuclear weapons does not matter, because the pattern of international relations will quickly adjust to nullify the consequences of the acquisition of nuclear weapons by one nation. The view, fashionable in France, that the world would be more stable if all nations were possessed of nuclear weapons somehow commensurate with their importance as prizes to potential enemies is equally suspect as an over-simplification. The plain truth is that it seems to be most sensible to pursue all ways of reinforcing the natural restraints of the spread of nuclear weapons. The difficulty is that there can be no single recipe for doing this, but only an awkward mixture of several ingredients, some political and some technical.

What, then, can be accomplished by technical devices for limiting the spread of nuclear weapons? There are obvious limits to what can be done. A device such as the test ban is less than perfect not merely because of the possibility, now remote, that violations might not be detected, but also because abrogations by individual nations of the international treaty to ban tests in the atmosphere entail no formal penalties. Only if the number of exceptions to the rule was indecently great would the other signatories of the treaty abandon it. From this it follows that an extension of the treaty on tests to inhibit underground tests would be valuable but not crucial. Persuading China to sign the existing treaty—a political task—would be more valuable than solving the technical problems which still prevent an agreement on underground tests.

Similar problems attend other proposals of a technica character for limiting the spread of nuclear weapons. Thus the several proposals that there have been for nuclear-free zones in parts of the world as different as Latin America and Central Europe are open to the general criticism that they refer either to groups of nations which are unlikely to acquire nuclear weapons anyway, or to parts of the world where international tensions are acute, and where a simple declaration of intent would be unconvincing if it were not accompanied by full-blooded inspection.

It is the same with the proposals for an international treaty under the terms of which nuclear powers would undertake not to help disseminate nuclear weapons, and other nations would agree not to acquire them. There are obvious but formidable difficulties in inspecting such a treaty, and indeed it seems now to be agreed that any agreement would have to rest on a declaration of good faith. But then there are the problems of how nuclear powers falsely accused of helping with the spread of weapons can publicly prove their innocence. There is the difficulty of knowing what will happen if signatories abrogate the treaty by a sudden declaration. And there is the over-riding unwillingness of the nations not now possessed of nuclear weapons to sign an instrument which will perpetuate what frequently appears as the overweening arrogance of the existing nuclear powers. Here, too, the existence of problems should not destroy the will to solve them, but progress must be slow.

In these circumstances, it may well be that the best contribution which technical people can make to the restraint of the spread of nuclear weapons lies in the design of procedures for controlling the uses to which fissile material is put. The International Atomic Energy Agency in Vienna has been occupied for several years in working out means of supervising research reactors, and some progress has now been made towards dealing with power reactors. By all accounts, there is a good chance of working out an effective system. Though it will certainly need several years before the international supervision of the IAEA could so win the trust of the nuclear powers that the Agency could take over the supervision now vested in a host of bilateral treaties, that goal is well worth working towards. Sooner or later, fears about the spread of nuclear weapons will inhibit the full use of civil nuclear power if convincing international supervision does not exist. And there is always the possibility that diplomatic ambitions will reach—as they did a decade ago—towards an inter-

national agreement to refrain from the manufacture of military nuclear explosives. The cut-off, as it is called, would be a more valuable agreement than any now in prospect.

WRONG REPENTANCE

WHAT seemed a week ago to be the near certainty that the European Launcher Development Organization would be torpedoed has in the event been transformed by compromise. The British Government seems to have recognized the force of the arguments against what amounted to its withdrawal from ELDO, and the Minister of Aviation at Paris a week ago played a much less intransigent part than he had at first announced. It is now likely that there will be a reapportionment of the costs of running ELDO in such a way that the three principal partners—Britain, France and West Germany—will pay roughly equal shares. There is also to be a technical examination of the programme of ELDO, though at this stage it is not clear whether this will be a thorough reappraisal of the somewhat shaky plans at present being followed, or merely an exercise in face-saving. More should be known when European ministers resume their debate on the future of ELDO on July 10. Meanwhile there is no doubt that the retreat of the British Government from its previously ill-prepared position is to be reckoned a sign of grace, though one that does not entirely cancel out the ostentatious tactlessness of the original decision to withdraw, for practical purposes, from ELDO.

The chief cause for continuing regret is that the survival of ELDO is now made to seem conditional on the reduction of the cost to the United Kingdom. With luck, the British Government can now expect to reduce the cost of its involvement in ELDO by just less than a third, and this is no doubt significant. But it is still a fact that the commitment is open-ended. It is also as true now as it was a week ago that the present plans of ELDO cannot bring the economic and technological benefits which the original sponsors of the organization looked for. The launching rocket based on *Blue Streak* is inconveniently cumbersome for launching the kinds of scientific satellites with which European work in this field would best begin, and at the same time is inconveniently small for launching communications satellites. Fallen as it has between two stools, it is no wonder that ELDO has found it hard to decide why it exists. The best outcome of the crisis that there has been would be a coherent definition of a sensible long-term objective. It would have been better if the British Government had insisted on such a reappraisal, and not a redistribution of the financial burdens, as the price of its continued membership.

But what can Europe accomplish on its own? The most conspicuous error in the past few years has been the illusion that a research and development programme which is tiny by American and Russian standards, and intended to make use of a number of components designed for purposes now forgotten, should somehow enable Europe to attain a position of equality in space technology. Even if the first ELDO programmes had been outstandingly successful—and they have not been—it is not realistic to suppose that Europe would then have been able to embark on a comprehensive programme of satellite research, or to help substantially in the construction of an international system of communications by satellite. For a long time to come, the only attainable objective will be to lay the foundations in Europe of a technology already widely practised elsewhere.

GOOD ENOUGH FOR MOSES?

D-Days at Dayton

Reflections on the Scopes Trial. Edited by Jerry R. Tomkins. Pp. xii+173+8 plates. (Baton Rouge, Louisiana: Louisiana State University Press, 1965.) 5 dollars.

ANYONE attentive to theatre, cinema, or television in recent years knows that in 1925 a teacher named John Scopes was hauled into court for discussing evolution in a tax-supported school in Dayton, Tennessee. With the exception of Scopes himself, who played no part other than that of warm body, everyone involved sought public attention for a variety of motives. Two of the most flamboyant characters in a country that never lacks such seized the opportunity. The summer of 1925 was hot and dull, and it produced no important news. The desire for publicity was requited in excess. The two characters, William Jennings Bryan and Clarence Darrow, staged a debate marked on both sides by inanity exceptional even in the long history of inane debates. The debate was stricken from the court record, but became the only reason why this trial is remembered. Three teachers produced affidavits favouring evolution, but their evidence was not admitted in court and was (of course) given to the newspapers instead. The decision of guilty, obviously mandatory and desired by all parties before the trial even started, was reversed on a technicality. Scopes was never retried. A test of the constitutionality of the anti-evolutionary law, the only rational reason for the procedure, was never made. The law is still on the books in Tennessee and also in the other southern states of Arkansas and Mississippi, but no other effort has ever been made to enforce it. Indeed, the Scopes trial involved no such effort, being instigated by non-Tennesseeans hoping to nullify the law and not by any State or local authorities hoping to enforce it.

D-Days at Dayton is intended to provide judgement on the effects of the trial after 40 years. It contains the contemporaneous accounts of an iconoclastic reporter, H. L. Mencken, and the contemporaneous affidavits of the three teachers of science, W. C. Curtis, K. F. Mather and F.-C. Cole. The main offering, however, is a series of eight newly written essays by two ministers, a theologian, three scientists, a scientific journalist, and a former director of the American Civil Liberties Union. Some of these were present at the trial, but none had an active part in it and for some the only connexion is that they remember hearing about the trial when they were children. There is also an essay by Scopes himself, and this is extraordinary. Scopes apparently had little interest in the trial at the time, has virtually none now, and is most nearly moved by his belief that Bryan, his rabble-rousing, anti-intellectual prosecutor, was "the greatest man produced in the United States since the days of Thomas Jefferson".

The not very clearly expressed thesis of the editor and some contributors seems to be that the Scopes trial has current relevance because it marked the opening of a largely successful attack on anti-evolutionism in the United States. As one contributor (Carlyle Marney, a Southern Baptist minister and evidently a unique one) does point out, the thesis is flatly wrong on both counts: the battle against anti-evolutionary fundamentalism began long before 1925 and was far from won in 1965. The strongest argument is that Tennessee was so ridiculed that no other States dared be so foolish. But all the evidence suggests that Tennesseeans were delighted by the publicity and unconscious of the ridicule. And in fact to-day the teaching of evolution is prevented in an enormous number of school districts (locally almost autonomous in the United States) by devices much more effective than unenforced State laws.

This somewhat interesting but unconvincing and patchwork volume does nothing to alter the feeling that the Scopes trial was a farce and that its only present importance

is that it inspired a more successful and more frank farce, the play *Inherit the Wind*. A serious enquiry into present-day anti-evolutionism, not attempted by this book, is urgently needed. GEORGE GAYLORD SIMPSON

IN DEFENCE OF PERSONAL KNOWLEDGE

The Knower and the Known

By Marjorie Grene. Pp. 283. (London: Faber and Faber, Ltd., 1966.) 42*s*. net.

THE first thing to notice about *The Knower and the Known* is its dedication to Prof. Michael Polanyi; a direct pointer to an emphasis on personal knowledge, indeed an extension and defence of that outlook. Prof. Grene's contribution has come at an appropriate moment, and her analysis, coupled with a pleasant style, will repay careful study. There are three main sections: knowledge as conjecture; the structure of experience; and the complexity of things. At the end is a competent discussion of the late Sir Ronald Fisher's *Genetical Theory of Natural Selection*, which forms a separate appendix. If there is one quality which these pages rightly place on a pedestal, it is that of intellectual humility. Descartes believed that he had solved every problem which had confronted, or could confront, the human race, while the genius of Newton had produced the perfect picture of a mechanical cosmology. Yet, all this was to predict and, if need be, to falsify, but not to understand. Whitehead once remarked that it was the medievalists who walked by reason, whereas we walk by faith. Nevertheless, credulity will always seek to understand, and that is what personal commitment means, as contrasted with sheer objectivity. If we are to recover from dead things, then we have to reckon with the unspecifiable element in knowledge, which is never explicit. It is possible, naturally, to cogitate in terms of billiard balls, hard lumps of matter, and so forth, but this is not comprehension, which involves an element of passion, which lies at the root of all great discovery. Aristotle's taxonomy was magnificent, and his logic superb, yet devoid of commitment or revelation.

This throw-back to the Stagirite is no accident: Grene is at home with the philosophy of the Lyceum; one can almost hear the steps of the peripatetics in the background. An epistemology fitted for the study of the living there must be, if existence in an arid waste is to be avoided. This connotes a large measure of responsibility in research; discovery is not detached spectatorship.

Now the odd feature about all this—as everybody will realize who has worked in any branch of higher education during recent years—is that the present generation of students has sensed this very thing without any trained ratiocination or prompting. There is a widespread reluctance to enter the (purely) mathematical sciences; the urge is to do something more in keeping with the needs of the living. Hence the pressure on the biological and social sciences. That there is a lurking danger in this, nobody can deny. (Lord Rutherford is reported as having observed, on one occasion, to Sir Gowland Hopkins, "How delightfully easy it must be to be a biologist".) If there are many unable, or unwilling, to face a mathematical discipline, there are a few who are looking towards a new kind of differential equation, capable of dealing with systems of giant molecules. It remains to be seen whether this attitude will prove constructive or destructive: whatever happens cannot obliterate the quest for personal knowledge, but it may well shift the burden of appreciation from a direct aesthetic appeal to one of more abstract type. In which case, the battle for subjective entailment will begin all over again.

Prof. Grene, in her copious references, seems to have overlooked the particular approach of the late Dr. Agnes Arber in this very context of morphology. She demonstrates the influence of Spinoza as a turning-point in the exploration of form.

Perhaps this volume reaches its zenith in Chapters 8 and 9, where the discussion ranges over the multiplicity of forms, time and teleology. We are still bidden—almost coerced—'to understand', but the price of doing so is clearly revealed. In terms of psychology this is very close to the Gestalt-concept, the apprehension of the whole in terms of its parts, and not only so, but the explicit recognition that an aim is a function of the means taken to attain it. The transition from here to teleological processes is easy in principle; few ventures have generated greater heat than this in scientific argument. To the naturalist, ends, goals, and even purposive behaviour, stand out sharply against the strict 'yes–no' of classical thought. Nobody expressed the fundamentals of the situation better than the late E. S. Russell (*The Directiveness of Organic Activity*), though not in the exact idiom of the author of *Personal Knowledge*. Probably such tendencies in natural systems can be transferred to the creative arts, wherein theories of minimal energies can suggest, even if they cannot prove, the existence of an *extremum*, in which satisfaction is found for eye or ear. It was not for nothing (p. 204) that Kekulé dreamt of snakes chasing their tails, which is alleged to have been the basis of his discovery of the benzene ring. Quite recently, in Holland, a concept of this character has produced a number of patterns of surprising beauty, dependent on the crystallographic symmetry of close-packing.

All in all, there is little doubt that we are moving away from mechanics as a powerful element in methodology (never to be confused with logic) in brave attempts to create a new theory of knowledge which will abandon explicit knowing as unattainable in this life. Having made this sacrifice (if ever we do), a happier epistemology may arise on the embers of determinism in the seventeenth century sense. Volumes like the one now under review will help. It should appeal to a wide circle of readers, who would do well to keep it near at hand if any of them are directors of research. For no discovery has ever been made without zeal—zeal to believe that there is a problem to be solved, and joy of a passionate kind on reaching journey's end. Prof. Polanyi himself pleads for a strong measure of patience and forbearance with existing theories before they are deliberately overthrown. Savants are not professional iconoclasts, and reverence for tradition is at least an element in true scholarship.

On p. 252, Prof. Grene refers to the problem of pain, and considers that "it makes life wholly present, and therefore wholly meaningless". One may doubt whether this view, plausible as it sounds, represents much more than a superficial reaction. To judge from their writings, this was not the experience of the saints and mystics (experts in the pursuit of personal knowledge) who, far from being deprived by it of their transcendence, found in it their finest hour. F. I. G. RAWLINS

AN IMPERFECT ANALOGY

The Railroad and the Space Program

An Exploration in Historical Analogy. Edited by Bruce Mazlish. (Technology, Space and Society.) Pp. xix+223. (Cambridge, Mass., and London: The M.I.T. Press, 1965.) 7.50 dollars; 57*s*. net.

IN 1962 the U.S. National Aeronautics and Space Administration, wishing to find out more about the probable long-term effect of its space activities, made a modest grant for an academic study of the subject. A committee of the American Academy of Arts and Sciences decided that the study should take the form of a historical analogy with some previous enterprise, and Bruce Mazlish, the editor of this book, chose to direct attention towards the development of the American railroad. After

a long introduction, in which Dr. Mazlish himself discusses historical analogy, deftly but inconclusively, we are treated to seven separate surveys of United States railroad history. These reveal in convincing detail the economic, social and political effects of the railroad expansion, but tell us virtually nothing about the likely effects of the space programme. Each contributor dutifully mentions space exploration at the end of his chapter, but often only to say that any analogy is doubtful. In short, the question that NASA wished to see answered is never properly tackled at all: the authors are like a pack of industrious detectives who efficiently scour the ground while their quarry lurks in a tree above.

Although the book does not fulfil its declared aim, the studies of railroad history may be of interest to economists and historians. Stylistically, the book is often heavy with detail: there are, for example, tables giving United States steel production yearly from 1871 to 1890, the proportion devoted to railways, etc. But there are also illuminating insights. For example, R. W. Fogel challenges the conventional view that the improvements in transportation brought about by railroads were the key to American industrialization. He prefers to emphasize that the diversion of resources into railroad construction delayed the advent of motor transport by several decades. Is there a lesson here for the space explorers? It is difficult to tell, because the analogy between rail and space travel is so imperfect: both often leave behind a plume of steam, it is true, but rail travel merely improved transportation between known areas, while space exploration opens up new areas. This fundamental difference sabotages the book's few real attempts at pursuing the analogy. D. G. KING-HELE

HYDROLOGY—CLASSICAL AND MODERN

Modern Hydrology

By Raphael G. Kazmann. (Harper's Geoscience Series.) Pp. xiv+301. (New York: Harper and Row, 1965.) 79*s*.

THE inception of the International Hydrological Decade in 1965 and the water shortages of the same year have helped to stimulate an interest in one branch of the geosciences—hydrology. In his introduction to *Modern Hydrology*, the editor quite rightly states that an up-to-date, authoritative but readable work is needed by newly interested administrators, lawyers, legislators and planners as well as by academics and professional hydrologists. The comments that follow will help indicate whether the book under review fulfils the undoubted need.

The dust cover claims the book to be "a succinct but thorough discussion of the hydrologic cycle, the development of water resources, and the effects of this development on future projects". Its organization of the material is refreshingly simple; after an introductory chapter, the basic components of the hydrological cycle are examined in turn, the two concluding chapters being an account of water resources development, and final summation. Within the terms of reference given, the contents of the chapters on "Precipitation" (22 pages), "Evaporation" (14 pages), "Surface Water" (70 pages) and "Ground Water" (80 pages) are no more than adequate. One must deplore the absence of material in both text and illustrations from sources outside the United States in a work dealing with the principles, methodology and practical application of hydrology, and presumably aimed at a world-wide market. The meat of the book is contained in the chapters on "Water Resources Development" (38 pages) and "Summation and Outlook" (26 pages). The remarks of the author on the organization and financing of large-scale water development works are forthright and thought-provoking. They are directly applicable to the present British scene where belated enthusiasm for barrage schemes and large-scale redirection of river water must be tempered by the high costs of such projects.

The purport of the title is emphasized many times throughout the book. Prof. Kazmann's thesis is that the concept of 'classical hydrology' is dependent on the assumption that the data are 'clean', that is, the result of natural circumstances alone. Such conditions are becoming increasingly less common because the numerous side-effects resulting from man's activities exert a modifying influence on the hydrological data, which are the foundations of modern hydrology. He goes further; given a continuation of the process, the data would become so 'dirty' that it would be impossible to distinguish between natural hydrological events and those induced by man in the form of dams and diversions, surface- and groundwater abstraction, and sewage disposal. This point of view seems over-pessimistic. Man's activities have been complicating hydrological data for centuries past, and indeed only rarely can water development projects be undertaken without incurring some form of hydrological side-effect. One might go so far as to say that most data have been of the dirty variety since scientific hydrological measurement first began.

Prof. Kazmann has attempted to cover too wide a field in too little space, and at the same time has tried to satisfy too many diverse interests. His central theme is the effect of man's activities on the hydrological cycle, and the significance of modern versus classical hydrology. The book would have been improved had it been more specifically directed to those ends. While *Modern Hydrology* will make a stimulating and worth-while addition to library shelves as a work of reference, it is unlikely to supersede any of the accepted hydrological text-books.

G. P. JONES

SOIL BIOLOGY

Bodenbiologie

Von Georg Müller. Pp. 889. (Jena: Veb Gustav Fischer Verlag, 1965.) 153*s*. 5*d*.

BODENBIOLOGIE, which was intended to fill a gap in the German literature as well as to supplement the more general literature of soil science, has certainly achieved its purpose. The author is to be congratulated on covering the extensive field of soil biology so thoroughly. Soil biology is the newest and perhaps most important branch of the soil sciences; it is developing rapidly and any attempt to integrate the separate studies of soil bacteriology, mycology and zoology into a unified discipline must be welcomed.

Prof. Müller divides his book into five main sections: soil organisms; methods, considerations of numbers and occurrence of soil organisms with reference to site conditions; activities of soil organisms; and influence of cultivation practices on the life in the soil. In the first section the morphology, systematics, ecology and physiology of the soil microflora (bacteria, fungi, algae and lichens) are discussed in much detail followed by a similar treatment of the soil fauna (from protozoa to mammals).

The section on methods concentrates rather on procedures for counting and isolating organisms, but also deals with all the usual laboratory techniques for studying the organisms in culture and refers also to soil sampling and to statistics. The effects of a variety of soil factors and conditions on different organisms and their populations in soil are treated in the third section. The fourth large section is an account in detail of the biochemical activities of organisms with more emphasis on the microflora (of which more is known) than the fauna, and attention is paid to the part played by different organisms in mineralization, humification and weathering processes. An important fifth section describes the effects of various treatments such as irrigation, cultivation, fertilizing

plant protection and soil inoculation measures on the different kinds of soil organisms. Finally some reference is made to soil sickness. Literature references take up about eighty pages; there is a useful index and also a list of all the genera and species of organisms mentioned.

Although not the only recent book on this subject, this volume is probably the most comprehensive at present. The book is well produced and bound and not expensive in view of its size. Misprints and errors are very rare (Beijerinck, of course, was a Dutchman, not a Dane). The text is reasonably up to date and moreover contains a fair number of references to Russian and Eastern European work as may be expected of an East German book. That it is written in German may unfortunately limit its usefulness, but it can be recommended to all advanced students and research workers, biologists and non-biologists alike, in the field of soil science.

N. WALKER

STEROIDS IN THE POLARIMETER

Selected Constants

Optical Rotatory Power. 1*a*. Steroids. By J. Jacques, H. Kagan and G. Ourisson. Edited by S. Allard. (Tables of Constants and Numerical Data, 14. Associated Organization of the International Union of Pure and Applied Chemistry.) Pp. 1031. (London, Paris and New York: Pergamon Press, Ltd., 1965.) 258 F.

THE steroids form a closely-knit group of organic compounds which have been in the forefront of natural product chemistry for more than thirty years. The reasons for this lie in their biological and economic importance (particularly in the pharmaceutical industry), and in the peculiar appropriateness of their rigid structure for investigations of reaction mechanisms, and of many physical properties. Steroid chemists have been well provided with text-books, with forums for discussion, and with specialized collections of data and materials.

Among the most valuable of these collections has been the catalogue of optical rotations of steroids, published in 1956. *Selected Constants: Optical Rotatory Power* is the second edition of this collection of data on optical rotation, to which have been added the melting-points of most of the compounds listed. Optical rotations are important as criteria of purity and also as characteristic constants for structural and stereochemical problems.

Although expensive, this work is essential for all laboratories working in the steroid field—and highly desirable for other laboratories which are interested in the general relations between optical rotation and structure. The literature is covered up to 1961, structures being corrected up to 1964 where necessary. About twenty-one thousand compounds are listed, in contrast to eight thousand compounds in the previous edition.

The introduction, in both French and English, deals with the nomenclature of steroids. The compounds are catalogued under their molecular formulae with full references (6,300 in all); the book includes extremely thorough indexes to authors and substances.

References to the important new technique of optical rotatory dispersion are given briefly. These items and data on circular dichroism will need to be dealt with more fully in the next edition.

Since optical rotation values are measured for nearly all new steroids, this book really constitutes a one-volume catalogue of steroids; it is therefore the easiest place in which to begin a literature search. The French authors are to be congratulated sincerely on carrying forward the good work of the first edition with their prolonged and meticulous labours.

The book is so valuable, and the method of indexing so simple, that the authors and editor might well ask all colleagues working in the steroid field to assist in the preparation of future editions in the following way. Authors of original papers could readily provide details of new compounds on a standard form, which would present the data on optical rotation ready for the preparation of a third edition or of a supplement. It would be the work of a few minutes to provide the data for each compound in this way, and we should all know that in doing this we were speeding up (and perhaps making a little cheaper) the next edition of this invaluable index and reference book.

W. KLYNE

VARIATION AND GENETICS OF BACTERIA

Bacterial Genetics

By Werner Braun. Second edition. Pp. xiii+380. (Philadelphia and London: W. B. Saunders Company, 1965.) 70*s*.

THE first edition of *Bacterial Genetics* was published in 1953 when text-books in this field were almost non-existent. To-day, however, the reader is confronted with a choice of good texts each covering much the same material. It is against this background that Prof. Braun's revised book has to be evaluated. Both editions of *Bacterial Genetics* have had as an explicit aim the presentation of ". . . the more important findings and principles of bacterial genetics to those primarily trained, or being trained, in bacteriology . . . ". Much of bacterial genetics is complex and too frequently communicated in a confusing manner. Furthermore, data tend to be submerged in a bewildering jargon with the result that the subject becomes largely unintelligible to the non-specialist. Prof. Braun is to be congratulated on making his book clear and palatable to those not primarily trained in genetics and in attempting to relate genetic discoveries to other aspects of bacteriology such as pathology and physiology.

The recent progress of bacterial genetics has been enormous and this second edition of *Bacterial Genetics* is, in large measure, a new book although the layout of the first edition is retained. Part 1 is devoted to introductory considerations, cytology and certain molecular features including mutation and mutagenesis. Part 3 deals with genetic transfer systems and "gene action" and its regulation. The middle section discusses "Representative Mutant Types" and "Population Changes" and, although the approach here is necessarily itinerative, the material is offered interestingly and will make particularly apposite reading for the bacteriologist. Several features of this book appealed to me, notably the careful definition of terms, the cross-referencing within the text, the inclusion of technical details and the insistent distinction made between working hypotheses and established facts. The book is comprehensive but several topics have received but brief consideration. Thus, although the reader is directed to other sources for additional details, this brevity can be misleading; for example, the statement that the life-time of messenger RNA is only a few minutes requires qualification. Also unsatisfactory is the abrupt termination of the book; we are suddenly halted during a discussion of feedback inhibition and allosterism. A more satisfying conclusion might have pursued the theme of regulatory mechanisms and cellular differentiation. Occasionally terms are used rather inconsistently (for example, nucleus and nucleoid), quoted data are at variance (for example, proportion of RNA that is ribosomal) and cited references incorrect. Little mathematical treatment is included. However, these are minor criticisms of an otherwise admirable book. Prof. Braun has accomplished his stated aims and his book deserves to be read by both geneticists and bacteriologists. *Bacterial Genetics* will be especially useful to those bacteriologists and other microbiologists who are not aware of the immense impact and relevance of genetics in their own fields.

ALAN T. BULL

Joseph Priestley

Adventurer in Science and Champion of Truth. By F. W. Gibbs. (British Men of Science.) Pp. xii + 258 + 20 plates. (London and Edinburgh: Thomas Nelson and Sons, Ltd., 1965.) 42*s*. net.

JOSEPH PRIESTLEY is a scholarly and readable account of this remarkable Englishman, the dissenting minister and teacher whose experimental discoveries immediately speeded up the progress of science, but whose advanced political views were widely accepted only a generation after his death.

Priestley did not contribute to science until 1765, when at thirty-two he began to compile his history of electricity. He soon turned to chemistry and started to work on gases in the winter of 1767–68, five years before the publication of his first great paper on "Different Kinds of Air". The important chemical work of the 1770's and early 1780's is well described by Dr. Gibbs, who clearly shows how Priestley was repeatedly forced to grapple with unfamiliar and difficult problems arising from his discovery and examination of a dozen new gases. Priestley quickly contrived methods for saturating mineral waters with carbon dioxide, and for generating hydrogen on a large scale in order to fill balloons.

Not only was he a great chemist but Priestley was also an accomplished linguist and biblical scholar. The book throws new light on his persecution for supporting the French revolution and his subsequent exile in America.

Dr. Gibbs has not only given an account of Priestley's life and work based on a very thorough study of printed and manuscript sources, but he has also described the political, religious, social and scientific background in both England and America. The many illustrations, including a number of political cartoons of the period, add to the value of a book which supersedes the earlier biographies of Priestley and can be recommended to all scientists interested in their intellectual ancestor.

W. A. SMEATON

The Evolution of the Nuclear Atom

By G. K. T. Conn and H. D. Turner. Pp. 266 + 13 plates. (London: Iliffe Books, Ltd.; New York: American Elsevier Publishing Company, Inc., 1965.) 55*s*. net.

THIS is the first of four volumes attempting to show the development of modern ideas on molecular, atomic and nuclear structure. It ranges from Faraday's first studies on electrical discharges in gases up to the Rutherford–Bohr theory of 1913–14. About 90–95 per cent of the material consists of original papers or excerpts from original papers by the principal figures of the time. Most of the quoted passages are from the decisive work of such people as Crookes, Thomson, Rydberg, Geiger, Marsden, Rutherford, Moseley, Chadwick and Bohr, but there are also a number of excerpts on some of the less successful ideas, such as Hertz's view that the cathode rays were a form of radiation, and there is a chapter on the more abortive theories of atomic structure proposed by Jeans, Thomson, Nagaoka, Rayleigh and Schott. These various passages are linked by a sketchy narrative in which the authors have made little attempt at commentary. Their intention has been to let the original authors speak for themselves.

The stated aim of the book is to "provide a stimulus to young research workers", but it is difficult to imagine this being its effect. Young research workers are not likely to read it. It cannot be denied that the momentous papers of Thomson, Rutherford, Bohr and the others make powerful reading even half a century later, yet their collective effect in this presentation is just not inspiring. This sort of material requires much more in the way of historiography to make it interesting.

W. M. CURRIE

Atomic Handbook

Vol. 1: Europe. Edited by John W. Shortall. Pp. xvi + 845. (London: Morgan Brothers (Publishers), Ltd., 1965.) 140*s*.; 20 dollars.

THIS publication represents the fruitful result of several years discussion both within and without the Nuclear Public Relations Contact Group. Information on what is going on in atomic physics is invariably the most difficult to obtain, and Mr. Shortall, as editor, and Morgan Brothers (Publishers), Ltd., have done signal service in providing all concerned with atomic physics with *Atomic Handbook Volume 1: Europe*.

The directory has been designed to give answer to who is doing what and where in atomic energy. It is claimed that it has been written "to serve as a management tool to locate collaborators, follow trends, prevent waste of resources and to facilitate interchange and contact". Essentially, then, it is a guide to people and their work. It provides an invaluable source of names, positions, addresses and telephone numbers of key people concerned with the day-to-day business of atomic energy. This first volume includes details of some 4,000 people in twenty-six countries, eighteen international organizations and almost a thousand industrial firms.

It is pleasing to note that the *Atomic Handbook* will be revised at least every 2 years and also that it is intended shortly to produce successive volumes covering other areas of the world. We all wish the enterprise well, and humbly thank all concerned for taking on the herculean task of making European atomic physics accessible.

Plant and Machinery for the Separation of Air by Low Temperature Methods

Design Atlas. By I. P. Usyukin, I. G. Aver'yanov, S. Gorokhov, A. M. Gorshkov, A. V. Zakharov and N. K. Yelukhin. Edited by I. P. Usyukin. Translated by S. S. Akerib and A. D. Norris. Translation edited by M. Ruhemann. Pp. viii + 185 + 164 plates. (London and New York: Pergamon Press, 1965.) 250*s*. net.

IN this *Design Atlas*, 171 of the 185 pages (each 18·5 × 12·5 in. in size) are devoted to flow diagrams of air separation plants, plant layouts or detailed drawings of equipment for use in such plants. Although mainly concerned with designs of Russian plants and equipment, some information about plants designed in western Europe and the United States is included.

The *Atlas* is attractively produced and the drawings are clearly presented. The publishers have ensured the technical accuracy of the work by securing the services of Dr. M. Ruhemann as editor. While the price seems high it is understandably so in view of the work involved in preparation and reproduction.

The foreword to the Russian edition says that the *Atlas* is designed as a text-book for higher technical institutions and will also be used by staff working in establishments which use low-temperature air separation plants. While this may be true in Russia, one doubts whether either of these groups will find the book useful in western countries. To start with, while in Russia air separation experts are trained as such at undergraduate level, in the West we tend to train men in broader fields and let them specialize later. Furthermore, any leading cryogenic engineering company tends to tailor its plants to suit customer requirements and to incorporate new ideas and items of equipment as they mature. Since this is probably also true of the Russian Oxygen Design Bureau, it is unlikely that the *Atlas* contains the very latest Russian designs. Certainly the non-Russian designs given are far from being "the most important now existing" as claimed in the foreword; they are inevitably far from comprehensive or up to date.

The cost of producing a gas from the atmosphere is dependent almost entirely on power consumption and capital outlay. Frequently the potential customer has to

balance a higher power cost against lower capital outlay. The *Atlas*, which quotes power consumptions but makes no reference to initial plant cost, does not help him in this respect.

Established experts in the design of air separation plants will find the Russian designs of considerable interest, despite the absence of information as to the reasons for the selection of particular cycles, techniques or items of equipment. They may well conclude that there is little to choose between the states of knowledge in this field in Russia and in other countries. N. BOOTH

Luminescence

l'Électron et la Lumière Matière et Photoluminescence. Par G. Monod-Herzen. Pp. viii+278. (Paris: Dunod, 1965.) 39 francs.

THIS volume is divided into two distinct sections. The first deals with the relationship between electromagnetic radiation and the electron, and the second with the general phenomena of photo-luminescence.

In the first section, a chapter is devoted to a description of the electron and its properties while at rest, while moving with linear velocity and while being accelerated. A second chapter deals with the emission of radiation as a result of electron impact on matter, distinguishing between phenomena resulting from primary and secondary processes. Here weight is given to the emission of X-radiations. This naturally leads on to a discussion of the behaviour of electrons in atoms and a brief survey of atomic spectroscopy. This section is brought to a close by a short, but comprehensive, reminder of the main features of molecular spectroscopy.

The second section of the book, which is the larger one, commences with a chapter which explains simply the processes which lead to luminescence and phosphorescence. A brief mention is made of excitation leading to ionization and incandescence.

The remainder of this section deals with luminescence phenomena exhibited by atoms and by molecules in the gas, liquid, glassy and crystalline phases. There is some mention of experimental methods for studying these phenomena but the principal emphasis is on the descriptive understanding of the underlying mechanisms. There is a particularly good account of luminescence in the crystalline phase, and the effect of impurities and imperfections.

This volume can be recommended as a good readable work, splendidly illustrated with well-chosen diagrams and with the necessary mathematical treatments, which have been kept to a minimum. There are very few references so that the book, while a very good introduction to the field, would not be appropriate to the advanced reader who wished to follow up specific points in more detail.

J. C. ROBB

Vision in Vertebrates

By Katharine Tansley. Pp. vii+132+16 plates. (London: Science Paperbacks and Chapman and Hall, Ltd., 1965.) 12*s*. 6*d*. net.

SO many books on vision have been published recently that the need for a further one may be questioned. This small book on *Vision in Vertebrates* fills a gap left by other authors who have concentrated on the vision of the human being, although paradoxically much of our knowledge about the physiology of human vision has been derived from investigations on other vertebrates.

Many interesting contrasts and comparisons of anatomical features between a wide variety of vertebrate eyes are given, along with a full list of sources. Sufficient description of the function of each part of the eye is given to enable the non-specialized reader to understand the possible significances of these anatomical differences. Some excellent photomicrographs are included to illustrate the histological structure, although detailed labelling of the structures would help the reader unfamiliar with microscopic sections.

In a work of this nature it is almost inevitable that teleological arguments will be used to reason from structural differences to function. While this approach does help to add interest and coherence to the assembly of species differences, it may lead in many cases to misleading and improbable conclusions. For example, it is a well-established principle of physical optics that the maximum resolving power of an optical system is determined only by the absolute size of the pupil and not by the focal length of the eye.

This lucid book provides a useful and stimulating introduction to the startling variations of adaptation which the vertebrate eye has undergone in order to match each animal to its visual environment. F. W. CAMPBELL

Recent Progress in Hormone Research

Edited by Gregory Pincus. (Proceedings of the 1964 Laurentian Hormone Conference.) Pp. viii+679. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 192*s*.

THIS is the most recent volume of an annual publication which has come to play an important part in the literature of endocrinology. The value of the series lies in fulfilment of two major functions. First, it contains texts of the papers delivered at the Laurentian Hormone Conference by international authorities invited to review recent progress and present-day thinking in their particular fields. Secondly, discussions at the conference are edited and presented in detail, complete with references. These often bring new ideas introduced in the papers into interesting perspective, and sometimes include explanations of difficulties concerning those ideas which colleagues have found puzzling. In some cases such presentation may one day prove invaluable to the historian.

The topics on which papers were invited at this conference, held in September 1964, were: (1) recent advances in thyroid chemistry and physiology; (2) hormones in normal and pathological physiology; (3) pituitary hormones; (4) steroid sex hormones; (5) comparative endocrinology; and (6) neurohumors.

The standard set by papers at these conferences is high, and it is somewhat invidious to select any for special mention; but among those in this volume are several which were found particularly memorable, either on account of exceptional lucidity of presentation or because of the thought-stimulating nature of their subject-matter. W. S. Peart reviews the functions of renin and angiotensin, and although a later review by this author has since appeared, this paper contains material not duplicated elsewhere and was followed by a valuable discussion of the interaction of the renal hormone system with aldosterone secretion and sodium balance in man. C. Gemzell gives an account of his experience with the use of human gonadotrophins to induce ovulation, and K. J. Ryan and O. W. Smith discuss the biogenesis of steroid hormones in the human ovary. J. J. Christian, J. A. Lloyd and D. E. Davis review experimental work, and observations on animals in the wild, concerning the role of endocrines in the self-regulation of mammalian populations. This has fascinating implications in the field of human social history and seems certain to provoke a great deal of further work. J. Axelrod reviews the metabolism, storage and release of catecholamines, and finally E. W. Sutherland, I. Øye and R. W. Butcher discuss the role of cyclic adenosine monophosphate as a secondary hormone 'messenger' in the action of adrenaline, suggesting that it may also be involved in the action of other hormones.

These and the many other worth-while papers included (a total of fourteen) make the volume, which is handsomely produced, worth its price to almost any biological library. It is a pity that it will probably prove too expensive for most private buyers. J. A. PARSONS

EVOLUTION OF PRIMATE SOCIETIES

By DR. J. H. CROOK and J. S. GARTLAN
Department of Psychology, University of Bristol

RESEARCH on primate behaviour has tended to concentrate on similarities rather than differences between taxa, thereby allowing authors to make generalizations concerning the evolution of primate and human societies based on the characteristics of single species[1]. Recent developments in field investigation[2], however, now provide a range of information clearly demonstrating the contrasts that exist even between species the behaviour and ecology of which appeared superficially alike previously. The number of species sampled remains small but enough is known to permit a preliminary examination of the range of social systems uncovered and their functional significance in the habitats concerned. Analysis of function provides some understanding of the selection pressures responsible for adaptive change in social structure, and, given information regarding relevant ecological change in past time, allows the construction of evolutionary hypotheses.

The present approach is based first on the ethological and ecological results of recent field investigations[3]; secondly, on a methodology of evolutionary analysis of social systems used in recent ornithological work[4,5]; and, thirdly, on recent research in the palaeoecology and palaeontology of African primates[6]. Our purpose is to direct attention to patterns of relationships inherent in the data now available and to begin the task of ordering it. The hypothesis put forward gains strength from two aspects not treated here, namely cases of parallel adaptive radiation among African, Malagasy and Asian primates and intra-specific adaptations to local differences within habitats of single species. Detailed field investigations will, in due course, test the ideas suggested.

Grades of Social Organization

Ornithological investigations have shown that patterns of social organization determining population dispersion are intimately linked to species ecology—the whole interrelationship of behavioural features being co-adapted to certain aspects of the environment forming major selection pressures. In Table 1 we attempt to allocate species recently investigated to a series of 'Grades'[7] representing 'levels' of adaptation in forest, tree savannah, grassland and arid environments respectively. Anatomical investigations of fossil and living material reveal a progressive adaptive radiation from forest-dwelling insectivorous primates to larger open country animals predominantly vegetarian. It is not surprising, therefore, to find correlated trends in the behavioural data[8]. Table 1 reveals a shift from insectivorous, nocturnal forest animals with markedly solitary habits and a population dispersion based on aggressive contacts through a range of fruit- or leaf-eating forms (diurnal; very small family groups or larger parties showing defensive behaviour of a variety of types in 'territorial' encounters with neighbours but, otherwise, little intra-group aggression, sexuality or inter-male competition) to vegetarian browsers of open country normally living in well-structured troops or herds, usually in home ranges and showing much sexuality (often seasonal) and inter-male competition.

The aboreal grades contain several species while fewer are allocated to those of open country. In Table 1 this reflects a large species contingent from Petter's investigations on the lemurs[9]; however, in general, the numbers of forest species do exceed those of open country forms. This is because of the availability of numerous niches in forest where primate populations have specialized in terms of diet, vertical zonation, daily rhythms, etc. The limited ranges of the population units and their confinement to forests means that geographical barriers impose a more extensive speciation than in open country where species show great adaptability in exploiting a considerable range of habitats.

In common with their insectivorous ancestors, the co-adaptations of Grade I primates are clearly related to their nocturnal insect-hunting habits. Two leaf-eating-

Table 1. ADAPTIVE GRADES OF PRIMATES (SEE TEXT)

Species, ecological and behavioural characteristics	Grade I	Grade II	Grade III	Grade IV	Grade V
Species	*Microcebus* sp. *Chierogaleus* sp. *Phaner* sp. *Daubentonia* sp. *Lepilemur* *Galago* *Aotus trivirgatus*	*Hapelemur griseus* *Indri* *Propithecus* sp. *Avahi* *Lemur* sp. *Callicebus moloch* *Hylobates* sp.	*Lemur macaca* *Alouatta palliata* *Saimiri sciureus* *Colobus* sp. *Cercopithecus ascanius* *Gorilla*	*Macaca mulatta*, etc. *Presbytis entellus* *Cercopithecus aethiops* *Papio cynocephalus* *Pan satyrus*	*Erythrocebus patas* *Papio hamadryas* *Theropithecus gelada*
Habitat	Forest	Forest	Forest–Forest fringe	Forest fringe, tree savannah	Grassland or arid savannah
Diet	Mostly insects	Fruit or leaves	Fruit or fruit and leaves. Stems, etc.	Vegetarian-omnivore Occasionally carnivorous in *Papio* and *Pan*	Vegetarian-omnivore *P. hamadryas* occasionally also carnivorous
Diurnal activity	Nocturnal	Crepuscular or diurnal	Diurnal	Diurnal	Diurnal
Size of groups	Usually solitary	Very small groups	Small to occasionally large parties	Medium to large groups. *Pan* groups inconstant in size	Medium to large groups, variable size in *T. gelada* and probably *P. hamadryas*
Reproductive units	Pairs where known	Small family parties based on single male	Multi-male groups	Multi-male groups	One-male groups
Male motility between groups	—	Probably slight	Yes—where known	Yes in *M. fuscata* and *C. aethiops*, otherwise not observed	Not observed
Sex dimorphism and social role differentiation	Slight	Slight	Slight—Size and behavioural dimorphism marked in *Gorilla*. Colour contrasts in *Lemur*	Marked dimorphism and role differentiation in *Papio* and *Macaca*	Marked dimorphism. Social role differentiation
Population dispersion	Limited information suggests territories	Territories with display, marking, etc.	Territories known in *Alouatta*, *Lemur*. Home ranges in *Gorilla* with some group avoidance probable	Territories with display in *C. aethiops*. Home ranges with avoidance or group combat in others. Extensive group mixing in *Pan*	Home ranges in *E. patas*, *P. hamadryas* and *T. gelada* show much congregation in feeding and sleeping. *T. gelada* in poor feeding conditions shows group dispersal

forms are exceptions requiring further explanation. The switch to frugivorous or leaf-eating habits in Grades II and III is linked with diurnal activity and the formation of larger social groups. As with many birds, this development is linked to the change from a diet requiring individual hunting to food sources often locally distributed and at which social responses allow congregation for exploitation in common. 'Family bands' of very small size and larger multi-male social units in forest are often markedly 'territorial', in that defensive behaviour involving displays and/or marking in relation to neighbouring groups is shown. While the behaviour may defend a discrete area, this has not often been demonstrated; nevertheless, it does ensure an over-dispersion of population units.

The small size of social units in many forest frugivores is probably related to limiting conditions of food supply occasioned by the relatively stable conditions of tropical rain forest. A non-seasonal climate with a moderately constant availability of various fruits presumably allows increase in numbers to a ceiling imposed by periodic food shortages due to local food crop failures. In this situation the addition of young to the population may be difficult—recruitment necessarily balancing mortality. Breeding appears to be non-seasonal and this may explain the low frequency of copulations observed in some of these species[10,11]. The 'territorial' behaviour of forest groups may be interpreted as ensuring an adequate provisioning area for the individuals comprising them. It remains difficult, however, to apply this argument to all leaf-eaters.

In the dry forest, savannah and steppe conditions of Grades IV and V marked climatic seasonality with a harsh period of aridity, and food shortage probably imposes high seasonal mortality rates especially on old or infirm animals. In the rainy season, food for the remainder is superabundant and vigorous seasonal breeding[12] can replenish the population without risks of failures from food shortage in rearing young. It is as yet unclear whether the timing of the breeding seasons confers advantages during pregnancy, lactation or in terms of food supply for young animals; however, in principle, the effects with regard to survival may be the same.

Outside the forest the social units are generally larger and this appears to result from open country conditions of predation and food supply affecting ground dwelling populations. Savannah primates face a number of predators and scattering undoubtedly decreases the chances of individual survival. Groups of *Papio* baboons and *Rhesus* monkeys show a marked cohesion permitted ecologically by local food abundance allowing congregation without risk of over-exploitation.

One of the effects of increased group size in open country terrestrial primates is marked competition between males for females and the consequent intra-sexual selection of male characteristics. The open nature of the terrain allows every troop member to be aware of its companion's activities, and the cohesion of most troops makes this doubly sure. The seasonality of mating activities further means that many males are particularly active sexually at the same time, when, in the absence of structural equilibration in the troop, fierce competition and fighting would result. Again some Japanese observations[13] suggest that with increased numbers the socionomic sex ratio of *Macaca fuscata* troops increases so that there are fewer females to be shared among the males. This, they observe, may be a factor occasioning the splitting off of small groups from a troop and the exclusion of 'all male' parties. Finally there is a possibility that female 'open-country' primates may be sexually attractive for longer periods per oestrus cycle than is the case for forest animals[14]. All these factors enhance intra-sexual selection producing sexual dimorphism in size, appearance, and also in behaviour. The marked aggressiveness of male baboons and rhesus monkeys results in differential access to females, and is correlated with the promiscuous behaviour of oestrus females prior to their mating exclusively with the troop despot at about the time of greatest receptivity.

The increased size and aggressive nature of these males are considered to have been pre-adaptive to their role in troop defence. Males disperse around the periphery of moving troops and co-operate against common danger (see, for example, refs. 15 and 16). We do not think size and aggressiveness originated directly as a response to predation (compare with refs. 17 and 18), but the organization of the troop and male co-operative tendencies in defence undoubtedly did so.

Within Grade IV we have included not only typical forest fringe and savannah woodland species such as *Papio cynocephalus* and *Macaca* sp. but also a number of forest forms, which, in secondary habitats provided by human destruction of woodlands, show social organization in some degree similar to these. Characteristic of all the members of this Grade is a marked adaptability revealed in investigations of the species in contrasting habitats. The *Cercopithecus aethiops* population on the rich but small Lolui Island has 'territorial' behaviour not apparent in unlimited but impoverished conditions where the animals have much larger home ranges[19]. Similar, though often less investigated, accounts are available for the langur (*Presbytis entellus*), chimpanzee and baboon. These variations in social systems appear owing to the plasticity of intra-group relations possible in these forms—a plasticity of undoubted survival value in forest-fringe environments.

Grade IV systems seem therefore to be the result of (i) open country conditions of food supply and predation favouring increase in group size, and (ii) intra-sex selection increasing size and aggressiveness of males, thereby producing (iii) marked group structuring and, in some forms, the protective functions of males acting in unison against predators. Changes from Grade IV to Grade V are related to the occupation of habitats in which food supplies are less abundant, and, at least seasonally, more sparsely and infrequently distributed in the environment. In such areas it would be advantageous for groups of Grade IV animals to fragment into smaller, more widely ranging parties to avoid local over-exploitation of food. Indeed there is evidence from *Papio cynocephalus* populations in arid African areas and from *Macaca fuscata* in northern Japan that group size decreases at the limit of the species geographical range. This is probably in part the result of a low population density, their group organization remaining typical of Grade IV. Small groups of this kind are, however, not very efficient population units. The presence of several large males, only functional in mating and playing no part in rearing young, results in the consumption of much food not used in maintaining the species. Furthermore, the role of the male in protection is less efficient in small groups. In these habitats the 'one-male groups'[20] of *Papio hamadryas*, *Erythrocebus patas* and *Theropithecus gelada* are more adaptive in that less food per reproductive unit goes to individuals not involved in rearing young. The exclusive possession of a 'harem' probably increases inter-male competition for females still further and the occurrence of 'all male' population units indicates a considerable degree of exclusion of potential reproductives from breeding. The intense intra-sexual selection is again doubtless the main factor increasing the dramatic appearance of *P. hamadryas* and *T. gelada* males through the growth of large capes on the backs of these animals[21]—an apparent increase in size not requiring much utilization of energy for its maintenance and providing the animals with a distinctly ferocious appearance enhanced by demeanour and facial expressions.

The differently co-adapted characteristics of the three Group V species suggest clearly the interaction of selection pressures from food shortage, predation and habitat

topography in slightly different environments. Thus *T. gelada* in the Ethiopian mountains forms herds in good feeding conditions that split into separately foraging harem groups as conditions deteriorate[16]. Sleeping sites on ever-present cliffs are super-abundant. *P. hamadryas*, although forming 'one-male groups', apparently shows less dispersal because of the limited number of sleeping sites available (rock cliffs, outcrops, etc.) in the single area so far investigated in detail[20]. The patas monkey of open savannah grasslands never forms herds, individual 'one-male groups' keeping apart[22]. There are no rocky sleeping sites and plenty of predators. At night the animals disperse into separate trees and re-assemble in the morning. The male does not adopt the aggressive defence tactics of *Papio* and *Theropithecus;* rather he plays the part of watchdog, showing alert and diversionary behaviour in relation to predators. He does not exert dominance over the group in the same way as the other two species.

The behavioural features of the Grade V species are thus maintained by three main environmental selection pressures the characteristics and range of lability of which are relatively constant within the three habitats. They contrast markedly with the differing values of these same pressures, maintaining the Grade IV organizations in a richer habitat. Signal flow diagrams relating the environmental input of information and the adaptive response of the population in terms of numerical size, population dispersion and social structure are prepared for each of these types. A generalized diagram to which the characteristics of any one of these systems may be fitted is shown in Fig. 1.

The Great Apes and Man

Clearly in Table 1 the Pongidae do not fit easily into categories imposed primarily by investigations of cercopithecid primates. Nevertheless, *Hylobates*, in spite of its adoption of brachiation, has much in common with other tree-top frugivores. Similarities between *Gorilla* and *Pan* to other animals in their Grades are much less clear—a function in part doubtless of their pongid status. Reynolds[23] has clarified the adaptive significance of contrasts in social systems between these two genera.

The development by *Pan* of a simple tool-using culture[24,25] suggests a social condition close to that of early hominids, but we do not accept the suggestion[26] that this necessarily implies a regression from a more human condition. Palaeontological investigations suggest a radiation of early hominids from dryopithecine stocks in circumstances similar to those controlling the transitions from Grade IV to V in the cercopithecids. While *Ramapithicus* and *Paranthropus* were herbivores[27], *Australopithecus* shows evidence from dentition of a more omnivorous food habit including meat eating. Early hominids faced with seasonal food shortage in savannahs probably had initially a social organization not unlike that of the chimpanzee and perhaps formed later either groups guarded by weapon-bearing males or moved in one-male family units—the males playing a part of guardian and watchdog. Possession of weapons would have substituted for gross sexual dimorphism in size. The creatures doubtless retained the ability to congregate in conditions of plenty and in sparsely distributed rocks or tree shelters. These would soon have become bases for hunting raids. Shortage of vegetable food would have accelerated the adoption of weapons for killing animal prey while the trend towards bipedalism, already providing speed in escape from danger, allowed the use of arms in wielding weapons and speed in the chase. The fact that with *Pan* 'all-male groups' occur suggests a source for the loyalty and co-operation necessary in the development of group hunting expeditions. Certainly the biomass of animals suitable for hunting was abundantly present in the grassland savannah of Pleistocene Africa[28], but probably only in *Australopithecus* did an increased reliance on meat lead to major changes in social structure[29].

Sexual Selection

The form of a primate social system is a function not only of selection pressures of the environment. As we have seen, sexual selection, especially in terrestrial societies, plays an important part. Wickler[30] has recently stressed this factor as an ultimate determinant of morphological signals functional in the sexual and agonistic behaviour of certain primates. It would seem that the structures concerned have been derived from skin colour changes occurring in certain phases of the oestrus cycle and for which no signal function is known[31]. Wickler suggests that the coloured buttocks normally present in certain species function in protecting the less dominant males from the despots during quarrels in the troops. Weaker or junior males 'present' to larger animals thereby stimulating a mounting gesture rather than aggression. He considers this to be a case of 'intra-specific mimicry', the male

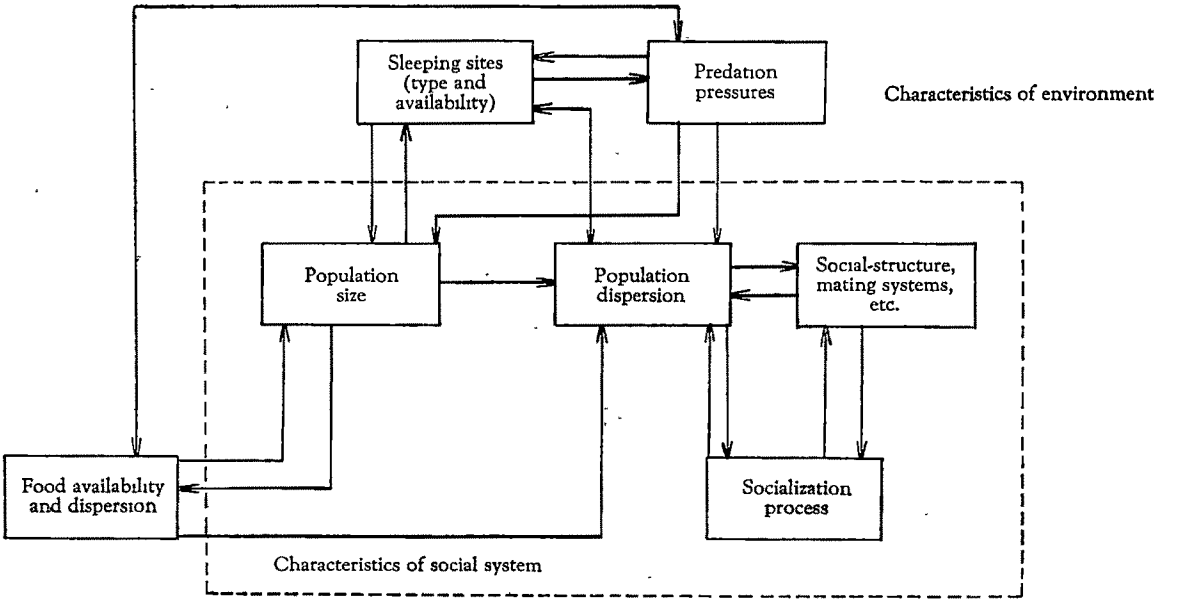


Fig. 1. Diagram showing the role of habitat factors maintaining a specific social system through time in a given environment

buttocks being copies of the feminine original. Again the curiously shaped patch of bare skin on the chest of *Theropithecus gelada* in both sexes, which undergoes cyclical changes including peripheral vesicle formation in the female[32], has a resemblance in pattern and colour to the perineal skin of the female at oestrus. Wickler considers the chest patches to be substitutes for signals given posteriorly in presentation for mating and he implies, though he does not state, that the chest patches of the males function in a similar way as the coloured buttocks of other male baboons.

Recent investigations (by J. H. C.) in Ethiopia have shown that males do indeed examine the chests of females in oestrus prior to certain copulations, probably those occurring early in an oestrus cycle. Quantitative data show, however, that the colour changes on chest and ischial areas are not in phase. Lactating mothers and non-oestrus females have the reddest behinds. It seems odd that if the red chest colour is attractive to males the perineal areas should be most red at precisely the time when mating would be inappropriate. Long nipples in the mid-line of the chest, assumed by Wickler to be signal imitations of the vulva, appear to arise in the course of suckling. Preparous females have small nipples spaced apart, but, when in oestrus, are nevertheless frequently mated. The role of nipple shape and position in sexual signalling prior to copulation is thus in doubt.

The gelada spends a considerably greater time sitting down than does *Papio doguera* in the same area. A chest signal as an indicator of female sexual condition would therefore be more appropriate than an ischial one. It does not appear essential, however, for the signal to be an exact copy of the ischial design—and indeed we find that it is not. In view of the baby and juvenile geladas' prolonged interest in the nipples—with which they play as well as from which they obtain nourishment—adult concern with this area is readily understandable and any colour changes are likely to develop a significance. The extent to which chest patches really copy the behind remains largely subjective. There is no evidence that the chest patch of the male gelada functions in a manner analogous to Wickler's suggestions for the buttocks in males of other species.

Wickler's hypothesis deserves critical scrutiny as it could have considerable significance in the investigation of human sexual aesthetics. The shape of the human mammary glands is not apparently 'essential' for it is not found in the great apes. Possibly they have acquired their function as a sexual releaser in a manner analogous to the evolutionary process suggested for the female gelada's chest. The development could have arisen in co-adaptation with the adoption of the upright bipedal stance in the early Hominidae and the use of the frontal position in mating. However, it is not so much the shape of the human breasts that is the critical sexual releaser as the visual stimulus provided by the nipple and the areolar zone around it[33]. While "intraspecific mimicry" might account for the shape of these organs it seems that normal Darwinian sexual selection (that is, inter-sexual selection) could account for the enhancement of structural features around the nipples. Further research into the significance and evolution of such signals in both non-human primates and man will be of considerable interest.

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DRUG ADDICTION* IN BRITAIN NOW

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An Interdepartmental Committee on Drug Addiction was set up in 1958 by the Minister of Health and the Secretary of State for Scotland to review the advice on morphine and heroin addiction† that had been given to the Ministers in 1926 and to consider whether any further steps should be taken.

In its report, published in 1961[1], the Committee concluded that addiction should be regarded as an expression of mental disorder and not as criminal behaviour, that satisfactory treatment demanded suitable institutions, but that, as the problem was small, the establishment of specialized institutions was not practicable. It also concluded that a system of registration of addicts would be neither desirable nor helpful as "the incidence of addiction to dangerous drugs was very small and there seemed no reason to think that any real increase was occurring", and that the supply of drugs to addicts through "irregularities in prescribing" by doctors was infrequent and not such as to justify further statutory controls.

In 1964, the Committee was reconvened to consider whether "in the light of recent experience" the advice given in relation to the prescribing of addictive drugs by doctors three years earlier needed revising. Thus, the

* The World Health Organization Expert Committee on Addiction-producing Drugs (ref. 5) now recommends the substitution of the term "drug dependence" for both "drug addiction" and "drug habituation" for it is not proved possible to formulate satisfactory definitions of the two older terms and the component in common is a dependence, psychic or physical or both.

† An addict is defined in the 1965 report as "a person who, as a result of repeated administration, has become dependent upon a drug controlled under the Dangerous Drugs Act and has an overpowering desire for its continuance, but who does not require it for the relief of organic disease".

Committee confined its attentions to the part played by medical practitioners in the supply of "dangerous drugs"—that is, drugs controlled under the Dangerous Drugs Act, such as morphine, heroin, pethidine and cocaine. It did not concern itself with the abuse of amphetamines, barbiturates and similar drugs, which is probably growing apace, so that, although the report is titled "Drug Addiction"[2], it deals with probably less than 1,000 people, 753 known addicts plus a few who get their supplies from illicit sources, whose number "cannot be precisely estimated", though it is very unlikely to exceed the number of those known.

Despite the restricted nature of the enquiry, disquieting facts were revealed. Between 1959 and 1964 the total number of known addicts to "dangerous drugs" rose from 454 to 753; the rise was chiefly due to increased use of heroin (68 in 1959, 342 in 1964) and of cocaine (30 in 1959, 211 in 1964). The age distribution of addicts had also altered; in 1959, 11 per cent, and in 1964, 40 per cent were less than 35 years old. The increase in addiction was largely in London, but there was suspicion of a similar trend in one or two other large cities.

Smuggled and stolen drugs did not, the Committee decided, provide a significant source. The major source of supply for these addicts was "not more than six doctors" (heroin can be prescribed legally in medicine in Britain, unlike many other countries in which it is banned). The problem can be defined thus: there is a substantial increase in addiction to heroin and cocaine, chiefly in London, supported by up to six doctors acting "within the law and according to their professional judgement" and "out of a sense of duty—because they felt that the treatment facilities elsewhere were inadequate". Whatever the six doctors may think, it is hard to resist the conclusion that they are, innocently, if naïvely, providing addicts with enough drugs for them to give away or sell to others, and the *British Medical Journal*[3] accepted that this is not improbable. The Interdepartmental Committee was quite clear on this when it stated that the facility for legally prescribing these drugs for addicts "has now been abused with the result that addiction has increased". If this is so, the question arises whether the long-held and still cherished right of doctors to prescribe whatever they think fit for any patient should be maintained inviolate. The Committee faced this problem and also the question of whether, if doctors were prohibited from prescribing drugs to addicts, for their addiction, this might lead to an "organized illicit traffic", such as does not at present exist in Britain, and the absence of which is attributed to the fact that addicts could be legitimately supplied by the medical profession.

The Committee decided that the existing problem of heroin and cocaine addiction justified the following measures:

(1) Addicts should be formally "notified" to a central authority as with certain infectious diseases.

(2) Special treatment centres should be set up, at least in the London area, and in other areas Regional Hospital Boards should make provision for treatment of addicts in selected hospitals in their regions.

(3) Statutory powers of the staff of treatment centres to detain addicts should be confined to the period of short-lived discomfort of drug withdrawal when the addict feels a specially strong incentive to terminate treatment. It is recognized that, beyond this, compulsory treatment "seems to meet with little success".

(4) Withdrawal of the drug from the addict is only the first step in treatment. Relapse is common "on returning to their old haunts". Therefore, facilities for long-term rehabilitation to prevent relapse are essential.

(5) The right of any doctor to prescribe heroin and cocaine to an addict for his addiction should be withdrawn and such prescribing should only be permissible by the medical staff of a treatment centre. Indeed, even in an emergency such as, for example, when an addict cannot get to a treatment centre or becomes ill, the Committee considers that the doctor in charge should seek authorization from the centre before he orders or administers such drugs.

(6) The Committee emphasizes that its recommendations apply to heroin and cocaine alone, at present, and only to supply to addicts.

(7) These recommendations, if implemented, would create two statutory offences: failure to notify an addict, and supply of a "restricted" drug to an addict by a doctor who is not a member of the staff of a treatment centre. It is considered that where "the central authority" alleges improper prescribing by a doctor, this should be investigated by "a tribunal of his professional colleagues" (the Disciplinary Committee of the General Medical Council) and not by a court of law "as the issues involved are primarily those of professional judgement and conduct".

The first reaction of the medical profession[3,4] was that it should not be necessary to restrict all doctors because of abuse by only six, and this is understandable. However, it has become plain that drug addicts are a group of patients who are peculiarly difficult to handle, that they spread their addiction among their associates and that "therapy by non-specialists is virtually impossible"[4].

As a result of this report, the medical profession is likely to find that refusal to consider even this slight restriction of its freedom to prescribe will fail to carry conviction. Some central control of prescribing for addicts is probably both inevitable and desirable. A reasonable solution might be to extend the right of such prescribing to doctors who accept the guidance of a specialist at a treatment centre which the addict should visit from time to time. In any case, geographical considerations will probably render it impossible to confine prescribing to doctors on the staff of only a few treatment centres.

Presumably any law that was framed in the light of the Committee's recommendations would allow for other drugs to be put on the restricted list by the Ministers if evidence accumulated that addicts had turned to, say, pethidine or methadone, and evidence of prescribing abuse was found.

The Committee interpreted its terms of reference "as meaning that we were not being invited to survey the subject of drug addiction as a whole", and they have probably done as much as could be expected of eight people meeting eight times. It is to be hoped that the Ministers who convened the Committee and gave them, whether deliberately or not, such a restricted brief will now set up a study group of much wider membership and with much wider terms of reference to investigate the whole problem of drug abuse in society. The Interdepartmental Committee was concerned about the wider problem sufficiently to exceed its terms of reference for a moment and to write of drug abuse generally that "this feature of contemporary life deserves thorough study so that remedial action on all relevant fronts may be planned with full knowledge and understanding". This somewhat ponderous phraseology obscures the urgency of the matter. But the Committee evidently felt a sense of urgency, even though it has not given cogent expression to it.

The problem of drug abuse is growing particularly among the young, and it will not be a short or easy task to get the knowledge of its "complex variety of social, medical and psychological factors" that is essential to deal with this destructive force in our society. The sooner a comprehensive study is put under way, the better.

[1] *Drug Addiction: Report of the Interdepartmental Committee* (H.M.S.O., London, 1961).
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edited by **Berni Alder, Sidney Fernbach**
Lawrence Radiation Laboratory, Livermore, California
and **Manuel Rotenberg**
University of California, La Jolla, California

May 1966, 246 pp., 92s.

The Chlorophylls:

PHYSICAL, CHEMICAL, AND BIOLOGICAL PROPERTIES

edited by **L. P. VERNON** and **G. R. SEELY**
C. F. Kettering Research Lab, Yellow Springs, Ohio

Reviews recent developments in the study of physical, chemical, and biological properties of chlorophylls. This treatise is divided into four sections; the chlorophylls as chemical entities; the chlorophylls in real and colloidal solution, and in the solid state *in vitro;* the biosynthesis, organization, and properties of chlorophylls in plant and bacterial cells; and the photochemical and photophysical behavior of chlorophylls *in vitro* and *in vivo*.

August 1966, 679 pp., 200s.

Fiber Optics

PRINCIPLES AND APPLICATIONS

by **N. S. KAPANY**
Optics Technology, Inc., Palo Alto, California

This volume presents a complete discussion of the field of fiber optics. The subject material ranges from basic principles to geometrical and physical optics of fibers, from waveguide effects to fiber optics technology, and from fiber drawing to assembly and testing.

Late 1966, about 375 pp., approx. 100s.

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G. E. Research Lab, New York

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Volume 4

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U.S. Naval Research Laboratory, Washington, D.C.
Harry Suhl
Department of Physics, University of California, La Jolla, California

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by **Conyers Herring**
Bell Telephone Laboratories, Murray Hill, New Jersey

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September 1966, about 425 pp., 116s.

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D. A. YOUNG, Imperial College of Science and Technology, London

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OBITUARIES

Sir Neil Hamilton Fairley

THROUGH the death of Sir Neil Hamilton Fairley, who died at the age of seventy-five on April 19, tropical medicine lost one of its greatest scientists and physicians. He carried out a series of brilliant pieces of research on a wide variety of subjects, was responsible for establishing the academic Department of Tropical Medicine in the University of London, played a large part in re-establishing the Hospital for Tropical Diseases, London, after the Second World War, and was president of the Royal Society of Tropical Medicine and Hygiene from 1951 until 1953. He was a valuable member of committees and sat on many connected with tropical medicine, where he was the undoubted leader of his field for many years.

Fairley was born in 1891 in Australia, and graduated with first-class honours from the University of Melbourne in 1915, when he joined the Army and immediately made his mark as a research worker by investigating cerebro-spinal meningitis, which was then creating a great problem among Australian troops. In 1916 he was posted to Egypt and there appreciated the enormous problem which schistosomiasis caused and the difficulties which it presents in diagnosis. He set to work to devise immunological methods of detecting it, and the complement-fixation test and skin-testing antigens which he brought into being are still extensively used and have provided the basis for much further work on the subject. Tests based on his work are used in epidemiological surveys, and much of modern schistosomal control work results from his findings. At this time he also carried out a series of classical investigations on the evolution of schistosomal lesions in animals.

After the First World War, Fairley studied tropical medicine at the London School of Hygiene and Tropical Medicine and worked at the Lister Institute before returning in 1920 to Australia as first assistant at the Walter and Eliza Hall Institute, Melbourne. In 1921 he went as Tata professor of tropical medicine to Bombay and there pioneered investigations on tropical sprue. He carried out detailed clinical and pathological studies of this disease and reached the then novel conclusion that high protein and low fat diets should be used for its therapy. Their introduction enormously improved the prognosis of the disease which until that time had been serious and not uncommonly fatal. Illness interrupted his work, and after a period of convalescence in Britain he returned to the Walter and Eliza Hall Institute. Fairley undertook research on the important problem of snake bite, for which treatment was then largely empirical and very unsatisfactory. He measured venom yields of Australian snakes and carried out detailed studies in animals of the effects of venom. His work enabled anti-venines to Australian snakes to be produced and their use standardized and rationalized.

In 1928 he became physician to the Hospital for Tropical Diseases, London. The pathogenesis of black-water fever was poorly understood at that time and his work on it was of outstanding importance, He demonstrated that when erythrocytes haemolysed, a hitherto unknown pigment, methaemalbumin, was produced. He carried out other investigations of haemolytic anaemia associated with malaria and malnutrition in Macedonia, and this phase of his research culminated in his election to fellowship of the Royal Society in 1942.

At the outbreak of the Second World War, Fairley was invited to become consultant to the Australian Forces. In the Middle East he was responsible for the introduction of sulphaguanidine for the treatment of bacillary dysentery and thus ushered in a new era in the management of this disease. At this time malaria was causing enormous casualties in the Far East and as much as anything else had defeated operations in Burma and New Guinea. The Land Headquarters Medical Research Unit, Cairns, Queensland, was set up to investigate it, its control and prevention, and Fairley was appointed director. He built up a very strong research team and again carried out classical work, perfecting the use of sub-inoculation techniques and not only demonstrating that malaria could be effectively controlled with mepacrine but also pointing the way to a later demonstration of the pre-erythrocytic phase of the malaria parasite—a discovery in which he participated. These investigations were detailed and complex; throughout Fairley was meticulous in carrying them out and brilliant in his use of deductive reasoning. Without his findings, it seems certain, the war in the Far East could not have been won. This work crowned his career and together with earlier achievements won for him international acclaim, a knighthood and many honours. Among the latter were the award of the Buchanan Medal of the Royal Society, the Manson Medal of the Royal Society of Tropical Medicine and Hygiene and the Moxon Medal of the Royal College of Physicians of London.

Few have done more in the field of scientific medicine than Fairley and certainly none has been kinder or more considerate than he was. His generosity and gentleness of nature were almost as internationally known as was his scientific work. Through his research, teaching and his association with large numbers of young men in his investigations and hospital work he had a tremendous influence for good on tropical medicine, an influence which will long continue. A. W. WOODRUFF

NEWS AND VIEWS

Dragon Resurgent

AFTER many ups and downs, the *Dragon* project to develop a high-temperature gas-cooled reactor is within sight of success. Hopes are high that the construction of a commercially viable reactor could begin within two or three years. At a recent symposium, Mr. C. A. Rennie, executive of the *Dragon* project, raised the prospect of competition between the *Dragon* and two other reactor systems now being developed in Britain—the advanced gas-cooled reactor and the fast breeder reactor—by saying that high-temperature reactors could generate electricity economically and could use only slightly enriched fuel as well. The first 20 MW (thermal) high-temperature reactor is now operating at Winfrith Heath, Dorset, and the development programme is financed jointly by Britain and eleven other countries.

When it was first planned in 1958, the *Dragon* reactor was intended to work with low fuel enrichment, but further work suggested that a highly enriched mixture of uranium and thorium would be necessary. But now the pendulum has swung back again and low enrichment is thought feasible. This has the attraction that the output of European thermal diffusion plants for enriching

uranium could be used. Another development of which the *Dragon* people are proud is the development of coated particle fuels which retain a large proportion of the fission products. This has made it possible to dispense with or to simplify the arrangements for preventing the escape of fission products into the reactor cooling cycle.

The *Dragon* programme is at present intended to end in March 1967 and will by then have cost £25 million. An extension of three years at a cost of £6 million is at present being considered. About a third of the total cost has gone on the construction of the reactor experiment, a third on running the reactor, and a third on research and development. Mr. Rennie claims that in its present state of development the high-temperature reactor will need less research and development to bring it to a commercial viable state than will the obvious competitors of this system.

Physics Teaching in Britain

THE Institute of Physics and the Physical Society has set out to give physics teachers in British schools and technical colleges a greater sense of community with professional physicists. One manifestation of this is the appearance of a new journal *Physics Education* intended to provide a certain amount of practical help for the classroom teacher but primarily to inform him (or her) of innovations in teaching technique. Pleased though the physicists were last week with their new venture, there were chemists to be heard saying that the new journal follows some years after the appearance of *Education in Chemistry*, designed to cater for the needs of chemistry teachers in schools and similar institutions.

Prof. K. W. Keohane, professor of physics in the Chelsea College of Advanced Technology and consultative editor of the new journal, says in his introduction that he intends to cater for the interests of teachers of physics "at all levels, from the upper forms in schools to the universities, colleges of education, technical colleges and indeed industry". Prof. Keohane is about to become the co-ordinator of the Nuffield Foundation Science Teaching Project and he says, in his introduction, that the atmosphere created by curriculum development projects like that of the Physical Sciences Study Committee in the United States and the Nuffield Foundation in Britain have made timely the launching of the new journal. He argues that there will be a need for "regular and continuous servicing" of the new curricula and effort by educational authorities to ensure that teachers are enabled to keep up not only with rapid advances in knowledge but also with developments in the curriculum and teaching method. Universities, the argument goes, should watch out for the influx of students from the schools brought up on a refreshing curriculum.

The new journal is only a part of the plans of the Institute and Society for fostering interest in physics education. A joint committee with the Royal Society, called the Committee on Physics Education, with Sir Nevill Mott as chairman, is plotting other schemes. One of these is an attempt to create regional centres to which physics teachers in schools may look for help, advice and continuing opportunities for training. The first of these centres is likely to emerge around the Department of Physics of the Manchester Institute of Science and Technology.

Nuffield Science Teaching

THE Nuffield Foundation has announced that Prof. Kevin Keohane, head of the Department of Physics at the Chelsea College of Science and Technology, is to become co-ordinator of the Nuffield Foundation Science Teaching Project from August 1, 1966. The work of the Project is at a particularly interesting stage, partly because the developments of the past four years are about to culminate in the publication of the books which embody O-level courses in physics, chemistry and biology, and partly because systematic trials of sixth form and combined science courses are about to begin. Prof. Keohane will combine responsibility for the Science Teaching Project with some of his present duties at Chelsea. From the point of view of the College, the appointment serves to emphasize the interest which has been taken at Chelsea in the past two years in fostering links between academic institutions and the schools. The College has, for example, been concerned to find ways of helping with the training of science teachers and has at various times considered the possibility of starting a unit for this purpose.

Atomic Energy Commission

THE E. O. Lawrence memorial awards of the Atomic Energy Commission have been presented to the following for contributions to nuclear science: Harold M. Agnew, Los Alamos Scientific Laboratory, for development of nuclear weapons; Ernest C. Anderson, also of Los Alamos, for research in nuclear medicine and biology and for development of liquid scintillation counting; John R. Huizenga, Argonne National Laboratory, for work in nuclear fission processes; Murray Gell-Mann, professor of physics at California Institute of Technology, for contributions to the theory of elementary particles; and Paul R. Vanstrum, Oak Ridge Gaseous Diffusion Plant, for technical administration and leadership in developing isotopic separation for uranium.

Canadian Science Council

THE Canadian Government has established a council to advise on science policy, called the Science Council of Canada, under the chairmanship of Dr. O. M. Solandt, vice-president for research and development of de Havilland Aircraft of Canada, Ltd. Dr. Solandt is well qualified for his new post by his previous experience as the first chairman of the Defence Research Board of Canada, which he helped to plan and which was formed in 1947. Since then, he has been in charge of research and development at the Canadian National Railway, and was elected chancellor of the University of Toronto for two years beginning in July 1965. The vice-chairman of the Science Council of Canada will be Dr. Roger Gaudry, at present rector of the University of Montreal, but with previous industrial experience in pharmaceuticals.

Rockefeller Animal Behaviour

THE Rockefeller University and the New York Zoological Society have jointly established an Institute for Research in Animal Behavior at New York. The Institute will not be a building but rather a group of scientists attached to one or other of the sponsoring institutions which will collaborate in research and teaching in animal behaviour. One of the facilities available to the new Institute will be the New York Zoological Society's collection of living animals.

They also plan to make a number of joint appointments to the faculties of the two institutions. Prof. Donald R. Griffin has been appointed director of the Institute. He will be joined later this summer by Prof. Peter Marler, from the University of California at Berkeley, and by Drs. Fernando Nottebohm, Richard Penney and Roger Payne. The Rockefeller University hopes that the work of the new Institute will form part of the overall development of teaching and research in the behavioural sciences which have been organized in recent years by Dr. Carl Pfaffmann, and that this part of the University's interest will be helped by the appointment of Prof. Neal E. Miller, a psychologist, to the faculty.

Effect of Dietary Lipids on Blood

A SYMPOSIUM was held in Cambridge on March 17 and 18, 1966, under the chairmanship of Prof. A. C.

Frazer (Britain) and Prof. M. J. L. Dols (The Netherlands), to discuss the present position with regard to the effect of dietary lipids on blood. The meeting was sponsored by the International Federation of Margarine Associations. In the first session Dr. H. J. Thomasson (Netherlands) presented investigations of blood lipids in a Trappist community under different dietary regimens. Dr. E. H. Ahrens, jun. (United States), discussed the possible mechanism of long-term blood lipid changes and described the investigations of his group into sterol balance. The second session was concerned with short-term changes and papers were given by Dr. D. S. Fredrickson (United States), who described changes in blood lipid pattern resulting from alterations in the diet, with particular reference to lipoproteins, and by Dr. D. S. Robinson (Britain), who discussed the lipid transport function of lipoproteins and the possible role of lipolytic enzymes. The third session was devoted to a discussion on the effects of lipids on white blood cells and erythrocytes. Dr. P. Elsbach (United States) described the differences between the behaviour of granulocytes and macrophages towards lipids, and Dr. J. de Gier (The Netherlands) described some effects of fats on red blood cells.

In the final session, the possible effects of dietary lipids on blood platelets and blood coagulation were the subjects of discussion. Prof. G. V. Born (Britain) described the possible relationships between lipids and blood platelet behaviour and Prof. R. G. Macfarlane (Britain) discussed the blood-clotting mechanism and the possible points at which lipids might be expected to have some significant effect.

In all, there were thirty-eight participants in the symposium, representing fifteen different countries. Original material presented at this meeting will be published independently by the respective authors in appropriate scientific journals.

Pig Trypanosomiasis in Africa

PIG trypanosomiasis, a possible source of human trypanosomiasis or 'sleeping sickness', is the subject of a monograph by Dr. L. E. Stephan, of the School of Public Health and Medicine of the American University, Beirut, Lebanon (*Pig Trypanosomiasis in Africa*. Commonwealth Bureau of Animal Health, Review Series No. 8. Farnham House, Farnham Royal, Buckinghamshire. 15*s*.). The review includes the geographical distribution of pig trypanosomiasis, the characteristics of the five species which infect the pig, and their mode of transmission, the chemotherapy and chemoprophylaxis of these infections, and an especially valuable chapter on the pig as a reservoir of human trypanosomiasis. Dr. Stephan concludes that further investigation is needed before any decision can be made on this point.

South African Museum

THE Minister of Education, Arts and Sciences decided that as from April 1, 1964, the South African Museum should consist of two sections: the South African Museum (Natural History) and the South African Museum (Cultural History). The Cultural History Section has moved into the old Supreme Court building. Two new galleries have been built for natural history, and extensions to the Marine Biology Department and a new palaeontological laboratory are in progress. Notable additions to the collection of photographs include prints of the Bushmen of Tsumkwe Pan, S.W. Africa, and a set of negatives of tribal studies taken *c*. 1900.

Technology and the U.S.S.R.

IN a written answer to the House of Commons on May 24, Mr. Frank Cousins, the Minister of Technology, outlined his visit to the U.S.S.R. earlier that month and commented on his discussions with Mr. Kosygin on the value of exchange in science and technology between Britain and the U.S.S.R. He had visited the U.S.S.R. at the invitation of Academician V. A. Kirillin, chairman of the State Committee for Science and Technology, to discuss with representatives of the Soviet Government their methods for managing and deploying technological resources and for introducing new technology into industry. He had been able to see some of the advanced technological work being carried out in research institutes and discussed methods of training technologists. During his visit, Mr. Cousins and Mr. Kirillin had discussed the organization and operation of the State Committee and its working relations with the Academy of Sciences, the Ministry of Higher Education and with Gosplan. Mr. Kirillin had described the methods used by the Soviet Government for allocating priorities to technological projects and for estimating their value, and for exploiting technological resources. He had also outlined the role of Soviet technology in economic planning, the training of qualified technologists and the dissemination of scientific and technological information.

Surveying Sand and Gravel Resources

IN a written answer in the House of Commons on May 26, the Minister of Land and Natural Resources, Mr. F. Willey, stated that he was giving priority to a survey of sand and gravel resources to the extent needed to supplement existing deposits potentially capable of economic exploitation. The Chelmsford and Ipswich areas were being studied first, and the aim was to establish potentially workable reserves, a category between possible resources as shown on geological maps and resources proved by detailed exploration by the industry. The first year's work would provide an opportunity to develop appropriate techniques and the field work would be done on an agency basis by the Institute of Geological Science.

Grants for Research

ON May 25 the Minister of State, Mr. G. Roberts, stated that of 1,078 applications for research grants to the Science Research Council in the fiscal year 1965–66, 832 were granted in whole or in part. For the Medical Research Council the corresponding figures were 516 and 377; for the Agricultural Research Council, 73 and 56; and for the Natural Environment Research Council, 101 and 75.

Dipeptide Sequences

MR. BEN BLOOM, of the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland, is seeking help in collecting information about amino-acid sequences in portions of polyamino-acid molecules. He is constructing a digital computer programme to evaluate the frequency of the occurrence of dipeptides and undertakes that information sent to him at Building 10, Room 9*B*-09, National Institutes of Health, Bethesda, Maryland 20014, will be fully acknowledged in any publication that may eventually be made. Bloom's work is in part inspired by that of H. B. Bull (*Arch. Biochem. Biophys.*, **112**, 208; 1965), who found in an analysis of 1,304 dipeptides in eight protein molecules that, of the 400 possibilities, ten were favoured and eight occurred less frequently than would have been expected.

University News: Belfast

DR. CHARLES DOW has been appointed to the chair of comparative pathology, and Dr. H. B. Gilbody to a second chair of physics.

Liverpool

THE University of Liverpool has just announced that Dr. K. McCarthy, senior lecturer in bacteriology, has been

appointed to the chair of bacteriology. He will take up his appointment on October 1, 1966, when Prof. A. W. Downie retires.

Massachusetts Institute of Technology

WILLIAM B. KEHL has been appointed associate professor of electrical engineering at the Massachusetts Institute of Technology and associate director of the M.I.T. Computation Center. Dr. John Ross has been appointed a professor of chemistry and, for five years, head of the Department of Chemistry.

Appointments

THE trustees of the British Museum (Natural History) have appointed Dr. H. W. Ball to be keeper of the Department of Palaeontology in succession to Dr. E. I. White, who retires on June 30; and Mr. R. Ross to be keeper of the Department of Botany in succession to Mr. J. E. Dandy, who relinquishes his post on September 30 to devote more time to research.

Announcements

DR. A. R. SANDAGE, of Mount Wilson and Palomar Observatories, has been awarded the Gold Medal of the Pontifical Academy of Sciences. This award was instituted by Pope John XXVIII to honour a young scientist who has made distinguished contributions to science.

DR. ALAN T. WATERMAN has received an award created by the National Academy of Sciences in the name of the late Edwin Bidwell Wilson to honour a civil servant especially distinguished in the field of science and public affairs. The award was presented during the Vicennial Convocation of the Office of Naval Research in Washington.

THE American Geophysical Union has made the following awards: the *William Bowie Medal*, to Dr. L. R. Slichter; the *John A. Fleming Medal*, to S. E. Forbush; the *James B. Macelwane Award*, to Dr. D. L. Anderson.

A SYMPOSIUM on "Aromaticity", sponsored by the Chemical Society, will be held in the University of Sheffield during July 6–8. Further information can be obtained from the General Secretary, The Chemical Society, Burlington House, London, W.1.

THE second conference of the Institute of Information Scientists will be held at Jesus College, Oxford, during July 11–13. Further information can be obtained from Dr. D. J. Campbell, 54 Hurst Rise Road, Cumnor Hill, Oxford.

THE seventh general assembly of the International Union of Crystallography will be held in Moscow during July 12–19. Further information can be obtained from the General Secretary of the Union, Rekencentrum der Rijksuniversiteit, Grote Appelstraat 11, Groningen.

A symposium on "Statistical Mechanics and Thermodynamics", arranged by the International Union of Pure and Applied Physics, will be held in Copenhagen during July 11–16. Further information can be obtained from Prof. T. A. Bak. H. C. Ørsted Institute, Universitetsparken 5, Copenhagen Ø.

AN international symposium on "Naturally Occurring Phosphoric Esters", arranged by the Chemical Society, will be held in Newcastle upon Tyne during July 10–12. Further information can be obtained from the General Secretary of the Chemical Society, Burlington House, London, W.1.

AN international summer school on "The Autonomic Nervous System" will be held by the Netherlands Central Institute for Brain Research, Amsterdam, during July 10–23. Further information can be obtained from Dr. J. P. Schadé, Central Institute for Brain Research, IJdijk 28, Amsterdam.

AN international symposium on "Reaction Mechanisms of Inorganic Solids", arranged by the Chemical Society, will be held in the University of Aberdeen during July 11–16. Further information can be obtained from the General Secretary of the Chemical Society, Burlington House, London, W.1.

A CONFERENCE on "Chemically-grown Surface Films", arranged by the Electron Microscopy and Analysis Group of the Institute of Physics and the Physical Society, will be held in the University of Strathclyde during July 7–8. Further information can be obtained from the Meetings Officer, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

THE first international conference on "Haemorheology" will be held in the University of Iceland, Reykjavik, during July 10–16. Further information can be obtained from the Scientific Organizing Committee of the conference, Hemorrhage and Thrombosis Research Laboratories, Veterans Administration Hospital, East Orange, New Jersey.

ERRATUM. Sir Harry Melville points out that the U.K. Science Research Council postgraduate awards are already tenable at universities outside Britain, contrary to the implication on June 4 (*Nature*, 210, 977; 1966).

THE NIGHT SKY IN JULY

All times are in Universal Time

MOON

New Moon	18d 05h
Full Moon	2d 20h

CONJUNCTIONS WITH THE MOON

Venus	15d 23h, 4° S.
Mars	16d 17h, 3° S.
Jupiter	— — —
Saturn	9d 07h, 2° N.

PLANETS

Times of rising (*R*) and setting (*S*) during the month

Name	*R*/*S*	Beginning	Middle	End	Mag.	D_g (10^6 miles)	Zodiacal position
Mercury	*S*	21h 45m	Unfavourable for observation		+0·9	60	Cancer
Venus	*R*	1h 50m	1h 45m	2h 00m	−3·3	129	Taurus
Mars	*R*	Unfavourable	2h 15m	2h 05m	+1·8	230	Gemini
Jupiter	—	Unfavourable for observation			—	577	Gemini
Saturn	*R*	23h 40m	22h 40m	21h 40m	+1 1	851	Pisces

D_g is the distance of planet from the Earth on the 15th of the month

OCCULTATIONS OF STARS BRIGHTER THAN MAGNITUDE +6 AT GREENWICH

Star	*R*/*D*	Time	Mag.
ω Sgr	*R*	4d 01h 03·4m	+4·8
τ Tau	*R*	15d 02h 29 7m	+4 3

(*D*, disappearance; *R*, reappearance)

METEORS

Name	Active period	Date of maximum	Radiant	Remarks
Perseids	July 27–Aug. 17	Aug. 12d	+46° R.A., +58° Dec.	Favourable

OTHER PHENOMENA

4d 21h, Venus 4° N. of Aldebaran.

A MECHANISM FOR THE GENERATION OF ACOUSTIC-GRAVITY WAVES DURING THUNDERSTORM FORMATION

By A. D. PIERCE and S. C. CORONITI

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DURING the past three decades, a number[1-7] of investigators have reported experimental evidence which would seem to indicate that thunderstorm activity may be related to ionospheric disturbances and, in particular, to sporadic *E*. Roberts *et al.* have provided more recent evidence in their data of path losses of 21·6 Mc/s radio waves transmitted from Panama and received at Stockbridge, New York[8]. They report a noontime disturbance which was detected on various days of August and September, 1964. This noontime disturbance represented a marked decrease in absorption below normal levels. Onset tended to occur between 1115 and 1215 E.S.T. The disturbance duration was about 1 h. We find this evidence particularly suggestive as the path passed over a portion of Florida especially noted for intense thunderstorm activity. One of our colleagues, W. J. Breitling, has checked on thunderstorm activity on August 19 (which was one of the two dates explicitly cited by Roberts *et al.* for which the noontime disturbance was observed) and found that a thunderstorm did exist in a region near the propagation path. Furthermore, the fact that thunderstorms tend to occur during the summer months and during the early afternoon[9] would seem to corroborate the reported times of the noontime disturbance occurrence. Although this evidence is highly circumstantial (as is also that of the previous investigators), it does tend to reinforce our belief that further investigation should be devoted to possible mechanisms by which thunderstorms can exert an influence on the ionosphere and that additional experiments should be carried out that would provide more conclusive evidence.

In this article we shall suggest a mechanism by which energy can be propagated from a thunderstorm during its early stages to the ionosphere and by which an ionospheric disturbance can be created The mechanism involves the generation of acoustic-gravity waves by air movements in the thunderstorm and the propagation of these waves to ionospheric levels.

The belief that acoustic-gravity waves propagating upwards from the troposphere may be responsible for a number of types of ionospheric disturbances has become increasingly widespread in recent years. Hines[10] has shown that many or most of the observational data pertaining to irregularities and irregular motions in the upper atmosphere can be interpreted on the basis of atmospheric gravity waves (that is, acoustic-gravity waves) and has discussed the possibility that these waves may originate in the troposphere. Gossard[11] has investigated the propagation of these waves from the troposphere and has found that a window can exist for wave periods between 10 min and 2 h through which fairly large amounts of energy can sometimes flow out of the troposphere. Although Gossard discussed several mechanisms by which such waves could be generated, he did not suggest any mechanism by which a thunderstorm could generate acoustic-gravity waves.

In describing our suggested mechanism, it is convenient first to review certain known features of thunderstorm formation. During the initial stage of the thunderstorm cell[9] (that is, the cumulus stage) an updraught extends throughout most of the cell. These updraughts are generally of the order of 10 ft./sec, but may locally (particularly at times early in the mature stage) exceed 100 ft./sec. The driving force for the updraught would seem to be an instability of the lower atmosphere. If this instability occurs in a region near the ground where locally the potential temperature decreases with height, then the air in the lower layer rises, causing the updraft, until a level is reached where the potential temperature (which will not change in an adiabatic expansion) is equal to that of the ambient air at the same altitude. The cooling of the air as it rises may cause condensation of water vapour and this condensation gives up energy to the air which in turn tends to increase the potential temperature and cause the driving force of the updraught to be more intense. The equilibrium height of the unstable air then increases due to the fact that the potential temperature in a stable atmosphere increases with height.

As the unstable air rises to its equilibrium height, it will overshoot this and then be pulled back. The net result will be an oscillation of the air at an altitude approximately the same as that of the cloud tops. The period of the oscillation should be about the same as the local Brunt period[12] (that is, that predicted for free vertical oscillations of the atmosphere).

Such an oscillation has been observed by C. E. Anderson[13], who gives an example of a curve of the vertical velocity of a cumulus cloud top versus time derived from measurements on photographs of developing clouds. The particular example given by Anderson shows an oscillation with a velocity amplitude of 330 ft./min and a period of 11·8 min superimposed on a steady rise of the air. Anderson's measurements are particularly suggestive as an 11·8-min period is of the correct order of magnitude for Brunt's period in any realistic atmosphere at an altitude below the tropopause.

The total energy associated with the oscillation observed by Anderson can be readily estimated. Taking $\frac{1}{2}\rho_0 V^2$ as the order of magnitude of the energy per unit volume, where ρ_0 is the ambient density and V is the peak oscillation velocity, we find the energy per unit volume to be of the order of 1 erg/cm^3. (The value of ρ_0 used in this estimate was 5×10^{-4} g/cm^3.) If we next assume the oscillation to extend over an area roughly 10 km by 10 km and over a height interval of 3 km, we can estimate the total energy of the oscillation to be 3×10^{17} ergs. Even if this estimate were too large by a factor of 10^3, an energy of 10^{14} ergs would still be considerable.

Because viscosity has negligible effect on oscillations of such a long period, the principal mechanism for the dissipation of this energy should be in its transport by wave motion (that is, by acoustic-gravity waves). These waves should have a period roughly equal to the local value of the Brunt period at source altitude.

The attainment of realistic estimates of the amplitudes of the resulting disturbance at distances removed from the source altitude is difficult because of the complicated manner in which atmosphere temperature gradients affect the propagation. Although we are at present considering this problem, some very rough estimates can be made which indicate that the effects on the ionosphere may be considerable. To make these estimates we consider the oscillation of the air at cloud top heights as producing waves which propagate according to the laws of ordinary acoustics. In the example reported by Anderson, the fluctuations in pressure at cloud top heights would be

estimated as being of the order of 7 per cent ambient. If we ignore gravitational dispersion, reflexions, refraction, etc., the ratio of the fluctuations in pressure to the ambient pressure of a travelling wave will vary inversely to the square root of the ambient pressure. Thus the ratio should increase by roughly a factor of ten as the wave travels upwards by 40 km. Even at ground level, the oscillation should be significant, as an oscillation of much less than 0·1 per cent can be detected by microbarographs. (However, the wave oscillations at ground-level may be obscured by the pressure fluctuations due to turbulence created by the thunderstorm formation.)

If these simple estimates were valid, then the wave would be a shock by the time it reached the ionosphere. We do not expect this to be the actual case for reasons which will be discussed later. However, even a pressure fluctuation of 1 per cent ambient at ionospheric levels would have a significant effect on radio wave propagation as the relative fluctuations in electron density will tend to equal those of the pressure—especially in the lower ionosphere where the collision frequency is much greater than the wave frequency.

A more careful analysis of this mechanism will have to take into account the propagation characteristics of acoustic-gravity waves—which are considerably different from those of ordinary acoustic waves. It is possible that considerable reflexion can occur at intermediate altitudes. This reflexion is particularly large at wave periods close to the local value of the Brunt period. Because the wave period at the source altitude is virtually identical to the local Brunt period, the reflexion process will be particularly strong there. However, because at frequencies near the Brunt frequency propagation is more favourable in the direction of increasing Brunt frequency and as, near tropopause altitudes, the Brunt frequency is increasing with height, the propagation should be predominantly upward. The group velocity of the wave near the source altitude may be expected to be very small and also to be nearly horizontal. Considerable geometrical spreading should be expected at ionospheric heights which would tend to reduce the magnitude of the effect from that expected for a wave propagating vertically upwards. It is possible also that some ducting may occur at intermediate altitudes. All these considerations make it difficult to say with any certainty that the mechanism we suggest will cause appreciable ionospheric perturbations. Nevertheless, it does show considerable promise and merits further investigation.

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REMOVAL OF MAGNESIUM FROM UNDERGROUND WATERS USING LIME

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THE problem of the production of sufficient fresh water for the needs of mankind is one which has steadily become more pressing, and considerable effort is now expended in research aimed at desalination. In Australia, generally acknowledged as the driest continental land-mass, this difficulty has always been acute, especially for the watering of domestic animals, and much marginal land is always in a state of hazard. The position is sometimes so critical that large areas may be dependent on waters whose concentration of salts lies close to the toxic level, and certainly well in excess of tolerated maxima of city supplies anywhere in the world. It is hardly surprising that authorities have hesitated to give decisions on maximum tolerable levels, but it is interesting to note that the better-watered areas have much lower 'suggested tolerable levels' than the semi-arid regions[1]. Such waters, in the dry regions, nearly always have their source in underground aquifers. These waters contain variable quantities of soluble salts, depending on the nature of the aquifer, and the rate of fill and movement in this porous layer. The most common ions in solution are sodium and chloride, but there are others also. One of these, magnesium, is especially harmful to animals. For this reason it is unfortunate that many hydrological authorities report their chemical analyses merely as chloride. From this they calculate the percentage of sodium chloride present, assuming quite arbitrarily that all the chloride is balanced by sodium. Using this type of calculation, many waters which appear safe are in fact highly dangerous to stock, especially to young animals.

It is surprising that so little attention appears to have been given to the magnesium content of underground waters. We have on several occasions found underground waters, from bores in Western Victoria, Australia, which have contents of from 400 to 1,000 parts per million magnesium after a long period of dry weather. This is within the range toxic for farm animals on dry summer pastures[2,3] and harmful effects were noted in stock drinking these waters, cattle and young sheep being more susceptible than older sheep.

A high concentration of magnesium in drinking-water may have a deleterious effect on animals in a number of ways. Initially, the presence of magnesium salts in water may make the animal disinclined to drink. Peirce[3] found that sheep suddenly presented with water of high salinity showed loss of weight and a marked decrease in food intake, whereas, if the salinity was increased gradually over 3 to 6 weeks, the same levels of salinity could be tolerated without nutritional disturbance. Field observations in Western Victoria confirm that animals moved to waters containing a high concentration of magnesium show at first a marked disinclination to drink.

These waters certainly have a most unpleasant taste to human beings. The most important harmful effect of the magnesium ion appears to be its local action in the alimentary canal after ingestion. Sheep and cattle drinking water of high magnesium content frequently contract diarrhoea. This is not surprising, as Peirce[3] found that sheep drinking water containing 0·20 per cent magnesium chloride (510 parts per million magnesium) had an average daily intake of 2·2 g magnesium, whereas a cathartic dose of magnesium sulphate for man contains about 1·5 g magnesium. The loss of fluids and nutrients from diarrhoea is likely to be a major cause of the nutritional disturbances caused by an excessive intake of magnesium. In addition, a direct depressing effect of the magnesium ion on the absorptive epithelium of the intestine cannot be excluded. Magnesium toxicity is unlikely to be due to excessive absorption leading to a blood plasma level in the toxic range. Magnesium is poorly absorbed from the alimentary canal and is rapidly excreted by normal kidneys. The highest plasma levels observed by Peirce[3] in sheep drinking water containing 0·50 per cent magnesium chloride (1,280 parts per million magnesium) were about 4 m.equiv./l. compared to the normal for sheep of about 2·5 m.equiv./l. General toxic effects in man (loss of reflexes, cardiac and respiratory failure) are not seen until a plasma level of 6 to 12 m.equiv./l. is reached[4] compared to the normal for man of about 2 m.equiv./l.

The source of the magnesium content of underground water is two-fold. Normally, rain-water contains proportions of salts with sodium : magnesium ratios generally similar to that of the ocean, from which they are derived by various mechanisms, such as spray, bursting bubbles, etc.[5]. Such solutions are then believed to become more concentrated by ion-filtration processes involving clay membranes[6–8]. The second source lies within the rocks themselves. Basic rocks such as basalt and also many shales contain large quantities of magnesium and, taken together, these two probably make up the predominant rock type over large areas of the Earth. A proportion of this magnesium is taken into solution during leaching, especially if the *p*H is low. The adage of Plinius, "*Tales sunt aquae, qualis terra per quam fluunt*" (waters take their nature from the rocks through which they flow), is certainly true of waters which have percolated through such rocks. The sum total of the various hydrological processes is to give a certain degree of stratification in aquifers, with fresher water overlying more saline liquids. When the subterranean water is overdrawn, the more saline bottom layers rise to give pollution. This is a common occurrence in dry spells, but even in winter it has been observed to take place if large amounts of water are withdrawn at any one time.

The removal of salts from water may be carried out by various methods such as distillation, dialysis and ion-exchange. All these are still comparatively expensive, especially for small-scale production. Many of the waters at present unsuitable for stock would become tolerable if the magnesium content could be lowered. Such a process does already exist and a considerable technological industry is, in fact, founded on the removal of magnesium from sea water by lime. The principle is simple. The solubility of magnesium hydroxide is several orders of magnitude lower than that of calcium hydroxide, or calcium carbonate in the presence of carbon dioxide. Exchange of calcium for magnesium is rapid at the interface between solid and liquid, and because of the low solubility of calcium hydroxide in water, little excess calcium is added to the water, even on long standing. In any case, excess solution of calcium hydroxide raises the *p*H, and subsequent absorption of carbon dioxide from the atmosphere gives a scum and finally a precipitate of calcium carbonate, while the *p*H falls to tolerable levels.

Experiments were conducted on a series of synthetic solutions and finally on bore waters, from basaltic terrain, containing toxic levels of magnesium. The addition of various quantities of lime to such systems gave efficient removal of magnesium in quite short periods of time (30 min), provided sufficient lime was added to give replacement on a 1 : 1 basis. The greater the amount of lime added, the more rapid the replacement. The replacement is understandably more rapid with stirring and takes longer in quiescent solutions. Another technique which has been used is the use of a filtration medium. After a large number of additives had been tried, a mixture of lime and coarse sawdust was found to act as an excellent filtration bed, and it allowed a constant passage of water. Depending on filter size, flow speed, etc., varying amounts of magnesium are removed. In filtration experiments it is important to have an inert but porous extending additive, or else clogging of the filter by fine-grained magnesium hydroxide rapidly occurs. The results of a series of experiments on one bore water have been tabulated in Table 1.

Table 1 EXTRACTION OF MAGNESIUM FROM AN UNDERGROUND WATER BY TREATMENT WITH LIME

	1	2	3	4	5	6	7	8
Calcium	6·2	5·7	7·2	11 0	10 4	8·8	18·3	37·4
Magnesium	21·3	20·8	18·4	14·3	15·4	17·3	5·9	0·3
Calcium + magnesium	27·5	27·5	25·6	25·3	25 8	26·1	24·2	37·7
*p*H	8·7	10 0	10·2	10·3	10 0	10·5	10 7	12·3

Values are given in molar parts per million to facilitate comparison.

(1) Bore water from Penshurst, Victoria, Australia, aquifer under basalt (2–5) Water after treatment with calcium oxide, 0 5 g/l , stirred 10 min, 30 min, 100 min, 270 min respectively. (6, 7) 1 g/l., stirred 10 min and 30 min respectively. (8) 2 5 g/l., 30 min stirring.

The replacement of magnesium by calcium does not present any problem, for both Heller[9] and Peirce[10] have shown that animals do not suffer any ill effects on waters high in calcium. In fact, such waters may provide an excellent source of calcium for animals the diets of which are deficient in this element. One additional advantage which may frequently arise is the precipitation of a portion of the calcium as the mineral gypsum, for waters containing high contents of magnesium are often also high in sulphate. Although the solubility of calcium sulphate is higher in sodium chloride solution than in pure water, precipitation of gypsum does occur when there is excess sulphate. In technology, use is made of the reduction in solubility of gypsum in sulphate solutions by adding 0·5 per cent of sodium sulphate to concentrated brine solutions to precipitate gypsum[11].

It may be argued that waters high in soluble matter should not be used. Such advice is of small assistance to the pastoralist whose livelihood is threatened, because owing to drought or other causes the water on which he depends has become more saline. It is believed that a fuller appreciation of the chemical nature of waters in the drier parts of the world would reveal many sources, at present thought valueless, to be capable of use after treatment with lime. A survey of the literature on Australian water sources has shown that waters rejected as unsuitable by pastoralists are nearly always high in magnesium and often sulphate as well. It should be pointed out that this rejection is based not on theory but on practical experiment.

Because of the ready availability of lime in any agricultural or settled community, the cost of treating water by this method is small and no complicated equipment is necessary.

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DATING GEOMAGNETIC POLARITY EPOCHS IN RÉUNION

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IN most palaeomagnetic investigations of Tertiary rocks the directions of natural remanent magnetization (NRM) are found to be approximately parallel or antiparallel to the direction of the present geomagnetic field. The rocks are said to possess normal or reversed polarity respectively. This bimodal distribution of polarities may be caused either by reversals in polarity of the geomagnetic field or by the rocks possessing the property of self-reversal.

Theoretical considerations indicate that rocks containing certain magnetic minerals may spontaneously acquire a direction of magnetization opposite to that of the ambient field. There are no laboratory tests at present available which can establish whether a rock had the property of self-reversal at the time it became magnetized. Other tests have to be used to distinguish between the two possible explanations of the observations.

If it can be shown that rocks of the same age have the same polarity throughout the world then this would constitute strong support for the hypothesis of geomagnetic field reversal. By contrast, if self-reversal were of importance in producing the bimodal distribution of polarities, then no consistent pattern of age versus polarity would be expected. Hence, a combination of isotopic dating and palaeomagnetic studies should provide a means of distinguishing between the two hypotheses.

Recently, by using these two techniques, a surprisingly consistent pattern of age versus polarity has emerged[1–5]; this is strong evidence in favour of the hypothesis of geomagnetic field reversal. The investigations have been directed mainly at establishing the relations during the past 4 m.y., and for this period four polarity epochs have been named[4,5]. In addition, at least two events have been recognized[4,5], an event being a short-period change in polarity during an otherwise normal or reversed epoch. The duration of an event may be of the order of 0·1 m.y.[5].

These investigations are important for a proper understanding of the origin and behaviour of the geomagnetic field; they should also form the basis for using polarity measurements as a means of correlating rocks that cannot be dated by isotopic or palaeontological methods.

Most of the available data have been obtained on rocks from the northern hemisphere; it is of some importance to make similar investigations on rocks from the southern hemisphere. This article presents palaeomagnetic and age results on rocks from the island of Réunion in the Indian Ocean. We are concerned here mainly with geomagnetic polarity epochs; full discussion of other aspects of this work will be given elsewhere.

Réunion is situated at 55° 30′ E., 21° S. at the south-west extremity of the Mauritius–Seychelles Ridge. The island is built of two volcanoes. Piton des Neiges, an extinct volcano, forms the north-western two-thirds of the island, and Piton de la Fournaise, which is still active, occupies the south-eastern part. Various aspects of the geology of Réunion have been described previously[6–9]. Both volcanoes are built predominantly of basaltic lavas which have tholeiitic affinities. The main basaltic shield of Piton des Neiges is covered by an extensive veneer of feldsparphyric basalt, hawaiite and mugearite, termed the "differentiated series" by Upton and Wadsworth[8].

More than one hundred samples were collected from unweathered, well-exposed outcrops on both volcanoes. Specimens were oriented by means of a Brunton compass. Isotopic dating was carried out on more than thirty-five of the samples used in the palaeomagnetic investigations.

The whole-rock technique of potassium-[illegible] was used as previously described[10,11]. Argo[illegible] med by isotope dilution and potassium by f[illegible] tometry. The experimental uncertainty in the measured ages generally is less than ±5 per cent at the 95 per cent confidence level, except in those cases where the air argon correction exceeds 85 per cent, when the error may increase to more than ±10 per cent. Estimates of the experimental error are given in the list of results, based on the quality of the mass spectrometer runs, the air correction, and the reproducibility of both argon and potassium determinations, as most results were measured in duplicate. Only essentially holocrystalline, unaltered rocks were used in the dating to minimize the possibility of loss of radiogenic argon. The consistency of dates determined on rocks the relative stratigraphic positions of which are known provides good evidence that the measured ages are correct within the limits of experimental error.

Palaeomagnetic measurements were made with a short-period astatic magnetometer. A series of fifteen test specimens were thermally demagnetized in steps up to 600° C, and by alternating fields up to 700 oersted (peak). These tests showed that the rocks generally are magnetically somewhat unstable. The thermal decay curves are variable, and commonly show low blocking temperatures. In many cases the intensity of NRM was reduced to less than one-half at 150 oersted (peak) during alternating current demagnetization. Furthermore, when left in the laboratory, the direction changed by as much as 15° over a period of 6 weeks. Most samples had a substantial component of temporary viscous magnetization which, however, could be removed after alternating current treatment to 150 oersted (peak). After alternating current demagnetization, the directions fall into two reasonably well-grouped clusters of normal and reversed polarity which approximate closely to the magnetic dipole field direction, when sign is disregarded. There are two exceptions: the magnetic direction of *RU*13 moves towards the reversed group on alternating current demagnetization, but does not quite reach it. Sample *RU*80 has an anomalous direction of magnetization, but would fall within the normal group if it is assumed that there was a misorientation of 180° in the strike line. This appears to be confirmed by the fact that another sample from the same lava flow has normal polarity.

The results are listed in Table 1, and plotted in Fig. 1 together with previously published data. The nomenclature of the polarity epochs follows that of Cox *et al.*[4,5]. Only those results are given for which the magnetic and age measurements were made on the same sample. Those samples measured palaeomagnetically, but not dated, can readily be correlated with the dated samples from the stratigraphy and polarity.

All the samples from Piton de la Fournaise have normal polarity. The indicated ages on seven samples from this volcano range from 0·04 to 0·35 m.y. Owing to the low proportion of radiogenic argon in the runs the dates have uncertainties of between ±10 and ±80 per cent (Table 1), the larger error relating to the younger samples. Similarly all specimens from the differentiated series of Piton d[illegible] Neiges possess normal polarity, and the measured dat[illegible] which are generally of good precision, lie in the range 0·10–0·35 m.y. The age data confirm that Piton de la Fournaise was erupting before Piton des Neiges became extinct.

as of the main dome building phase of Piton des ...es, termed the "oceanite series"[8], can be divided into three distinct groups on the basis of the age measurements. It is not known whether this indicates three major periods of eruption or whether it is the result of biased sampling because of the restricted accessibility of the outcrop of the oceanite series.

The youngest group of lavas of the oceanite series are dated at between 0·43 and 0·58 m.y. (six samples), and indicate that the hiatus between this series and the differentiated series was of short duration. Lavas of this group consistently have normal polarity.

The results from the lavas of Piton de la Fournaise, the differentiated series and the younger lavas of the oceanite series of Piton des Neiges provide a large body of data that show that in the age range 0·04–0·58 m.y. the polarity was normal. Fig. 1 emphasizes that, prior to the present work, very few results were available for this part of the time scale. These rocks clearly belong to the Brunhes normal polarity epoch.

A second group of lavas from the oceanite series have measured ages lying between 0·97 and 1·18 m.y., based on samples from seven flows. Five of the specimens yield dates in the range 1·07–1·18 m.y., and have reversed polarity. These results are consistent with previously published data; the rocks can confidently be assigned to the Matuyama reversed epoch. Sample *RU*13 (*GA* 1255), dated at 1·02 ± 0·03 m.y., was magnetically somewhat unstable, but also appears to belong to the reversed polarity group. The remaining lava of this age group is a distinctive columnar olivine basalt that crops out on the road to Takamaka. Field observations suggest that the lava occurs adjacent to the edge of a valley eroded in the oceanite series; the valley was afterwards filled by lavas of the differentiated series. The directions of magnetization measured on three different samples from this particular olivine basalt agree to within a few degrees and indicate normal polarity. Two of the specimens were collected by one of us, and the third was obtained at a later time by Dr. M. W. McElhinny. All three samples were dated (Table 1), and the results agree closely at 1·01 ± 0·05 m.y. The rocks are holocrystalline; *RU*93 and *RU*182 are quite free of alteration, but *RU*92 showed incipient alteration which may account for the poor reproducibility of the age measurements on this sample. Because of the consistency of the results we are very confident of both the polarity and age measurements.

Table 1. Potassium–Argon Dates and Palaeomagnetic Polarity Results on Rocks from Réunion (*N*–Normal Polarity; *R*–Reversed Polarity) (Errors quoted for Dates are Estimates only, Equivalent to about the 95 per cent Confidence Level)

Field No.	Sample No (*GA*)	Potassium–Argon age (m.y.)	Polarity
	Piton de la Fournaise		
*RU*97	1287	0·036 ± 0·03	*N*
*RU*80	1292	0·05 ± 0·01	(*N*)
*RU*86	1283	0·087 ± 0·02	*N*
*RU*87	1262	0·088 ± 0·01	*N*
*RU*88	1263	0·18 ± 0·02	*N*
*RU*70	1290	0·33 ± 0·04	*N*
*RU*76	1291	0·36 ± 0·04	*N*
	Differentiated series—Piton des Neiges		
*RU*105	1264	0·104 ± 0·003	*N*
*RU*106	1265	0·137 ± 0·007	*N*
*RU*68	1277	0·15 ± 0·02	*N*
*RU*95	1286	0·20 ± 0·015	*N*
*RU*66	1279	0·212 ± 0·015	*N*
*RU*31	1257	0·227 ± 0·007	*N*
*RU*90	1284	0·25 ± 0·01	*N*
*RU*34	1258	0·29 ± 0·03	*N*
*RU*49	1294	0·29 ± 0·03	*N*
*RU*45	1260	0·35 ± 0·02	*N*
	Oceanite series—Piton des Neiges		
*RU*41	1259	0·43 ± 0·07	*N*
*RU*53	1295	0·43 ± 0·03	*N*
*RU*85	1282	0·45 ± 0·04	*N*
*RU*36	1293	0·50 ± 0·15	*N*
*RU*82	1281	0·54 ± 0·03	*N*
*RU*46	1261	0·58 ± 0·02	*N*
*RU*92	1299	1·05 ± 0·10	*N*
*RU*93	1285	1·01 ± 0·03	*N*
*RU*182	2000	0·97 ± 0·05	*N*
*RU*13	1255	1·02 ± 0·03	(*R*)
	1271	1·07 ± 0·04	*R*
	1276	1·10 ± 0·10	*R*
	1275	1·15 ± 0·03	*R*
	1280	1·18 ± 0·07	*R*
	1274	1·18 ± 0·15	*R*
	1278	1·96 ± 0·07	*R*
	1270	1·97 ± 0·07	*R*
	69	1·97 ± 0·06	*N*
		2·00 ± 0·10	*R*
		2·03 ± 0·05	*R*
		2·03 ± 0·05	*R*
		2·04 ± 0·05	*N*
		1·98 ± 0·06	*N*

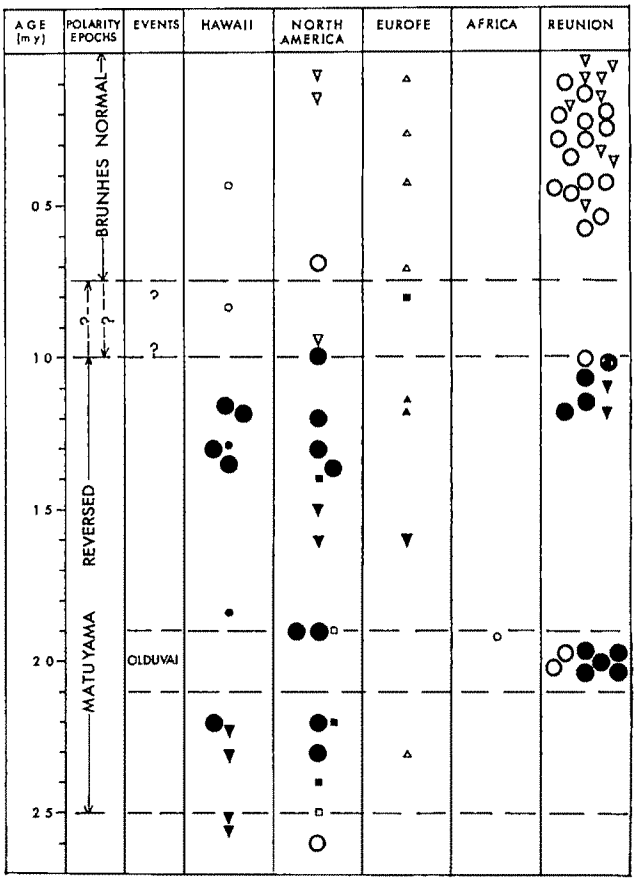


Fig. 1 Plot of potassium–argon dates versus magnetic polarity for rocks from Réunion, together with previously published data from elsewhere within the age range 0 to 2·6 m.y. See refs. 1, 3, 4, 5, 15 for sources of data. Open symbols, normal polarity, closed symbols, reversed polarity. Large circle, data appear to be satisfactory. Small circle, date determined on different sample from that on which palaeomagnetic measurement was made, although generally from the same formation. Triangle, polarity determined using a hand compass. Inverted triangle, uncertainty in age determination greater than 10 per cent. Small square, detailed information not published

These results suggest that a change from normal to reversed polarity occurred at about 1·0 m.y. The data available are too few to decide whether this is the boundary between the Brunhes normal and Matuyama reversed epochs. The results from Réunion and elsewhere indicate that normal polarity prevailed from the present back to about 0·7 m.y. ago, and that the polarity was reversed from about 1·0 m.y. to at least 1·9 m.y. ago (Fig. 1). The boundary between the two epochs must lie between 0·7 and 1·0 m.y., but because of somewhat conflicting evidence the relationships are not clear in this time range. The relevant data may be briefly reviewed. The normally magnetized Bishop Tuff, California, is dated at about 0·71 m.y.[12], and basalts with reversed polarity from Victoria and France are dated at 0·81 ± 0·03 m.y.[13], suggesting that a change from normal to reversed polarity occurred at 0·75 ± 0·07 m.y. A date of 0·84 ± 0·04 m.y. was recorded on a rock from East Maui, Hawaii[10]; it was inferred that this rock had normal polarity because other samples from the same volcano had this polarity[14]. Basalt of the Devils Postpile, California, has normal polarity and was dated at 0·94 ± 0·16 m.y.[3], and the Big Pine

obsidian is reversely magnetized and has a measured age of 0·99 ± 0·04 m.y.[15].

These data suggest that either the boundary between the Brunhes normal and Matuyama reversed epochs occurred at 0·75 ± 0·07 m.y., with a short interval of normal polarity (that is, an event) at close to 1·0 m.y., or that the boundary lies at 1·01 ± 0·03 m.y. and the reversed polarity of rocks dated at 0·81 m.y. records an event in an otherwise normal epoch. Another possibility is that self-reversal is partly responsible for the somewhat confused picture. Clearly many additional data are needed in the age range 0·7–1·0 m.y. to distinguish between the alternative explanations.

One of the most important findings from our work in Réunion concerns the third and oldest group of lavas from the oceanite series of Piton des Neiges. The eight samples which were used for the age determinations all give dates agreeing closely at 2·0 ± 0·1 m.y. Of these rocks three have normal polarity and five show reversed polarity. Two of the rocks of normal polarity were from the same lava flow about 30 m apart. However, several other samples from the same sequence also have normal polarity, and although they were not dated it is obvious that they must have essentially the same age. The age data are not precise enough to distinguish between the two groups, but at one locality it appears that rocks with reversed polarity conformably overlie rocks with normal polarity. According to the dates these samples belong to the Matuyama reversed epoch, which extends from 1·0 m.y. or younger to 2·5 ± 0·1 m.y.[1,2,4,5]. Hence, from Réunion we have evidence for normal polarity within the Matuyama reversed epoch, apparently indicating a normal event of short, but uncertain, duration. This can be correlated with the Olduvai event[4,5], which was first suggested from palaeomagnetic studies[16] on a basalt from Olduvai Gorge, Tanganyika, dated at 1·9 m.y.[17]. Our data provide strong confirmatory evidence for the reality of the Olduvai event, which we confidently date at 2·0 ± 0·1 m.y. Its existence also seems to be confirmed by work in the Pribiloff Islands, Alaska[5], but the data have not been published, so that no accurate comparison with the present investigation can be made.

The combined palaeomagnetic and age data from Réunion generally are in good agreement with results from other parts of the world. The results lend a[illegible] strong support for the hypothesis that geomagnetic [illegible] reversals are the main cause of the bimodal distribution of polarity. Our investigations emphasize the need for detailed work on rocks the ages of which lie between 0·7 and 1·0 m.y.

Although the main purpose of this article is to discuss polarity epochs a brief summary of some of the conclusions from the dating seems desirable. Piton des Neiges was active at least 2·0 m.y. ago and eruptions continued until as recently as 0·1 m.y. ago. The marked change in composition of the lavas of Piton des Neiges from basalts of tholeiitic affinities to alkali lavas occurred over a short interval of time of the order of 0·1 m.y. or less. This suggests a close genetic association between the two lava series, in agreement with conclusions reached from strontium isotope studies[9,18]. The data also indicate that Piton de la Fournaise was active at least during the period when the lavas of the differentiated series of Piton des Neiges were being erupted.

We thank R. Fanucci and E. Hugot for assistance while one of us (I. McD.) was in Réunion. Dr. M. W. McElhinny kindly collected some additional samples for us. J. A. Cooper, I. H. Ingram and J. Wasik provided technical assistance with the age determinations.

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RADIO EMISSION OF GASES IN A HIGH-VOLTAGE DISCHARGE

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IT is well known that electric sparks are excellent sources of high-frequency radio noise. In the present experiments, radio emission from sparks in helium, argon, hydrogen, nitrogen, ammonia and air were measured to determine whether any characteristic emission spectra exist in the radio range for these gases when in the presence of a strong electric field and accompanying discharge.

The spark was drawn between two nickel electrodes in a 250-ml. spherical glass vessel. The entering gas continually flowed upward between the electrodes and left through an exit port in the top of the vessel. The electrodes were 3/8-in. cold-rolled nickel rods tapered to an 18° solid angle. The electrodes were threaded through nuts in the sidearms of the vessel. The sidearms were detachable so that the gap between the electrodes could be easily adjusted. The nuts in the sidearms were connected to the outside through tungsten rods which passed through the glass.

The power supply for the greatest number of runs was a 60-cycle high-voltage transformer having a 115 V input and 7,500 V output at a root mean square current of 0·089 amp. The power to this transformer was supplied by a 20-amp 'Variac' to control the voltage. A few runs were made with a high-frequency spark to determine if the characteristic radiations existed with a high-frequency power source. The power source for these experiments was a 27·132 Mc/s diathermy machine.

The glass reaction vessel was surrounded by a radio shield consisting of a grounded copper-screen wire box. The antenna was a combination dipole and wire loop antenna for the range from 0·19 to 1·65 Mc/s and a wire loop antenna for the range of 1·65 to 30 Mc/s. The antennae were mounted inside the radio shield close to the react[illegible] vessel and parallel to the electrodes. The radio r[illegible] was a five-band 'Hallicrafter' model *S*-118 equip[illegible] a standard 'Hallicrafter' *S*-meter kit to me[illegible] strength. The radio ground was common[illegible] wire shield and the transformer.

The gases used were as supplied b[illegible] and no attempt was made to purif[illegible] cases the gases contained less than

Table 1

Gas	Peak emission frequencies: Peak frequency Mc/s	Ratio of peak strength to base signal strength: Mean	Range	Comment
Argon	0 32	1 2	1·1–1 3	Possible
	0·70	1·2	1 1–3 0	Possible
	1·3	1 6	1 1–2 0	Definite
	2·8	1·2	1·1–2 5	Possible
	7·0	1·6	1·2–3 0	Definite
	9 0	1·6	1 2–3 0	Definite
	13 0	2 1	1 2–4 0	Definite
	17·0	15 0	1·1–2 0	Definite
	21·0	2·2	2 0–3·0	Definite
Helium	1·1	1·8	1 2–4 0	Definite
	2 0	1·5	1·2–5·0	Definite
	2·6	1·3	1·0–4·0	Possible
	9·0	1·8	1·5–3 0	Definite
	13 0	1 9	1 5–2 0	Definite
Hydrogen	None			
Nitrogen	0·25	3·3	2 0–9 0	Definite
Ammonia	0 25	2·5	2 0–10,000	Definite
Air	0 25	6 0	5 5–3,000	Definite

The *S*-meter was set at zero by removing both the antenna and the ground wires and shorting out these terminals. The entire five bands were then scanned for background without either spark or antenna and the frequency which gave the lowest signal was determined. Using the variable potentiometer in the kit, this quietest point was then set to zero on the *S*-meter. From time to time this procedure was undertaken to adjust for shifts in the zero of the *S*-meter.

Before each run 4 or 5 l of the gas to be investigated were passed through the reaction vessel to ensure that the gas within the vessel was essentially the same as that in the supply tank. Sufficient voltage was then placed across the electrodes to initiate a discharge. If the 7,500 V available was insufficient to initiate a discharge, a 'Tesla' coil-leak detector was used.

After initiation, the power was set at the desired level by adjusting the input voltage. The apparatus was then run for at least 1 h to allow for stabilization. After the period of stabilization, the signal power, as indicated by the *S*-meter, was recorded as a function of frequency over the range from 0·19 to 30 Mc/s. This measurement was then repeated several times during the next hour to check for drift. The power to the discharge was then shut off. Immediately following this, the reading was repeated in order to determine the background intensity.

The characteristic frequency of each observed peak and its signal strength relative to the adjacent radio noise level are presented in Table 1 for each of the gases investigated. Figs. 1, 2 and 3 are typical curves of the observed radio emission for the gases. The discontinuities in the curves are caused by band changes in the radio receiver. The dipole antenna was designed for use in the first two bands, and consequently the signal strength in these bands appears higher than in the other bands. In general, the sensitivity of the antenna receiver combination decreased with increasing frequency, as is shown by the decline in the base line evident in the figures.

Figs. 1 and 2 show some characteristic emission curves for argon. Table 1 shows that argon exhibits six definite emission frequencies. These are at 1·3, 7·0, 9·0, 13·0, 17 0 and 21·0 Mc/s. There are also three possible peaks at 0·32, 0·7 and 2·8 Mc/s. In general, these peaks are weak and did not appear as definite on some occasions as the other peaks.

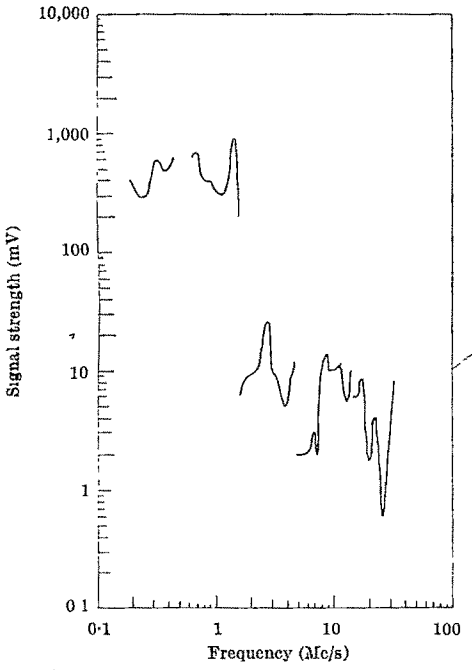


Fig 1. Radio emission spectrum of argon

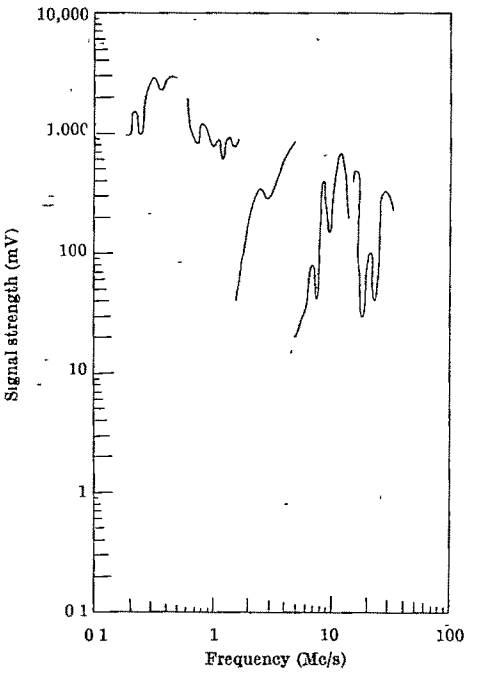


Fig. 2. Radio emission spectrum of argon, wet, short electrode gap

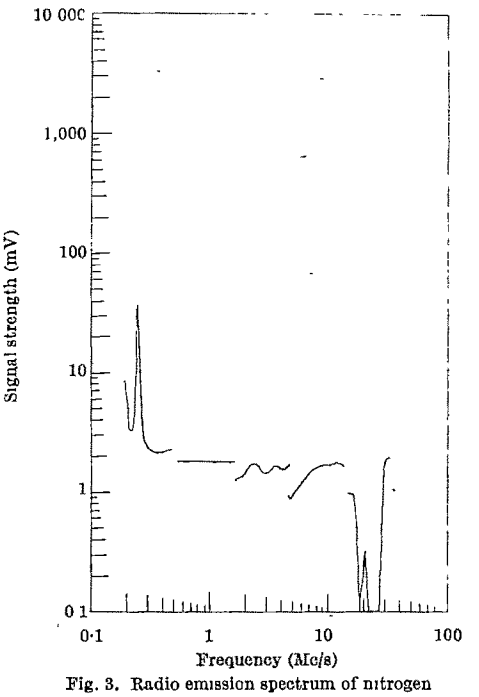


Fig. 3. Radio emission spectrum of nitrogen

Helium exhibits four definite characteristic emission frequencies. These are at 1·1, 2·0, 9·0 and 13 Mc/s. There is also a possible peak at 2·7 Mc/s; however, it is somewhat weaker than the others and could not be detected on several occasions.

As indicated in Table 1, hydrogen shows no characteristic peak frequency. A uniform radio noise was observed across all five bands. The signal strength decreased with increased frequency, due to the antenna, similar to the levels of base noise in Figs. 1 and 3.

For air, ammonia and nitrogen, only one characteristic frequency was observed. This occurs at 0 25 Mc/s. Since ammonia decomposes in the discharge to nitrogen and hydrogen, and air is four-fifths nitrogen, it seems that the 0·25 peak is characteristic of nitrogen. On occasion a peak near 0·25 Mc/s appeared in the argon discharge. This may have been due to a trace amount of nitrogen in the argon. This argument is supported by the relatively strong signals obtained at 0·25 Mc/s as shown in Table 1.

In the majority of runs the cold electrode gap was 2·5 mm and the power input was 290 W. This corresponded to an average field strength of about 6,400 V/cm. In several cases the gap distance was reduced to 1·5 mm and the corresponding field strength was about 8,500 V/cm. The effect of changing the electrode gap is illustrated in Figs 1 and 2. The general conclusion is that changing the gap changes the noise level, but does not affect the position of the peaks.

Figs. 1 and 2 also indicate the general drift in signal strength, since they represent two runs that were made several months apart. The base level varies with the physical conditions of the electrodes. Large changes in the base level can be effected by the simple expedient of cleaning the electrodes with a wire buffing wheel. Fig. 2 also shows that there is little, if any, effect of a small amount of moisture on the emission of the radio signals.

The effects of changing pressure and power level were also investigated. Increasing the power supply simply increases the noise level, but does not shift the characteristic frequencies. Reducing the pressure reduces the signal strength and does not change the peak emission frequencies.

In several runs the diathermy was used as a power source for the spark discharge in helium at one atmosphere. Over the first four bands, the diathermy discharge gave a low noise level and no peaks. In the fifth band, which contains the diathermy's rated signal frequency of 27·132 Mc/s, the signal increased uniformly and went off scale on the *S*-meter as the diathermy frequency was reached. The radio emissions observed for the 60-cycle discharge are apparently not excited by the high-frequency discharge. This may be because the frequency of the discharge is higher than any frequency of the emission peaks presented in Table 1. In addition, the mean field strength is in the order of one-half that of the 60-cycle discharge. The mean electron energies may be considerably less because the field reverses much more frequently.

It appears that the observed radio emission frequencies are a submacroscopic phenomenon. This hypothesis is based on the failure of the observed frequencies to shift with changing pressure or with changing electric field strength and power. If the frequencies were a plasma resonance effect, they would be expected to shift with pressure. If this were a system resonance effect, different characteristic frequencies for the different gases would not be expected, and a shift with changing field strength would be expected.

The various mechanisms by which plasmas are known to emit radiation are[1,2]: radiative recombination, bound electronic transitions, radiation in free-free transition bremsstrahlung, and possibly from magnetofluid-mechanical waves. It seems quite unlikely that recombination and bound transitions would involve energies as low as those corresponding to the observed radio frequencies.

The radiations studied by radio astronomers at present contain only one singular wavelength, the 21-cm line (1,420 Mc/s) emitted by hydrogen in the neutral state[3]. This line corresponds to hyperfine transitions of the neutral atom between its two possible spin states. The 21-cm line for hydrogen and the corresponding hyperfine lines for other substances ordinarily lie considerably above the radio frequencies observed in the present experiments.

Macroscopic phenomena in plasmas that involve electromagnetic radiation are electron and ion resonance and magnetic waves. Substituting the root mean square current and voltage in equations for the electric conductivity of slightly ionized plasmas indicates that the degree of ionization is 10^{-4} (ref. 1). For the gases at atmospheric pressure used, this corresponds to electron and ion concentrations of 10^{-15}/c.c. Thus the electron and ion concentrations vary from near zero when the field reverses to slightly more than 10^{15} at peak field strength.

The fact that 60-cycle current gives the radio signals while 27·132 Mc/s current does not may indicate that the heavy ions rather than the electrons are responsible for the signals. For the average concentrations mentioned above, the corresponding plasma electron frequency is 10^{-12}/sec (1), and the plasma frequency for argon is 10^{-9}/sec. Both these values are well above the observed frequencies. In addition, it is difficult to see how macroscopic plasma resonance frequencies which are usually determined for radio and microwave transmission and absorption can be expected to be responsible for discrete radio emission.

Since the gas collision frequency is in the order of 10^{-10}/sec whatever process is emitting, the radio signals must withstand several thousand collisions. In addition, the build-up and reversal of the electric field every 1/120 of a second is so slow that it seems impossible for resonance coupling to take place between the slowly varying field and the radio emission process.

Alfvén waves have been observed in gas discharges when strong magnetic fields are used[4]. It seems unlikely that they have any connexion with the observed signals, as they are restricted to frequencies which are small compared with the ion cyclotron frequencies in the magnetic field. Since no magnetic field was applied, the only field present is that which arises from the flow of current. There is little reason to believe that these magnetofluid-mechanical waves could account for the observed radio signals.

During the build-up and decay of the electric field, the electrons will receive varying amounts of energy from the field. For argon some of the electron velocities will pass through the critical value required in the Ramsauer effect[2]. At one atmosphere, an electron, on the average, will gain 0·30 eV between collisions from the field in the case of the 290 W. For argon[2] the minimum cross-section of 1 Å^2 occurs for electron energies of 0·55 eV and the maximum cross-section of 26 Å^2 occurs at 13 eV for the bombarding electron energy. As the field strength changes some electrons should pass through the region of almost complete transparency corresponding to the minimum in the collision cross-section. The Ramsauer effect does not occur in helium because the strength of the potential well is insufficient to produce a phase difference of 180° for the partial reflexion.

If the electron collision cross-sections pass through a minimum and a maximum, as they would for argon, the electric conductivity, which is inversely related to the cross-section, would also show considerable variation during the build-up and decay of the electric field. Such a change in cross-section could also permit an apparent emission peak in the otherwise continuous bremsstrahlung which arises from free-free transitions between electrons and heavy particles[2]. While this somewhat involved argument might be applied to argon, it will not, apparently, suffice for helium and nitrogen. Thus the radio strength

bremsstrahlung interactions can hardly be expected to provide an explanation for the observations.

The Stark effect[5] offers a potential explanation of the data. The splitting of the spectral lines in an electric field is not symmetrical about the original line, and the energy shift is given by the product of the field strength and the dipole moment. However, available data on hydrogen, helium and sodium indicate that the energy levels between the split spectral lines are considerably larger than the energy of the observed radio signals.

Cathode sputter arising from the collisions of ions and atoms with the electrode surface may be the cause of at least some of the radio signals. There is little information available from theory or experiment to allow a decision on the contribution that cathode sputter may make. Previous investigations have reported sputter rates in the order of the collision frequency of the particles with the surface[2]. If this is the case, sputtering rates at one atmosphere are several thousand times above the observed frequencies.

The particle-surface energy levels are very closely spaced near the top of the potential well, and it is possible that radio frequency transitions may occur between bound states near the top of the well for particle-surface interaction[2]. It seems unlikely that these will be sufficiently discrete to explain the observed signals. On the contrary, it appears that the transitions, if they occur, will give nearly continuous signals much like the bremsstrahlung emission.

Some support for the cathode sputtering argument is found in the fact that polishing the electrodes increased the background radio emission level. Since polishing did not influence the location of the emission peak frequencies, it is difficult to see how this particle-surface interaction can provide an explanation for the observations.

Since radio signal strength readings were taken over long periods of time, and a given frequency was often observed for many minutes, the results reported are average values which represent a great number of occurrences of the emission phenomena. None of the aforementioned radiation emission processes offers a satisfactory means of explaining these observations and it appears that the observed emission process arises by some mechanism not previously considered or reported.

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LASER INTERFEROMETRY OF A DROPPING MERCURY ELECTRODE

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AND

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SINCE the advent of the uniphase output, low wattage C. W. gas laser, the long path-length interferometer has become practical for the study of phenomena in the liquid phase. The study of concentration polarization around working electrodes has already received attention in thin cells, but the laser light source with its high degree of spacial and temporal coherence now permits the use of a thick enough cell to contain a dropping mercury electrode. Using such a long-path interferometer (identical to short-path models described previously[1,2], but substituting a laser for a sodium vapour lamp) the first qualitative results on transport mechanisms around the dropping mercury electrode are described here.

The original Ilkovic equation (where $i_d = 607\, n\, D^{\frac{1}{2}}\, C\, m^{\frac{2}{3}}\, t^{\frac{1}{6}}$, i_d is the average diffusion current, n is the number of faradays per mole, D is the diffusion coefficient of the electroactive substance, C is its concentration, m is the rate of mercury flow, and t the drop time), represented by curve 1, falls far short of describing the experimental current-time relationship[3], curve 2, plotted in Fig. 1 (taken from Markowitz and Elving[4]).

The main deficiencies of the equation are the assumptions that (1) during the initiation of drop formation a constant concentration is always encountered; (2) the rate of flow of mercury is constant; (3) mass transfer is by diffusion and migration only (and not by convection). The mathematical treatment of Matsuda[5] (curve 3 on Fig. 1) has shown that the discrepancies are not due to lack of mathematical rigor but to inadequate assumptions regarding the physical situation.

Without the knowledge of precise physical data, Markowitz and Elving made logical corrections to two of the aforementioned assumptions (assuming depletion of electroactive material around the capillary tip, and following the Lingane and Loveridge[6] method to account for the effect of convection on the current) and derived expressions plotted as curves 4 and 5, Fig. 1, which approximated the experimental data much better than either the Ilkovic or Matsuda formulae. Curve 4 differs from curve 5 in that in curve 4 a planar rather than spherical spacial distribution of a parabolic concentration gradient is used.

Curve 2 represents the experimental data of Taylor, Smith and Cooter[3]. The solution was $3{\cdot}018 \times 10^{-3}$ M Cd^{2+}, the diffusion constant assumed was $7{\cdot}2 \times 10^{-6}$ cm²/sec and the mercury flow was 2·305 g/sec, giving a drop life time of 3·392 sec.

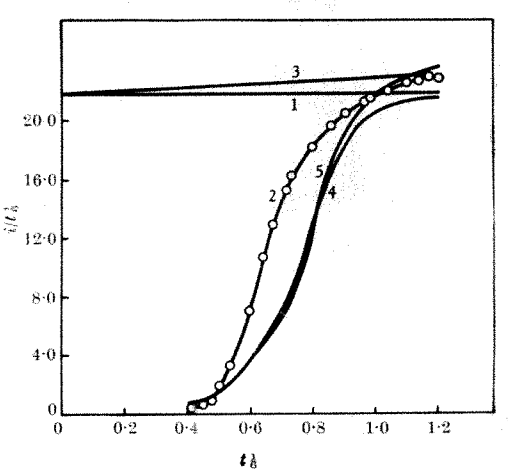


Fig. 1. Theoretical and experimental current-time dependence at the dropping mercury electrode taken from Markowitz and Elving (ref. 4). Curve 1 is the simple Ilkovic equation. Curve 2 shows the experimental results of Taylor, Smith and Cooter (ref. 3). Curve 3 is Matsuda's equation (ref. 5). Curves 4 and 5 are those of Markowitz and Elving using a plane and spherical approximation of an initial parabolic concentration gradient, respectively

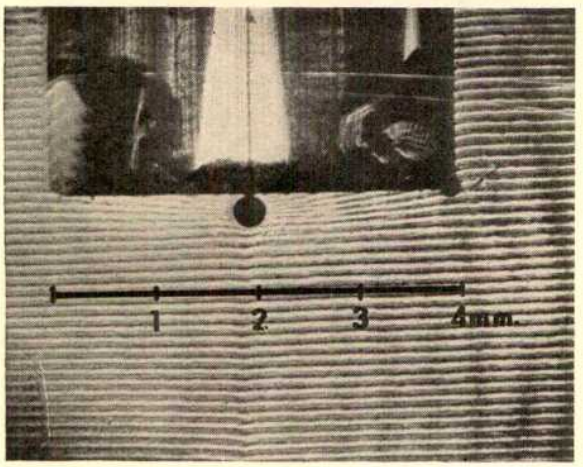


Fig. 2. Maximum concentration observed around a mercury drop. The solution is 0·1 N lead nitrate, 1 N potassium chloride at −1·40 V. The concentration of lead nitrate is about zero at the mercury-solution interface at the top of the drop

Elving (to whom we are indebted for the suggestion of the form of this work) and Markowitz assumed the concentration encountered by an emerging drop was always 2/3 C_0, where C_0 is the bulk concentration of the electroactive species. Fig. 2 is an interferogram taken with a motion picture camera in 0·1 N lead nitrate with 1 N potassium chloride supporting electrolyte at −1·4 V. The spacial distribution is far from spherical or planar but does have the shape which would be expected if a convective upward flow of electrolyte around the capillary was occurring, and this is what has been observed by following the movement of suspended rosin particles.

Fig. 3 is an interferogram taken when an attempt was made to start the camera, mercury flow and current simultaneously. The salt bridge is shown at the right. Potassium chloride is diffusing out of it into the less concentrated cell solution (0·1 N lead nitrate, 1 N potassium chloride, electrolysed at −0·45 V, drop time 1¼ sec) and convection upward is occurring, distorting the diffusion perturbation to the fringes to the right at the bottom of the frame and carrying some of the lower refractive index solution up between the salt bridge and the capillary. It should be noted that the concentration change at this unfavourable voltage (just within the range of the normal polarographic wave for lead ion) is very small compared to that in Fig. 2 at −1·45 V, the top of the polarographic wave.

Fig. 4 is a series of interferograms: 4*a* was taken of the same drop as Fig. 3, 1¼ sec later as the drop is about to fall, and 4*b* is $\frac{1}{16}$ of a sec after this. The drop has fallen and its 'ghost' is just visible. A very small concentration gradient over the whole bottom area of the capillary is just visible (breaks in the first and second double fringes, and the ninth, tenth, eleventh, twelfth and thirteenth below the capillary are optical defects in the cell) and has the same convection-influenced form as Fig. 2.

Fig. 4*c* was taken, 1¼ sec later, of the next drop as it is about to fall. The general, slight slope in the concentration gradient is now slightly increased, but the gradient immediately around the drop is much more pronounced. Comparing this with Fig. 2 (and assuming that the refractive index of lead nitrate is greater than that of copper sulphate at 6328 Å as it is known to be at 5893 Å), the concentration of lead ion is near zero at the upper part of the drop in Fig. 2 and about 2/3 C_0 in Fig. 4*c*. Fig. 4*d* was taken $\frac{1}{16}$ of a sec after Fig. 4*c* and shows the new drop already forming.

We believe that this is the first interferometric motion picture work done on the dropping mercury electrode, although high-speed motion pictures have been used to correlate drop formation and electrical phenomena[7].

Improvement in photographic techniques and measurement of the refractive index of lead nitrate solutions at the laser wave-length will soon make it possible to obtain quantitative results. Meanwhile, there would seem to be

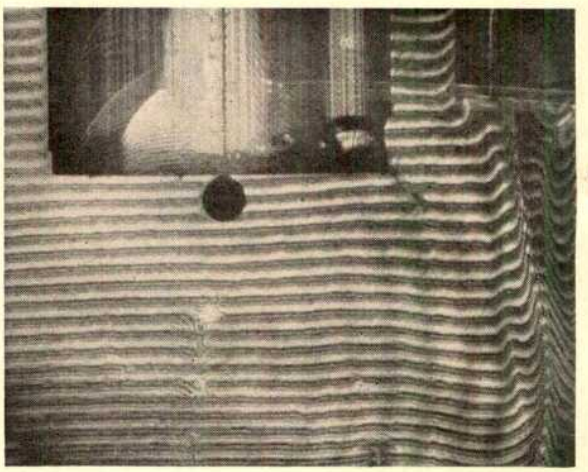


Fig. 3. The first drop of mercury to flow into the cell. The camera and current (voltage −0·45 V) were turned on at the same time as the mercury began to flow. The salt bridge is seen to the right of the capillary. The solution is the same as Fig. 2

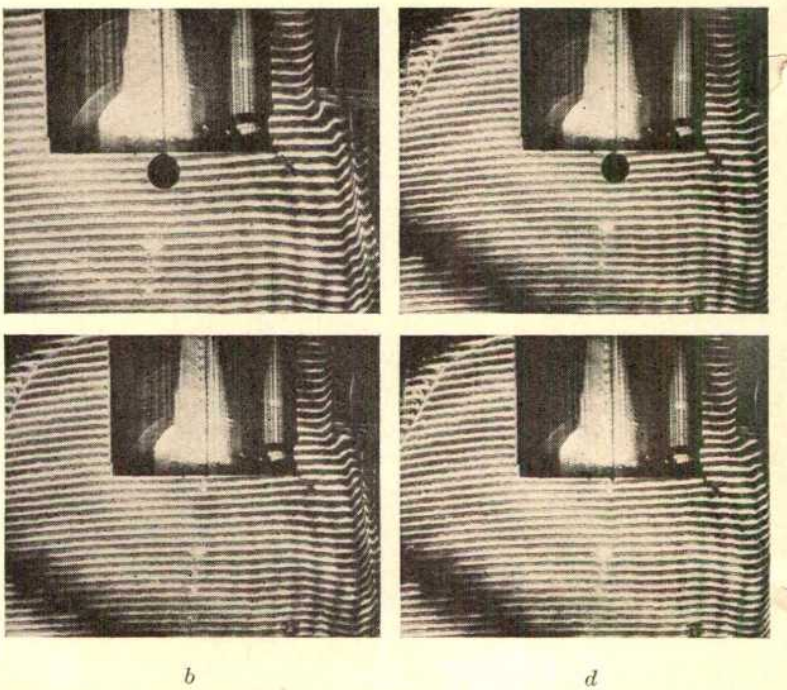


Fig. 4. A series of interferograms taken subsequent to Fig. 3. Frame *a* was taken 1¼ sec after Fig. 3, and frame *b* was taken $\frac{1}{16}$ of a sec later showing the ghost of the drop after it has fallen and very little concentration change. Frames *c* and *d* are photographs taken of the next drop again 1¼ sec and $1\frac{1}{16}$ sec later showing the increase in concentration gradient with successive drops

no doubt that concentration polarization, not spacially spherical or constant with time of electrolysis around the drop, is dependent on applied voltage and natural convection.

The experimental work was done by P. Winkelaar and J. Wojno and supported by the National Research Council of Canada, whom we thank.

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ELECTROLUMINESCENCE OF TRANSFORMER OIL AND LIQUID PARAFFIN IN HIGH D.C. FIELDS

By E. JONES and L. ANGERER

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RELATIVELY little information is available regarding the electroluminescence of insulating oils. Darveniza and Tropper[1,2] have described electroluminescence phenomena in transformer oil, its constituents and in simple organic liquids containing fluorescent materials, when they are subjected to high d.c. stresses. Emission of light, conduction current and stress relationships were investigated and also the effect of added impurities on the breakdown voltage of the solutions.

The study has been continued, for some time, at this Laboratory and the results presented here give more information about these phenomena. The test liquids used were transformer oil and liquid paraffin. Anthracene and 1,4-*bis*-2-(5-phenyloxazolyl)-benzene (POPOP) were used to study their effect on conduction current and light output.

The test samples were prepared by filtering through a No. 5 sintered glass filter and degassing to a pressure of 10^{-3} mm mercury. The test cell had a capacity of about 10 ml. and two nickel electrodes of 5 mm diameter were used to form the test gap. An eleven-stage photomultiplier was placed adjacent to a synthetic quartz window, perpendicular to the common axis of the spheres, and a microscope with a magnification of 100 was used for visual observation.

All tests were preceded by an electrical prestressing period in an attempt to stabilize the conditions during the tests. The effect of prestressing on the conduction current was similar to that reported by other workers[3–5]—2 h being needed to obtain reproducible measurements. The photomultiplier anode current was much greater at the beginning of the prestressing period and decreased with time in an approximately exponential manner, the mean value of the light output decreasing by several decades. In most cases, reasonable steady values for mean light output could be obtained after a period of 20–30 min of prestressing. Further stressing, for as long as 6 h, resulted in only small further decrease of about 30 per cent in the light output.

The photomultiplier had an *S*13 cathode with the spectral response covering from 1600 to 7000 Å and was connected to a Vibron electrometer by means of a cathode follower. A high-speed oscilloscope was used for a study of the photomultiplier anode current waveshape and a d.c.—10-c/s pen-recorder was used to record the fluctuations of light output.

The photomultiplier anode current consisted of a series of discrete pulses even when the liquid was highly stressed. The number of pulses increased with the applied stress and it was found that the mean value of the anode current was proportional to the pulse count. Fig. 1 shows typical conduction current and light pulses as seen on the oscilloscope screen at different stresses. The shape of the individual pulse appeared to be a function of the detecting circuitry. The use of a soda glass filter gave no appreciable change of the mean value of the photomultiplier current.

From time to time a sudden increase of the anode current of the photomultiplier was observed; this decayed in a generally exponential manner in a few minutes. Furthermore, even under the most stable conditions, a small variation of the anode current, about its mean value, was always present. Fig. 2 shows such an increase of light output with consequent decay and the fluctuation of light output about its mean value.

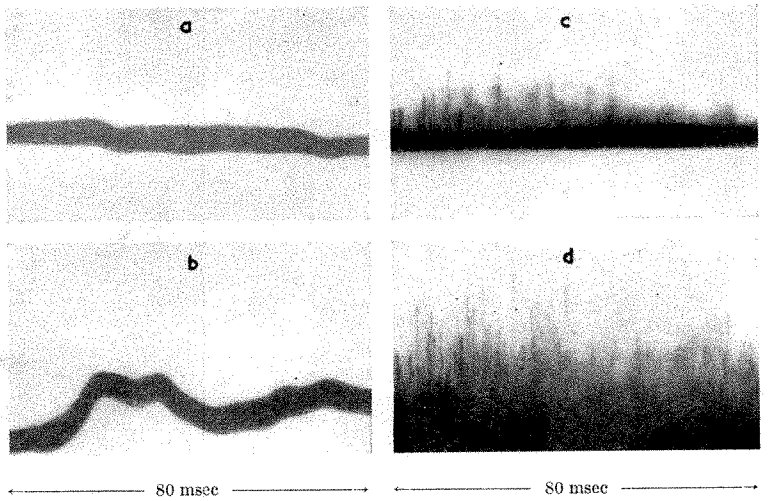


Fig. 1. Fluctuation of conduction current and light output pulses at different constant stresses. *a* and *b*, Fluctuation of conduction current at stresses of 200 kV/cm and 600 kV/cm, respectively. *c* and *d*, Light output pulses at stresses of 200 kV/cm and 600 kV/cm, respectively

From many tests a straight line for $\log_{10} B$ (light output) mean stress characteristics was obtained for several decades, but in some cases a pronounced curvature was also observed. Several minutes of stressing were required, at each value of applied stress, to allow the light output to settle to a stable value before the measurements were taken. The light output was observed to be lower for ascending values of stress than for descending values. Accidental breakdowns were found considerably to affect the light output. The effect was observed to be most marked with the first breakdown—subsequent breakdowns had less effect, providing there was an adequate prestressing period of the sample after a breakdown.

For nearly all the tests plots of $\log_{10} B/\log_{10} I$ (light output versus conduction current) yielded linear relationships

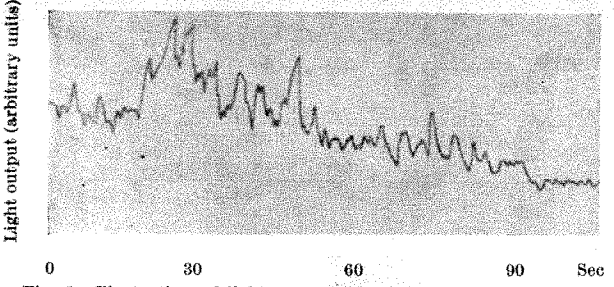


Fig. 2. Fluctuation of light output (photomultiplier anode current) under constant stress of 600 kV/cm. The figure shows a sudden increase in light output which decayed in about 90 sec

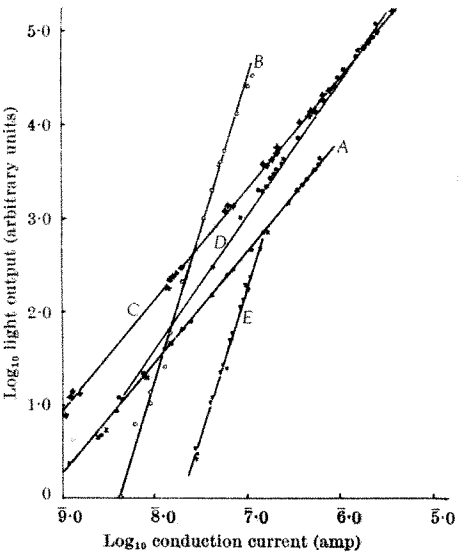


Fig. 3. $\log_{10} B/\log_{10} I$ (light output-conduction current) characteristics. *A*, Liquid paraffin and transformer oil; *B*, liquid paraffin containing POPOP at a concentration of 2×10^{-5} mole/100 g (28μ, 51μ, 81μ and 125μ gaps); *C*, transformer oil containing POPOP at a concentration of 2×10^{-5} mole/100 g (125μ gap); *D*, transformer oil containing POPOP at a concentration of 2×10^{-5} mole/100 g (51μ gap); *E*, liquid paraffin containing POPOP and dissolved nitrogen at atmospheric pressure (125μ gap)

over several decades. This is in agreement with the results reported by Darveniza and Tropper[2] and by Kalinowski and Dera[6]. Hysteresis effects were relatively small with no significant consistency. There was also no significant change of the slope of the characteristics for different gap settings. As can be seen from Fig. 3, pure liquid paraffin and transformer oil gave the same characteristics for all gap settings. Straight line $\log_{10} B/\log_{10} I$ characteristics were also obtained for solutions of liquid paraffin and transformer oil containing POPOP at a concentration of 2×10^{-5} mole/100 g of solvent but the slope of the characteristics was considerably changed. The presence of POPOP in both solutions resulted in an increase of conduction current followed by a considerable increase of light output.

Visual observations of the gap revealed that at very high stresses a visible luminescence could be observed in transformer oil and in solutions containing POPOP. For lower stresses no visible luminescence was noted in either liquid. In the case of pure liquid paraffin and transformer oil at high stresses, occasional transient spots of light have been observed—the light output under these conditions varying appreciably about its mean value.

Luminous spots in insulating oil gaps, subjected to high d.c. stresses, were also observed by Dakin and Berg[7], who attributed them to the luminescence of transformer oil molecules excited by electrons emitted from points of high localized electric stress on the electrodes. Similar phenomena were reported by Eidel'berg[8], who investigated the electroluminescence of castor oil in high a.c. fields. Two types of luminescence were observed: bright spots, at lower stresses, which flashed up and vanished very quickly, and at higher stresses, weak steady luminescent spots the brightness of which increased with applied stress. Both phenomena were also simultaneously observed.

A hysteresis effect was reported by Tumerman and Czajakow[9], who found that this effect can be reduced by increasing prestressing period or increasing the frequency of applied voltage. These authors also reported the difference between light signals as obtained from paraffinic wax and liquid transformer oil induced by uni-directional trapezoidal pulses. In transformer oil a series of short-duration pulses were observed, their frequency depending on the magnitude of applied voltage and the steepness of the trapezoidal wave front.

We thank Prof. W. F. Lowering for providing facilities and advice.

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COMMON MODE OF ACTION OF THREE AGENTS THAT DECREASE THE TRANSIENT CHANGE IN SODIUM PERMEABILITY IN NERVES

By BERTIL HILLE
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VALUABLE clues to the nature of the ionic permeability changes accompanying the excitation of nervous tissues will come from a detailed analysis of the effects of chemicals applied directly to nerve membranes. For two primary reasons the node of Ranvier of single myelinated frog fibres is a good preparation in which to study the mechanism of action of applied reagents: (1) the cell membrane is exceptionally naked at the node; (2) a complete biophysical description of nodal currents and voltages is possible. Electron micrographs[1] show that the only visible barrier between the bathing medium and the excitable membrane is a basement membrane and a loose collar of pseudopod-like processes of the adjacent Schwann cells. The membranes of these processes are not found to be in contact with the nodal membrane. In nearly every other common nerve preparation a uniform, closely adhering cellular layer bounded in turn by a basement membrane covers the nerve surface. The nakedness means that applied local anaesthetics, for example, have full effect within seconds and can be washed out almost completely[2]. The second advantage of the node follows the availability of a voltage clamping technique[3,4]. The Hodgkin–Huxley[5] equations for ionic currents of the squid giant axon modified by using different values for the

empirical rate constants account for the normal action potentials and other electrical responses of *Rana pipiens* nodes[6,7].

This article reports voltage-clamp analyses of the rapid, reversible effects of a powerful toxin, a local anaesthetic, and a tranquillizer. All three affect the sodium conductance in the Hodgkin–Huxley model. This change can be interpreted in terms of a physical model.

Data from voltage-clamp experiments on large fibres of the sciatic nerve of *Rana pipiens* were recorded and analysed with the aid of a computer. Every 50 μsec two digital samples, one of current and one of voltage, were stored using an on-line analogue-to-digital converter associated with a 'Control Data 160*A*' digital computer. Each voltage-clamp command pulse was applied in duplicate to the nerve, and the data of the duplicate runs were automatically added before being recorded on magnetic tape. The magnetic tape could be analysed immediately or at any later time with a 'Fortran' programme which was designed to separate the sodium, potassium, and leakage currents and to extract the kinetic and amplitude constants of the Hodgkin–Huxley equations. The computer programme could also draw graphs of the intermediate stages of the analysis such as: current-voltage diagrams, residual currents after subtracting leakage current, or sodium current after subtracting potassium current. One of these graphs is shown in Fig. 1. The separation of currents was achieved mathematically by fitting the Hodgkin–Huxley functions, and thus does not imply that the leakage current, for example, is carried only by ions other than sodium and potassium.

The membrane potential was held at −75 mV, approximately the normal resting potential. For the 25 msec preceding the variable command pulse the membrane was hyperpolarized to −120 mV. With this prepulse all steady-state inactivation of the sodium-carrying mechanism was removed. The standard experimental protocol was to measure the currents flowing in response to command pulses at nineteen equally spaced voltages from −67·5 mV to +67·5 mV absolute (each in duplicate) as well as to a pulse at −135 mV that measured the 'leakage' conductance. Successive 5-min periods in increasing concentrations of the experimental agent were alternated with 5-min periods in Ringer's. The preparation was maintained at between 3° and 10° C.

The drugs used were the puffer fish poison tetrodotoxin (gift of Sankyo Company, Ltd.), the local anaesthetic 'Xylocaine' (as the hydrochloride gift of Astra Pharmaceutical Products, Inc.), and the tranquillizer 'Compazine' (prochlorperazine ethane disulphonate), a close relative of 'Chlorpromazine' (gift of Smith, Kline, and French Laboratories). The drugs were applied as solutions in standard frog Ringer's solution buffered with *tris*-(hydroxymethyl) aminomethane at *p*H 7·2. At all concentrations a steady state effect was reached within the few seconds before the first measurements were made.

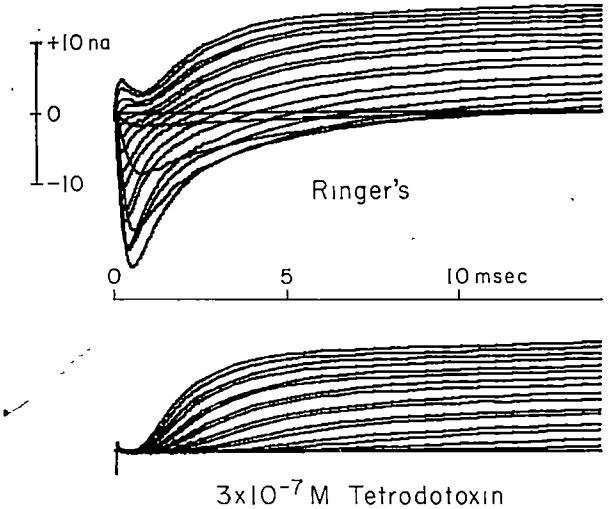


Fig. 1. Voltage-clamp currents plotted against time. The curves are drawn by the computer from raw data after subtracting the leakage current. Outward currents are positive. The nineteen clamp voltages were spaced at 7·5-mV intervals and spanned the range of membrane potentials from −67 5 mV to 67·5 mV. The two families of curves are the responses of the same node in a control solution and in tetrodotoxin

The effect of the agents on the current-clamped node was to raise the threshold, slow the rate of rise, and decrease the amplitude of the action potential without affecting the resting membrane potential. These changes are explained by a specific diminution of the sodium current seen in the voltage-clamp measurements. Fig. 1 is a plot of two families of ionic currents against time at the nineteen clamp voltages. The leakage component of the current has already been subtracted by the computer. In the upper family from an untreated node, the sodium current turns on and off in the first few milliseconds. At most depolarizations it appears as an inward current (negative on the graph) and at the largest depolarizations as an outward current. The outward potassium current turns on gradually as the sodium current wanes. In the lower family of curves from a node in 3×10^{-7} M tetrodotoxin, both the inward and outward sodium components are entirely absent. The same complete loss of sodium currents occurs with 5×10^{-5} M 'Compazine' and 10^{-3} M 'Xylocaine'.

Within the ionic hypothesis there are only three parameters whose variation could lead to a loss of the sodium current. The time constant of the turning on (τ_m) could be increased, the time constant of the turning off (τ_h) could be decreased, or the maximum available sodium conductance ($\bar{g}_{Na}$) could be decreased. To determine which of these changes occurred here we measured these parameters in normal nodes and in the same nodes treated with enough drug to approximately halve the sodium current: 3×10^{-9} M tetrodotoxin, 2×10^{-5} M 'Compazine' or 10^{-4} M 'Xylocaine'. In all cases the time constants were unchanged within the reproducibility of replicate clamp series in the same solution (about ±10 per cent at clamp-voltages that give fairly large currents), and no change was seen in the voltage dependence of steady-state sodium inactivation. Therefore, these three agents act primarily by reducing $\bar{g}_{Na}$.

While the reduction of $\bar{g}_{Na}$ is sufficient to explain the changes in the action potential we also looked for effects on the time constant (τ_n) and steady-state conductance (g_K) of the potassium component and on the conductance of the leakage component ($\bar{g}_L$). No changes were seen with tetrodotoxin up to 3×10^{-7} M, with 'Compazine' up to 5×10^{-5} M, or with 'Xylocaine' up to 5×10^{-4} M. At $3{\cdot}5 \times 10^{-3}$ M 'Xylocaine', $\bar{g}_K$ was reduced to 75 per cent of its original value without noticeable change in τ_n or $\bar{g}_L$. Higher concentrations of these reagents were not tried.

As the agents used here reversibly eliminated conduction in a nerve they may be said to have local anaesthetic action. This action was known before this investigation was started[8–10]. When the ionic basis of the action potentials of squid axons was clarified[5] it became apparent that non-depolarizing anaesthesia could be due to some kind of reduction of the transient inward sodium currents that underlie the rising phase of the spike. Shanes[11–12] reviews many studies that demonstrate (1) a summation of the effects of local anaesthetics and of treatments which decrease sodium currents: low external sodium, refractoriness, and depolarization by elevated external potassium or by applied electrical currents; and (2) an antagonism between local anaesthetics and treatments which enhance sodium currents: high external sodium, veratrine, and hyperpolarization by applied electrical currents. Thus our observations are not surprising. However, neither the specificity nor the precise nature of the sodium current reduction by anaesthetics has been previously described. Only recently, experiments with tetrodotoxin have demon-

strated that agents could be specific inhibitors of sodium currents[14]. Takata *et al.*[13] showed in giant axons from the lobster that tetrodotoxin reduces $\bar{g}_{Na}$ without affecting the sodium current kinetics. The present experiments agree with their observations and extend them to other agents. We expect that most local anaesthetics will be found to act in this way.

We believe that the principle of distinguishing physical components of biological tissues by their different chemical reactivities is a valid one and expect that it can be used to demonstrate the spatial as well as the functional separation of the formal parameters of the Hodgkin–Huxley equations. If this can be done, the formal parameters will seem less like an arbitrarily chosen set of variables of an abstract model and more like the one-to-one representations of physical properties of molecular constituents of the membrane. The specificity of tetrodotoxin has been used to argue that the sodium current channels are spatially separate from the potassium current channels[14]. We can extend this argument to 'Xylocaine' and 'Compazine'. The major effect of another compound, tetraethylammonium ion, is to reduce reversibly the delayed potassium currents of frog nodes[15,16]. A nerve in 6 mM tetraethylammonium chloride has almost no potassium currents, slightly diminished sodium currents, and 95 per cent of the normal leakage current. On the addition of 1·2 mM 'Xylocaine' we find little conductance remaining other than the leakage, which is unchanged. Thus the leakage channels, the sodium channels, and the potassium channels may be regarded as spatially separate, independent structures of the membrane, containing molecular configurations that react selectively with these reagents. Probably the reduction of $\bar{g}_K$ seen in high concentrations of tetraethylammonium ion or of 'Xylocaine' results from reaction with the potassium channel. These two compounds share some structural properties, but are different enough for 'Xylocaine' to favour the sodium channel over the potassium channel by a factor of about 100, while tetraethylammonium ion favours the potassium channel by a factor of more than 50.

The final subject of this article is a hypothesis regarding the mode of action of these compounds. We believe that the sodium channels of the nerve are closed or clogged individually by the binding of the anaesthetic molecule to a complementary structure in the channel. The molecule is active as a cation and approaches the binding site from the extracellular side of the cell membrane.

A binding equilibrium is suggested by the steady increase from no effect to nearly full effect over a ten-fold range of concentration with the steady state achieved rapidly and reversibly. A study of the apparent binding constants of various anaesthetic molecules in the isolated frog node could suggest the structure of a part of the sodium channel. The anaesthetic seems to be active as the cation because the guanidino group (pK about 12·5) of tetrodotoxin and the tertiary amino group of 'Xylocaine' and 'Compazine' (pK 7·9 and about 7·6, respectively) are largely ionized at pH 7·2. Perhaps the sodium channel bears a negative charge that attracts sodium ions, calcium ions, and other cationic molecules including the anaesthetics. The activity of the cationic form would seem to contradict the general clinical rule that local anaesthetics are more powerful in alkaline solution; however, it is recognized that this rule follows from the requirement of the free base for diffusion through layers of tissue to reach the site of action[9,17]. Indeed studies of the pH dependence of local anaesthesia clearly show that anaesthesia of isolated fibres is favoured by the cation while the anaesthesia of whole nerve trunks is favoured by the free base[18,19]. The molecules must be acting from the extracellular side because large extracellular charged molecules could not come to equilibrium with the axoplasm a few seconds after application. Truant[20] found that squid axons loaded with procaine are not blocked so long as the escaping procaine is washed away but are blocked if most of the surrounding fluid is blotted away and the anaesthetic accumulates outside the nerve. This effect can be repeated many times[12]. We have depicted the binding of the molecule as a clogging of the sodium channel because anaesthesia seems to decrease the available number of channels as reflected by the magnitude of $\bar{g}_{Na}$ without affecting the kinetic properties of those remaining.

I thank Dr. Frederick A. Dodge, jun., who designed most of the instrumentation, and Dr. H. K. Hartline, who made his computer available to me.

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PHOTOCHEMISTRY OF 5-BROMOURACILS: ISOLATION OF 5,5′-DIURACILS

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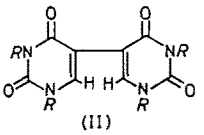
ALTHOUGH the increased ultra-violet sensitivity of bacteria[1,2], bacteriophages[3,4] and mammalian cells[5] attendant on the incorporation of 5-bromouracil (BU (I)) or 5-iodouracil into DNA in place of thymine has been widely observed, the mechanism by which this occurs remains uncertain. Previously, it was believed that the photochemical conversion of halogenated pyrimidines to uracil in the DNA might be a lethal event[6], but this hypothesis has been refuted by the work of Rupp and Prusoff[7] and that of Hotz[8].

The ultra-violet sensitization produced by these thymine analogues could be due either to their greater photochemical reactivity[9] or to the inability of existing cellular mechanisms to repair the type of damage (photoproduct) produced[3,4]. In either case, the isolation and identification of the photoproducts of 5-halogenouracils and the

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study of their photochemical mechanisms is essential to a complete understanding of the enhanced ultra-violet sensitivity.

From the irradiation of BU and 5-bromo-1,3-dimethyluracil (BDMU) in aqueous solutions with germicidal lamps, many photoproducts were isolated, identified, and quantitatively estimated (*Biochemistry*, in the press). Among these are two coupled products (II): 5,5′-di-1,3-dimethyluracil ($R = CH_3$, DMU-DMU) from BDMU and 5,5′-diuracil ($R = H$, U-U) from BU. (To avoid confusion between the 'single bonded coupled product' and the '*cyclo*butyl dimer', we propose to abbreviate the former as U-U, DMU-DMU, etc., and to change the latter from the present abbreviation (TT, UU, TT) to U=U, T=T, etc.)



(II)

This type of coupled product, with only one single bond between two pyrimidine rings, may be of significance in photobiology. This article briefly describes the isolation and identification of these two coupled products.

Methods and Materials

Chromatographically pure samples of BU and BDMU (I), prepared according to the method of Wang[10], were used. For BU, λ_{max}^{HOH} 276 mμ, $\varepsilon = 7{\cdot}30 \times 10^3$; λ_{min}^{HOH} 241 mμ, $\varepsilon = 1{\cdot}76 \times 10^3$. For BDMU, λ_{max}^{HOH} 283 mμ, $\varepsilon = 8{\cdot}57 \times 10^3$; λ_{min}^{HOH} 246 mμ, $\varepsilon = 1{\cdot}57 \times 10^3$. General Electric 'Germicidal' tubes (*G15T8*) were used as our sources of ultra-violet light: these emit mainly at 253·7 mμ. For spectroscopic investigations, 25 ml. of a 0·1 mM aqueous solution was placed in an 8-cm diameter Petri dish and covered with a quartz-plate (the depth of the solution was 5 mm). One lamp was used for irradiation at proper distances for various dose-rates and the solutions were agitated during irradiation. Dose-rates of ultra-violet light were calibrated according to the method of Latarjet *et al.*[12].

For the synthesis of 5,5′-di-1,3-dimethyluracil (II)[13], 4·38 g of BDMU was dissolved in 25 ml. of dry nitrobenzene under refluxing with stirring. Purified copper powder (4·38 g) was added portion by portion over a 4-h period. The reaction mixture was then allowed to reflux for 2 days with stirring. Afterwards, the reaction mixture was filtered and most of the nitrobenzene was removed under reduced pressure. The remaining few ml. of nitrobenzene were removed by steam distillation. The aqueous solution so obtained was filtered and was concentrated for crystallization to yield 0·4 g of the crude product. Material twice recrystallized from absolute ethanol gave a melting-point at 281°–282° C (subl.). λ_{max}^{HOH} 272 mμ, $\varepsilon = 15{\cdot}1 \times 10^3$; λ_{min}^{HOH} 244 mμ, $\varepsilon = 7{\cdot}2 \times 10^3$. Analysis for $C_{12}H_{14}O_4N_4$ was: calculated, C, 51·79; H, 5·07; N, 20·44; found, C, 51·69; H. 4·97; N, 20·21 (kindly undertaken by Dr. S. M. Nagy, Microchemical Laboratory, Massachusetts Institute of Technology).

Spectroscopic Study

Wacker[6,14] reported that the ultra-violet absorbancy of an aqueous solution of BU remains unchanged during ultra-violet irradiation up to a total dose of 2×10^5 ergs/mm². On the contrary, we observed a continued increase in ultra-violet absorbancy at about 240 mμ when a 0·1 mM aqueous solution of BU was irradiated at a dose-rate of 15, 30, 45, 60 or 90 ergs/mm²/sec with a final total incident light dose of $1{\cdot}5 \times 10^5$ ergs/mm². When BDMU, a model compound, was irradiated in an identical manner, the increase in a shorter wave-length region was also observed in all cases, and was more pronounced with lower dose-rate. In addition, a decrease in absorbancy at the maximum was also observed and, in particular, there was a 5 per cent decrease at high dose-rate (90 ergs/mm²/sec) with a total incident light dose of $1{\cdot}5 \times 10^5$ ergs/mm². These preliminary spectroscopic investigations clearly demonstrated that BU and BDMU do undergo photochemical changes even with a small dose of ultra-violet light. The absence of any appreciable decrease in absorbancy at the maximum suggests that the photoproduct has a similar absorption spectrum in the neighbourhood of 270 mμ. It is indicative that the photoproduct may still possess the pyrimidine ring. The increase in the 240 mμ region implies that the photoproduct must have high absorbancy in this region. U-U or DMU-DMU becomes the most likely candidate because they contain two uracil moieties and have high absorbancy at 240 mμ.

Isolation Study

(1) *Fractionation after irradiation.* 10 l. of a 1 mM solution of BDMU were irradiated in quartz tubes for 1·5 h at room temperature under conditions described previously[11]. During irradiation, the *p*H of the solution changed from about 6·0 to about 3·0 and the absorbancy at 283 mμ decreased about 55 per cent. The solution was titrated to *p*H 9 with sodium hydroxide to release the volatile acids and to prevent the loss of volatile acids during evaporation. The solution (*p*H 9) was concentrated to about 10 ml. in a rotary evaporator at below 40° and subjected to continuous ether extraction for 5 days. In this way three fractions were obtained, namely, the distillate fraction, the ether extract, and the aqueous phase. The present article deals only with the ether extract.

(2) *Separation and identification.* The ether extract was evaporated to dryness and the residue was dissolved in 2 ml. of chloroform. 3 g of alumina (*p*H 10, for chromatography, Merck) were mixed with this solution and the chloroform was evaporated in a gentle stream of nitrogen. The sample was transferred quantitatively to a water-jacketed column packed with 75 g of alumina ($1{\cdot}5 \times 50$ cm) and was treated with various eluents (see Table 1). Eluates were collected in 25 ml. fractions. Quantitative estimations were made after evaporation to dryness. The residue from each fraction was dissolved in water and the ultra-violet spectrum recorded. On the basis of the amount of residue and of the ultra-violet absorbancy, seven large fractions were obtained. Each fraction was evaporated to dryness on a rotary evaporator below 40° C and dried over phosphorus pentoxide. The residue was extracted three times with chloroform and filtered. The combined chloroform solution was evaporated to dryness and the residue dried *in vacuo* and weighed (see Table 1).

These products were crystallized from water or ethanol and identified by comparing their melting-points, mixed melting-points, ultra-violet, and infra-red spectra, with those of authentic samples of the various products and by elementary analyses.

Table 1. Isolation and Identification of Photoproducts of BDMU in Ether Extract by Alumina Column Chromatography*

Fraction No.	Identified as	Tube No of eluates	Eluted with ethanol-ether (v/v)	Amount obtained (mg) By weight	Amount obtained (mg) By E_{270} mμ	Percentage in total ether extract
1	—	1–34	0 100 (ether alone)	<0 1	—	<0·01
2	BDMU	35–63		258·5	284 9	26 3
3	*Sym*-dimethyl-oxamide	64–73		13 5	—	1 4
4	DMU	74–101	1 99	135·2	135 5	13 8
5	—	102–112	1 5 : 98·5	1 2	—	0 1
6	DMU-DMU	113–140		75 1	83·5	7·6
7	(Unidentified)	141–165		20 9	—	2·1
			Total recovery by weight			51·4 per cent†

* Results are the average from four separate irradiation experiments. Average amount of dried ether extract was 982 mg.
† Other products will be described in a subsequent article

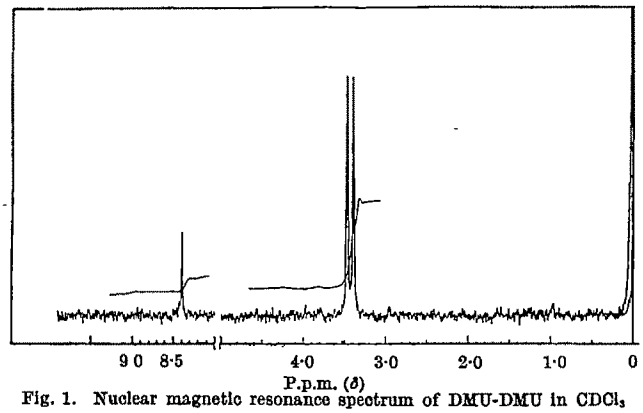


Fig. 1. Nuclear magnetic resonance spectrum of DMU-DMU in $CDCl_3$

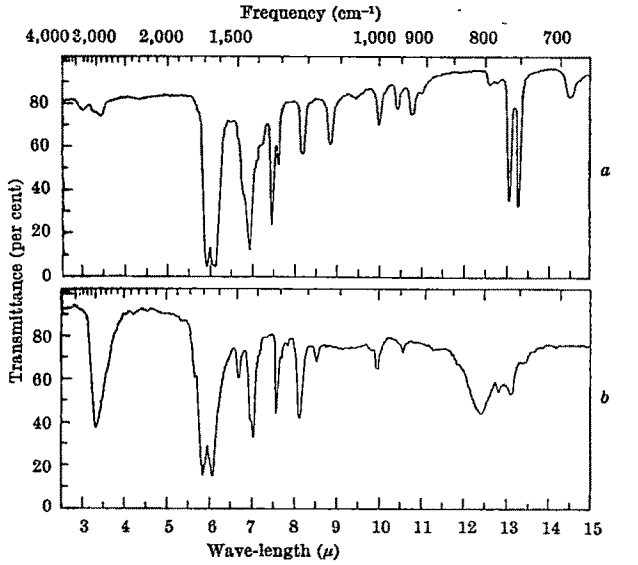


Fig. 2. Infra-red spectra of single-bonded dimers, DMU-DMU (*a*) and U-U (*b*) in potassium bromide pellet

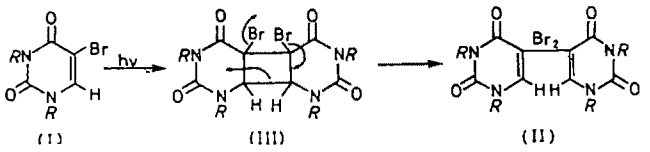
The nuclear magnetic resonance spectrum of DMU-DMU ($CDCl_3$) contains three singlets; one (δ8·42) is attributed to the proton at C (6) and the other two (δ3·40 and δ3·47) are for the methyl groups on N (1) and N (3) (Fig. 1). The singlet rather than a doublet for C (6) and the absence of a signal for a proton at C (5) (δ about 6) indicate that the compound is coupled through C (5) and C′ (5) atoms. This is compatible with the structure of the single-bonded coupled product. The ratio of the peak areas C (6): —N—CH_3 is 1 : 6 which is what would be expected for this compound. (Measurements were made by Mr. Martin Schweizer on a 'Varian *A*-60' spectrometer. Values of δ are given in p.p.m. from tetramethylsilane.)

A similar study on the irradiation of BU in an aqueous solution resulted in the isolation of the crystalline U-U. Its identity was established by comparison with DMU-DMU after exhaustive methylation with diazomethane. Infra-red spectra of these coupled products are shown in Fig. 2.

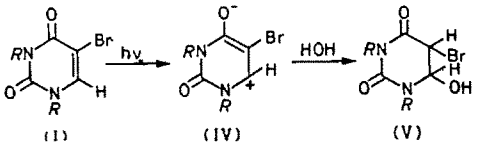
Photochemical Mechanisms

Three conceivable mechanisms for the photolysis of BU will be discussed in the following paragraphs. The first two are well known in the photochemistry of nucleic acids while the third has long been recognized in the action of visible or ultra-violet light on halogenated organic compounds.

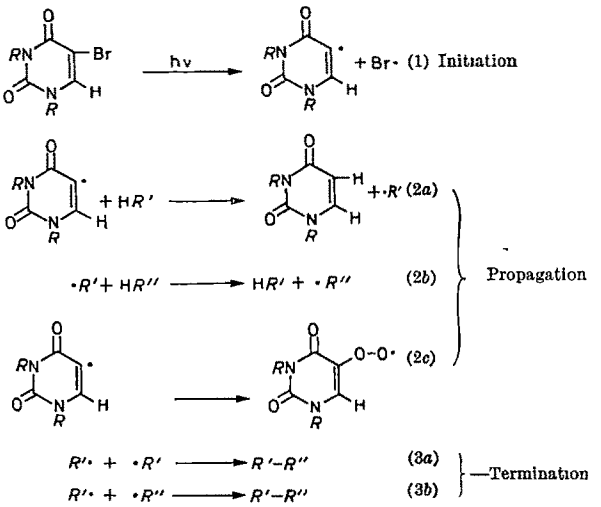
(*A*) The formation of *cyclo*butyl derivatives by way of the photodimerization process occurs most frequently in solid films[15–17] and in frozen aqueous solutions[15–19], but seldom in dilute (1 mM–0·1 mM) aqueous solutions. Even if the homodimer (III) of BU is formed, the electronic effect of the bromine atom would be expected to confer instability, with possible decomposition to 5,5′-diuracil derivatives (II) according to the following scheme[13b]:



(*B*) The formation of a water addition product (V) by way of a photo-zwitterion intermediate (IV) occurs in uracil derivatives. Water addition would have to result in a decrease of absorbancy at the maximum due to the saturation of 5,6-double bond[20]. The decrease in ultra-violet absorbancy should be reversed to various extents by acid, alkali or heat, because the water addition products are known to revert to the originals under such treatment[21]. Since heating of the irradiated solution of BDMU in dilute acid (0·1 N hydrochloric acid, for 20 min at 100° C) did not affect its absorbancy, it must be concluded that the water addition compound is not a final product. However, the possibility remains that the water addition compound may exist as a transient intermediate during irradiation. In fact, this situation could be analogous to that of thymine derivatives[22]. Lozeron *et al.*[23] have recently reported the identification of the water addition product from 5-fluorouracil irradiated in aqueous solutions.

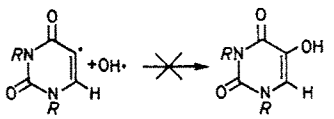


(*C*) The formation of radicals by way of the photo-dissociation process frequently occurs with halogenated organic compounds[24]. In this reaction, the homolytic cleavage of the C–Br bond results in the formation of Br· and uracil radicals (VI)—step (1). Uracil molecules may be produced by hydrogen abstraction from other surrounding molecules, and consequently a second radical (·*R*′) is produced—step (2*a*). The propagation sequence might then follow to produce ·*R*″—step (2*b*). Thus, a 'chain-like' reaction might occur and terminate with the reaction between two radicals to form single-bonded coupled products. Two radicals of the same species would give a homocoupled product—step (3*a*); and two different ones would give a heterocoupled product (3*b*).



It must be mentioned that, in the presence of oxygen in the irradiation medium, peroxide radicals could be formed—step (2c). The fate of these *R*-OO· radicals and also Br· from step (1) is a question that should be investigated further.

Another question that arises is whether hydrogen abstraction from the solvent molecules occurs in this chain reaction. If this did occur it would result in the formation of HO· which in turn would couple with uracil radicals to form 5-hydroxyuracil derivatives (VII) as shown. Thus, it is reasonable to assume that the presence of a 5-hydroxy compound would indicate the participation of water molecules in this chain reaction. However, the 5-hydroxy derivatives were neither isolated as photoproducts nor detected from the irradiation mixture of BU or BDMU by the 'Ferric chloride test' (*Biochemistry*, in the press). The results suggest that the 5-hydroxy compound is not a photoproduct and therefore water does not participate in this chain reaction.



(VI) (VII)

The homolytic cleavage of C–Cl, C–Br and C–I bonds requires 79, 66 and 57 kcal/mole, respectively[25], and the 253·7 mμ light would provide about 110 kcal/einstein which is, therefore, ample for initiating the chain reaction. Assuming photo-homolysis occurs with all these analogues in biological systems, the thermodynamical consideration would predict that the least potent sensitizing agent is 5-chlorouracil. The most pronounced one should be 5-iodouracil, the mechanism of which has been discussed by Rupp and Prusoff[26]. It is also interesting to note that 5-fluorouracil demands at least 105 kcal/mole for homolytic cleavage of the C–F bond. The requirement would decrease free radical formation. Indeed, the water addition reaction becomes predominant when 5-fluorouracil is irradiated with ultra-violet light[23].

The foregoing prediction is very similar to that of the findings in biological systems[27,28]. Bacteria pre-incubated with low concentrations of fluorouracil show enhanced resistance to ultra-violet irradiation[29]. This phenomenon may be due to the inability of fluorouracil to form free radicals. If this type of radical reaction should occur in DNA, it is conceivable that the uracil radicals produced by irradiation could attack their neighbouring purines, pyrimidines, or sugar moieties. Thus, different possible homocoupled products and heterocoupled products would result. In the absence of water participation in the radical reaction, the formation of various coupled products in the irradiated DNA may assume an even more important role. Moreover, from a stereochemical point of view, the formation of coupled products is much more favoured than *cyclo*butyl homodimers and heterodimers in DNA molecules. A similar type of intermediate has been proposed for the photo-oxidation of thymine derivatives[30–32]. Both observations could serve as a basis for further photobiological investigations related to thymine and 5-halogenated uracils.

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TRANSPORT OF TRITIUM-LABELLED *l*-HISTIDINE THROUGH THE SCHWANN AND MYELIN SHEATHS INTO THE AXON OF PERIPHERAL NERVES

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THE neurone is unique in a morphological sense in that its axonal processes extend from the cell body for long distances. When these delicate threads of cytoplasm are separated from the cell body, they rapidly waste and disappear. For this reason early workers realized that the cell body in some way maintains the morphological integrity of the entire process; and the cell body was called the "trophic" centre of the neurone, thus implying a nourishing function on the processes, a concept generally accepted to-day. Moreover, the agents of trophic control are believed to perfuse the axon in a continuous stream from the cell body[1,2]. Lapsed time photography supports the view of axoplasmic streaming and shows that the force of movement is provided by pulsations of the axon itself[3]; also, radioactive substances of various sorts have been demonstrated by biochemical and autoradiographic techniques to be incorporated at first in the cell body and to move thence into the axon[4,5]. The rate of movement has been determined in some instances and the possibility of disto-proximal streaming has been explored[6]. Therefore,

the ensemble of evidence at the moment supports the view that the cell body is the source of cytoplasmic constituents of the axon.

The question must now be asked, is it not possible and, indeed, logical that the incorporation of substances into the axon also occurs along its length by way of the sheaths that envelop it and, therefore, that the cell body is not the exclusive source of axonal stuff? Some of the early cytologists raised this question and speculated that the myelin segment is nutritive in function[7]. The question has been examined recently by Geren and Schmitt, who reasoned that the axonal surface must have some access to interstitial fluids and to products of Schwann cell metabolism for its own metabolic activity and to maintain the ionic and water balance required for propagation of the impulse[8,9].

We have evidence to show that the amino-acid, *l*-histidine, can be followed through the sheaths of the axon and thence into the axoplasm itself. Therefore, the cell body, albeit a predominant source, is not an exclusive one for substances in the axon.

Twenty-eight newts, *Triturus viridescens*, were injected intraperitoneally with 40–50 μc. of tritium-labelled *l*-histidine in 0·05 ml. of 0·01 N hydrochloric acid. Then brachial spinal nerves 3 and 4 were removed 1 h to 8 days afterwards, fixed in Carnoy's fluid containing magnesium ion for about 1 h, and sectioned at 5μ. The pieces included proximal segments of nerves 3 and 4 and their ganglia which were cross-sectioned in the case of nerve 3 and cut longitudinally in the case of nerve 4, and a distal piece of nerve extending from the brachial plexus to the elbow which was sectioned longitudinally. In some instances the nerves were transected just distal to the ganglia before the animals were injected with the radioactive substance; and the part distal to the cut was fixed 12 and 24 h after injection. For light microscopy the nerves were coated with the photographic emulsion Kodak '*NTE*-3', and exposed at low temperature for 3 weeks before developing; the sections were then stained with carmalum.

One hour after injection the radioactive label could be detected around and in the nucleus of the sheath of Schwann of the intact and also of the transected nerve. The axon did not contain any label and the myelin sheath was not involved. Ganglion cell bodies were well labelled as were the cells of two other tissues, skin and liver, selected for comparison with nerve. By 2 h some of the *l*-histidine label appeared in the axons. The axons and sheath nuclei of transected nerve were indistinguishably labelled from normal ones. In both normal and transected nerve the label was as heavy in distal as in proximal regions.

In subsequent hours the intensity of the label in the axons increased and the silver grains were often arranged along the axis of the individual nerve fibres. By 24 h the axonal region appeared about as heavily labelled as the Schwann nuclei; and the distribution and intensity of the tritium label in nerves separated from their cell bodies appeared indistinguishable from those of normal nerves. The label in the ganglia and the fibres of intact nerves, including Schwann nuclei, persisted until about the fifth day when it appeared to fade, but an obvious, although sparse, label could still be seen after 8 days when the experiment was terminated

Autoradiographic investigations of nerves 4, 24 and 72 h after injection of tritiated *l*-histidine were also made with the electron microscope. Animals were injected intraperitoneally with 500 μc. of aqueous *l*-histidine. A proximal and distal segment, the former including the spinal ganglion and the latter extending to the elbow, were fixed in osmium tetroxide, embedded in 'Epon 812' and prepared for electron microscope autoradiography[10]. Sections (500–1000 Å) were coated with monolayers of Ilford '*L*4' or Kodak '*NTE*' emulsions. Developed grains were counted and expressed as a density of radioactivity, namely grains per unit area of underlying tissue. The density of radioactivity was compared in sections of equal thickness coated and developed under identical conditions[11].

Four hours after injection of radioactive *l*-histidine, developed grains could be seen over all the components of the nerve fibres. The density of radioactivity in the axis cylinder was the same in the proximal and distal segment of the nerve. The Schwann nucleus and the myelin sheath showed a higher density of label than the axis cylinder. By 24 h the density of developed grains was about equal for the three components along most of the nerve. At this time the radioactivity of the axons in the proximal segment near the ganglion was higher than in the terminal segment, probably reflecting the addition of radioactive material streaming from the neuronal cell body into the axis cylinder. The density of radioactivity was reduced over all the components of the neurone 72 h after injection, but the disparity in intensity of labelling between the proximal and distal axoplasm was even more marked.

Electron microscope autoradiograms of thinner sections (500 Å) coated with monolayers of Kodak '*NTE*' emulsion for optimal resolution showed that the label in the axis cylinder was randomly distributed over the axon and not preferentially associated with a discrete organelle, such as vesicles or mitochondria—although these were sometimes labelled. The technique was, however, inadequate to distinguish the radioactivity of neurofilaments from surrounding axoplasm. The label in the myelin sheath was also scattered throughout the wrappings and was not localized in any part, and thus did not indicate any preferential pathway from the Schwann cell body inwards.

The results support the conclusion that *l*-histidine entered the nerve fibre first by way of the Schwann cell nucleus and perhaps its perikaryon and thence traversed the myelin sheath, and was finally carried into the neuroplasm of the axon. While these investigations were underway, evidence became available that radioactive lysine may also take this route into the axon[12]. Consequently, we wish to assert that the myelin sheath, in addition to its at present recognized function in saltatory conduction, serves to transport substances of metabolic importance into the axon. The mechanism of transport within the myelin is unknown to us. It is possible that the labelled substance moves within the membranes of the myelin sheath or between the interfaces of the wrappings. Robertson has suggested that the clefts of Schmidt-Lantermann represent more obvious waves of cytoplasm of the sheath of Schwann moving between the paired membranes of the myelin wrappings[13]. Perhaps, it is such streams of moving cytoplasm that carry substances inward toward the axon. Or, perhaps, the solid state of the myelin structure provides a mechanism for molecular transport.

Myelin once formed is considered to be metabolically inert since cholesterol, cerebroside, cephalins and sphingomyelin show little metabolic turnover[14]. The presence of incorporated histidine in myelin shows that the substance of myelin does change whether or not some of the incorporated amino-acid is, indeed, part of myelin structure or only a substance in passage.

We have as yet no information about the form in which the *l*-histidine, which eventually reaches the axoplasm, traverses the enveloping membranes of the neurone. The amino-acid may be incorporated into a substance of higher molecular weight within the Schwann nucleus and cytoplasm and be transported in this form through the myelin membranes to the axolemma and thence into the axoplasm. Or, perhaps, only molecularly free histidine penetrates the axon after being transported inward through the membranes that surround the axon. But, whatever the form of histidine before it reaches the axoplasm, it must traverse the myelin sheath, which must now be considered to function as a pathway for metabolic

substances from the extracellular fluid to the axonal cytoplasm. Therefore, the axon is fed by two streams, one originating in the cell body and moving within the axon itself and a second by way of the enveloping sheaths.

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PERSISTENCE OF AN ORGAN-SPECIFIC ANTIGEN IN ORGAN AND TISSUE CULTURES OF HYPERPLASTIC HUMAN THYROID GLAND

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THE organ culture system has been used to investigate *in vitro* the thyroid gland of several mammalian species[1–3] including man[4–7]. The persistence of the human thyroid organ-specific antigen in tissue (cell) cultures has been investigated with the immunofluorescence technique using human auto-immune antibodies to an organ-specific microsomal antigen[5,8], but there are no conclusive reports of investigations on this antigen in organ cultures of human thyroid glands.

In this article we report short-term maintenance of hyperplastic human thyroid gland tissue *in vitro* in organ culture. The persistence of the human thyroid organ-specific antigen in organ cultures and disaggregated tissue (cell) cultures of the same glands was compared in a concurrent investigation.

The thyroid glands were obtained at operation for thyrotoxicosis on seven patients (one male and six female, age range 23–50 years). In all patients, pre-operative diagnosis was made on clinical assessment and this was confirmed with studies of iodine-131 uptake in five. Pre-operative medication (methyl-thiouracil or carbimazole, followed by iodine) had been given to all patients. The diagnosis was confirmed by histological examination in all cases. Approximately 12 g of tissue was required for each experiment, 2 g for organ culture and 10 g for tissue culture.

For organ culture, a modification of the method described by Hoorn and Tyrell[9] was used. The base of a sterile 2 in. diameter plastic Petri dish ('Nunclon', Sterilin, Ltd., Richmond, Surrey) was scored by means of a scalpel blade. Ten to twenty explants of thyroid gland, each measuring approximately 4 mm^3, were placed in each dish and 1·2 ml. of chemically defined culture medium ('T.C. 199', Glaxo) was added. The culture fluid was changed every 3 days in the early experiments and daily in the later experiments. Usually ten to twelve Petri dishes were prepared from each thyroid gland. The preparations were incubated at 37° C in a mixture of 5 per cent carbon dioxide in 95 per cent air. The explants were usually investigated as frozen sections, but the contents of occasional Petri dishes were fixed in 4 per cent neutral buffered formaldehyde in saline, and paraffin sections were stained with haematoxylin and eosin. Substantial portions of the explants were considered to have survived, as judged by conventional histological criteria, until about 7 days *in vitro*, as the acinar epithelium appeared intact (Fig. 1)—although necrotic changes were often seen in the stroma of the explants. By 14 days, many explants were necrotic and others exhibited only small foci of survival. The colloid persisted even in necrotic explants.

The sera to be used for immunofluorescence investigation of organ culture explants were selected with an immunofluorescence test using unfixed frozen sections of uncultured human thyroid gland. Five sera containing strong anticytoplasmic antibody (from patients with thyroid disease) and five sera which did not contain antithyroid antibodies (from patients with other diseases) were used in the investigation. Fresh frozen sections were prepared from thyroid organ culture explants frozen on blocks of human or rat liver as the thyroid microsomal antigen is not damaged by this procedure[10,11]. The sections (5μ) were stained with the indirect immunofluorescence method using positive and negative sera with the methods described previously[12]. The appearances of the histologically preserved thyroid tissue in explants stained with anticytoplasmic sera were closely similar to the well-known pattern seen in sections of uncultured thyroid gland[10]. The cytoplasm of surviving thyroid epithelium was brightly stained with some accentuation around the nuclei which were completely unstained (Fig. 2), but there was no specific staining in necrotic epithelium. The stroma was always unstained. The colloid was stained if the

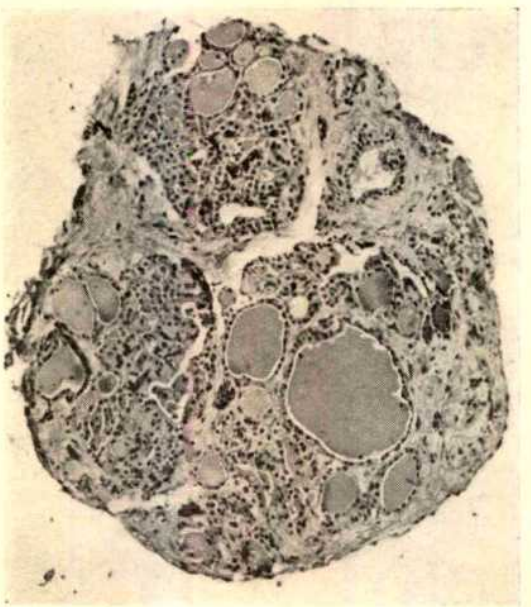


Fig. 1. Human hyperplastic thyroid explant maintained in organ culture for 7 days. (× 100) (stained with haematoxylin and eosin)

serum also contained anticolloid antibody. When sera without anticytoplasmic antibodies were used, faint diffuse non-specific staining was seen throughout the explant (Fig. 3).

The sections were examined independently by both of us, without prior knowledge of the serum used, and the organ-specific antigen was considered to be present when a clear distinction could be made between sections stained by positive and negative sera. The quantities of tissue available did not permit daily sampling of organ cultures, and Table 1 shows the last day on which antigen could be detected and does not represent a negative end-point.

Table 1. PERSISTENCE OF THYROID ORGAN-SPECIFIC ANTIGEN *in vitro*

Experiment No.	Tissue culture: Last day antigen detected	Tissue culture: First day antigen not detected	Organ culture: Last day antigen detected
1	3	4	8
2	2	3	14
3	1	5	7
4	3	5	9
5	3	4	10
6	3	5	8
7	1	3	14

In a few cases, the distribution of immunofluorescence staining in sections of explants maintained *in vitro* for periods of 5, 7, 11 and 14 days was photographed monochromatically and in colour. The appearances were then compared with those seen after haematoxylin and eosin staining of the same or of an adjacent cryostat section. Cells with positive cytoplasmic fluorescence always showed histological survival with preservation of cellular and nuclear detail and no group of cells was identified where convincing histological survival was not accompanied by positive immunofluorescence staining.

The following experiments were undertaken to demonstrate that the immunofluorescence staining was immunologically specific. Anticytoplasmic sera were treated with equal volumes of absorbants—protein solutions or microsomes suspended in an equal volume of saline—for 2 h at 37° C followed by 24 h at 4° C before being used to stain 5-day explants. Absorption with fresh human hyperplastic thyroid gland microsomes prevented staining, whereas absorption with microsomes prepared from other fresh human tissues (gastric mucosa, duodenal mucosa, kidney and prostate) or with purified human thyroglobulin (38 mg/ml.) or serum albumin (50 mg/ml.) did not significantly reduce staining intensity. Two strong anticytoplasmic sera were fractionated by chromatography on 'DEAE Sephadex (*A*50)' and various fractions were used separately as first stage in the immunofluorescence staining of organ-culture sections; bright staining was seen with the IgG (7·0*S* γ-globulin) fractions but not with other serum fractions. The specificity of the second stage of the immunofluorescence reaction was also investigated. An immunoglobulin was involved in this stage as the fluorescein conjugate had been prepared from the IgG fraction of an immune goat anti-human IgG serum. Absorption of the conjugate with human IgG (50 mg/ml.) removed its staining capacity—whereas absorption with rabbit IgG or human serum albumin (50 mg/ml.) did not alter its properties. Fluorescein-conjugated rabbit anti-human serum albumin and fluorescein-conjugated goat anti-rabbit IgG sera gave negative results. On the basis of these findings, and on the reproducibility of the indirect immunofluorescence staining method with anticytoplasmic sera and constant absence of staining with negative sera, we concluded that immunofluorescence staining was immunologically specific for demonstration of the organ-specific thyroid cytoplasmic antigen.

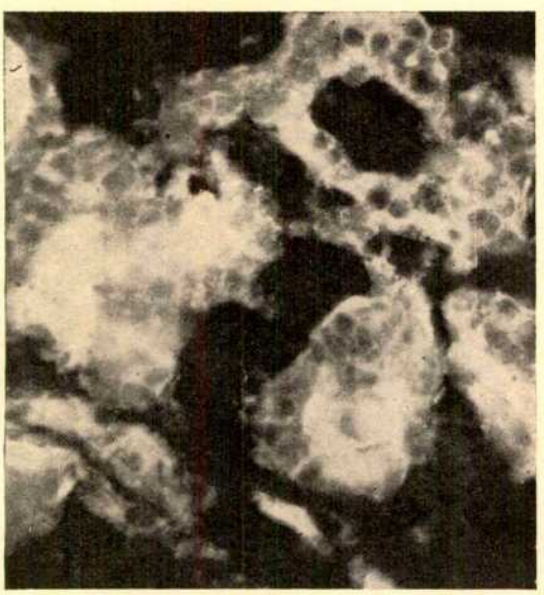


Fig. 2. Immunofluorescent staining of cytoplasm of epithelial cells of explant maintained in organ culture for 4 days. (× 400) (Anti-cytoplasmic antibody)



Fig. 3. Absence of immunofluorescent staining of culture shown in Fig. 2. (× 400) (Normal human serum)

The histological appearances suggested that explants were surviving *in vitro*, while the lack of fluorescence in necrotic areas of explants seemed to indicate that cytoplasmic antigen did not persist after cell death. To investigate the possibility of antigen persistence in necrotic tissue further, 1·0 cm (approximately) cubes of thyroid gland were well moistened with culture medium and incubated in the gas mixture. Histological examination of the tissue masses showed central necrosis from 24 h onwards and autolysis was almost complete by 3 days. Indirect immunofluorescence staining of frozen sections showed that the organ-specific cytoplasmic antigen was lost from this tissue 24 h after removal of the gland from the patient.

Concurrently with the organ culture experiments, tissue (cell) cultures of the thyroid gland were set up, the method differing little from that of Pulvertaft *et al.*[13]. Our haematoxylin and eosin-stained preparations showed partial monolayers of cells identical with those described previously. In previous immunofluorescence investigations on tissue culture preparations, it was found that organ-specific cytoplasmic staining could not be obtained on intact cells[4]. Attempts have been made to disrupt the cell membrane by air-drying the monolayers[5] or by brief fixation with acetone[6], but these methods have serious

theoretical disadvantages in view of the lability of the thyroid organ-specific microsomal antigen[11]. Accordingly, we developed a simple freezing technique to allow penetration of antibodies into the cells; coverslips were washed with gentle agitation in phosphate-buffered saline, dried by blotting the edge on filter paper and snap-frozen by laying the underside of the coverslip on a block of carbon dioxide snow for about 30 sec. Batches of coverslips (usually twelve) were stained after 14, 24, 36, 48, 72, 96 and 120 h in tissue culture with the methods used for organ culture sections.

Immunofluorescence staining was attempted after 7 h in culture, but very few cells remained adherent to the coverslips by the end of the staining process. From 12 to 14 h onwards more cells adhered to the coverslips and immunofluorescence assessment was possible. After 24 h in culture, antigen was seen throughout the cytoplasm in the distribution reported previously[8], but after longer periods in culture (usually about 36 h) it was only seen in a narrow zone around the nucleus. There was no specific staining with normal human sera. The longest duration *in vitro* at which definite cytoplasmic staining was still evident in cells stained by sera containing anticytoplasmic antibody is shown in Table 1. The antigen was considered to have disappeared when we could not detect any difference between technically satisfactory cell preparations stained with anticytoplasmic and normal sera. In experiments 3, 4, 6 and 7 there was an interval between these times because the tissue-culture preparations examined on the intervening days were technically imperfect and could not be assessed.

The times of persistence of the thyroid organ-specific antigen in tissue culture and organ culture systems are compared in Table 1. In the organ cultures, the antigen was demonstrated in all viable cells and was never seen in necrotic cells whereas the antigen was lost from tissue culture cells while they were still apparently viable on histological examination. Furthermore, the antigen was demonstrated after longer periods in organ culture than in tissue culture. There have been no previous reports of investigations of the persistence of the antigen in organ cultures but our findings in tissue cultures confirm the results reported by others[5,8] who used the same method. Slightly longer persistence (less than 1 week) was reported[14] with a different tissue culture technique but even this was shorter than our experience with the organ culture technique. In a biochemical investigation, Hagmuller and Leslie[6] have presented evidence that organ-cultured human thyroid tissue can fulfil the specific metabolic functions of thyroid gland more efficiently than dissociated thyroid cells. Our finding of longer persistence of the organ-specific antigen in organ cultures commends further the use of this technique for metabolic investigations on thyroid tissue *in vitro*.

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PROPERTIES AND STABILIZATION OF THE LYSOSOMAL MEMBRANE

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ALLISON, Magnus and Young[1] have recently studied the role of lysosomes in the photosensitization of cells, and from these results Allison[2] has presented a unifying hypothesis, implicating lysosomes in carcinogenesis. He suggests that radiation, oncogenic viruses and carcinogenic chemicals have a common action in causing damage to the lysosomal membrane, so releasing the lysosomal enzymes which in turn attack the genetic material of the cell. If the DNase enzymes can operate without impairing the capacity of the cells to divide, they could produce genetic changes (probably deletions) resulting in the somatic mutations which lead to cancerous growth.

If such a hypothesis is correct, the properties and stabilization of the lysosomal membrane are of considerable interest. The available evidence suggests that the membranes bounding lysosomes resemble other lipoprotein unit membranes; many of the agents that disrupt lysosomes also labilize the membranes of erythrocytes and mitochondria[3]. This article emphasizes the similarities between the bounding membrane of lysosomes, the mitochondrial membrane and the plasma membrane of nerves by reference to their specialized properties, and suggests the molecular properties of the lysosomal membrane that are responsible for the maintenance of its integrity.

A hypothesis has been developed elsewhere[4-6], together with supporting evidence[7], that the cation-permeability of the membrane of sense organs and other input components of excitable cells is governed by an ATPase mechano-enzyme system sited in the membrane which is able to change its molecular configuration as a function of ADP–ATP balance. Changes in effective pore diameter and/or position of charged groups would produce a modification of selective cation-permeability. This system corresponds with that shown to be present in the mitochondrial membrane[8], where physical changes (probably of shape or symmetry[9]) are produced reversibly and rapidly by normal reactants of the respiratory chain[10]. It is not the ATPase of the active cation pump. A protein with actomyosin-like properties has been isolated from mitochondria; it underwent conformational changes (as shown by light-scattering and viscosity-change experiments) with ATP and showed ATPase activity[11,12]. Subsequent investigations[13,14] have emphasized the close similarity between this protein and the actomyosin system of muscle. More recently, an actomyosin-like ATPase has been identified in the plasma membrane of the nerve cells from crayfish nerve cord[15] and rat brain[16]. The presence of such a system would then account for the changes in axon length[17] and in the light-scattering properties of the axon membrane[18] which follow nerve activity. Its enzymatic activity would also produce (in part) the changes in liberation of inorganic phosphate which follow nerve activity[19].

Agents that normally sensitize lysosomes (such as anthracene, euchrysine) can produce photodynamic damage to cell membranes[1]. Allison *et al.* describe two

major types of photosensitization. Some dyes (rose bengal) remain at the cell surface; absorption of light of appropriate wave-length causes immediate photo-oxidative damage to the cell membrane with loss of potassium ions. Other substances (neutral red, anthracene) are concentrated in the lysosomes, and light absorption causes escape of lysomal enzymes and cell damage. The presence of oxygen is necessary for photodynamic damage, but photons of these wave-lengths are harmless if no photosensitizers are present. A parallel situation is found when the *Sepia* axon is stained with neutral red; the membrane immediately begins to depolarize (that is, sodium permeability increases) when locally illuminated, again only in the presence of oxygen[20]. During this time, uncoordinated miniature potentials are superimposed which may reveal the quasiquantal recruitment of multiple active foci or patches in the illuminated area of the membrane[20]. As depolarization proceeds, the miniature potentials synchronize into regular oscillations of increasing amplitude, eventually initiating spikes. The maximum amplitude and maximum rate of development of this potential are both dependent on the light intensity. The lysosomal and excitable membranes are therefore both sensitive to this form of photodynamic increase in permeability. Blue-violet light also rapidly immobilizes spermatozoa after the addition of euchrysine. If a 10μ beam of light is directed on the tip of the tail, only that part of the tail is immobilized[1]. The light would presumably be acting on the ATPase of the contractile machinery[21,22].

Although a number of agents labilize the lysosomal membrane, far fewer have been described with a stabilizing action. Antihistaminics (such as benadryl) are an example of a stabilizer[3,23], although at dosages too toxic to be of clinical use at present[2]. Antihistaminics (benadryl, histadyl) also act as stabilizers for nerves[24], that is, they produce little change in membrane potential, but markedly decrease depolarizing changes in the membrane potential brought about by alteration of the potassium ion concentration and other experimental means. These agents are therefore able to resist changes in membrane permeability and to block conduction in frog sciatic nerves[25,26]. Similarly, antihistaminics inhibit the configurational changes in the mitochondrial membrane, producing stabilization[27–29].

There is evidence[6,7] that the ATPase enzyme complex of the excitable membrane is associated with a non-specific cholinesterase component. It is probable that in lysosomes, in mitochondria and in the excitable cell membrane, antihistaminics stabilize and inhibit phosphoprotein turnover[30] by combining with this cholinesterase component; antihistaminics can produce cholinergic block and can correct certain effects of the anticholinesterase DFP, in addition to their action on the mammalian central nervous system[31]. In any event, histamine is a powerful dilator of capillaries; the action is a direct one on the contractile mechanism, the response being independent of innervation. After large doses, the capillaries dilate to such a degree that their permeability is altered. Antihistaminics antagonize this action by straight competition with histamine for the receptor sites[31].

Allison[32] has drawn my attention to other properties of lysosomes. Granules, apparently identical with lysosomes, have been shown to undergo relatively rapid displacements over dimensions that are outside the range of Brownian movement[33], an observation which accords with the presence of a contractile protein.

Metal ions (such as zinc, cadmium and iron) are carcinogenic, again probably operating by way of the lysosomes[32]. These same ions are also potent in accelerating the breakdown of amine granules isolated from the adrenal medulla[34], with a consequent liberation of catecholamines. These amine granules are also dependent on an ATPase system[7,34,35] and it is probable that synaptic transmitter release, catecholamine granules and lysosomes have many features in common[7].

It is therefore suggested that the integrity of the lysosome membrane is governed and maintained by an ATPase mechano-enzyme complex. The effect of many of the labilizing agents on the lysosomal membrane[3] (some with carcinogenic activity) can be explained in terms of a direct or indirect action on an ATPase system[7]. Examples of such agents are phospholipase (destroys ATPase activity[36]), DOC (see refs. 37, 38 and 39), 2,4-dinitrophenol (action on mitochondrial ATPase[40]; modifies membrane permeability[7]), anoxia (depolarizing action on the nerve membrane[24]) and thyrotropin (see refs. 7 and 8).

If the theory implicating lysosomes in carcinogenesis should prove correct, the molecular properties of the lysosome membrane and the means by which it could be stabilized would be of importance. By analogy with the systems present in the mitochondrial and excitable cell membranes, the enzyme activity of the ATPase complex of the lysosomal membrane depends on the integrity of a phospholipid component[36], it is not inhibited by cardiac glycosides, its sensitivity to cations is known and it depends on an active sulphydryl group[41]. Among suitable stabilizing agents (although not necessarily of clinical value), factors influencing ATP supply could have an indirect effect and other possible candidates could be the many stabilizing agents discovered for the excitable cell membrane, such as procaine, calcium ions, physostigmine, sulphanilamide, thiophene-2-sulphonamide and Diamox.

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Escherichia coli $B_{s-1}\gamma R$: A STRAIN RESISTANT TO X-RAYS AND SENSITIVE TO ULTRA-VIOLET LIGHT

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INJURIES caused by X-ray and ultra-violet irradiation are partially reversible. Although the damage caused by the two effects differs in nature (dimers of pyrimidine bases in DNA are induced only by ultra-violet light but never by X-rays)[1,2], dark repair after both forms of injury requires similar conditions[3].

Many examples can be given to demonstrate a parallelism of sensitivity to ultra-violet and X-ray irradiation and recovery after injuries of both kind in bacteria: (1) the cross-resistance to ultra-violet light and X-rays found in various mutants of *E. coli*[4]; in strains of *E. coli* grown, before irradiation, in a glucose media[5], or obtained as a result of treatment with camphor vapour[6]; in various strains of *Bacillus subtilis*[7]; (2) the fact that treatment of radio-resistant bacteria with acriflavine inhibits their recovery and increases their sensitivity to both kinds of irradiation[3]; (3) the displacement of thymine in DNA by 5-bromouracil makes bacteria more sensitive to ultra-violet and X-ray irradiation[8,9]; (4) the transfer of increased X-ray resistance from a radio-resistant donor to a sensitive recipient on conjugation is accompanied by a transfer of ultra-violet resistance[10]; (5) the finding that bacteria cells revive phages irradiated by both ultra-violet and X-rays, although in the latter case this recovery is less strongly pronounced[11,12]; (6) that caffeine, which is a recovery inhibitor, represses a host cell reactivation at both kinds of irradiation[12,13].

Until now, however, there has been no direct evidence as to whether the recovery of ultra-violet and X-ray injuries is produced by identical or different mechanisms. The possibility of obtaining mutants capable of recovering only from one kind of injury seemed to offer an approach to solving this problem. This article discusses our work with the mutant *E. coli* $B_{s-1}\gamma R$, which is resistant to X-ray irradiation yet sensitive to ultra-violet light.

Bacteria of the strain *E. coli* B_{s-1} were cultivated during continuous cobalt-60 irradiation (2,800 r. per 24 h) for 3 months in Petri dishes at 37° C in a nutrient medium containing eosin-methylene blue. The transfer of bacteria was repeated daily (Sunday excluded). The bacteria obtained are designated as *E. coli* $B_{s-1}\gamma R$. The sensitivity of bacteria to ultra-violet light, X- and α-rays was checked periodically and compared with the radiosensitivity of the parent strain B_{s-1} and radio-resistant mutant B/r. Bacteria were grown for 18 h at 37° C on nutrient agar under γ-irradiation and after 10 passages without γ-irradiation. Ultra-violet irradiation of bacteria suspended in water was performed in Petri dishes on mixing. A *BUV*-30 lamp was used as a source of ultra-violet light (2537 Å; dose-rate—120 ergs/mm²/min). The sensitivity to ultra-violet light in a great number of subcultures of *E. coli* $B_{s-1}\gamma R$ was determined according to Greenberg's method[14]. The technique of X- and α-ray irradiation was described by Paribok *et al.*[15]. The host cell reactivation of ultra-violet irradiated phages *T*1 was studied in the following way: the phage *T*1 was irradiated in buffer. The diluted phage was preliminarily adsorbed on bacteria of the strains *B*, B_{s-1} and $B_{s-1}\gamma R$ (multiplicity was less than 1 : 100) and then seeded by the agar layer method. Negative colonies were calculated on the following day.

Fig. 1 shows the change in sensitivity ($L.D._{90}$) to X-rays and ultra-violet light exhibited by the strain $B_{s-1}\gamma R$ when grown under γ-irradiation. Survival curves for ultra-violet, X- and α-irradiation are given in Figs. 2 and 3. The changes in the resistance of *E. coli* $B_{s-1}\gamma R$ to ultra-violet light and X-rays were not identical.

X-ray resistance increases and after 2·5–3 weeks of cultivation under γ-irradiation attains a level of the strain B/r (Figs. 1 and 2), differing from the latter only by the factor 1·14 (factor of differences between the strains B/r and parent B_{s-1}–3·0). The results of two experimental series performed at 20-day intervals proved to be identical. Changes in the ultra-violet sensitivity of *E. coli* $B_{s-1}\gamma R$ cultivated over a period of 3 months under γ-irradiation are insignificant. $L.D._{90}$ was increased from 4 ergs/mm² (parent strain B_{s-1}) to 6 ergs/mm² (*E. coli* $B_{s-1}\gamma R$). The strains B/r and $B_{s-1}\gamma R$ differ by a factor of 52, whereas differences between the strain B/r and parent culture B_{s-1} are characterized by a factor 77 (see Figs. 1 and 2). The properties of *E. coli* $B_{s-1}\gamma R$ are inherited. An increased resistance to X-rays on insignificant changes in ultra-violet sensitivity is retained after ten passages without γ-irradiation. The sensitivity to ultra-violet light has been investigated in 700 substrains of *E. coli* $B_{s-1}\gamma R$ with inherited properties. The technique of double ultra-violet irradiation on nutrient agar[14] applied in this case made it possible to discern radio-resistant (confluent growth) and radiosensitive organisms (which either do or do not form single colonies). It was found that among 700 substrains there was not one which was more resistant to ultra-violet light than a parent culture *E. coli* $B_{s-1}\gamma R$. The strain *E. coli* $B_{s-1}\gamma R$ as well as the initial strain B_{s-1} does not enhance the recovery of ultra-violet irradiated bacteriophage *T*1 (Fig. 4).

The sensitivity of *E. coli* $B_{s-1}\gamma R$ to α-particles is almost identical to that of the strains B_{s-1} and B/r (factor 1·2) (Fig. 3). It has been shown that after α-irradiation (in the presence of oxygen during irradiation) no recovery of irradiation injuries occurs with *E. coli*[15].

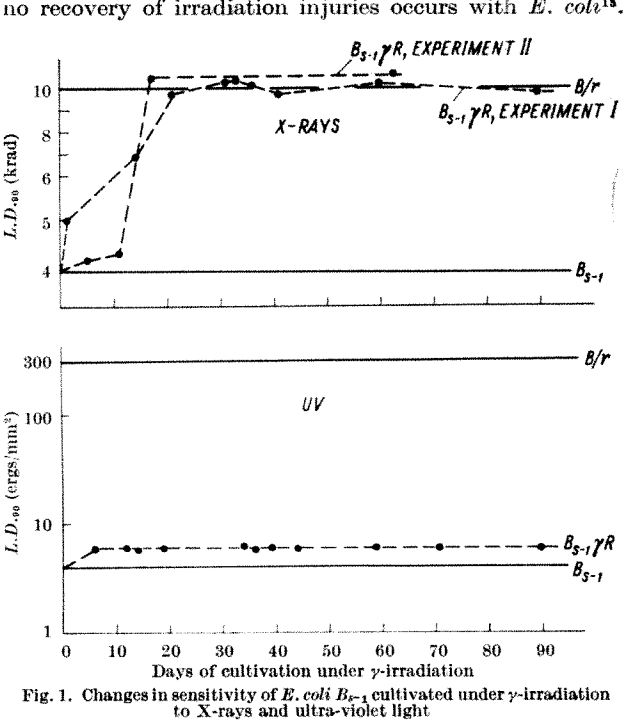


Fig. 1. Changes in sensitivity of *E. coli* B_{s-1} cultivated under γ-irradiation to X-rays and ultra-violet light

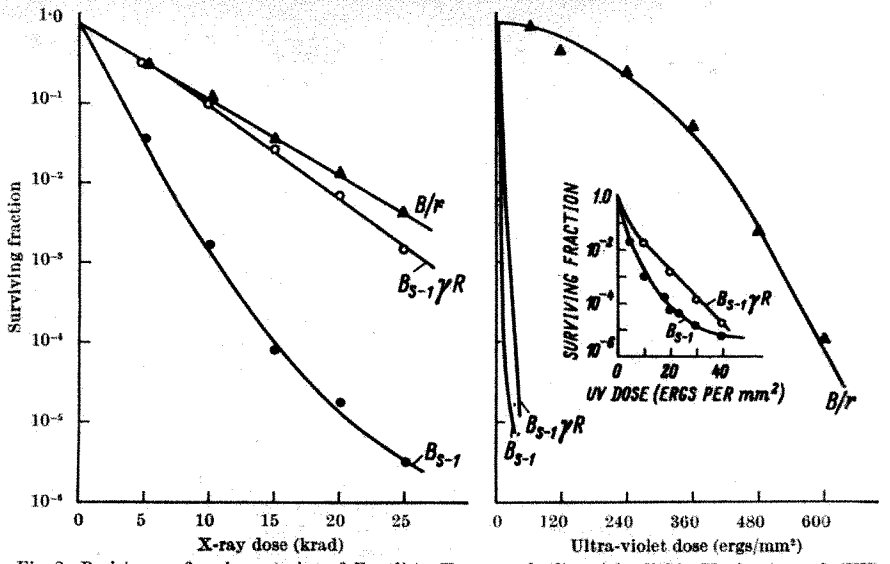


Fig. 2. Resistance of various strains of *E. coli* to X-rays and ultra-violet light. The inset graph (UV) shows the low dose portion of the survival curves of strains B_{s-1} and $B_{s-1}\gamma R$

Significant differences in the sensitivity of different strains of *E. coli* to ultra-violet light (factor 77) and X-rays (factor 3·4) in the presence of a similar sensitivity of the same strains to α-irradiation (factor 1·33) indicates that a high resistance to ultra-violet light and X-rays is due to post-irradiation recovery in radio-resistant variants of *E. coli*[16]. Hence this high resistance of *E. coli* $B_{s-1}\gamma R$ to X-rays demonstrates the capability of a mutant to repair injuries induced by X-ray irradiation. The ultra-violet sensitivity of the mutant $B_{s-1}\gamma R$ differs insignificantly from that of the strain B_{s-1}, and does not revive the ultra-violet-irradiated phages *T*1. Thus this mutant, as well as the initial strain B_{s-1}, does not make good injuries induced by ultra-violet light. This capacity of one strain to repair X-ray injuries coupled with its inability to revive ultra-violet damage shows that the recovery mechanisms of these injuries must at least differ in some respects.

The results obtained can be used to explain the following facts: (*a*) the character of the dose curves obtained with pigmentless strains of *Micrococcus radiodurans* after X-ray irradiation varies, yet this is not so in the case of ultra-violet irradiation[17]; (*b*) *Paramecia* isolated from radio-active natural sources have proved to be more resistant

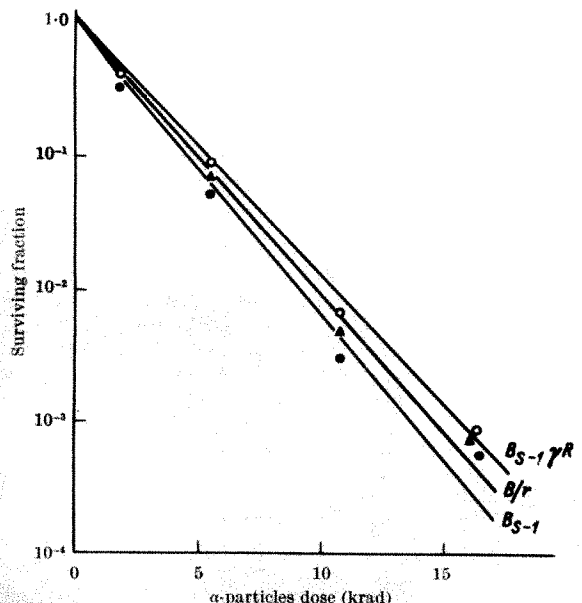


Fig. 3. Resistance of various strains of *E. coli* to α-particles

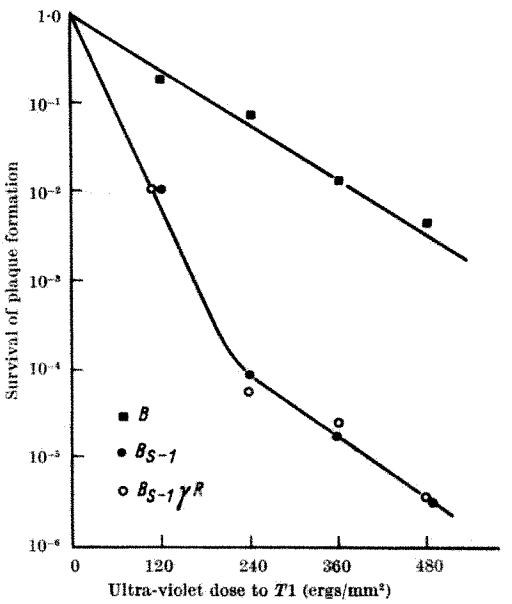


Fig. 4. Survival of bacteriophage *T*1 after ultra-violet irradiation as determined in bacteria strains *B*, B_{s-1} and $B_{s-1}\gamma R$ as hosts

to X-rays, yet have retained their increased sensitivity to ultra-violet light[18,19].

I thank E. A. Valdstein for her assistance in experiments on host cell reactivation of phages.

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ADAPTATION OF MEASLES VIRUS TO THE CHORIO-ALLANTOIC MEMBRANE OF CHICKEN EMBRYOS *in ovo*

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THE development of measles vaccines[1,2] was made possible by Enders and his associates, who propagated measles virus in human and simian kdiney cultures[3], adapted the Edmonston strain of the virus to chicken embryos inoculated by way of the amniotic sac[4], and cultivated that strain in monolayers of chicken embryo cells[5]. Within the past 2 years, vaccines incorporating either live or inactivated measles virus have become generally available.

Live-virus vaccines prepared from the Edmonston strain after thirteen to twenty passages in cultures of chicken embryo tissue proved to be highly immunogenic when administered to measles-susceptible children. However, as many as 40 per cent of these children reacted with temperatures of 103° F or higher, and about 50 per cent developed a rash[6]. In attempts to eliminate these reactions, human gamma globulin was administered at the same time as the vaccine[6-8], or the vaccine virus was further attenuated by additional passages in cultures of chicken embryo tissue at various incubation temperatures[9-10]. Both these approaches to the problem significantly reduced the incidence of reactions to measles vaccination, but did not entirely eliminate them[11].

This laboratory attempted to eliminate the reactions by further attenuating the virus in a different way, by adapting it to the chorio-allantoic membrane (CAM) of the chicken embryo. CAM adaptation of measles virus had already been unsuccessfully attempted by other investigators[4], but nevertheless seemed possible because another myxovirus, that of canine distemper, which is taxonomically proximal to measles virus, had been adapted in this way. Distemper virus adapted to the chicken embryo when passed by way of the CAM produced characteristic inflammatory and necrotic lesions on the CAM, and after further CAM passages became completely attenuated for its naturally susceptible hosts[12,13].

This article reports the adaptation of the Edmonston strain of measles virus to growth on the chicken embryo CAM. Serial CAM-passage eventually produced inflammatory and necrotic lesions on measles-infected CAMs which resembled those produced by canine distemper virus.

The measles virus used for CAM adaptation was derived from Enders vaccine *B* (ref. 1), and was received here at the thirteenth level of passage in chicken embryo tissue culture. Before CAM adaptation was begun, the virus was serially passed ten additional times in monolayers of chicken embryo cells. The first three of these passages were incubated at 37° C, the other seven at 32° C. The cells used for the first five of these passages were of unknown RIF status. The embryo cells used for the other five passages came from an RIF-free flock of chickens developed here from a commercial flock intensively tested for RIF[14]. The fifth and seventh passage levels of the virus were treated with a chicken RIF antiserum for 45 min at 37° C before further passage. The level with which CAM adaptation was initiated (the tenth passage in this laboratory, or the twenty-third passage altogether) was shown to be free of RIF and had a measles virus titre of $10^{4.5}$ *T.C.I.D.*$_{50}$ per ml. in cultures of an established line of cercopithecus monkey kidney cells (*BS-C*).

The *BS-C* cultures used for titrating measles virus were grown in stationary tubes in a medium consisting of Earle's balanced salt solution, 0·1 per cent lactalbumin hydrolysate and 2 per cent horse serum, and after 2 days were renewed with a medium consisting of Medium 199, without glutamine, containing 1 per cent horse serum. To titrate the virus in either a 20 per cent suspension of infected CAM or tissue culture fluid, tenfold dilutions were prepared in Hanks's balanced salt solution, and each culture tube was inoculated with 0·1 ml. of a virus dilution. After 4–5 days' incubation at 37° C, the medium in the tubes was renewed. On the seventh day, end points were calculated by the formula of Reed and Muench[15].

Neutralization by a measles antiserum prepared by hyperimmunizing horses with measles virus that had been passed six times in HeLa cells was used to identify the virus. This antiserum had a serum neutralizing end point against approximately 100 *T.C.I.D.*$_{50}$ of measles virus of the order of 1 : 4,000. Neutralization of th[illegible] was tested either in *BS-C* cultures or on the CA[illegible] chicken embryos.

For CAM passage of the virus, 8-day-old embr[illegible] the aforementioned RIF-free flock were inoculate[illegible] at the base of an artificial air sac formed on the side of the egg. Inoculated eggs were incubated for 7 days, at the various temperatures specified later, and on the seventh day were opened aseptically. CAMs were examined for macroscopic changes, and infected portions were removed, pooled and used to prepare a 20 per cent CAM suspension in sterile distilled water containing 100 μg each of streptomycin and neomycin. Until the twenty-fifth serial CAM passage, each embryo was inoculated with 0·5 ml. of infected CAM suspension. The inoculum in subsequent passages was 0·2 ml.

To subculture CAM-adapted virus, Blake or Povitsky bottles containing cell monolayers were prepared from 9-day-old RIF-free chicken embryos, overlaid with a medium consisting of Earle's solution, 0·5 per cent lactalbumin hydrolysate, 10 per cent calf serum and 100 μg each of streptomycin and neomycin, and incubated for 24 h at 27° C. The monolayers were then renewed with the same medium as used for growth except that the calf serum was omitted, infected with a 20 per cent suspension of infected CAM by inoculation in the proportion of 5·0 ml. to each 300 ml. of overlaying medium, incubated for 7 to 10 days at 34° C and gathered for virus by pooling fluids and cells.

The virus was followed in several lines of CAM passages, at various incubation temperatures, but only the line giving continuous virus propagation is described in detail here. The first eleven passages of this line were incubated at 37°–38° C, and at all passage levels tested except the seventh, the virus titre was within the range of $10^{2.5}$–$10^{4.0}$ *T.C.I.D.*$_{50}$ per ml. of 20 per cent infected CAM suspension (Table 1). Subsequent passages of this line were incubated at 34° C, and at all but the twenty-first passage level the virus titre was within the range of $10^{2.5}$–$10^{5.5}$ *T.C.I.D.*$_{50}$ per ml. (Table 2). It cannot be ascertained whether the titres of less than $10^{1.6}$ obtained for both the seventh and the twenty-first passage levels of the virus were actual or whether they reflected virus inactivation as a result of manipulation, but in both cases the titre of the virus at the next passage level was within the expected range.

No significant change was visible macroscopically in the infected CAMs until the fifteenth passage, when transparent areas of swelling, gelatinous in appearance, could be seen. By the eighteenth passage, infected portions

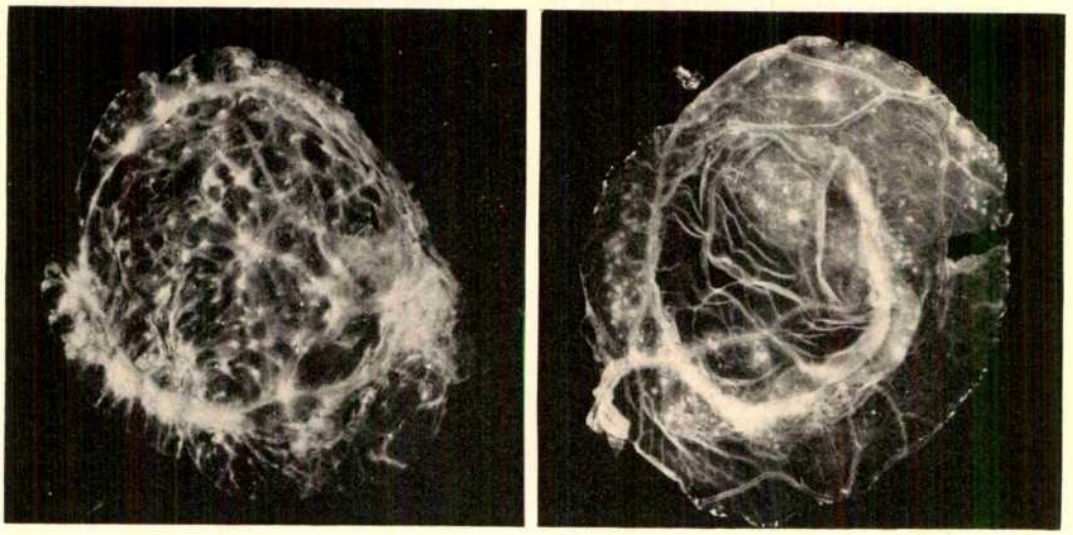


Fig. 1. Left, CAM-adapted canine distemper virus (Cabasso strain). Right, CAM-adapted measles virus (Edmonston strain). Note ring-like formation of inflammatory and necrotic lesions and discrete plaque-like lesions on both chorio-allantoic membranes

of the CAMs contained frank inflammatory and necrotic [redacted] which became more pronounced with continued [redacted] of the virus. The lesions appeared as large, [redacted]d opaque areas, most often in the form of a ring [redacted]e of contact between shell membrane and CAM, [redacted]ialler, discrete plaque-like lesions both inside and outside this ring. These lesions were macroscopically indistinguishable from CAM lesions produced by chicken-embryo-adapted canine distemper virus (Fig. 1), and histological examination of CAMs infected with measles virus or distemper virus substantiated the similarity of the lesions. Both viruses produced focal epithelial plaques that consisted of proliferating epithelial cells with oedema, degeneration and sometimes vacuolization. There were also marked changes in the underlying stroma, with many neocapillaries and fibroplasia, and in addition a few cytoplasmic Shorr-positive inclusions.

Table 1. ADAPTATION OF MEASLES VIRUS TO THE CHORIO-ALLANTOIC MEMBRANE (CAM): INFECTIVITY TITRE OF VIRUS AT PASSAGES INCUBATED AT 37°–38° C

CAM serial passage No.	Titre (log. base 10) ($T.C.I.D._{50}$/ml. of 20 per cent infected CAM suspension)
1	Not tested
2	⩾2·5
3	3·0
4	Not tested
5	4·0
6	2·5
7	< 1·0
8	2·5
9	
10	Not tested
11	

Table 2. ADAPTATION OF MEASLES VIRUS TO THE CHORIO-ALLANTOIC MEMBRANE (CAM): INFECTIVITY TITRE OF VIRUS AT PASSAGES INCUBATED AT 34° C

CAM serial passage No.	Titre (log. base 10) ($T.C.I.D._{50}$/ml. of 20 per cent infected CAM-suspension)	CAM changes evident	Identified by measles-antiserum neutralization	Subcultured in chicken embryo monolayers ($T.C.I.D._{50}$/ml.)
12	5·5			
13	4·5			
14	3·5			
15	4·0	Areas of "gelatinous" swelling	X	
16	4·0			
17	5·1			
18	5·5			5·0
19	5·0		X	
20	4·0			
21	< 1·0			
22	4·0			
23	4·5	Inflammatory lesions		
24	4·5			
25	5·0			
26	4·5			
27	5·0			
28	4·0		X	5·0
29	4·5			5·5
30	5·0			4·9

Changes in incubation temperature seemed at times to interfere with the CAM propagation of measles virus. For example, when virus representing the fifth CAM passage at 37°–38° C was passed and the infected embryos were incubated at the same temperature, the titre of the virus was $10^{2\cdot5}$ $T.C.I.D._{50}$ per ml. of 20 per cent CAM suspension, but when infected embryos in which the fifth-passage virus was subcultured were incubated at 35° C, no virus was detectable by titration. Similarly, when virus had been successfully passed ten times at 34° C and was then passed five times at 37° C, virus was detectable only after the first of these latter passages.

CAM-adapted virus was identified by measles-antiserum neutralization at the fifteenth, nineteenth and twenty-eighth passage levels (Table 2). All three passage levels were completely neutralized when mixed with antiserum in *BS-C* cultures. The antiserum also suppressed CAM lesions produced by nineteenth passage virus in the presence of normal serum, and neutralized the infected fluid from subcultures of twenty-first passage virus in chicken embryo cells. Antiserum to respiratory syncytial virus, which was used as a control, did not neutralize fifteenth passage virus. The development of measles antibody by three rhesus monkeys that had no antibody before their intramuscular inoculation with harvest from chicken-embryo tissue subcultures of the thirtieth CAM passage of the virus served further to identify it as measles virus. At thirty-five and fifty-one days after inoculation with 0·5 ml. of virus (50,000 $T.C.I.D._{50}$), each monkey had measles haemagglutination-inhibiting antibody within the titre range of 1 : 64–1 : 128. In contrast, three rhesus monkey controls kept in the same cages as the inoculated monkeys for 51 days after their inoculation remained free of measles antibody. This finding also indicated that the CAM-propagated virus had not regained the ability of measles virus to spread among monkeys kept in close contact.

Four passage levels of the CAM-adapted virus (the eighteenth, twenty-eighth, twenty-ninth and thirtieth) were subcultured in monolayers of chicken embryo cells (Table 2). No cytopathic effect was evident in any of these subcultures, but they all had infective titres within the range of $10^{4\cdot9}$–$10^{5\cdot5}$ $T.C.I.D._{50}$ per ml. of pooled infected fluid and cells after 7–10 days of incubation.

In summary, after propagation in live chicken embryos inoculated by way of the amniotic sac for six serial passages, and in cultures of chicken embryo tissue for twenty-three passages, the Edmonston strain of measles virus was adapted to growth on the chorio-allantoic

membrane (CAM) of 8-day-old chicken embryos free of RIF and was propagated through thirty serial CAM passages. The incubation temperature was 37°–38° C for the first eleven CAM passages and 34° C for subsequent passages. The CAM-propagated virus was identified as measles at the fifteenth, nineteenth and twenty-eighth CAM passage levels, and was also identified after subculture of the twenty-eighth passage virus in chicken embryo cell monolayers. Inflammatory and necrotic lesions began to appear on the infected portions of CAMs at the level of the eighteenth CAM passage of the virus and increased in intensity as passage continued. The taxonomical proximity of the viruses of measles and canine distemper gains support from the similarity of the CAM lesions they produce when adapted to the chicken embryo. Canine distemper virus became completely attenuated for ferrets, mink and dogs when CAM passage of that virus was continued after lesions had appeared on infected CAMs. Clinical trials of the CAM-adapted measles virus will determine whether that virus also was further attenuated by serial passage following the appearance of lesions on infected CAMs.

We thank Dr. S. W. Nielsen for histological examinations of infected CAMs, Mr. Walter Olson for able technical assistance, and Mrs. Esther Chasan for help in the preparation of the manuscript.

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PENICILLAMINE THERAPY FOR RHEUMATOID LUNG DISEASE: EFFECTS ON PROTEIN SULPHYDRYL GROUPS

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THE rheumatoid factor, a macroglobulin (mol. wt. 1,000,000) possessing many of the attributes of an auto-antibody, is frequently detectable in the sera of patients with rheumatoid arthritis[1]. It has been reported that these patients also have decreased serum protein sulphydryl content[2–5]. We recently suggested that the subnormal serum protein sulphydryl content could contribute to the formation of abnormal, disulphide-linked proteins such as the rheumatoid factor, due to an impairment in the mechanism for regenerating and maintaining protein sulphydryl groups[3].

Thiol compounds such as *d*-penicillamine are known to dissociate polymeric proteins by cleaving disulphide bonds[6–10]. It might be expected, therefore, that the therapeutic administration of *d*-penicillamine to patients with rheumatoid arthritis would result in an increase in the serum protein sulphydryl content and a decrease in the rheumatoid factor titre (Fig. 1). The purpose of this investigation was to determine what changes were brought about in serum protein sulphydryl content before, during, and after *d*-penicillamine therapy, and to relate these findings to the clinical course of the disease and to changes in rheumatoid factor titre.

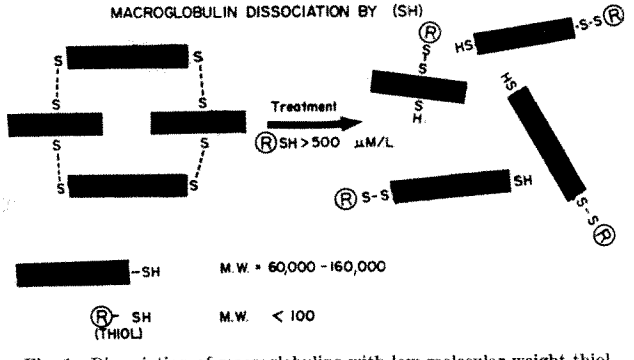


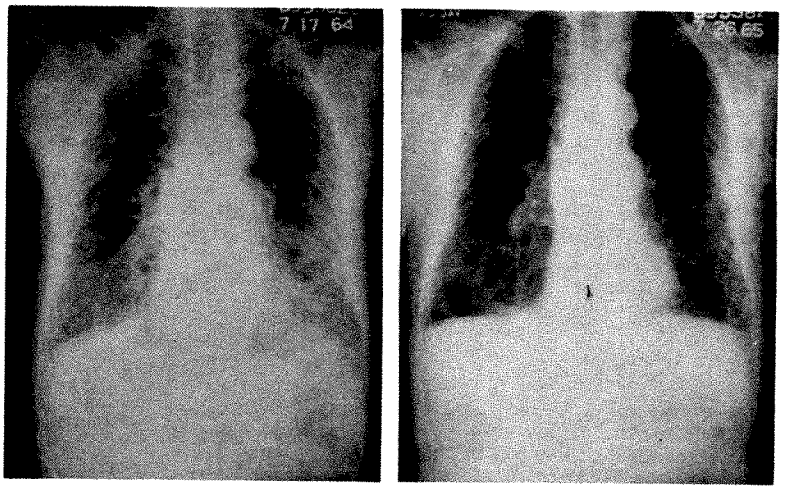
Fig. 1. Dissociation of macroglobulins with low molecular weight thiol compound (®SH). The solid rectangles represent peptide chains interlinked by S–S bonds. Dissociation results in increased serum protein SH

Three patients with rheumatoid lung disease were observed at bi-weekly intervals for 6–14 months. All the patients had abnormal pulmonary function, incomplete oxygen saturation of the arterial blood, and progressive lesions of the lung (these were demonstrated by chest X-ray). Neoplastic and infectious aetiologies were ruled out by appropriate investigations, and the pulmonary lesions were attributed to the underlying rheumatoid process[11]. Open lung biopsies were performed on each patient, and the histopathology of the lesions disclosed the formation of lymphoid follicles, interstitial and parenchymal lymphocytic and plasmacytic infiltrations, including involvement of the pulmonary vessels (Fig. 2). The vascular lesions included a spectrum of inflammatory, hypertrophic and occlusive changes. Serum protein sulphydryl content and rheumatoid factor titre were determined at bi-weekly intervals before, during and after *d*-penicillamine therapy. (*d*-Penicillamine ('Cuprimine') was kindly supplied by Dr. E. Alpert of Merck, Sharp and Dohme, and administered in dosage of 1–3 g per day supplemented with 50 mg pyridoxine per day.) Electrophoretic and ultracentrifugation investigations were also regularly performed.

Serum protein sulphydryl content was determined by a modified polarographic technique[3]. Spectrophotometric methods (that is, 5,5-dithio*bis*-(2-nitrobenzoic acid) (DTNB)[12], parachloromercuribenzoate (*p*CMB, more recently designated as *p*-hydroxymercuribenzoate)[13] and *N*-ethylmaleimide (NEM)[14]), for determining sulphydryl content, were used to confirm the findings obtained by the polarographic method. Total protein and albumin–globulin fractions were determined by the biuret and sodium sulphite fractionation methods[15]. The protein sulphydryl content (µmoles sulphydryl/mole of protein) was calculated on the basis of the molecular weights of 60,000 for albumin and 160,000 for globulin. Ultracentrifugation was performed with the use of a Beckman model *E*

analytical ultracentrifuge[16]. Rheumatoid factor titre was determined by tube incubation of serum with latex-coated γ-globulin[17].

Within 4 weeks of commencing the *d*-penicillamine therapy, the patients reported relief of pain, stiffness and shortness of breath. Subjective clinical improvement was confirmed objectively by investigations of the articular and pulmonary functions. Increased oxygen saturation of arterial blood and resolution of pulmonary infiltrates (as demonstrated by chest X-ray) were observed (Fig. 3). A further lung biopsy, performed 3 months after initiating penicillamine therapy in one patient, disclosed considerable resolution of the lymphocytic infiltrate and of the inflammatory changes involving the pulmonary vessels. Both the rheumatoid factor titre and the macroglobulin fraction decreased during penicillamine therapy. (A decline in rheumatoid factor titre averaging four tube dilutions was observed during therapy. In one patient the titre decreased from 10,240 to 320 on 5 months of sustained penicillamine therapy.)



a *b*

Fig. 3. Radiographic investigations of a patient with rheumatoid lung disease. Bilatera llower lobe infiltrates are noted to resolve with penicillamine therapy (*a*) before therapy; (*b*) progressive resolution of pulmonary infiltrates at the eleventh month

Serum protein sulphydryl content was noted to increase by more than 50 per cent during therapy and to decrease after withdrawal of the medication (Table 1). Patients were maintained on penicillamine supplemented with pyridoxine for intervals of 2–7 months. Therapy was discontinued in two patients because of undesirable side reactions (that is, nephrosis and bone marrow depression) which were attributed to the medication.

Table 1. EFFECTS OF PENICILLAMINE THERAPY ON SERUM PROTEIN SULPHYDRYL (SH) CONTENT AND RHEUMATOID FACTOR TITRE OF THREE PATIENTS WITH RHEUMATOID LUNG DISEASE

	No.* of determinations	SH (μmole/l.)	SH (μmole/g total protein)	SH (mole/mole protein)	Average rheumatoid factor titre tube dil.
Before therapy	7	237	3·2	0·32	7
During therapy	24	382	5·1	0·50	3
After therapy	17	282	3·9	0·38	5
Percentage of increase		61	60	56	
Normal subjects (18)	34				
Average		512	7·5	0·61	
Range		400–600	5·5–8·6	0·50–0·72	

* Serial determinations performed at 2–4 week intervals.

The serum protein sulphydryl content was noted to be decreased initially by about 30 per cent in the three patients reported here. (Serum protein sulphydryl content determined in 8 M urea confirmed that the decreased serum sulphydryl content could not be attributed to unreactive sulphydryl groups.) Similar findings have also been observed in more than 150 patients with clinically active rheumatoid disease. It is suggested that the inability to maintain and regenerate sulphydryl groups might contribute to the formation of abnormal, disulphide-linked proteins such as the rheumatoid factor (PSSP) and that these abnormal proteins can be dissociated with thiol administered or endogenous low-molecular-weight thiol compound (RSH). PSH represents the protein with the reduced disulphide, that is, free sulphydryl group (Fig. 1 and equation (1)):

$$PSSP + RSH \rightleftharpoons PSSR + PSH \quad (1)$$

It was observed that rheumatoid factor titre decreased and the serum protein sulphydryl content increased following *d*-penicillamine therapy. It has also been noted that some patients with high rheumatoid factor titre have a high incidence of systemic lesions (that is, pulmonary and neurovascular lesions[18]). Thus it is suggested that there is a relationship between the severity of rheumatoid disease, rheumatoid factor titre and subnormal protein sulphydryl content.

The formation of abnormal disulphide-interlinked proteins, such as the rheumatoid factor, may be caused by impaired biochemical reduction of disulphide bonds (ref. 10 and equation (2)):

$$PSSR + NADPH \rightleftharpoons PSH + RSH + NADP^{+} \quad (2)$$

(NADPH is reduced nicotinamide adenine dinucleotide phosphate.) The oxidation of sulphydryl groups resulting in the formation of disulphide bonds is favoured by the presence of divalent trace metals such as copper, and by the high oxygen tension and by the alkaline *p*H of the blood (ref. 10 and equation (3)):

$$2PSH + \text{oxidant} \rightleftharpoons PSSP + 2H^{+} + 2e + \text{reductant} \quad (3)$$

The expenditure of energy is, therefore, necessary to reverse the oxidation of sulphydryl groups and to maintain the reduced state. The formation of abnormal proteins such as the rheumatoid factor and the clinical activity of rheumatoid arthritis may have a common origin—a biochemical disturbance in the mechanism for regenerating and maintaining serum protein sulphydryl groups. Such a disturbance might contribute to the process of the disease in the following ways: (1) The loss of sulphydryl groups could change the physical and chemical configuration of structural proteins and enzymes, thus altering or destroying their function. (2) The inability to reduce disulphide bonds and to regenerate sulphydryl groups could result in the formation of abnormal proteins (that is,

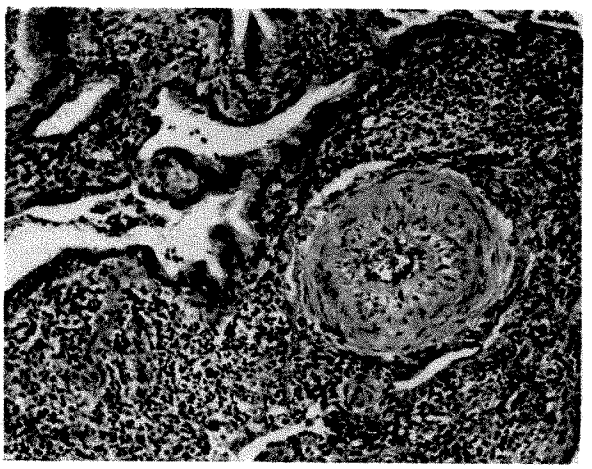


Fig. 2. Photomicrograph of lung biopsy illustrating the lymphocytic and plasmacytic infiltrate about a pulmonary vessel and a small bronchus. The wall of the artery is thickened and the lumen is almost occluded (× 30)

antigenic substances) or adversely affect the synthesis of immune globulins (by altering the specific disulphide inter-linkages of protein sub-units, that is, the *H* and *L* chains composing the immune globulins), causing formation of 'auto-antibodies'.

The mechanism(s) for the therapeutic action of *d*-penicillamine is not known; however, the following possible modes of action have been postulated:

Immunosuppressive. Suppression of γ-globulin production has been reported in animals with the administration of *d,l* penicillamine[19,20]. However, the immunosuppressive effect of the racemic compound has been attributed to the *l*-isomer, which exerts an antimetabolic effect on pyridoxine. The patients in this investigation were placed on pyridoxine supplements and recent reports indicate that *d*-penicillamine is not significantly antagonistic to pyridoxine[21,22]. Therefore, immunosuppression seems an unlikely mechanism.

Chelation. Penicillamine is an effective chelator of metal ions, and it is possible that removal of these metals could exert a beneficial therapeutic effect[23]. Copper and other trace metals have been reported to be elevated in the serum and synovial fluid of patients with rheumatoid arthritis[24]. These divalent metals are known to catalyse the oxidation of sulphydryl groups[25]. Thus, penicillamine could retard the formation of disulphide bonds by chelating metals and preventing the subsequent formation of abnormal disulphide-linked proteins.

Exchange-dissociation. Penicillamine has been shown to cause dissociation of disulphide-linked immune globulins *in vitro* and *in vivo*[7-9,26]. It was recently reported that several patients with diagnosed cysteinuria excreted penicillamine-cysteine disulphides following penicillamine therapy[27,28]. This substantiates the hypothesis that *d*-penicillamine can act *in vivo* through an exchange dissociation with disulphide bonds.

Although the exact mode of action of *d*-penicillamine has not been elucidated, serum protein sulphydryl concentrations were shown to increase with therapeutic administration of this drug. Changes in serum protein sulphydryl concentrations were accompanied by improvement in the patient's clinical condition, a decrease in the concentration of rheumatoid factor, and reduction of immunogenic cellular pulmonary infiltrate.

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COMPARISON OF CHANGES IN THE CHEMICAL COMPOSITION OF RABBIT CARTILAGE MATRIX WITH AGE AND FOLLOWING INTRAVENOUS PAPAIN

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THE intravenous administration of papain solution in young rabbits has been shown to produce marked softening of the ear cartilage accompanied microscopically by considerable reduction of the metachromatic cartilage matrix[1]. Adolescent or mature rabbits were originally thought to be refractory to these effects of papain[1]. Recently we have been able to demonstrate that papain produces changes similar to the aforementioned ones in rabbits of all ages, but with diminished intensity[2-4]. We have also shown that these gross and microscopic changes are due to a marked depletion of chondromucoprotein (CMP) from the cartilage matrix and a change in the ratio of nitrogen to hexosamine in CMP as a result of the injection of papain.

In the course of these investigations, it was noted that the CMP content of the cartilage of normal rabbits varied, depending on the weight and age of the animals. These age-related changes appeared to be similar to the ones observed following the intravenous administration of papain.

This article deals with a detailed comparison of the changes in the composition of normal rabbit cartilage with ageing and with those found following papain injection.

All experiments were performed on New Zealand albino rabbits of both sexes. A total of 144 animals were used. Of these, 63 animals were used as normal controls, while the remainder were injected with papain. The weight of the animals ranged from one to approximately 5 kg. They were divided into three major groups.

The first group were the very young rabbits that were obtained right after the end of lactation, and they weighed 800–1,300 g. They are classified in this report as "young rabbits". The second group of animals weighed between 2–3 kg, and they are called "adolescent rabbits". The third group consisted of old animals repeatedly bred, and they weighed between 3·5 and 5 kg. They are called "adult rabbits".

The animals were maintained on Purina Rabbit Chow for at least a 2-week period before any experiments were undertaken. All rabbits were weighed accurately at the time that they were purchased, immediately before the injection of papain, and at the time that they were killed. No significant changes in weight were observed after the injection of papain.

A 2 per cent papain solution was prepared for injection from a highly purified powdered papain (donated by the Sterling-Winthrop Research Laboratories, Rensselaer, New York). Crystalline and crude papain have been

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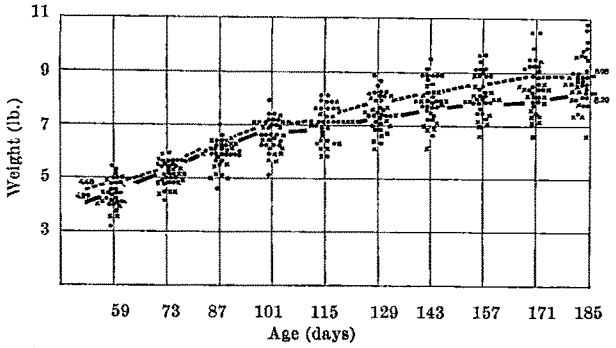


Fig. 1. Comparison of weight increase with ageing in normal New Zealand albino rabbits. Average: thirteen bucks, ———×, twenty-five does, — — — —●

used in the past and have been shown to be as effective. It was preferable not to use crystalline papain because of its high rate of oxidation and the very high cost. The papain was prepared by mixing 2 g of powdered papain with 100 ml. 0·15 M sodium chloride in water. This was placed on a magnetic stirrer for 30 min and then filtered through pre-sterilized Boerner bacteriological centrifuge filters. This produced a straw-coloured, clear solution that was free of bacteria.

35 mg of papain per kg body weight were injected in the marginal vein of the ear of the rabbits. The cartilagenous plate of the ear was used for the isolation of the CMP. This is because the cartilage from the ear can be obtained easily without delay and can be quite easily freed of skin and perichondrium by a few simple manipulations.

All animals were killed by air embolism at the end of each experiment. The ears were removed promptly after death and the skin and perichondrium were stripped as rapidly as possible. They were minced, weighed, and then homogenized in a 'Virtis 45' high-speed homogenizer at 45,000 r.p.m. and 4° C.

The chondromucoprotein was extracted from the homogenate by the methods of Malawista and Schubert[5]. The extracted CMP was dried and portions were analysed for nitrogen content by a micro-Kjeldahl method, and the hexosamine content of the CMP was determined by a modification of the Schloss method[6]. The molar ratio of nitrogen to hexosamine in the extracted CMP was calculated from these values. The results obtained were plotted against the weight of the animal, and the data were analysed for statistical significance using the least squares method of linear correlation.

Since the precise age of all the animals could not be determined, it was necessary to establish some uniform criteria for the age of the animals. From tables that we obtained from commercial breeders, animal food manufacturers, and from the U.S. Government Department of Agriculture, it was established that a direct relationship exists between the age and the weight of the rabbits. This was also confirmed by X-ray investigations of the epiphyseal plate of the animals.

Fig. 1 shows the relative increase in weight of New Zealand albino rabbits with ageing and was used as the main criterion for the selection of the rabbits used in these experiments. In Figs. 2–5, the weight of the animals is shown, and their age can be extrapolated by referring to Fig. 1.

Table 1 gives a summary of the changes that occurred in the ear cartilage matrix of three groups of rabbits, as specified earlier in the article. It should be pointed out here that while the maximum changes (that is, the maximum depletion of the cartilage matrix following administration of papain) in the very young rabbits occurred 24 h after papain, in the adolescent rabbits the same changes occurred 48 h after papain; while in the adult rabbits they occurred 72 h after papain. The details of these experiments have been described elsewhere[2–4].

Table 1

	Young rabbits*		Adolescent rabbits*		Adult rabbits*	
	Control	After papain	Control	After papain	Control	After papain
mg CMP/g wet cartilage	37·4	22 0	44 82	32·23	40 21	33·55
mg hexosamine/CMP	8 6	6·6	7·47	6 43	7 46	6·76
mg nitrogen/g CMP	7 7	9 2	8 39	9 35	8 69	9·45
Nitrogen/hexosamine molar ratio	11·5	17·8	14·79	19·55	15·29	18 27

* For details of the effect of papain on these three groups, see refs. 2, 3 and 4

Table 1 shows that in all groups papain produced a statistically significant decrease in the amount of CMP extracted from the ear cartilage. It can also be seen that there was a decrease in the hexosamine content of the extracted CMP. Conversely, there was an increase in the nitrogen content of the extracted CMP and an increase in the molar ratio of nitrogen to hexosamine in CMP. It is also interesting to note that the changes that occurred following papain, even though of a decreasing magnitude as the age of the animals progressed, are statistically significant for all groups.

Fig. 2 shows that the amount of the extracted CMP decreased as the weight and age of the animals increased. The solid line represents the line of least squares for the plotted points ($P_{(t)} < 0{\cdot}001$).

Fig. 3 shows the correlation between the hexosamine content of the extracted CMP and the weight and age of the rabbits. The hexosamine content of the extracted

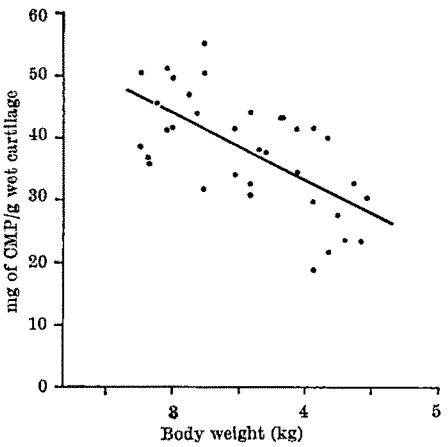


Fig. 2. Changes in the amount of CMP/g wet cartilage with ageing. Solid line represents the line of least squares for the points shown ($P(t) < 0{\cdot}001$). Each point represents one animal

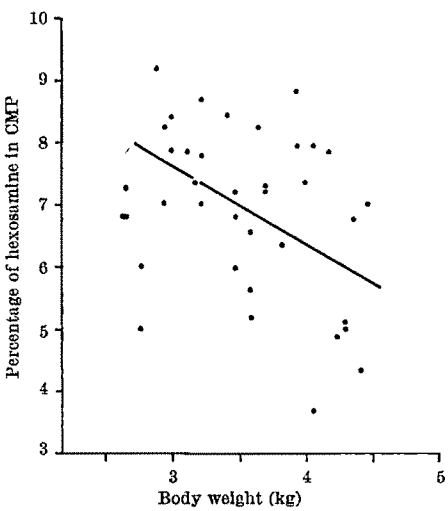


Fig. 3 Changes in the percentage content of hexosamine in the extracted CMP with ageing. Solid line represents the line of least squares for the points shown ($P(t) < 0{\cdot}005$). Each point represents one animal

CMP decreased as the weight and age of the rabbits increased. The solid line represents the line of least squares ($P_{(t)} < 0{\cdot}005$).

Fig. 4 shows the correlation between the nitrogen content of the extracted CMP, and the weight and age of the rabbits. It can be seen that the nitrogen content of the extracted CMP increased as the animals increased in weight and age. The solid line represents the line of least squares ($P_{(t)} < 0{\cdot}005$).

The correlation between the nitrogen to hexosamine molar ratio and the weight and age of the rabbits is shown in Fig. 5. The nitrogen to hexosamine molar ratio increased as the weight and age of the animal increased. Again the solid line represents the line of least squares ($P_{(t)} < 0{\cdot}025$).

The data presented here support results published by other investigators[7-9] that cartilage matrix undergoes chemical changes with ageing. These changes consist of a decrease in the amount of extractable CMP and a decrease in the amount of hexosamine in the extracted CMP. An increased nitrogen content in the extracted CMP is also found, with a consequent increase of the molar ratio of nitrogen to hexosamine.

These changes are very similar to those produced in the cartilage matrix of rabbits by the intravenous administration of papain.

It is interesting that the maximal changes in the cartilage matrix after papain required longer periods of time as the age of the animals increased. Thus the young rabbits showed maximal response to papain 24 h after injection. In the adolescent group these changes occurred 48 h after injection, and in the adult animals they did not reach their maximum until 72 h after injection of papain.

Concurrently, the percentage change between control and papain-injected animals decreased as the age of the animals advanced. The delay in the response to the injection of papain and the decrease in the percentage change could be explained on the basis of the data recently published by Stockwell and Barnett, who showed that ageing reduces the permeability of cartilage[10]. On the basis of these data, it could be concluded that papain diffuses with diminished ease through the cartilage matrix as the animals age and, therefore, it not only produces smaller chemical changes in the matrix, but also requires a longer time to achieve these changes.

Matthews[11] and, recently, Bollet *et al.*[12] have reported on the chemical changes occurring in the cartilage of persons with degenerative arthritis. Both authors found a decreased hexosamine content in the CMP of the degenerated cartilage. We also found that this occurred as a result of the administration of papain to our experimental animals. The animals used in our experiments were not followed for long periods after the administration of papain to determine whether or not they would develop

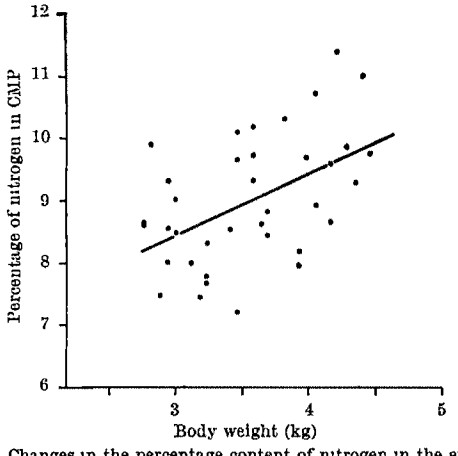


Fig. 4. Changes in the percentage content of nitrogen in the extracted CMP with ageing. Solid line represents the line of least squares for the points shown ($P_{(t)} < 0{\cdot}005$). Each point represents one animal

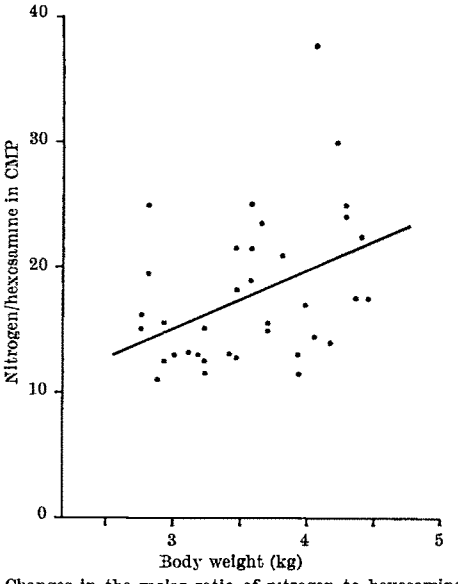


Fig. 5 Changes in the molar ratio of nitrogen to hexosamine in the extracted CMP with ageing. Solid line represents the line of least squares for the points shown ($P_{(t)} < 0{\cdot}025$). Each point represents one animal

degenerative changes in the joints. However, as pointed out by Bauer and Bennett[13], the early changes in degenerative arthritis are merely an accentuation of the normal ageing process.

Supporting this possibility are data published by Fell *et al.*[14-18] and Thomas *et al.*[19]. Using excessive quantities of vitamin A *in vivo* and *in vitro*, they have been able to produce chemical changes in cartilage similar to those produced with papain. The basis of these changes has been reported to be the release of an intracellular protease induced by the excessive vitamin A, thus indicating that such an enzyme exists within normal chondrocytes.

By comparing these published data with our present findings, we feel that the action of an intracellular papain-like enzyme in the pathogenesis of degenerative arthritis is a distinct possibility and that further attention should be given to its eventual isolation, and its implications to the pathogenesis of degenerative arthritis.

To sum up, the amount of chondromucoprotein (CMP) present in the cartilage matrix as well as the hexosamine content of such CMP were found to decrease with the ageing of normal rabbits. Identical changes were also found after the intravenous injection of papain in rabbits. These changes are postulated to be produced by the presence of an endogenous papain-like enzyme. The relationship of these changes to degenerative arthritis is discussed.

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"FERTILE" INTESTINE NUCLEI

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NUCLEAR transplantation experiments carried out on *Xenopus laevis* since 1958[1] have shown that nuclei from the majority of differentiating endoderm cells[2] and from fully differentiated intestine cells[3] can support the formation of normal muscle and nerve cells after transplantation to enucleated eggs. We describe here some adult frogs which are derived from transplanted intestine nuclei and some of which are fertile. This result is of interest since it shows that genetic factors required for the formation of a fertile adult frog are not lost in the course of cell differentiation, at least in the case of some intestine cells.

Preparation of nuclear-transplant frogs. Cells from the intestinal epithelium of *Xenopus laevis* tadpoles which had just started feeding were dissociated and their nuclei transplanted into unfertilized eggs enucleated by ultraviolet irradiation as described previously[4]. The development promoted by intestine nuclei is often more normal if serial nuclear transfers are carried out using a first-transfer blastula to provide nuclei for serial transplantation[3]. The reason for this apparent improvement in development is thought to be the variable quality of *Xenopus* eggs laid in the laboratory[5]. Of the intestine nuclear-transplant frogs described below, all but one were obtained by serial nuclear transplantation (Table 1). The nuclear transplantation experiments which yielded these frogs were carried out in Oxford by J. B. G. and the majority of the mating records were obtained in Geneva by V. U.

Nuclear-transplant frogs derived from intestine nuclei. The embryos and tadpoles which grew into the frogs listed in Table 1 were normal as larvae, and the adult frogs do not differ from other frogs reared in the laboratory, whether from fertilized eggs or from other kinds of transplanted nuclei. In an attempt to demonstrate that these nuclear-transplant frogs are normal in respect of all cell types, they were mated to wild-type and other nuclear-transplant frogs, an activity promoted by the injection of gonadotrophic hormone (300–500 I.U. for females, and 100–150 I.U. for males). Table 1 shows that five of these seven intestine nuclear-transplant frogs are fertile. The females produced the numbers of eggs expected of frogs reared in the laboratory. One female and one male were not shown to be fertile although they contained apparently normal ovaries and testes. Among frogs reared from eggs laid in the laboratory, it has generally been found that about 5 per cent (17/386) are sterile.

The intestine nuclear-transplant frogs which gave fertile eggs in some matings failed to do so in other matings; the question therefore arises whether they are less fertile than normal. Under laboratory conditions, the fertility of *Xenopus laevis* is variable and only rarely are 100 per cent of the eggs fertile. This is likely to be due partly to the unnatural conditions provided in the laboratory (especially diet), and partly to the induction of mating by hormone injection at unnatural seasons and frequencies. We must therefore compare the fertility of nuclear-transplant frogs with that of other *Xenopus laevis* reared to maturity in the laboratory. Even this can provide only an approximate comparison, since the fertility of frogs reared in the laboratory is affected by a number of factors such as the age of the frogs, degree of crowding, temperature, and season, and these conditions are not known to have been the same for the frogs which we compare in this article. We consider here two measurements of fertility: (*a*) the percentage of matings which yielded at least a few fertile eggs (Table 2), and (*b*) the percentage of total eggs laid which were fertile (Table 3).

Table 2 shows the proportion of matings in which at least ten eggs started cleaving normally. The female nuclear-transplant frogs derived from intestine nuclei (row 1) participated in fertile matings with the same frequency as wild-type frogs reared in the laboratory or imported from Africa (rows 6 and 7). Furthermore, this is also true of frogs reared from the F_1 progeny of the intestine transplant-frogs (row 4). Male nuclear-transplant frogs obtained from intestine nuclei (row 1) failed to fertilize eggs as often as males usually do, but this result has been obtained with only two frogs.

Table 3 provides another measurement of fertility. Here we have excluded all matings in which no fertile eggs were produced, and the percentage figures show what proportion of eggs laid started to cleave normally. The female nuclear-transplant frogs (Table 3, rows 1–3) from intestine nuclei are as fertile in this respect as other frogs reared in the laboratory. Male nuclear-transplant frogs from intestine nuclei (row 1) as well as from tadpole nuclei (row 2) appear to be subnormally fertile, but the number of males concerned is small.

Table 1. REPRODUCTIVE CAPACITY OF FROGS OBTAINED FROM TRANSPLANTED INTESTINE NUCLEI OF *Xenopus laevis*

Number and sex of frog	Genetic constitution	Origin of transplanted nuclei: First transfer donor*	Origin of transplanted nuclei: Serial transfer donor*	Reproductive capacity of nuclear-transplant frogs†: First mating (per cent)	Reproductive capacity of nuclear-transplant frogs†: Subsequent matings (per cent)
♀ 1	Diploid, 1-*nu*	Stage 46	Stage 8 (Blastula)	85 (2000)	0 (270); 0 (1500); 20 (3000); 0·1 (1900); 10 (3800); 20 (5200)
♀ 2	,, ,,	,, ,,	,, ,, ,,	20 (2000)	50 (300); 15 (1800); 30 (2500)
♀ 3	,, ,,	,, ,,	,, ,, ,,	0 (1800)	1·8 (2200); 45 (1700)
♀ 4	,, ,,	,, ,,	,, ,, ,,	20 (570)	
♀ 5	Diploid, 2-*nu*	Stage 47	Stage 10 (Gastrula)	0 (1000)	0 (650;) 0 (370)
♂ 6	Diploid, 1-*nu*	Stage 46	—	0	0; 0; 3; 0; 15
♂ 7	,, ,,	Stage 45	Stage 16 (Neurula)	0	0

* All first transfer donor nuclei were taken from intestine cells of feeding tadpoles (stage 45–47), and serial transfers were made from endoderm nuclei. Developmental stages are those of Nieuwkoop and Faber (ref. 6).
† The percentage figures show the proportion of the total eggs laid which were fertile; most of these developed normally, and some were reared into adult frogs. The figures in brackets show the total number of eggs laid during the ovulation.

Table 2. FREQUENCY OF NUCLEAR-TRANSPLANT FROG MATINGS WHICH YIELDED FERTILE OFFSPRING

	Origin of frogs (Nieuwkoop and Faber stages (ref 6))	Total number and sex of frogs mated	First matings which included fertile eggs (per cent)	All subsequent matings which included fertile eggs. (Number of matings in brackets) (per cent)
(1)	Intestine nuclear-transplant frogs (intestine nuclei of feeding tadpoles stages 45–47)	5 ♀s 2 ♂s	60 0	69 (1)3 33 (6)
(2)	Tadpole nuclear-transplant frogs (endoderm nuclei of hatching and swimming tadpoles; stages 33–42)	12 ♀s 2 ♂s	50 50	81 (27) 80 (5)
(3)	Embryo nuclear-transplant frogs (endoderm nuclei of blastula to heart-beat embryos; stages 8–26)	24 ♀s 7 ♂s	58 57	74 (23) 77 (13)
(4)	F_1 progeny of intestine nuclear-transplant frogs	28 ♀s 27 ♂s	65 68	89 (9) 72 (11)
(5)	F_1 progeny of tadpole nuclear-transplant frogs	14 ♀s 16 ♂s	86 81	100 (6) 100 (10)
(6)	Frogs reared from eggs laid in the laboratory by imported frogs	29 ♀s 32 ♂s	86 87	76 (88) 82 (110)
(7)	Frogs imported from Africa	72 ♀s 20 ♂s	61 75	68 (244) 68 (88)

Table 3. PERCENTAGE OF FERTILE EGGS IN SUCCESSFUL MATINGS OF NUCLEAR-TRANSPLANT FROGS

	Origin of frogs (Nieuwkoop and Faber stages (ref. 6))	Number and sex of frogs mated	Fertile eggs: First mating (per cent)	Fertile eggs: Subsequent matings (per cent)
(1)	Intestine nuclear-transplant frogs (stages 45–47)	5 ♀s 2 ♂s	42 (3) 0 (0)	17 (3) 9 (2)
(2)	Tadpole nuclear-transplant frogs (stages 33–42)	8 ♀s 1 ♂	18 (3) 2 (1)	18 (19) 9 (3)
(3)	Embryo nuclear-transplant frogs (stages 8–26)	23 ♀s 7 ♂s	34 (14) 36 (4)	31 (40) 35 (11)
(4)	F_1 progeny of intestine nuclear-transplant frogs	19 ♀s 21 ♂s	35 (13) 35 (13)	16 (8) 19 (8)
(5)	F_1 progeny of tadpole nuclear-transplant frogs	13 ♀s 16 ♂s	18 (12) 17 (13)	11 (6) 8 (10)
(6)	Frogs reared from eggs laid in the laboratory by wild-type frogs	28 ♀s 31 ♂s	25 (24) 52 (23)	32 (46) 46 (90)

These figures apply only to matings in which some fertile eggs were obtained. The percentage of fertile eggs in each mating is very variable, ranging from 2–99 per cent, and different numbers of eggs were obtained in each mating. The average values for each group of frogs are not significantly different.
The figures in brackets in the last two columns show the number of successful matings on which the percentage is based. Matings which took place between two nuclear-transplant frogs are recorded in the results for each parent frog.

We make no attempt to describe here the quality of development of fertile eggs provided by nuclear-transplant frogs, since this varies considerably from one mating to another even with the same frogs. It is sufficient to state that we have observed no early developmental abnormalities characteristic of the progeny of any intestine nuclear-transplant frogs, and that at least some of the progeny of the frogs have been reared into mature males and females of normal fertility (row 4 of Tables 2 and 3).

The frogs described earlier are known to have been derived from intestine nuclei and not egg nuclei for two reasons. First, all except one of the intestine nuclear-transplant frogs were obtained from the injection into unfertilized wild-type eggs of nuclei from tadpoles heterozygous for the anucleolate mutation[1]. Since all but one of the frogs in Table 1 were diploid and one-nucleolated, their nuclei must be mitotic products of the transplanted nucleus, and not of an unsuccessfully irradiated egg-nucleus. There are no germ-cells in the intestine in stage 45–47, since these have all migrated to the germ-ridge about 24 h before this stage[6]. The nuclei used for transplantation almost certainly came from differentiated epithelial cells of the intestine since, when dissociated, these are visibly larger and different from other cell types in this organ owing to their possession of yolk at this stage. Study of the intestinal epithelium at stages 45–47 reveals only columnar epithelial cells with a striated border for absorption and a small proportion (about 1 per cent) of gland cells.

In Amphibia it has been generally found that only a small proportion of nuclear-transplant embryos reach advanced developmental stages, especially when the transplanted nuclei are taken from differentiating cells. The intestine nuclear-transplant frogs described here were obtained from experiments in which a total of 120 intestine nuclei were transplanted. However, in other experiments with over 600 intestine nuclear transfers, none of the resulting nuclear-transplant embryos became sexually mature. Most of these intestine nuclear-transplant frogs were obtained by serial nuclear transplantation, and the seven frogs can be seen from Table 1 to have resulted from the transplantation of four original intestine nuclei only two of which have been demonstrated to yield fertile frogs. It is permissible to draw definite conclusions from these very small numbers, since all but one of the seven frogs discussed were obtained from genetically marked donor nuclei. The existence of fertile nuclear-transplant frogs shows that at least some intestine nuclei have promoted normal cell differentiation without accumulating dominant mutations of essential genes. Furthermore, the genetic analysis of nuclear-transplant frogs has shown that transplanted somatic nuclei capable of supporting normal development do not differ markedly from typical germ-cell nuclei in respect of recessive mutations[7].

The seven adult frogs described here show that nuclei from differentiated intestine cells can support the normal formation, after transfer to enucleated unfertilized eggs, of adult male and female frogs, some of which are fertile. The processes involved in permitting the activity of those genes required for the differentiation of an intestine cell, and in repressing those not so required, cannot therefore entail the irreversible loss or inactivation of genes needed for the differentiation of other cell types.

We thank Dr. A. W. Blackler, A. Droin, and J. Reynaud for permitting us to include in Tables 2 and 3 the results of some nuclear-transplant frog mating tests carried out by them, and Dr. A. W. Blackler for supervising the care of the intestine nuclear-transplant frogs at an early stage in their life. One of us (J. B. G.) thanks the Medical Research Council for financial support.

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LETTERS TO THE EDITOR

ASTRONOMY

Probable Value of the Time of Rise for the Sunspot Cycle No. 20

IT has been shown[1-5] that the time of rise T_R, that is the time interval between the minimum of solar activity and the next maximum, is a basic parameter for determining the variation of values of the various indices of solar activity (relative sunspot numbers, areas of the sunspots, umbrae and faculae, etc.) within each sunspot cycle and from cycle to cycle. Therefore, knowledge of the value of T_R for future sunspot cycles enables a prediction to be made of the probable variation of the values of these indices of solar activity during the corresponding cycles.

King-Hele found[6] that the value of T_R for the current cycle No. 20 will be equal to $T_R = 3{\cdot}4$ years. Therefore, if we take into account the fact that the minimum of cycle No. 20 occurred in 1964·7, we should expect the maximum of this cycle to happen in 1968·1. This result is based on the hypothesis of the existence of "a 7-cycles recurrence tendency in the time of rise" postulated by King-Hele[7].

In the present report a new approach is used to study the variation of the values of the time of rise T_R from cycle to cycle of solar activity. For this purpose the data given by Waldmeier[8] are used: these are summarized in Table 1.

Table 1

Cycle No.	Yr of maximum	Time of rise T_R(yr)	Cycle No.	Yr of maximum	Time of rise T_R(yr.)	Cycle No.	Yr of maximum	Time of rise T_R(yr.)
−12	1615·5	4 1	−1	1738 7	4 7	10	1860·1	4 1
−11	1626·0	7 0	0	1750 3	5 3	11	1870·6	3 4
−10	1639·5	5 5	1	1761 5	6 3	12	1883 9	5 0
−9	1649 0	4·0	2	1769·7	3 2	13	1894·1	4 5
−8	1660 0	5 0	3	1778·4	2 9	14	1907 0	5·3
−7	1675·0	9 0	4	1788·1	3 4	15	1917 6	4·0
−6	1685·0	5 5	5	1805·2	6·9	16	1928·4	4 8
−5	1693 0	3·5	6	1816·4	5·8	17	1937·4	3 6
−4	1705 5	7·5	7	1829 9	6 6	18	1947 5	3·3
−3	1718·2	6·2	8	1837 2	3 3	19	1957 9	3·6
−2	1727·5	4 0	9	1848·1	4·6	(20)	(1968·7)	(4 0)

In Fig. 1 the continuous line gives the values of T_R for cycles Nos. 7–19 and the broken line the values of the same parameter for the cycles Nos. −5 to 6. The data concerning the latter group of cycles are scant and, therefore,

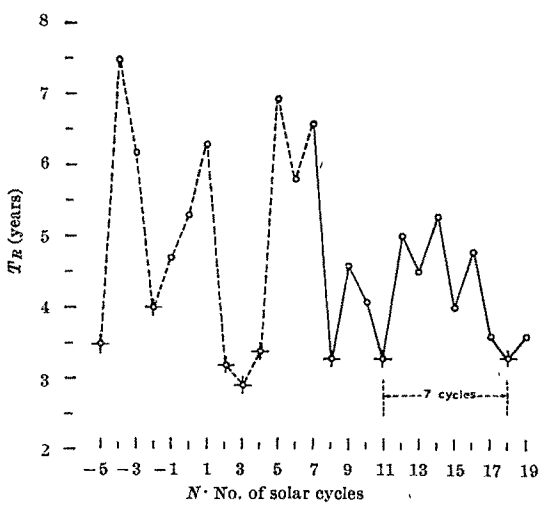


Fig. 1

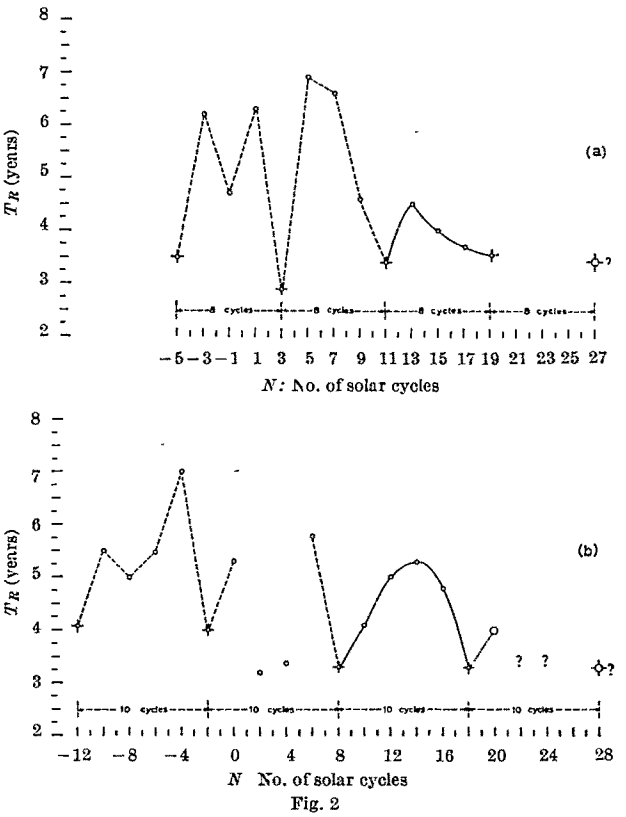


Fig. 2

care should be exercised in taking into account the corresponding values of T_R. It can be seen in Fig. 1 that the minima of T_R (marked with the symbol -⊙-) correspond to cycles Nos. −5, −2, 2, 3, 4, 8, 11 and 18. If we disregard cycles Nos. 2 and 4, then the time interval contained between the successive minima of T_R is correspondingly equal to 3, 5, 5, 3 and 7 cycles. Therefore, the values of T_R do not seem to show a constant periodicity. If we consider the two groups formed by cycles Nos. 4–11 and 11–18 only, then we have the seven-cycles recurrence tendency found by King-Hele. This tendency is, however, destroyed, if we also take into account the previous cycles Nos. −5 to 4.

The situation becomes quite different if we consider the values of T_R corresponding to cycles with even and odd current number, that is, cycles with the same polarity in the sunspot magnetic fields, separately.

Figs. 2*a* and 2*b* show, respectively, the values of T_R corresponding to sunspot cycles No. $N = 2n + 1$ ($n = -3$ to 9) and $N = 2n$ ($n = -6$ to 9). From these figures we see that the values of T_R show in this case a more or less smooth variation with minima occurring every eight (Fig. 2*a*) and ten (Fig. 2*b*) cycles respectively. Only the values of T_R corresponding to cycles Nos. 2 and 4 (Fig. 2*b*) are disproportionally low. If we take into account that these values are more or less precise (Xanthakis, in the press) this means that probably cycles Nos. 22 and 24 should also show low values of T_R, as compared with the values corresponding to cycles Nos. 20 and 26.

It is worth mentioning that a similar periodicity with periods respectively equal to eight and ten cycles is also

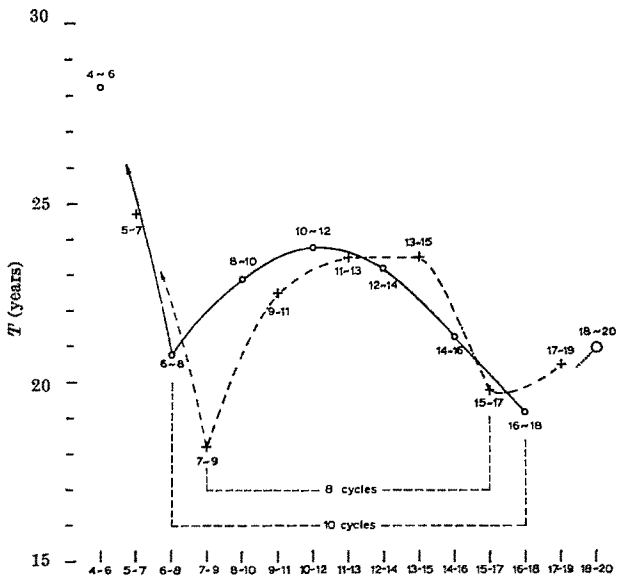


Fig. 3

found if we consider the time interval between the successive maxima of cycles Nos. $N = 2n + 1$ ($n = 2$–9) and $N = 2n$ ($n = 2$–9). This is clearly shown by Fig. 3, where the continuous line represents the time interval between the maxima of cycles No. $N = 2n$ ($n = 2$–9), that is, cycles Nos. 4–6, 6–8, . . ., 16–18, while the broken line represents the same interval for cycles No. $N = 2n+1$ ($n = 2$–9), that is, cycles Nos. 5–7, 7–9, . . ., 17–19.

We thus conclude that the cycles with different polarity in the sunspot magnetic fields also show different variation of the time of rise T_R as well as of the time interval between the successive maxima.

Unfortunately, even on the basis of these periodicities it is not possible to determine with certainty the value of T_R which corresponds to the current cycle No. 20, because of the fact that the amplitude of the variation of the values of T_R changes from one eight- or ten-cycle period to the other. From Fig. 2*b* it can be guessed, however, that the value of T_R for cycle No. 20 will probably not be less than 3·5–4 years. Therefore, if we take into account that the minimum of cycle No. 20 occurred in 1964·7, we come to the conclusion that the next maximum will happen not earlier than in 1968·2–1968·7. We can get an analogous result also from the continuous line of Fig. 3 that gives the variation of the time interval between the successive maxima of solar activity for the cycles No. $N = 2n$ ($n = 2$–9). From this figure we can guess that the time interval between the maxima of cycles Nos. 18–20 will probably be not lower than 21 years. Therefore, if we take into account the fact that the maximum of cycle No. 18 occurred in 1947·5, we conclude that the maximum of cycle No. 20 will not occur earlier than 1968·5.

In a forthcoming paper the probable values of the relative sunspot numbers (R) and the areas of the sunspots (A), umbrae (u) and faculae (f) for the successive years of the cycle No. 20, computed with the help of analytical relations (Xanthakis, in the press), will be given.

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METEOROLOGY

Polar Lobe of the Quasi-biennial Stratospheric Wind Oscillation

EVIDENCE was first reported in 1960 (by Reed[1] and Ebdon[2]) of a wind oscillation in the tropical stratosphere possessing a quasi-biennial rhythm in the zonal component. As more adequate wind data became available during the following years, the existence, beyond the 1953–59 epoch, of an equatorial oscillation of about 26 months average period and 20-knot maximum amplitude (at *c.* 25 km height) was established. (A comprehensive review has already been provided by Reed[3] of the stratospheric zonal wind oscillation and the similar cycles in stratospheric temperature and total ozone which were later discovered.)

The basic features of the structure of the wind oscillation are: (i) a vertical downward propagation of the (temporal) maximum amplitude at a rate of about 1 km per month and disappearance at the tropopause level; (ii) maximum amplitude at the equator and rapid amplitude attenuation to 15° N. and S.

Reed demonstrated that phase relations between zonal wind, temperature and ozone (serving as an indicator for meridional-plane circulations in the lower stratosphere) meet the criteria of a perturbation of the tropical stratosphere in thermal-wind balance.

Tucker[4] found evidence of quasi-biennial cycles in the divergence of eddy-transports of momentum in the tropical belt of the stratosphere. His results helped to elucidate the problem as to how, with west winds maintained for many months, absolute angular momentum is conserved in the vicinity of the equator.

No adequate theories have so far been propounded to explain the origin of the oscillation of tropical winds which is complicated by features such as asymmetry in pole-ward attenuation and the emergence of similar periodicity in the data for some middle- and high-latitude stations.

Although the sparseness of observations in southern middle latitudes means that a less conclusive analysis can be made than in the northern hemisphere generally, soundings in the Australasian and South American sectors, and over Antarctica, have yielded evidence of a more pronounced extratropical oscillation. There is, for example, a sharp increase in the temperature amplitude at the 50–60-mbar levels (*c.* 20 km) from middle latitudes to Macquarie Island (54° S.)[5], and a similar increase to Hallett (72° S.)[6] and McMurdo Sound (78° S.)[7]. Furthermore, Sparrow and Unthank[8] have reported a comparatively strong quasi-biennial zonal wind oscillation at 60,000 and 70,000 ft. over Hobart.

The existence of a polar lobe would have an important bearing on external theories which postulate a direct drive of the tropical oscillation by extraterrestrial effects such as fluctuations in ultra-violet radiation, possibly connected with the spectrum of sunspot number (Shapiro and Ward[9]). (Such theories must also deal with the meridional interactions between the polar and subtropical stratospheric régimes.) Impulsive modes of heat transfer associated with the breakdown of the stratospheric polar-night vortex in late winter or spring may have a particularly important bearing on the processes involved. Investigations conducted at the International Antarctic Meteorological Research Centre in Melbourne[10] have provided some evidence of alternating modes in stratospheric spring warming during the years 1958–62.

The purpose of this report is to discuss some novel features of the wind régime in high southern latitudes. There is evidence that during 1958–62 marked 24-month cycles occurred in the meridional wind component at 60,000 ft. over Wilkes (Australian Antarctic Research) Station at 67° S. This can be seen in Fig. 1 from the

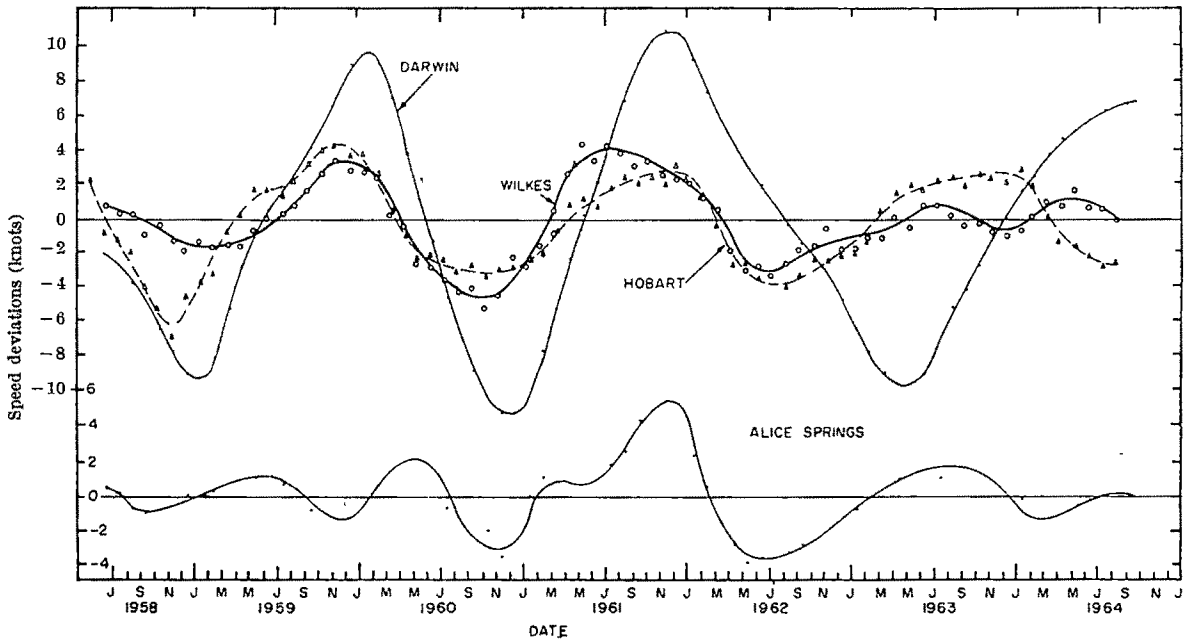


Fig. 1. Oscillations of the zonal wind at 70,000 ft over Darwin (12° S) and Alice Springs (23° S.); at 60,000 ft. over Hobart (43° S.); and of the meridional wind at 60,000 ft. over Wilkes (67° S.) Ordinate scale gives the deviation of a 12-month from a 24-month moving average of mean monthly components, a filtering device for the suppression of short periodic fluctuations and the elimination of mean annual waves and long-term trends in the time series (July 1957–June 1965). A positive deviation indicates excess of west or south wind

curve for Wilkes—peaks and troughs of the 1959–62 oscillation occur between winter and late spring. The latter is broadly in phase with the zonal wind oscillation at 60,000 ft. over Hobart, but leads in phase the tropical zonal wind oscillation by several months. This is shown by way of example at 70,000 ft. over Darwin where it undergoes a considerable lengthening of period after 1962.

In the curve for the zonal wind at Alice Springs, which mainly demonstrates the pole-ward attenuation of the tropical oscillation, the occurrence of a 24-month cycle between 1960 and 1962 is apparent. While in phase with the tropical oscillation at Darwin, this subtropical oscillation is later damped away in a manner strikingly similar to the fading of the oscillation at Wilkes.

Attenuation at Wilkes and Alice Springs is apparent during the middle of 1963. This suggests the possibility of a connexion with the large reduction in direct solar radiation at southern hemisphere stations following the eruption of Mount Agung, Bali (8° S., 115° E.) on March 17, 1963, as reported by Dyer and Hicks[11] for Aspendale (Southern Australia) and Flowers and Viebrock[12] for the South Polar Station.

A detailed correlation analysis is being carried out of the coupling mechanism between quasi-biennial oscillations in polar and tropical latitudes of the stratosphere. An investigation of relevant connexions in the northern hemisphere would seem highly desirable.

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GEOPHYSICS

Transfer of Northern Hemisphere Fall-out to the Southern Hemisphere

THE detonation of a low-yield nuclear device in Western China at 1900 h, October 16, 1964 (New Zealand time), was reported, and tests for barium-140 were included in our routine investigations of fall-out at Gracefield, Wellington (41° 14′ S., 174° 55′ E.), to identify debris from this test. Barium-140 was detected in rainwater samples collected between December 1, 1964, and January 1, 1965, and the deposition was 50 ± 10 mc. per square mile. It would appear, therefore, that debris from this first Chinese bomb took between 45 and 76 days to reach Wellington.

Monthly samples of rainwater taken from January to May 1965 gave only background levels of barium-140, but for the sample collected between June 3 and July 2, 1965, the barium-140 deposition value was 30 ± 5 mc. per square mile. A second Chinese bomb test was reported to have taken place on May 14, 1965. Thus the debris from this second test took between 19 and 48 days to reach New Zealand.

Previous occurrences of barium-140 in New Zealand rainwater have been reported in this journal[1]. Rain samples collected between February 13 and March 26, 1960, contained barium-140 assumed to be from the French atomic bomb test at Reggan in the Sahara (27° N. 0°) of February 15, 1960. The barium-140 in the sample collected during May 1–3, 1960, followed the French low-yield explosion of April 1, 1960, and similarly an atomic bomb test on December 23, 1960, at Reggan gave barium-140 in the rain sample collected at Gracefield between January 24 and 31, 1961.

After the French tests, the Soviet Union began a series on September 1, 1961, using the Novaya Zemlya site (approximately 75° N., 60° E.) mainly for megaton yield tests and the Semipalatinsk area (50° N., 80° E.) for low and low-intermediate yield explosions. No barium-140 from the Soviet experiments was detected in monthly rain samples collected between September 1961 and April 1962, but, as was to be expected, new fall-out was evident in the May 1962 sample after American tests began on April 25, 1962, in the South Pacific area at

Christmas Island (4° N., 157° W.). Deposition of barium-140 at Gracefield reached 50,000 μc. per square mile for May 1962.

To summarize, barium-140 has been found in rainwater at Wellington a few weeks after low-yield nuclear explosions from test sites south of about 50° N. It is reasonable to assume that this trans-equatorial movement of nuclear debris takes place by way of the troposphere. It is surprising that barium-140 from low-yield tests at Semipalatinsk has not been detected in New Zealand rain. This test-site at about 50° N., 80° E., is roughly 10,000 miles from Wellington, bearing north-west. The Chinese tests, from which barium-140 has been noted, were reported as being carried out "somewhere in Sinkiang, Western China". The middle of this province is about 9,000 miles from Wellington, again bearing to the north-west. It seems likely that debris from the Semipalatinsk tests was injected into the stratosphere. Barium-140 from Reggan, more than 13,000 miles from Wellington lying west-north-west, has been detected here.

Strontium-90 deposition at Gracefield has shown a marked increase since about July 1964, as is shown in Fig. 1.

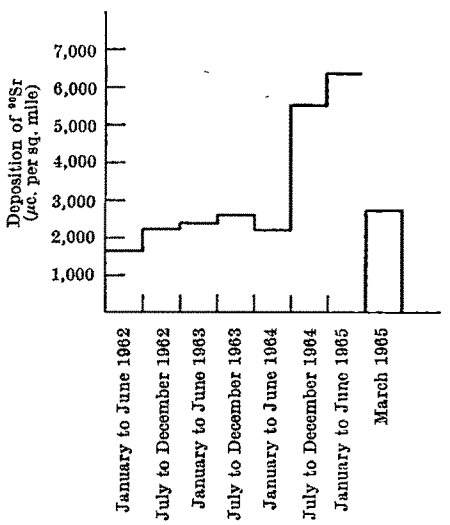


Fig. 1. Deposition of strontium-90 in rainwater at Wellington, New Zealand, 1962–65

The most likely source of this increase is the last major series of tests carried out from early August to the end of December 1962 by the Soviet Union, when many megatons of nuclear energy were put into the stratosphere. Whether this debris returned to the troposphere above the Northern Hemisphere, and crossed the equator in the troposphere, or whether the trans-equatorial movement was in the stratosphere cannot be determined from our available data. Another instance of the transfer is afforded by the United States *Starfish* shot of July 7, 1962. In order to investigate the movement of nuclear debris at very high altitudes, a device was detonated 400 km above Johnston Island (17° N., 169° W.) and cadmium-109, a radioisotope not normally present in nuclear debris, was included as a marker. As reported previously in this journal[2], cadmium-109 was detected in rainwater collected at Gracefield during September 1964, but could have been present in earlier samples. Here again we do not know whether the actual passage of the fall-out across the equator took place by way of the troposphere or the stratosphere, because we have not heard of cadmium-109 being detected in rainwater in the Northern Hemisphere.

I thank the Director of the Institute of Nuclear Sciences, Department of Scientific and Industrial Research, New Zealand, for his interest in these investigations.

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Isotopic and Alkali Measurements from the Vema Seamount of the South Atlantic Ocean

THE Vema Seamount is an isolated volcanic pile which rises from the ocean floor near the centre of Cape Basin at 30° 38′ S., 8° 21′ E., approximately midway between the Walvis Ridge and the South African mainland[1]. It is approximately 1,500 miles north-east of Gough Island and 2,300 miles south-east of Ascension Island, which lie on the mid-Atlantic Ridge, and 1,500 miles south-east of St. Helena Island, which lies close to the Ridge. Isotopic measurements have previously been obtained for samples from all three localities[2,3].

The Seamount was explored extensively by Simpson and Heydorn[1]. It extends for about 5 square miles at approximately 40 fathoms and consists mainly of tuffaceous material. The present sample was taken by Simpson from Collins Peak, which rises to within 14 fathoms from the main plateau. It is a dense green semi-glassy phonolite which was sent to Dr. Ian McDougall for potassium–argon dating, and yielded an age of 11 m.y.[1] which must be regarded as minimal because of the altered state of the sample. Analytical details are included here with his permission.

The lead was isolated by vacuum volatilization[4] and solvent extraction during which the approximate lead concentration (48 p.p.m.) was estimated. A steady lead-ion beam of approximately 5×10^{-11} amp was obtained in a 12-in. radius of curvature, 60° sector single-focusing mass-spectrometer, using single filament thermal-ion source and a single plate collector. Ratios were obtained by the usual magnetic peak switching procedures, using voltage-to-frequency conversion and digital printout[5]. The strontium isotope measurement was made on the same machine using procedures described elsewhere[6,7]. The strontium and rubidium abundance data were obtained by courtesy of Dr. A. J. R. White, using X-ray fluorescence with precise mass absorption corrections, and the potassium analysis was made by flame photometry[8,9]. A. Turek wrote the programme for treatment of the lead results; M. J. Vernon extracted the strontium sample. Results are listed in Table 1.

From the lead isotope plot of Fig. 1, it can be seen that this sample provides yet another example of an "anomal-

Table 1. ISOTOPIC AND GEOCHEMICAL MEASUREMENTS ON THE COLLINS PEAK PHONOLITE

Lead isotope data

206/204	207/206	208/206	207/204	208/204
19·82	0·8015	2 021	15·88	40 05

Potassium-argon data

Potassium (%)	*Argon-40 / Potassium-40	Argon-40 atm (%)	Age
2·80	$6{\cdot}459 \times 10^{-4}$	35·3	11 0 ± 0 3 (minimal)

$\lambda_\beta = 0{\cdot}584 \times 10^{-10}$ per year; $\lambda_k = 4\ 72 \times 10^{-10}$ per year; potassium-40 = $1{\cdot}19 \times 10^{-2}$ atom per cent

Rubidium–strontium data

Rubidium (μg/g)	Strontium (μg/g)	$^{87}Sr/^{86}Sr$
104	446	0 7034

Geochemical data

Potassium–rubidium	Approximate lead concentration (μg/g)	Estimated uranium-238 / lead-204
269	48	< 8·9

* Radiogenic portion.

ous" oceanic volcanic lead[2,10], in that it does not fall anywhere near the 11 m.y. isochron: it is enriched in lead-206 and in lead-208 to an extent slightly greater than that for the volcanics from Ascension Island[2]. A limit on the uranium content may be estimated, from the published range of potassium–uranium ratios on volcanic extrusives[11], to be less than 5·6 p.p.m. Thus, because the lead content is approximately 48 p.p.m., the uranium-238–lead-204 ratio must be less than 8·9. Since this is not greater than the accepted "primary ore–lead source" of about 8·99 (ref. 12) the "excess" lead-206 cannot have grown in since eruption and must be characteristic of the lead of the source material. The rock is an alkaline differentiate (phonolitic) as are some of the Ascension rocks (trachytic), but it is different from some of the other oceanic extrusives for which excessive lead-206 has been reported (such as Gough[2], Ascension[2] and Easter[10] Islands) in that its source does not lie on a seismically active mid-ocean ridge. However, if Wilson[13,14] is correct in his recent suggestions about the generation of the Atlantic Ocean and the phenomenon of transform faulting, then it follows that the Vema Seamount and also St. Helena may have been on or near the ridge at the time of eruption.

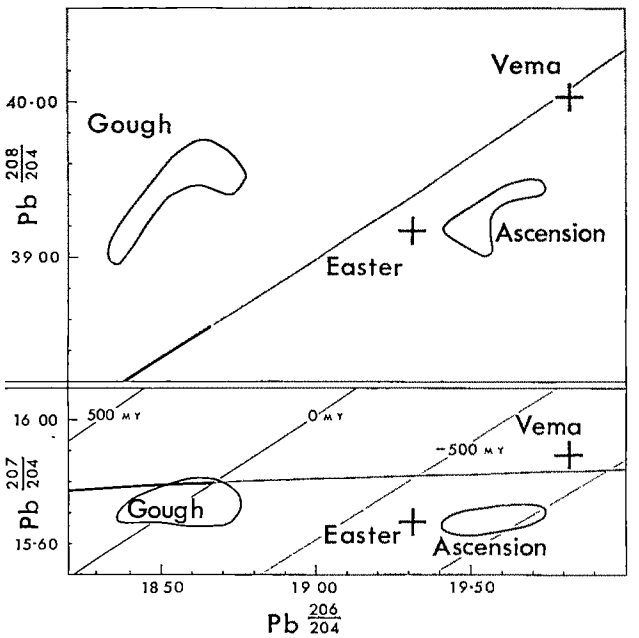


Fig 1. Lead isotope ratio plots of the Collins Peak phonolite showing its relationship with those from other oceanic volcanics The heavy line growth curve and isochrons, and the dotted extensions, have been constructed from the parameters·

primordial lead: $\frac{206}{204} = 9{\cdot}56$, $\frac{207}{204} = 10\,42$, $\frac{208}{204} = 29\,71$,

$t_0 = 4\,55 \times 10^9$ years, $V = 8{\cdot}99$; $W = 35\,55$

The strontium-87–86 ratio of 0·7034 ($^{86}Sr/^{88}Sr = 0{\cdot}1194$) lies within the narrow range of 0·702–0·706 reported for oceanic volcanics[2,6,15,16], being closer to the lower limit for those which are radiogenically unenriched. The low ratio of rubidium to strontium (0·23) and the low age preclude the possibility of any significant contribution to the strontium-87 by radiogenic decay.

Some enrichment of rubidium content over potassium could reasonably have been expected because of the comparatively high (2·8 per cent) potassium content. This is particularly so because its glassy nature suggests that the sample could be a residual liquid in which the rubidium would concentrate because of its low solubility in the lattice of crystallizing sodium-rich potassium feldspars[17,18]. Nevertheless it yields a potassium–rubidium ratio of 269 which cannot in any way be regarded as anomalous, although it is close to the lower limit of those reported for oceanic volcanics.

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GEOLOGY

Palaeocurrent Directions in the Upper Cambrian of North Wales

RECENTLY, Evans, Garrett and Whitaker[1] recorded some palaeocurrent measurements from the Maentwrog and Ffestiniog beds of North Wales. They suggest that their measurements show that currents came generally from the south-south-west during Maentwrog and Ffestiniog times. The authors make no acknowledgment of the work of Kopstein[2], who reached the same conclusion using, in part, the same criteria and localities (*op. cit.*, p 81, and appendix 3). I have made a detailed investigation of the palaeogeography of these and other Cambrian sediments in North and South Wales[3] and have recorded the presence of palaeocurrents from the south-westerly quadrant from widespread localities in the Upper Cambrian rocks of North Wales[4,5].

The statistical, sedimentological and structural problems involved in the production of a viable interpretation of the palaeogeography of these rocks are, however, far more complex than Evans *et al.* imply. Their map (ref. 1, Fig. 1) shows palaeocurrent readings based on the orientation of cross-stratification, but neither the exact type of cross-stratification nor the statistical significance of their results is indicated. They show, for example, westerly and south-westerly derived palaeocurrents based on cross-stratification measurements at localities to the south of Bethesda. I have made a detailed study of the exposures in question. Alpha, beta and xi cross-stratification types[6] are common and I have taken eighty palaeocurrent readings from cross-strata. The wide spread of these results suggests that any mean or resultant palaeocurrent direction deduced from measurements of cross-stratification at these localities will have little or no statistical significance. Bassett and Walton[7] have similarly studied the orientation of small-scale cross-lamination from upper portions of grit and siltstone beds from the Hell's Mouth Grits of St. Tudwal's Peninsula. The palaeocurrent directions showed a large standard deviation (± 57°). This indicated an absence of preferred orientation which was confirmed by a χ^2 test. They concluded that "this mean has little or no significance". The validity of palaeocurrent arrows deduced by Evans *et al.* from cross-stratification (ref. 1, Fig. 1) is, in the absence of statistical treatment, therefore open to question.

The record[1] of flute cast(?s) showing a south-westerly derived current at localities to the south of Bethesda is interesting. I have also observed the orientation of flute casts at these localities: my measurements (thirty-one) show considerable variation, but in general suggest that palaeocurrents travelled from a north-easterly quadrant.

Evans *et al.* also refer to asymmetric ripples in the Ffestiniog grits of Cwm Graianog. In addition to the asymmetric type mentioned by them, there are four other types—symmetric, flat topped, interference and cuspate. They describe the direction of the asymmetric ripples as anomalous because they do not lie at right angles to the palaeocurrent trend postulated from their observations on sole markings. They suggest that the asymmetric ripples may be due to surge waves set up by the nose of a turbidity current which are reflected back from the marginal slopes of the trough. This explanation presupposes that these beds are turbidites. It also implies that they were deposited in relatively deep water, since the turbidity currents are unlikely to have travelled far up the marginal slope of 'the trough'. Williams[8], however, has suggested that these beds were deposited in shallow water. I have found[3] sedimentological evidence which indicates, by comparison with modern environments, deposition within reach of wave or tidal scour. These sediments also contain a wide variety of trace fossils. The origin of many biogenic structures is uncertain, but Seilacher[9] has concluded that they can sometimes be used as an indication of the depth of water during deposition. I have observed numerous examples of 'trilobite tracks', *Repichnia* (Cruziana) and *Cubichnia* (Cruziana). These are characteristic of the Cruziana Facies which Seilacher (*op. cit.*) considers to be deposited above wave base. The biogenic and non-biogenic sedimentary structures both, therefore, indicate deposition in shallow water.

There also appears to be little evidence to suggest that these beds are turbidites. I would not question the conclusion of Evans *et al.* that they are greywackes but, as Cummins[10] has insisted, not all greywackes are turbidites. Dzulynski and Walton[11] have also emphasized that non-biogenic sedimentary structures found in turbidites also occur in non-turbidite sediments. They conclude that "the operation of turbidity currents is suggested above all by evidence for deep water conditions combined with indications of violent and episodic inflow of coarse clastics". The shallow water features associated with these grits clearly reduce the possibility of a turbidite origin. The evidence suggests that these are shallow water greywackes. Ripple formation would, therefore, probably be at least partly wind controlled[12]. The trend of the asymmetric ripples would thus not be expected to maintain a definite relationship to any sole markings that might be developed in these or other Ffestiniog sediments. These particular grits seem to be a local development confined to the north-western part of North Wales. They do not appear to be connected with the south-westerly derived palaeocurrents observed elsewhere[2–5].

Evans *et al.* make no mention of the structural problems involved in interpreting the palaeogeography of these rocks. They state, for example, that in re-orientating the palaeocurrent directions they have allowed for dip and, where possible, plunge. This method of re-orientation is clearly invalid if the arcuation of the cleavage in North Wales is due to refolding about a large-scale F_3 fold, as Helm, Roberts and Simpson[13] have suggested. Evans *et al.* produce no evidence on this point, which is, however, considered elsewhere by me[3].

It must therefore once again be stressed that the structural and palaeogeographical complexity of these sediments is such that it would be unwise to arrive at any regional conclusions on the basis of the evidence so far published.

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Quicksand Structures in the Bedded Tuffs of Great Langdale, Westmorland

QUICKSAND structures similar to those described by Selley (ref. 1, Fig. 1*a* and *b*) abound in the bedded tuffs (Ordovician) of Great Langdale. These sediments appear to have been highly susceptible to the type of behaviour suggested by Shearman[2] and called by him "'quicksand' movement". These authors described examples in Torridonian[1] and Devonian[2] sandstones where this process, involving the repacking of sand grains and the expulsion of excess pore-water from the sediment, had, they assumed, operated "penecontemporaneously". It appears that both authors consider that this process took place below a thin (less than 1 m ?) cover of sediment.

Fig. 1 illustrates a structure in the bedded tuffs thought to have been produced by "quicksand" movement. Laminated mudstone rests on sandstone. A rectangular embayment projects upwards into the mudstone. Within this embayment, and also along the base of the laminated mudstone, angular, sharply bounded fragments of the laminated mudstone occur dispersed in the sandstone. Sand grains have not been pressed into the mudstone fragments.

From this structure it is concluded that: (*a*) the sand was mobilized after the laminated mud had been compacted to mudstone; (*b*) mobilization must, therefore, have occurred beneath a sediment overburden thick enough to have compacted the mud to mudstone. This overburden would also have provided the hydrostatic pressure which enabled the mobilized sand to penetrate into the mudstone.

This structure shows that sand can remain loosely packed during the accumulation of sufficient sediment

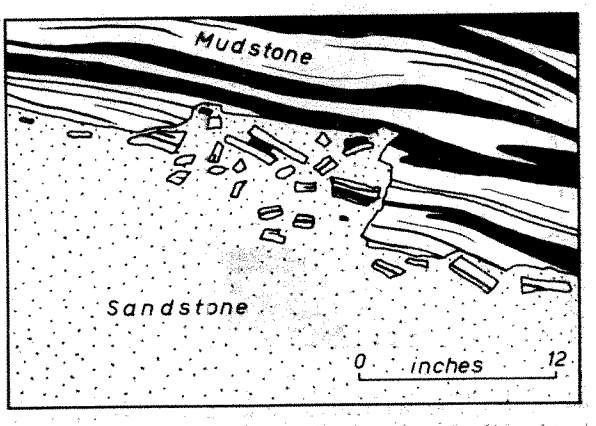


Fig. 1. Structure in bedded tuffs believed to have been produced by "'quicksand' movement". Simplified diagram from a photograph

overburden to compact overlying mud to mudstone. The behaviour of the sand in a manner called by Shearman "'quicksand' movement" may occur at a time so long after its deposition as to be outside the broadest interpretation of the meaning of 'penecontemporaneous'.

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Sandstones of Sublittoral (Neritic) Facies

A SANDSTONE unit which is considered to be unique to, and diagnostic of, a sub-littoral (neritic) environment occurs at many levels in the stratigraphic column. It shows several features which allow it to be clearly distinguished from units either associated with fluviatile sequences, or supposedly deposited from turbidity currents.

Characteristically, each unit (Fig. 1), which appears generally to be up to 1·0 m thick, is fine- to very fine-grained, and moderately to well sorted, with an overall upward decrease in the median grain size of the quartz of about 0·5φ. The base is sharp, frequently flat or undulose, and lacks sole structures, such as flute casts, which indicate a unidirectional current flow. Stratification is typically sub-horizontal or at a very low angle, whereas cross-stratification is much less common. Primary current lineation is difficult to establish because of the fineness of the grain, but has been observed in some instances. The top of the unit may be strongly bioturbated, giving a transition zone to the overlying clay grade sediment, or may show oscillation ripple (often ripple-drift), or a gradation by decrease in grain size to clay grade sediment. Where micaceous minerals are abundant, the upper part of the unit is finely laminated. Penecontemporaneous erosion has often removed the upper, finer-grained portion of a unit prior to the deposition of the succeeding sediment.

Fossils may be numerous on partings, although, apart from the trace fossils, they are not in a life position. The common fossiliferous sandstones, yielding, for instance, in the Lower Palaeozoics of Wales and the Welsh Borderland,

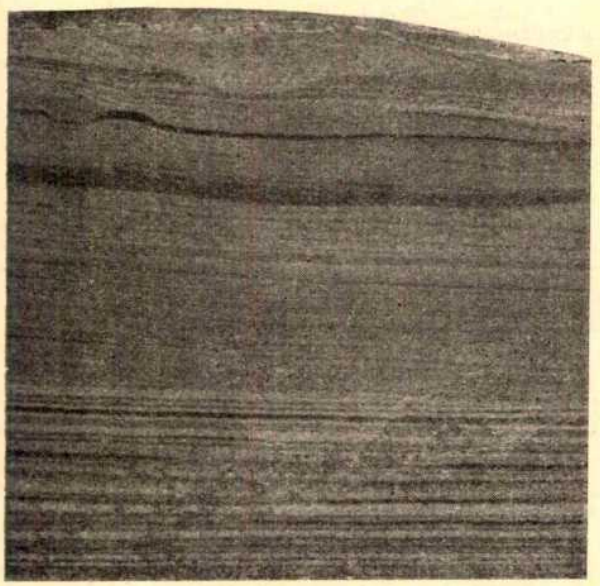


Fig. 1. Single unit (×1) from upper part of Baggy Beds (Upper Devonian), North Devon (*SS* 42254026). Face approximately east–west, and showing sub-horizontal stratification at base, grading into straight ripple-drift (section parallels ripple crests) with oscillation ripple above. A thin seam of clay grade sediment is present below a scoured surface marking the base of a second unit

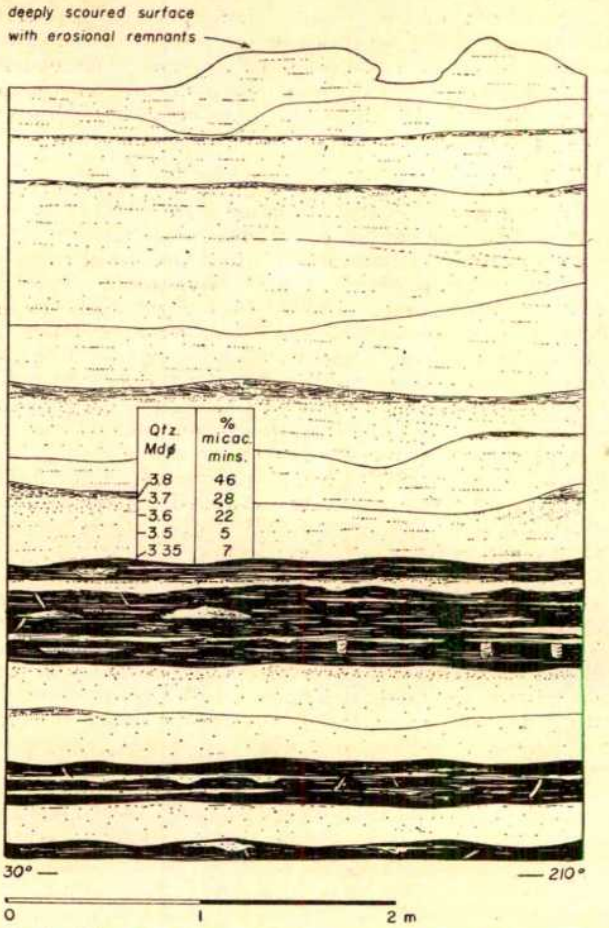


Fig. 2. Diagram of co-set developed in the Baggy Beds (Upper Devonian), North Devon (*SS* 42114039). The units are of well sorted, very fine, calcareous, feldspathic sandstone. The base follows several single units with interbedded siltstones and shales. The upper surface is strongly scoured and is followed by sandstones considered to represent a shallow water environment

a trilobite-brachiopod fauna, are associated with this sandstone unit, although the more fossiliferous sandstones generally show cross-stratification. Plant debris is uncommon at all levels. Although bioturbation is for the most part only developed at the top of the unit, it may involve more or even most of the unit. It is not generally possible to determine current direction in a unit, unless cross-stratification is developed. When this is only weakly developed a polarity of provenance may well be suggested by the main part of the unit. Load casting is uncommon. This is probably an indirect result of the degree of wave action.

Units occur either as single sets in a predominantly clay grade sequence, or grouped into co-sets (Fig. 2) of up to at least 55 ft. The latter may be bounded by gradational sequences of single sets, or, with a lower gradational sequence, the co-set may be followed, and probably truncated, by a sequence of sediments considered to represent deposition in shallower water or a fluviatile environment, as with the Fort Collins Member in the Lower Cretaceous of North America[1]. Co-sets seem to be less fossiliferous than individual sets.

The erosion which preceded deposition of the unit may in some cases have been sufficient to cut a series of parallel, and generally straight but sometimes sinuous, grooves of up to 1·2 m in width and 30 cm depth, as in the Baggy Beds (Upper Devonian) of North Devon and the Llandovery Sandstone (Silurian) of the Gullet Quarry, Malvern Hills[2]. In North Devon, on one strike surface 30 m across, more than thirty filled grooves are displayed, some of which merge locally in an irregular manner. The

original lateral extent of individual units is not known, and in several instances penecontemporaneous erosion has determined the present extent. The oil-producing co-set described by MacKenzie[1] has a blanket form recognizable over much of Wyoming and eastern Colorado, but farther south it appears to interdigitate with marine shales.

In several instances in the Baggy Beds, and in the contemporaneous Condroz Sandstone of Belgium[3], where the sediment is calcareous, penecontemporaneous erosion following deposition of the unit has scoured out irregularly shaped hollows 3–4 m in diameter, or removed the unit so as to leave only hummocky erosional remnants similar to the *talards* of Brittany[4]. The scoured surfaces may be followed by sand or clay grade sediment. The groovings and the erosional remnants recall the backwash channelling of recent intertidal mud flats[5], but no evidence whatsoever of emergence has been observed. They are also similar to the submarine scour structures described by Norrman[6] from Lake Vättern, Sweden, and by Seibold[7] from the Baltic, and attributed by both to wave action. There is no evidence that the course of the grooves was influenced by plant growth as in recent aggradational marshes, although in the Baggy Beds, colonies of the tube-building organism, *Diplocraterion*[8,9], may have determined their development to some extent.

Table 1. SOME DEVELOPMENTS OF THE UNIT

Ordovician, Llanvirn. Ffairfach group (iii), Llandeilo (Carmarthen) (*SN* 628212) (ref. 13);
Silurian, Llandovery. May Hill Sandstone, Malvern Hills (*SO* 762381) (ref. 2);
Wenlock. Buckover, near Tortworth (Gloucester) (*ST* 667907) (ref. 10);
Ludlow. Black Cock Beds, Pont-ar-llechau (Carmarthen) (*SN* 727247) (ref. 14);
Devonian, Downtonian. Above Ludlow Bone Bed at Ludlow (Shrewsbury) (*SO* 513741) (ref. 15);
Downtonian. Downton Castle Sandstone (Hereford) (*SO* 454750) (ref. 16);
Givetian. Ilfracombe Beds, Avill Sandstone (Somerset) (*SS* 940405) (ref. 17);
Famennian. Baggy Beds (Devonshire) (*SS* 4240) (refs. 8, 9);
Jurassic, Upper. Kellaways Rock, Cayton Bay (Yorkshire) (*TA* 0784) (ref. 18);
Cretaceous, Lower. Fort Collins Member and Skull Creek Shale of Dakota (ref. 1);
Eocene, London Clay, Whitecliff Bay, Isle of Wight (*SZ*639860).

Four characters may be considered especially diagnostic of the facies unit: (1) The unquestionably marine (though sometimes brackish) origin of the unit, as indicated by the associated fauna. (In the section showing the unconformity between marine Wenlock and the continental Upper Old Red Sandstone at Buckover, near Tortworth (Gloucestershire), the unconformity can readily be marked at the termination of the sandstone and siltstone units of the type described, although Curtis and Cave[10] comment that its position is not at first sight obvious.) (2) The form of the sole, and the style of penecontemporaneous erosion. (3) The graded bedding. This recalls the grading associated with turbidites and deep-sea sands[11], but the main part of the unit never shows such a considerable proportion of clay grade. The general absence of bioturbation in the main part of the unit suggests that sediment deposition was too rapid for organic colonization. (4) The form and variation of the upper part of the set.

No unidirectional current system such as a turbidity current or a river, can be invoked as the cause of the scour preceding and succeeding deposition of the unit, or can be considered to have operated during deposition of the upper part of the unit. In a marine sequence, only wave action remains as an effective agent which could have operated before and after deposition, and at least during the deposition of the upper part of the unit. Because of the amount of sediment represented by the main part of the unit it is unlikely that this was deposited without associated current action. Wave action has been invoked by Daly and Passega[12] as the cause of turbidity currents, but in these units there is no evidence that actual currents were produced. This could have been because the sedimentary slope was insufficient. The considerable thickness to which the units have accumulated in several examples indicates strong, though not necessarily local, sediment sources. These may have been deltaic, possibly associated with long-shore drift. In North America[1] co-sets can be oil producing, and individual units may well have been important in oil migration in connecting marine clay grade to coarser grade sequences.

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Kink-bands and Related Geological Structures

ANDERSON[1] has proposed that kink-bands develop in response to a maximum principal pressure acting within, or close to, a strong planar anisotropy, and that their orientation is controlled by shear movements on the intra-kink-band folia. He has also stated that these shears make an angle of $\frac{90 - \varphi}{2}$ degrees with the maximum pressure (P_{max}), while the kink-bands form angles of $\frac{90 + \varphi}{2}$ degrees with the same stress axis. This theory has been criticized[2] on the grounds that, in planar anisotropic rocks, the maximum principal stress cannot be given unless the kink-bands are truly symmetrical about the anisotropy, that the known variation in the angle formed by kink-bands about P_{max} was not wholly explained, and that the intra-kink-band movements are a secondary response to a primary direction of shear paralleling the kink-band. It was also stated that Anderson's figured example was not truly conjugate and that the kink-band system in the Ards Peninsula might not be symmetrically arrayed about the cleavage. Anderson's inadequate reply[3] to these criticisms merits a further communication.

Anderson[3] stated that he fully realized symmetrically arrayed conjugate kink-bands comprise a special case and that kink-bands are not necessarily produced by a maximum principal pressure acting exactly along the foliation. I agree that kink-bands result from a maximum pressure acting within or at an angle to the anisotropy, provided that the orientation of one or both of the potential shear-surfaces is such that the displacement would tend to 'open out' the individual folia (Fig. 1). The point which appears to have eluded Anderson is that should P_{max} not be contained within the planar aniso-

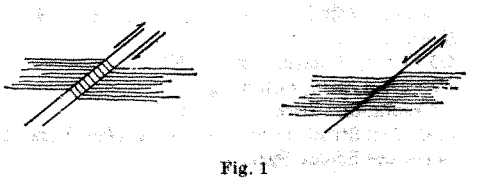


Fig. 1

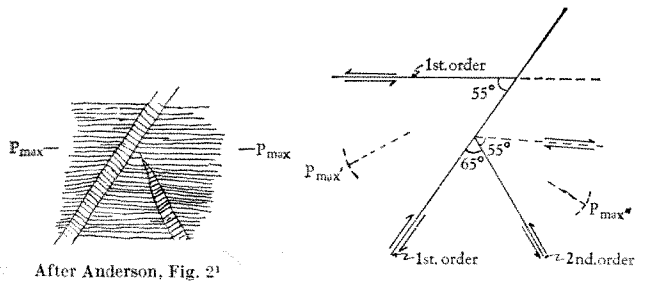
tropy, the consequent rotational strain will result in the kink-bands being asymmetrical about both the anisotropy and the maximum compressive stress. This is the fabric-type [F1 m_1] of Hoeppener[4], who stated that the precise locations of the least and maxima principal stresses cannot be given. It has been shown[5] that in strongly anisotropic rocks the surface of shear-failure will always lie in the anisotropy or between it and the nearest of the planes predicted by the Coulomb–Navier theory. Consequently, varying with the angle of impingement of the maximum compressive stress on the anisotropy, the angle between the plane of practical shear-failure and P_{max} will either be smaller or larger than in a corresponding isotropic system. In view of the difficulty of locating P_{max} in any but a perfectly symmetrical, truly conjugate system of kink-bands, it is meaningless to ascribe values of $\frac{90 - \varphi}{2}$ degrees and $\frac{90 + \varphi}{2}$ degrees to the respective angles made by P_{max} with the intra-kink-band shears and the kink-bands.

Regarding the orientation of the kink-bands in the Ards Peninsula, Anderson[1] wrote: "The vertical dextral kink-bands have a strongly preferred orientation striking N. 15° E., while *most* (my italics, B. M.) sinistral kink-bands strike at about E. 40° S.". Implicit within this statement is that the sinistral kink-bands are less strongly preferred. Thus, my conclusion[2] that the kink-bands are not truly symmetrical would seem justified, and my remarks concerning the statistical evaluation of regional data would appear apposite.

Johnson[6] coined the term conjugate folds to describe "paired reversed folds that are controlled by a pair of symmetrically arranged slip planes which are inclined towards one another and intersect parallel to the . . . fold axes". Should Anderson accept that kink-bands of differing orders or of differing phases of tectonism, provided that they fulfil the orientational requirements, may be conjugate, I am prepared to acknowledge his usage. However, Johnson, as is apparent from his diagram (Fig. 1, ref. 6), envisaged the synchronous development of the conjugate folds, and this is not evident in Anderson's Fig. 2 (ref. 1), where the dextral kink-band is dominant. Such a relationship has been interpreted by Moody and Hill[7] as indicating displacements of differing order, the lower order movement terminating against that of the higher order.

Anderson[3] questioned my usage of shear as applied to the sense of displacement paralleling a kink-band. In this I followed Ramsay[8], whose usage Anderson[1] noted without demur. He[1] also recorded Knill's conclusion that dextral kink-bands may be complementary to contemporary sinistral wrench faults without questioning the implication that kink-bands parallel the complementary direction of shear.

Applying Moody and Hill's[7] work on the development of lower order stress-systems, Anderson's Fig. 2 (ref. 1) may readily be explained in terms of first- and second-order structures. This is illustrated in Fig. 2; φ is taken as approximately 35°, being the value necessary to explain the angle between the dextral kink-band and the cleavage, whereas the angle with which the second-order maximum compressive stress impinges on the first-order displacement-surface is ascribed a value of approximately 87° (Moody and Hill[7] indicated that the angle may vary between 60° and 90°).



After Anderson, Fig. 2[1]

Fig. 2

Fig. 1 (ref. 2) was drawn to illustrate that "I have traced a kink-band along its strike into a single kink-plane . . ." (ref. 2). I readily accept Anderson's[3] correction that "The two kink-planes of the kink-band illustrated converge and pass into a shear-plane . . .", since it emphasizes that a single shear-surface may substitute for a kink-band a feature recorded by Anderson[1]: In some examples shearing has occurred along the kink-plane, but it is not an essential feature and probably post-dates the folding, while unsheared kink-planes passing into faults along their length are not common", but do occur. Thus, Ramsay[8] would appear justified in figuring and describing the axial surfaces of conjugate folds as conjugate shear-planes.

In Fig. 1 (ref. 2) the same sense of displacement is expressed by kink-band, shear-plane, and sigmoidal tension gashes, these structures all being symptomatic of a couple acting along the kink-band. In the development of the kink-band, the shear movement is accommodated by contrary rotation on the intra-kink-band folia which play an essentially passive role in the deformation. Actual displacement on the kink-planes may be expected when the intensity of shearing exceeds the rate of accommodation.

Having quoted extreme values of φ, Anderson[3] remained unable to explain, in terms of his theory, the full range[2] of the dihedral angle enclosed by conjugate kink-bands. He stated that values of less than 90° are uncommon, yet Ramsay[8] reported that kink-bands form on "one or more shear surface which usually (but not invariably) make an angle of less than 45 degrees to the principal stress axis", and by implication suggested that they might not affect the correctness of his conclusions. However, no matter what percentage of the total number of kink-bands they comprise, a theory regarding the mechanism of formation should encompass the available facts.

Anderson[3] concluded that experimental results of Paterson and Weiss[9] "are in good agreement with the geometrical relationships and mechanisms of formation I (T. B. A.) outlined . . .". Certainly, Anderson's modal values appear to have been duplicated, although it is perhaps more significant that the full range[2] of field relationships have yet to be reproduced, but I fail to see how he was able to claim support for his mechanism of formation. Anderson[1] stated that "In a conjugate set of kink-bands the small shear planes make an acute angle about the same major principal stress which bisects the obtuse angle formed by the kink bands. . . . In effect, while shear makes an angle of $\frac{90 - \varphi}{2}$ degrees with the major principal stress . . . kink-bands might be expected to make an angle of $\frac{90 + \varphi}{2}$ degrees with the same major principal stress"; yet in the excellent photographs of Paterson and Weiss (Figs. 2 and 3)[9] the intra-kink-band shears enclose P_{max} in angles exceeding 115°. Anderson presumably must advocate minus values of φ in excess of $-25°$ since he claimed support from this experimental work.

Finally, in his Fig. 6, Anderson[1] showed the intra-kink-band folia varying between 0° and approximately 48° in their angle with the anisotropy. If their orientation is a function of $\frac{90 - \varphi}{2}$, φ must be continuously variable between 90° and a minus quantity; if their orientation controls that of the kink-bands it is peculiar that they change their attitude as shearing proceeds while the kink-band remains obdurately constant.

Anderson's[1] theory was apparently formulated to explain the special case in the Ards Peninsula; it has no general application and I consequently doubt its specific application.

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PHYSICS

Ultrasonically induced Etching of Quartz

SOUND waves have been excited in quartz from 20 kc/s to 3,000 Mc/s. Frequencies of greater than 20 Mc/s are usually excited either by placing a quartz rod in a re-entrant cavity and exciting the surface layer[1], or by exciting 1–10 Mc/s quartz plates at high odd harmonics[2]. Both these methods suffer from the fact that the acoustic power produced is only of the order of mW/cm². A distinct improvement in power level is obtained if the quartz is excited at its fundamental frequency[3]. This frequency is, of course, dependent on the thickness of the quartz plate—the latter is equal to half the wave-length for this frequency in the quartz. Difficulties arise because for frequencies above 10 Mc/s the thickness of the quartz plate must be less than 0·3 mm. However, by conventional grinding techniques, using fine 'Carborundum' powders, thicknesses as low as 150μ can be achieved. To obtain thinner plates, of higher fundamental frequencies, is exceedingly difficult, and requires very expensive machinery. Quartz, because of its brittleness, is easily damaged during grinding, especially because of the large particle size of even the smallest 'Carborundum' particles used. Graded grit sizes average 13μ and may be as large as 40μ. Even after polishing the ground quartz, deep score marks are visible on its surface.

A simple solution to this problem devised in this department is the use of an etchant liquid agitated by 40 kc/s sound waves. The apparatus is shown in Fig. 1. The 40 kc/s transducer must be plated with a material resistant to the acid etchant used. In the case of quartz a suitable etchant is hydrofluoric acid, and the faces of the transducer are plated with copper. A mixture of 40 per cent hydrofluoric acid in water has been found to be the best for etching quartz. For appreciable etching to occur, the vibrating face of the 40 kc/s transducer must be only a short distance, h cm, from the quartz surface. The theory of acoustic streaming, which describes the production of a steady flow of fluid from an alternating pressure field, predicts that the streaming occurs very close to the vibrating surface. The streaming is especially marked in a thin layer of fluid on the vibrating surface. The thickness of this layer at 40 kc/s is calculated to be about 10μ, which is approximately equal to the experimentally established distance h already mentioned. This fact clearly implicates acoustic streaming as the agent for enhancing etching. Nyborg[4] has commented that acoustic streaming would greatly increase the rate of such mass transfer processes, and has ascribed the removal of loosely adherent films to boundary layer streaming[5]. This is precisely the effect needed here.

Two precautions must be observed. The first concerns the production of small bubbles in the sound field which cause large local increases in fluid velocity and thus in mass transfer processes occurring near their surfaces[6].

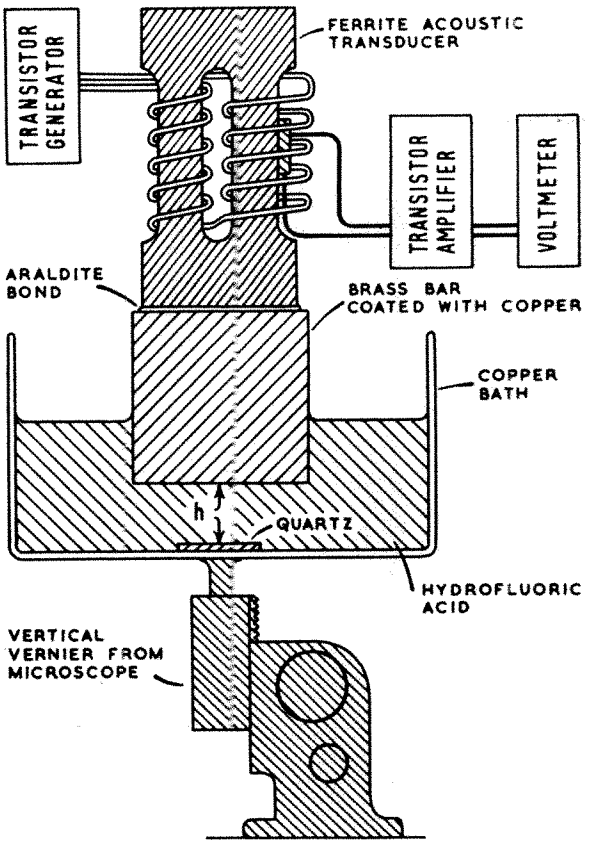


Fig. 1

This can be avoided by the use of very low acoustic powers from the transducer—certainly no higher than 0·5 W/cm²—or by establishing static pressures up to 10 atm. in the treatment vessel. Secondly, the diameter of the transducer head must be more than three times the diameter of the plates being etched. The reason for this is that the acoustic field must be relatively uniform over the quartz surface, for the sharp fall which occurs at the perimeter of the transducer must be avoided.

By this method, quartz sections 50μ thick have been readily obtained here for frequencies up to 90 mc/s. Plates for even higher frequencies could doubtless be obtained by the etching technique described, which could indeed be utilized for many purposes where the production of very thin plates is a requisite (for example, hard biological tissues, metals).

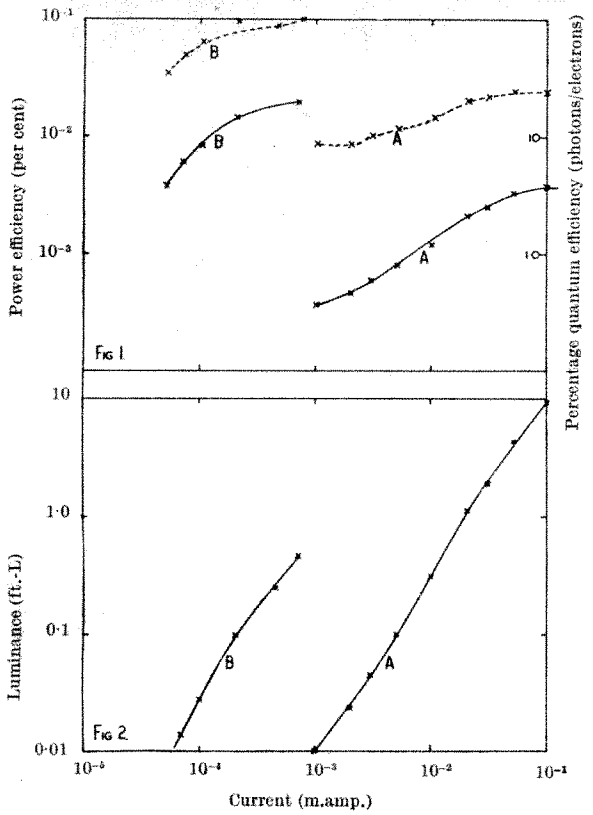
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Direct Current Electroluminescence in the Zinc Sulphide/Copper/Manganese System

WORK on electroluminescence at the AEI Central Research Laboratory has been aimed at producing layers for a solid-state image intensifier. These usually consist of a combination of an a.c. electroluminescent layer with a photoconducting layer. To avoid the problems associated with capacitive coupling we have concentrated our attention on electroluminescent layers which operate



Figs. 1 and 2. Efficiency and luminance in the zinc sulphide/copper/manganese powder phosphor system. *A*, Air fired; *B*, nitrogen fired. . . . . , Power efficiency; ——, quantum efficiency

under d.c. conditions. Initial investigations were directed at the production of thin films showing good d.c. electroluminescence; the results of these investigations have been reported elsewhere[1–3], but subsequent investigation has shown that brighter and more efficient cells could be prepared using powder phosphors.

Although there are many reports on the a.c. electroluminescent properties of zinc sulphide powders[4,5] (usually activated with copper and/or manganese and halogen), there is very little information on the d.c. electroluminescent characteristics of such materials. The bulk of the investigation of d.c. electroluminescence in zinc sulphide has been on thin films[6–8] because it was thought that thin films would be more efficient, have higher resolution, and yield more information as to the fundamental processes occurring, due to the fact that the effect of grain boundaries would be obviated. Although some workers have studied the d.c. electroluminescent properties of zinc sulphide, copper manganese activated powders[9,10], no quantitative details are available as to the efficiency of the systems. The lack of published data on powder d.c. electroluminescent phosphors is probably due to the great care that is required in the preparation of such phosphors. A phosphor showing good a.c. electroluminescence, for example, in the zinc sulphide copper chloride system can quite readily be prepared. To obtain some sort of electroluminescence it does not matter if an intergranular or a small surface excess of copper or halide is present, or if a very small proportion of highly conducting powder particles is produced, as an insulating layer is always placed between the electrodes. However, if such a phosphor is tested under d.c. conditions such irregularities would form 'shorts' and the panel would burn out immediately. With control of the starting material and careful control of the addition of halide, copper and manganese, the firing temperature and atmosphere and suitable particle size separation, useful d.c. electroluminescent phosphors can be prepared.

We have recently made d.c. electroluminescent cells consisting simply of such specially prepared zinc sulphide powders in an organic matrix sandwiched between two conducting electrodes; these electrodes were, respectively, glass coated with tin oxide and graphite. The quantum efficiency and brightness that can be achieved with these panels are illustrated in Figs. 1 and 2. It will be noted that the firing atmosphere has a marked effect on both the brightness and efficiency characteristics. The quantum efficiency that can be achieved with these panels can reach 20 per cent and the power efficiency 0·1 per cent. We have observed that the current voltage relationship is exponential over several orders of the applied voltage. All measurements were made at room temperature.

It can be seen that high efficiencies can be obtained with this system over a large range of current and voltage. It will also be noted that the efficiency of this system is considerably higher than that reported by Thornton[6] (using d.c. electroluminescent thin films), who reported an efficiency at 10 ft. L of 0·05 lm/W $\approx$ 0·01 per cent. It is felt that these preliminary results are very pertinent to the elucidation of the mechanism of the electroluminescent processes in zinc sulphide, and with further analysis will indicate the actual mechanism of the processes occurring.

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METALLURGY

Austenite–Martensite Interface Dislocatio in Stainless Steel

WHEN 18 chromium : 8 nickel austenitic stainless ste is plastically deformed at room and sub-zero tempera tures, ε (hexagonal closest packing) and α′ (body centred cubic) martensites are formed. The ε phase forms as the result of the propagation of Shockley partial dislocations on every second {111} γ plane and appears in the form of thin disks having a {111} γ habit[1]. The mechanism of the γ → α′ transformation in this steel is not yet entirely understood. Venables[1] suggested that α′ martensite nucleates from the ε phase and grows by the motion of a parallel grid of screw dislocations such as that postulated by Frank[2]. Bogers and Burgers[3] showed, by geometrical arguments, that the shears necessary for the transformation correspond to 'well-known' (that is, Shockley partial) dislocations and also suggested the possibility that the transformation proceeded by way of the motion of a parallel array of screw dislocations. Lagneborg[4] found that deformation induced α′ nucleated preferentially at the intersections of two ε disks or at the intersections of active slip planes with ε disks, and proposed that the martensite-like region existing near the centre of a Shockley partial dislocation was a favourable nucleation site. He did not discuss a mechanism for the subsequent growth of α′. To our knowledge, no direct evidence for

ated dislocation interfaces has been observed. a recent investigation of the effects of shock loading romium : 8 nickel stainless steel, an experimental ion was made that showed a matrix-martensite composed of an array of dislocations. This ication presents this observation and discusses ce to the previously postulated transformation ms.

pecimens used were type 302 austenitic stainless lates of the following composition (in weight per : carbon, 0·054; manganese, 1·04; phosphorus, ; sulphur, 0·012; silicon, 0·54; nickel, 9·49; mium, 18·26; molybdenum, 0·16; copper, 0·16; lt, 0·095. The plates were annealed at 1,066° C for nin, water quenched, and subsequently shocked to kbar pressure in a plane wave generator similar to that ribed by Dieter[5]. A photomicrograph of the shock-aded steel is shown in Fig. 1. The structure is very similar to type 302 stainless steel deformed by conventional methods[4].

For examination by transmission electron microscopy, samples were cut from the shocked plates and hand lapped to a thickness of 5 mil. Foils were then prepared by electropolishing the mechanically thinned samples in a chromic-acetic acid electrolyte using the window technique.

A transmission electron micrograph of a region containing both shock-induced α' martensite and austenite is shown in Fig. 2. The direction of shock propagation was perpendicular to the foil surface which was determined to be a few degrees from (001) γ. The martensite is in the form of needles and contains dislocations lying along {110} α' or {112} α' planes. Since a one-surface trace analysis was all that could be used, the exact slip system operating in the martensite could not be determined. A number of the needles appear to be along directions that are close to [110] γ. The orientation relationship between the shock-induced martensite and the austenite was found by electron diffraction to be close to the Kurdjumov–Sachs relationship; namely, $(111)\gamma//(011)\alpha'$, $[101]\gamma//[111]\alpha'$. This relation is also found in 18 chromium : 8 nickel stainless steel deformed by conventional means[4].



Fig. 1. Light micrograph of type 302 stainless steel shocked at 158 kbar at ambient temperature (× c. 170)

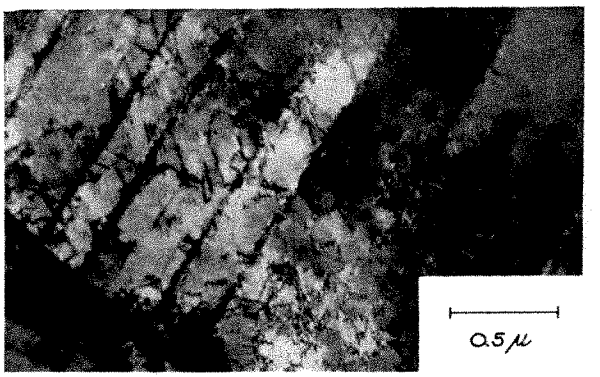


Fig. 3. Untransformed austenite in type 302 stainless steel shocked at 158 kbar at ambient temperature

The austenite in the vicinity of the martensite needles is heavily faulted on $(11\bar{1})\gamma$ planes. This was a common occurrence in most of the foils examined, provided a sufficient amount of martensite was present in the structure. Since the austenite in areas remote from shock induced α' martensite did not have the heavy faulted appearance (Fig. 3), it is believed that the stacking faults were a result of the propagation of martensite needles and were not due to the transient strain present at the shock front. Most of the habit planes were curved and contained a high density of dislocations which gave them the fuzzy appearance observed in Fig. 2. The habit plane AB in Fig. 2 is, however, relatively straight and contains resolvable detail. The habit plane was determined by one-surface trace analysis and knowledge of the foil thickness to be close to $(1\bar{1}1)\gamma$. The regular array of dislocations mentioned here is visible along this habit plane. An enlargement of the interface region at point C is shown in Fig. 4. A parallel grid of dislocations that appear to lie along the intersections of the $(11\bar{1})\gamma$ fault planes and the $(1\bar{1}1)\gamma$ habit plane is clearly visible. The presence of the observed interface dislocations can be explained by the following argument if it is assumed that the $(1\bar{1}1)\gamma$ habit plane of the growing α' needle contained a parallel array of dislocations with $a/2 <110>\gamma$ Burgers vectors moving on parallel $(11\bar{1})\gamma$ glide planes. When propagation of the interface ceased, the stresses holding the dislocations together relaxed thus allowing the dislocations to dissociate into two Shockley partial dislocations lying on $(11\bar{1})\gamma$ glide planes. One partial then glided into the austenite, creating the observed stacking faults while the other remained pinned due to the mismatch at the interface. The dislocations confined to the interface AB shown in Fig. 2 are believed to be pinned Shockley partials.

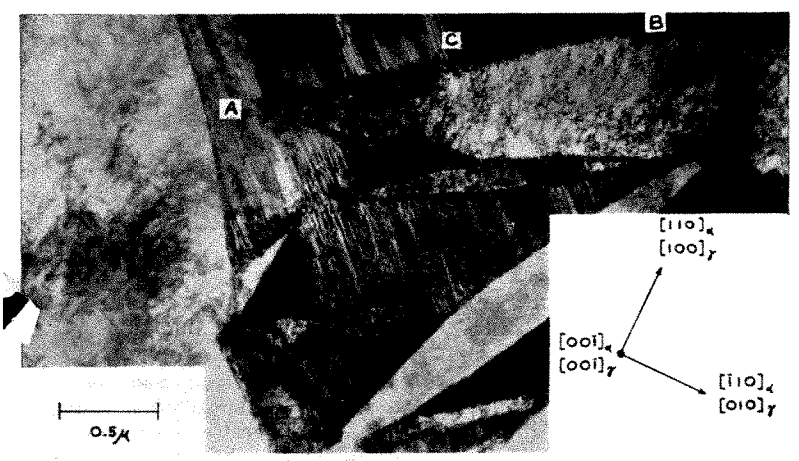


Fig. 2. α' martensite needles in faulted austenite matrix in type 302 stainless steel shocked at 158 kbar at ambient temperature

This work was supported by a contract with the Metals and Ceramics Division,

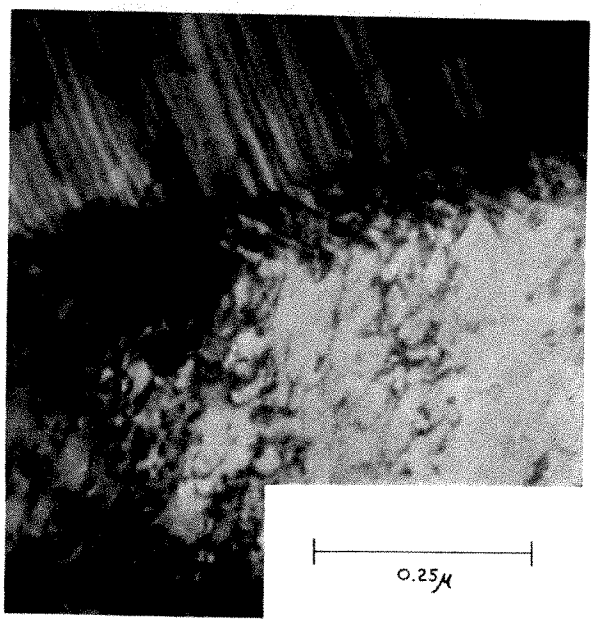


Fig. 4. Enlargement of habit plane at point C in Fig. 2

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CHEMISTRY

Effect of Crack Propagation Velocity on the Fracture Surface Energy of Poly(methyl methacrylate)

WE have measured values of the fracture surface energy (γ) at $+20°$ C of a sample of high molecular weight poly(methyl methacrylate) sheet ('Perspex', manufactured by Imperial Chemical Industries, Ltd., Plastics Division). In order to measure γ over a wide range of crack propagation velocities ($\dot{c}$), we used three different experimental techniques:

(1) A modification of the static loading method described by Van den Boogaart and Turner[1] in which the crack length was measured as a function of time. From the results it was possible to deduce the relation between γ and $\dot{c}$ by assuming a value for Young's modulus ($E = 2{\cdot}85 \times 10^{10}$ dynes/cm^2). This technique covered the range of velocities from comparatively slow rates ($\simeq 10^{-4}$ cm/sec) up to about 1 cm/sec. Beyond this the crack propagation rate increased rapidly and the specimen failed catastrophically.

(2) A modification of the cleavage technique described by Broutman and McGarry[2] which produced controlled crack growth at various lead screw speeds.

(3) Charpy impact tests on sharply notched specimens. γ was taken as the ratio of the energy to break to the area of the new surfaces created during crack growth. Although $\dot{c}$ was not measured accurately, it is between the pendulum velocity at the point of impact (240 cm/sec) and maximum possible crack propagation velocity (~ cm/sec). Thus it is substantially higher than in the o experiments.

Table 1

Experimental technique	Crack propagation velocity (cm/sec)	Fracture surface energy (10^5 dynes/cm
1	10^{-4}	3·65
2	$7{\cdot}4 \times 10^{-4}$	3·7
1	10^{-3}	3·65
2	$6{\cdot}8 \times 10^{-3}$	3·0
1	10^{-2}	4·0
1	3×10^{-2}	4·75
2	$3{\cdot}3 \times 10^{-2}$	5·8
1	10^{-1}	5·5
2	$1{\cdot}4 \times 10^{-1}$	5·1
1	3×10^{-1}	6·2
2	$7{\cdot}6 \times 10^{-1}$	8·9
2	4·3	6·8
2	33	5·3
3	>240	4·5

The results are given in Table 1.

The principal conclusion that can be drawn from these experiments is that γ, like all other mechanical properties of poly(methyl methacrylate), is rate-dependent. It would seem to have a maximum value of about 9×10^5 dynes/cm at about 1 cm/sec at $+20°$ C. This conclusion is qualitatively confirmed by the fact that crack propagation was stable at all in the static loading experiment. Stable crack propagation, in this experiment, implies that γ increases as $\dot{c}$ increases; a change to unstable, catastrophic crack propagation implies that a maximum value of γ has been reached.

In the static loading experiment the change-over point from stable to unstable crack propagation is readily seen by examination of the fracture surface. This makes it possible to calculate K_c by the equation given in ref. 1. K_c can also be calculated from the cleavage experiments by assuming that the maximum value of γ is 9×10^5 dynes/cm and that E is $2{\cdot}85 \times 10^{10}$ dynes/cm^2. $K_c^2 = E\gamma$ for plane stress. Both techniques give:

$$K_c = 1{\cdot}6 \times 10^8 \text{ dynes/cm}^{3/2}$$

Another implication of these results is that γ will be higher when measured on notched tension specimens than when measured in equilibrium cleavage experiments as found by Berry[3] for both poly(methyl methacrylate) and polystyrene.

It is well known that, however it is characterized and measured, the 'ductility' of poly(methyl methacrylate) increases as the temperature is raised above $+20°$ C; it reaches a peak value and then decreases again with further increase in temperature. We suggest, therefore, that the cause of the rate-dependence of the fracture surface energy may be the heat produced by the localized 'plastic' deformation at the tip of the propagating crack. As the crack-propagation velocity increases, the process changes from isothermal to adiabatic. It follows that as the crack-propagation velocity increases, the temperature at the crack tip increases and therefore the fracture surface energy first increases and then decreases.

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Structure of Desmosine and Isodesmosine

IN 1963 Partridge, Elsden and Thomas[1] reported the isolation of two new amino-acids named desmosine and isodesmosine from bovine ligamentum nuchae elastin. Since then the presence of these amino-acids has been demonstrated in elastins from various sources[2].

ıe basis mainly of close resemblances of the ultra-iet absorption and proton magnetic resonance spectra of these compounds with those of substituted pyridinium salts, it was proposed[3,4] that desmosine and isodesmosine are 1, 3, 4, 5 and 1, 2, 3, 5 tetra-substituted pyridinium salts.

Hantzsch[5] in 1886 showed that the treatment of *N*-methyl nicotinic acid with 6 N potassium hydroxide yields methylamine: Huff and Perlzweig[6] on steam distillation of *N'*-methylnicotinamide were able to recover CH_3–N≡ group from the ring as methyl amine (as shown in Fig. 1) in 97–98 per cent yield.

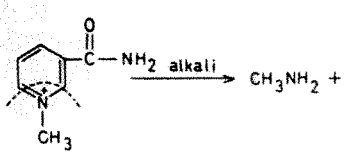


Fig. 1

In the light of these observations with nicotinic acid derivatives, the action of alkali on desmosine and isodesmosine was studied with the hope of liberating the pyridinium nitrogen in the form of amino group still attached to its side chain.

Some of the results of this study are summarized in Table 1. One of the compounds liberated by the action of alkali on both desmosine and isodesmosine has been identified as lysine. The liberation of lysine was confirmed by (1) ion exchange chromatography (*a*) on the short column of the Beckman-Spinco amino-acid analyser using 0·35 N citrate buffer (*p*H 5·28), and (*b*) with a Technicon amino-acid analyser using *p*H gradient from *p*H 2·75 to *p*H 6·1; (2) paper electrophoresis in pyridine-acetate buffer *p*H 3·5; (3) paper chromatography on a buffered, Whatman No. 3 *HR* paper using *n*-propanol, ethanol, and aqueous pyrophosphate buffer (50 : 25 : 25) as the solvent[7]. As shown in Table 1, lysine could be obtained in a 73 per cent yield from isodesmosine and in a 41 per cent yield from desmosine. The maximum yield of lysine from isodesmosine was obtained with 10 N sodium hydroxide at 110° C for 16 h in a sealed tube and from desmosine with 6 N sodium hydroxide at 110° C for 70 h.

Table 1. LIBERATION OF LYSINE FROM DESMOSINE AND ISODESMOSINE BY THE ACTION OF ALKALI

Treatment	Compound used	Lysine yield: Percentage of total	Lysine yield: Percentage of degraded compound
6 N sodium hydroxide at 100° C for 4 h	Desmosine	Trace	Trace
" " "	Isodesmosine	10·6	31·3
6 N sodium hydroxide at 110° C for 16 h	Desmosine	12·0	28·8
" " "	Isodesmosine	17·4	59·5
10 N sodium hydroxide at 110° C for 16 h	Desmosine	12·5	19·2
" " "	Isodesmosine	73·4	84·0
6 N sodium hydroxide at 110° C for 70 h	Desmosine	41·0	61·2
" " "	Isodesmosine	17·2	17·2

The liberation of lysine from desmosine and isodesmosine by the action of alkali provides independent proof of the pyridinium structure of both desmosine and isodesmosine. The ε-amino nitrogen of the liberated lysine is evidently the pyridinium ring nitrogen of the parent desmosine or isodesmosine. This also shows that the side chain attached to the pyridinium nitrogen in both cases is a 6 carbon side chain ending in α-amino carboxylic acid. These results provide additional support for the proposed structure[3,4] of desmosine and isodesmosine. This degradative method also provides a technique for determining the distribution of radioactivity in the desmosine and isodesmosine molecules after the incorporation of the activity from radioactive precursors. This technique is already being used to investigate the distribution of radioactivity in desmosine and isodesmosine after the incorporation of the activity from uniformly labelled lysine-^{14}C, and the results support the hypothesis[8,9] that four lysine molecules condense to form a desmosine or isodesmosine molecule.

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Variation of the Isothermal Compressibilities of Liquids with Temperature

THE effect of pressure (P) on the isothermal compressibilities (κ_T) of liquids is well expressed in most cases by the empirical equation (1) of Tait[1]:

$$\kappa_T = \frac{C}{L + P} \qquad (1)$$

For a given substance, C is a constant independent of temperature and L is a constant at a given temperature. Attempts in the past to give a formula for the variation of L, or the isothermal compressibility, with temperature have not proved satisfactory, and Gibson and Loeffler[2] found that the empirical polynomial (2) gave the best fit with their results:

$$L = L_{25} + a(t - 25) + b(t - 25)^2 \qquad (2)$$

where L_{25} is the value of the Tait constant L at 25° C, t is the temperature and a and b are constant for a given substance.

The simple relationship (3) between the isothermal compressibility and surface tension (γ) has been found to hold[3] for many liquids:

$$\kappa_T \gamma^{3/2} = \text{a constant } (1{\cdot}33 \times 10^{-8} \text{ cgs units}) \qquad (3)$$

The elimination of the surface tension and the molecular volume term from equation (3); the equation of Sugden for the parachor (V_p); and Katoyama's modification of the Eotvos equation leads to an expression[3] which can be written as equation (4):

$$\left(\frac{1}{\kappa_T}\right)^{5/9} = \frac{K(t_c - t)}{V_p^{2/3}} \qquad (4)$$

where t_c is the critical temperature and K is a constant which equals about 5×10^4 cgs units for unassociated substances with molecules not too far from spherical in shape.

The compressibilities of a number of organic substances which have recently been measured[4] over a range of temperature and earlier values[5] have been combined and used to test equation (4) which has not previously been tested. The regression equations of $\left(\frac{1}{\kappa_T}\right)^{5/9}$ on t were calculated, and results for the compounds for which most values of compressibilities have been given are shown in Table 1. The equation (4) clearly does not apply to water which is peculiar in that the compressibility goes through a minimum[6] at about 50° C. For all the other twelve compounds, the plots of $\left(\frac{1}{\kappa_T}\right)^{5/9}$ against temperature give satisfactory straight lines. The critical temperatures were taken from tables[5] and the parachors were calculated[7].

Table 1. VARIATION OF ISOTHERMAL COMPRESSIBILITY (κT) WITH TEMPERATURE (t)

Compound	Equation $\kappa T^{-5/9} \times 10^{-3} =$	Intercept on t-axis	Correlation	No. of data points	Slope $\times V_p^{2/3}$ ($= -K$)	t_c observe (° C)
Aniline	$-1{\cdot}58605t + 589{\cdot}229$	372·0	−0·999583	13	$-5{\cdot}978 \times 10^4$	426
Anisole	$-1{\cdot}49687t + 488{\cdot}361$	327·3	−0·987139	9	$-6{\cdot}092 \times 10^4$	369
Benzene	$-1{\cdot}51454t + 402{\cdot}024$	266·5	−0·991664	26	$-5{\cdot}233 \times 10^4$	288·5
Carbon disulphide	$-1{\cdot}54611t + 406{\cdot}744$	263·2	−0·991489	16	$-4{\cdot}151 \times 10^4$	273
Carbon tetrachloride	$-1{\cdot}4548t + 379{\cdot}438$	261·4	−0·997455	32	$-5{\cdot}134 \times 10^4$	283·1
Chlorobenzene	$-1{\cdot}36579t + 453{\cdot}056$	332·7	−0·993770	18	$-5{\cdot}242 \times 10^4$	359
Chloroform	$-1{\cdot}49868t + 386{\cdot}79$	258·7	−0·987434	25	$-4{\cdot}686 \times 10^4$	263
Ethyl alcohol	$-1{\cdot}23542t + 363{\cdot}836$	295·5	−0·998770	16	$-3{\cdot}128 \times 10^4$	243·1
Ethyl ether	$-1{\cdot}71215t + 287{\cdot}305$	168·4	−0·989415	9	$-5{\cdot}996 \times 10^4$	193·8
Ethylene chloride	$-1{\cdot}62768t + 441{\cdot}000$	271·1	−0·995244	16	$-5{\cdot}189 \times 10^4$	290
Methanol	$-1{\cdot}14523t + 345{\cdot}723$	302·4	−0·999020	13	$-2{\cdot}255 \times 10^4$	240
Toluene	$-1{\cdot}52568t + 414{\cdot}331$	271·9	−0·997583	18	$-5{\cdot}943 \times 10^4$	320·6
Water	$0{\cdot}10128t + 550{\cdot}423$	543·5	+0·234	21	$+1{\cdot}328 \times 10^3$	374

The two associated compounds, methanol and ethyl alcohol, give intercepts above the critical temperatures and values of K less than 5×10^4 cgs units. The intercepts for all the other compounds are below the critical temperatures (sometimes by as much as 10 per cent on T_c in °K) and the values of K vary between $4{\cdot}1 \times 10^4$ and $6{\cdot}1 \times 10^4$ cgs units. Thus the linear relationship, suggested by equation (4), between $\left(\frac{1}{\kappa_T}\right)^{5/9}$ and temperature is found to hold for most substances, but accurate values for compressibilities cannot be obtained from the equation, critical temperatures and parachors.

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Oxyfluorides and Basic Fluorides of Aluminium

EARLIER[1], we reported the fact that despite the numerous syntheses of aluminium oxyhalides of the type AlOCl, AlOBr and AlOI, by methods generally applicable to all these aluminium oxyhalides, no preparation of AlOF has been reported. After experimental investigations on a number of reactions that might by analogy lead to the formation of AlOF, we have concluded that the lack of information concerning AlOF is not accidental, but stems from the general inapplicability with respect to AlOF of most preparative methods for AlOX compounds. This results from the greater bond strength of aluminium–fluorine bonds as compared with other aluminium–halogen bonds, the high crystallization energy of aluminium fluoride as compared with the other AlX_3 compounds, and the enhanced ability of fluorine to form higher complex anions with elements such as arsenic. It is the latter characteristic which prevents the use of the $As_2O_3 + 3AlX_3 \rightarrow 3AlOX + 2AsX_3$ reaction with aluminium fluoride[1].

In studying various possibilities of forming AlOF we have noted some interesting relationships between aluminium oxyfluorides and their corresponding basic fluorides. This communication describes some of the difficulties inherent in AlOF formation, relates aluminium oxyfluorides to their basic aluminium fluorides, and finally reports on a successful route to AlOF formation.

Cowley and Scott[2] attempted to prepare aluminium oxyfluorides by the pyrolytic dehydration of basic aluminium fluorides, in the composition range $AlF(OH)_2$ to $AlF_2(OH)$. However, they found that preparations dehydrated at 500° C exhibited the same X-ray diffraction spectrum that was characteristic of the hydrated basic fluorides; the same X-ray diffraction spectrum also characterized the entire composition range of basic fluorides. They concluded that the dehydrated samples were in fact still basic fluorides, rather than true oxyfluorides, a conclusion which we are able to confirm, as will be described here. Cowley and Scott also found that at 600° C the basic fluorides decomposed to form aluminium fluoride, indicating that one could not prepare oxyfluorides by the pyrolytic route at higher temperatures. These results, combined with our previous results[1], in which it was shown that AlOF cannot be prepared by the reaction in water solution between AlOCl and silver fluoride, because of the polymerized structure of AlOCl in aqueous solutions, rule out the possibility of AlOF formation by aqueous paths.

AlF_3—oxide reactions. The most common method used to prepare AlOX compounds is the reaction between AlX_3 and an oxide. The simplest of these is the one using Al_2O_3 as the oxide. The high sublimation point of 1281° C for aluminium fluoride and the 2042° C melting point for Al_2O_3 creates experimental difficulties with this system, and it was decided to investigate this reaction by differential thermal analysis. However, at temperatures up to the sublimation point of aluminium fluoride, there was no reaction detectable by this technique, even at very slow scan rates.

Aluminium oxyfluoride formation was then attempted by reaction in a nickel crucible between aluminium fluoride and the lower melting oxide, B_2O_3, according to $B_2O_3 + 3AlF_3 \rightarrow 2BF_3 + 3AlOF$. A slow reaction rate was noted at 600° C; after 20 h only 22 per cent of the reactants had reacted, as determined by the loss of volatile material. At 700° C, complete reaction was noted in 1 h. It was demonstrated, by the absence of a green boron emission band in the flame spectrum of the non-volatile residue from the 700° C reaction, that the entire boron content had volatilized. The product had an experimentally determined composition of 31·5 per cent fluorine and 40·5 per cent aluminium, corresponding nearly to AlOF which has a theoretical composition of 30·7 per cent fluorine and 43·6 per cent aluminium. However, the X-ray diffraction pattern showed the presence of only aluminium fluoride and alumina. Furthermore, on hydration of the sample, by boiling down a suspension of the product in water, the characteristic X-ray diffraction pattern of the corresponding basic fluoride hydrate[2] was not observed. Neither was the basic fluoride pattern observed on hydration of the 600° C product. As will be described later, the basic fluoride X-ray diffraction spectrum is found when the true oxyfluoride is hydrated by the foregoing procedure. Accordingly, the reaction with boron oxide proceeded according to equation (1) and aluminium oxyfluoride was not formed:

$$B_2O_3(l) + 2AlF_3(s) \xrightarrow{700^\circ} 2BF_3(g) + Al_2O_3(s) \qquad (1)$$

Aluminium alkoxide reactions. A promising path to AlOF formation was believed to be by the reaction forming AlOCl from aluminium alkoxides, as shown in equation (2) (refs. 3 and 4). Anhydrous hydrogen fluoride was added to a solution of aluminium tertiary butoxide in

$$Al(OR)_3 + 2HCl \xrightarrow{\text{benzene}} AlCl_2(OR) + 2ROH \quad (2)$$

$$\Big\downarrow \Delta$$

$$AlOCl + RCl$$

benzene solution, in the molar ratio of 2 : 1, at a temperature just above the melting point of the solvent. The reactants were then allowed to stand overnight at room temperature, and the precipitate formed was filtered. It was evacuated under high vacuum at room temperature and then heated in several stages to 600° C. The product which had been heated to 200° C had the empirical formula $AlF_{1\cdot84}(OH)_{1\cdot13}(OC_4H_9)_{0\cdot025}$ based on analyses for aluminium, fluorine, carbon and hydrogen. It evidently included a small residue of organic material which had not yet decomposed, but its composition indicated that the fluorination was almost complete. However, unlike equation (2), tertiary butyl fluoride had not been evolved, because both fluorine atoms were still bonded to aluminium. The presence of the OH group indicates that decomposition of the organic intermediate had been by elimination of butene rather than butyl fluoride, leaving an H from the butyl radical attached to the oxygen. Thus when hydrogen fluoride is substituted for hydrogen chloride or the other hydrogen halides, the reaction proceeds by way of equation (3), forming the basic difluoride rather than AlOF. The product originally obtained was amorphous, but on hydration it gave the characteristic basic fluoride X-ray diffraction spectrum. Although AlOF cannot be prepared by this path, it does constitute a new and relatively simple path to the basic difluoride.

$$Al(OC_4H_9)F_2 \xrightarrow{200^\circ} AlF_2(OH) + C_4H_8 \quad (3)$$

At 600° C, the non-volatile product had the empirical formula $AlF_{1\cdot54}(OH)_{0\cdot46}O_{0\cdot5}$, based on analyses for aluminium fluorine, carbon and hydrogen. On hydration of the sample, no indication was obtained by X-ray diffraction for the formation of the basic aluminium fluoride. It was concluded that the non-volatile product was an equimolar mixture of AlF_3 + AlO(OH), which was consistent with the observed X-ray diffraction spectrum. This observation is also consistent with the reported thermal stability of basic aluminium fluorides[2]. The drop in fluorine content between the samples heated to 200° C and 600° C is by loss of fluorine as hydrogen fluoride. The data indicate that $AlF_2(OH)$ decomposes according to equation (4)—a point not made in ref. 2:

$$2AlF_2(OH) \xrightarrow{600^\circ} AlF_3 + AlO(OH) + HF \quad (4)$$

Fluorination of AlOBr. It had become apparent for reasons already described that the most likely method of preparing AlOF (and perhaps the only practical method) was by fluorination of an existing AlO*X* compound, although this could not be done in an aqueous medium. Using this technique, aluminium oxyfluorides with compositions ranging from AlOF to Al_2OF_4 have been prepared by reaction of AlOBr with BrF_3 in appropriate ratios. At present we can only make a preliminary report on these compounds, and further details will be published later when the oxyfluorides are better characterized and their properties better understood. However, a significant relationship between the oxyfluorides and basic fluorides of aluminium, especially in regard to their inter-[illegible] has been found and will be described.

[illegible] each of the oxyfluorides prepared [illegible] tion the [illegible] n [illegible] es [illegible] to [illegible]

basic fluorides by simple hydration, although in the reverse direction aluminium oxyfluorides cannot be readily obtained from the basic fluorides.

Each of the aluminium oxyfluorides prepared by the AlOBr + BrF_3 reactions were only sparingly soluble in water. In this respect they differ significantly from the other AlO*X* compounds, which are all very soluble in water.

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Specific Conductivity of High-purity Water up to 300° C

THIS communication reports work in progress on the direct measurement of the conductivity of high purity water from 100 to 300° C along the saturated vapour pressure curve.

A knowledge of the specific conductivity of water is important in the study of the conductivity of dilute salt solutions, particularly at temperatures above 100° C where it increases to almost two orders of magnitude greater than its value at 25° C. At present the only values of the specific conductivity available above 100° C are those due to Noyes *et al.*[1] at 100°, 156°, 218° and 306° C, which were obtained indirectly from a study of the hydrolysis of acetates, and those due to Wright, Lindsay and Druga[2], which were obtained from conductivity measurements on relatively impure water.

The water used had a conductivity varying from 0·06 to 0·12 μmho/cm at room temperature and was prepared by passing distilled water successively through a demineralization unit, four stainless steel stills and a de-aerator. The high-temperature conductivity cell is contained in a platinum-lined autoclave as shown in Fig. 1. Two methods of operating this cell were used. First, water was pumped continuously through the autoclave, with the pump stroke and outlet valve adjusted to keep the autoclave full of water. Secondly, the autoclave was filled to the level *CD* (Fig. 1), isolated and measurements made with water distilling from the outer compartment into the cell. At present the first method is unsatisfactory above 200° C owing to temperature instability, while the second method gave less satisfactory results than the first below 180° C.

The most uncertain error in the measured conductivity is that due to contamination of the water in the system. Contamination may arise from hydrolysis of organic materials carried over from the resin of the demineralization unit and not removed by the stills, from corrosion of the stainless steel in contact with the platinum lining and from decomposition of the polytetrafluoroethylene gasket. The contamination occurring in a flowing system is indicated by the difference in the outlet and inlet water conductivities. The flowing results in Fig. 2 were taken from regions where the outlet conductivity was below 0·35 μmho/cm and the maximum increase relative to the inlet conductivity was 0·21 μmho/cm. Some of this conductivity increase was certainly due to contamination occurring during the cooling of the outlet to room temperature and so the contamination occurring inside the autoclave is thought to be quite small.

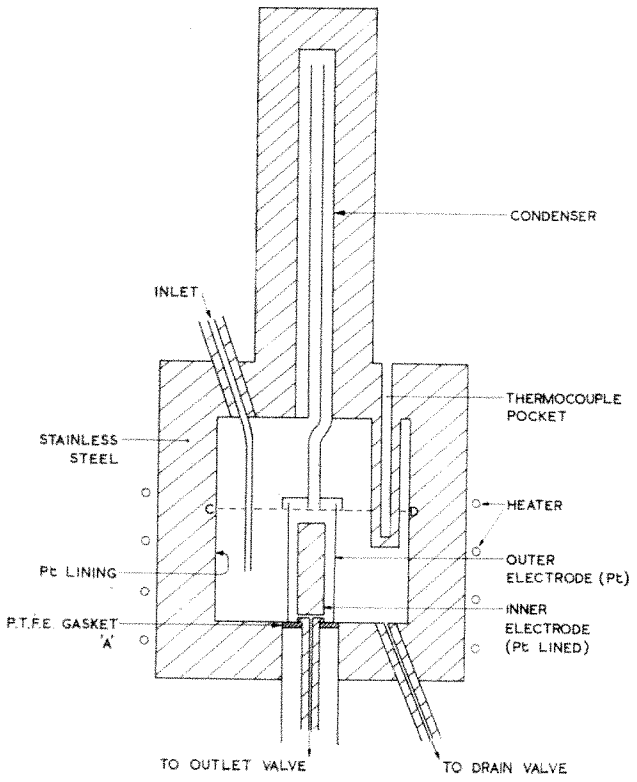


Fig. 1. Schematic diagram of autoclave

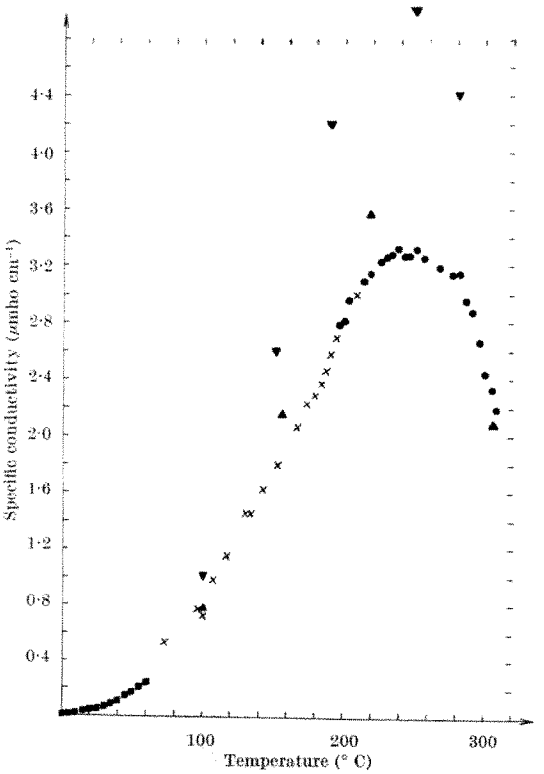


Fig. 2. Specific conductivity of water at saturated vapour pressure. ×, Present results, flowing; ●, present results, distilling; ■, Harned *et al.*[3]; ▲, Noyes *et al.*[1]; ▼, Wright *et al.*[2]

Preliminary experimental results are compared with previously reported data in Fig. 2. The estimated errors arising from contamination, and from temperature and cell constant errors, are ± 2 per cent below 200° C, ± 5 per cent from 200° to 280° C and ± 8 per cent above 280° C. The results are in good agreement with the data recalculated from the work of Noyes *et al.* and also wi[th] temperature data of Harned and Robinson[3].

Modifications to the apparatus are in progress to enable the conductivity to be determined with greater accuracy at temperatures up to at least 320° C and pressures up to 300 bars.

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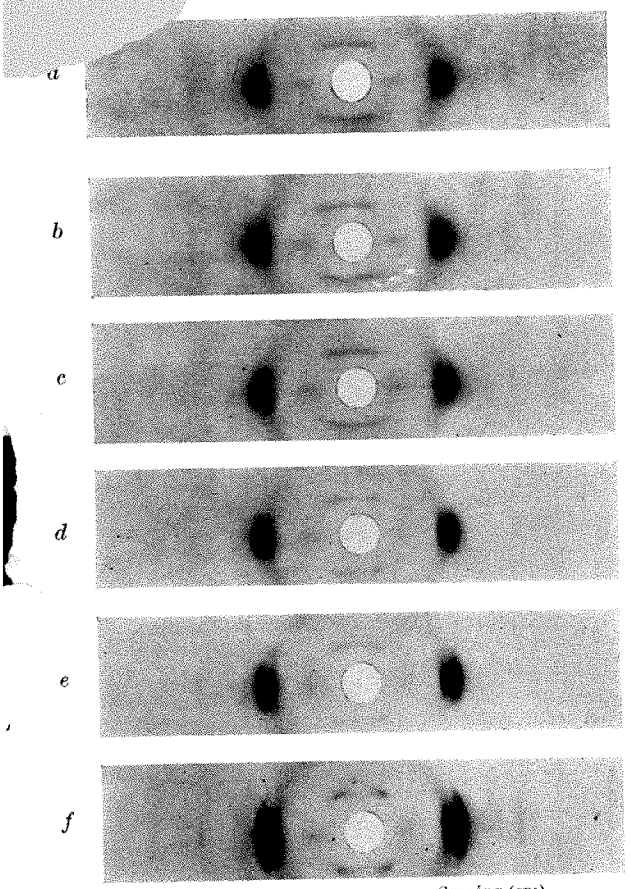
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Two-phase Model for Structure of Polymers

Attempts to relate polymer properties to structure have usually involved the assumption of a two-phase structural model: crystalline regions embedded in an amorphous matrix. The crystalline and amorphous regions in the structure are each presumed to have well-defined properties which do not vary as the proportions change in different polymers. With this model, the variables available to compare different samples of the same polymer are: crystallite size, shape and orientation correlation; orientation effects in the amorphous material; and the relative amounts of crystalline and amorphous material present. The last parameter mentioned is termed 'degree of crystallinity', and it is measured in various ways: by techniques involving X-ray diffraction, density, infra-red, nuclear magnetic resonances, chemical reactivity, and swelling measurements. The degree to which the results of these techniques correlate is a measure of the validity of the two-phase model. Farrow and Ward[1] found little correlation between crystallinity measurements on polyethylene terephthalate fibres by X-ray diffraction, density and infra-red techniques. Similar comparisons between crystallinity measurements on polypropylene by various techniques were reported by Quynn, Riley, Young and Noether[2] with similar inconsistencies. Nuclear magnetic resonance studies of polyethylene and polypropylene fibres by Hyndman and Origlio[3] indicated the existence of a distinct phase intermediate in degree of order between the crystalline and amorphous phases. Sobue and Tabata[4] found X-ray evidence for polymorphism in polypropylene.

These and other investigations indicate that the two-phase model as defined earlier does not apply. The open question at the moment is: how far from the two-phase hypothesis and in which direction must we go to be consistent with the data? There is great reluctance to abandon completely this hypothesis, as its predicted results, though not correct, are of the right order of magnitude.

We would like to point out the significance with respect to the two-phase hypothesis of the shifting in the positions of diffraction peaks in polymer X-ray patterns. This shift has been observed in some polymers when subjected to various physical treatments which tend to change the degree of order. There are various industrial quality-control tests on polymers based on this effect, but its interpretation has only been in terms of an empirical relationship between the physical properties and the amount of splitting of diffraction spots. Crystals can only change size, shape, orientation [illegible] ance; these [illegible]



Sample	Spacing (cm) d_{100}	$d_{010} = d_{110}$
a, Unannealed fibre	4·317	4·030
b, Annealed at 80° C	4·399	4·040
c, Annealed at 100° C	4·360	4·046
d, Annealed at 130° C	4·362	4·040
e, Annealed at 160° C	4·392	3·883
f, Annealed at 190° C	4·436	3·778

Fig. 1

between these kinds of regions are becoming somewhat obscure.

An obvious alternative is paracrystalline theory where the shifting of diffraction peaks is a necessary consequence of the statistical changes associated with varying degrees of order. According to this theory, a particular peak position represents an average lattice spacing. This suggests that, as the degree of order changes, one can measure the change in average lattice parameters.

Particularly accessible parameters for fibres are the projections of the inter-chain lattice vectors on to the cross-sectional plane perpendicular to the chain axes. If we make the approximation that each chain is equivalent, and (as is usually the case) the resultant Bravais cell contains but one chain, then in the X-ray fibre diagram there will be up to three particularly strong equatorial reflexions which result from Bragg planes passing through the axes of nearest neighbour chains. When the Bravais cell projection is a 60° rhombus, we have a hexagonal structure and only one such reflexion appears; when it is a general rhombus, we have a centred orthorhombic structure and two such reflexions appear; and when it is a general parallelogram, we have a monoclinic or triclinic structure and three such reflexions appear. The area of this latter Bravais cell projection is simply double the area of the triangle the three altitudes of which are the three measured spacings; the areas of the other Bravais cells are special cases of this. The vectors and angles of the projected Bravais cell can then be directly calculated. The area of the aforementioned triangle is the cross-sectional excluded area per chain, which is a [illegible] of density and consequently of degree of order.

We have observed a continuous variation in X-ray diagrams of nylon between a hexagonal pattern from material quenched from the melt to the standard pseudo-orthorhombic pattern in well-annealed material (Fig. 1). The cross-sectional area per chain was 19 per cent greater in the quenched material than in the annealed material. If we consider that there is complete rotational disorder of the planes of the chain zigzags in the quenched sample, and complete order in the annealed sample, then the percentage of rotational order in any intermediate sample could be defined in terms of a linear interpolation of the measured cross-sectional area per chain between the extreme values.

This approach might be considered as 'X-ray density' technique. A comparison between the density variation as measured by this technique with that measured in the standard way would give a good indication as to whether the material is homogeneously paracrystalline (as would be shown by good correlation) or whether there are truly amorphous regions in the structure which do not contribute to the crystalline pattern but do contribute to the total density.

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Nitroxide Radicals from Methylmethacrylate interacting with Oxides of Nitrogen

A CHARACTERISTIC three-line electron spin resonance spectrum has been detected by other workers when nitric oxide or nitrogen dioxide react with methylmethacrylate at room temperature[1]. Spectra for both fluid and rigid solutions of the radical were recorded and, by the use of ^{15}NO, the triplet was shown to be a consequence of hyperfine coupling to nitrogen[1]. Both *X*-band and *Q*-band spectrometers were used.

We have confirmed these results, derived the isotropic and anisotropic parts of the ^{14}N hyperfine coupling tensor for this radical (Table 1), and have derived details of the molecular orbital of the unpaired electron on nitrogen by standard procedures[2]. Using the values of 550 G and 34 G for the 2*s* and 2*p* atomic orbitals on nitrogen[2] we obtain $a^2_{2s}(N) = 0{\cdot}026$ and $a^2_{2p}(N) = 0{\cdot}70$.

These data show that the radical is planar, with the unpaired electron in a π-molecular orbital which is very largely made up of a 2*p*-atomic orbital on nitrogen. The isotropic splittings and the value of g_{av} are almost identical with those of triacetonamine N-oxide (Table 1), while the perpendicular and parallel results are closely similar to those for the radical $(Me_3C)_2NO$ studied by Edelstein, Kwok and Maki[3]. We therefore postulate that the radical involved has the formula $(R_3C)_2NO$. Furthermore, as no strong hyperfine splitting due to protons was detected, there can be no β-protons, that is, no protons bonded to the saturated carbon atoms directly linked to

Table 1. PRINCIPAL VALUES OF THE ^{14}N HYPERFINE AND *g*-TENSORS OF NITROXIDE RADICALS

(Hyperfine parameters are given in G)

Radical	$A_\parallel$	$A_\perp$	A_{iso}	$g_\parallel$	$g_\perp$	g_{av}
Methyl methacrylate radical	37	3·2*	14·5	2·003	2·008	2·0065
di-*t*-butyl nitroxide (ref. 3)	33·2	5·0*	15·4	2·0040	2·0076	2·0064
Triacetonamine N-oxide			14·6			2·0063

* Values estimated from $A_\parallel$ and A_{iso}.

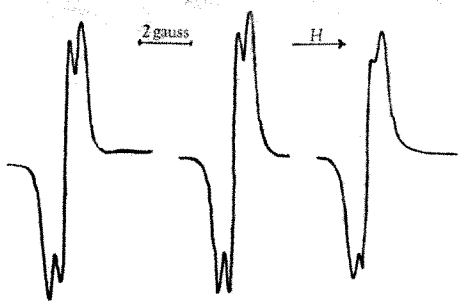


Fig. 1. Electron spin resonance spectrum of the nitroxide radical (I) under high resolution at room temperature

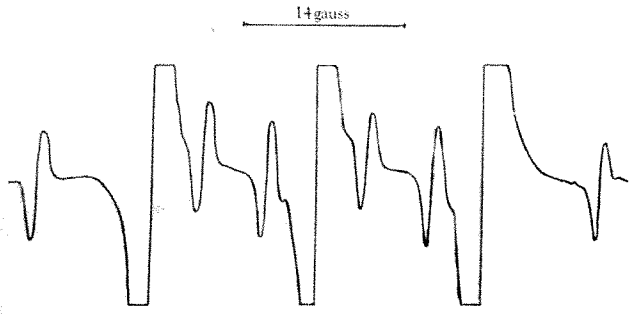


Fig. 2. Electron spin resonance spectrum at high gain showing features assigned to radical (II)

nitrogen. However, a small splitting of about 0·30 G has been resolved (Fig. 1), which is close to expectation for interaction with γ-protons[4]. Unfortunately, resolution is not sufficient to enable us to be certain of the number of γ-protons, but at least three of them are relatively strongly coupled.

This conclusion is strongly supported by the relatively high stability of the radical. Alkyl nitroxides[5,6] are indeed remarkably stable especially when the alkyl groups are bulky[4]. Perhaps the simplest mode of formation of such a radical would be:

$$2CH_2{=}C(Me)CO_2Me + NO \rightarrow \begin{array}{l} CH_2-C(Me)CO_2Me \\ \quad\quad\quad\quad\quad\quad NO \\ CH_2-C(Me)CO_2Me \end{array} \qquad (I)$$

Because nitric oxide does not give any detectable radicals directly with the olefine in the complete absence of the dioxide, we favour the alternative:

$$2CH_2{=}C(Me)CO_2Me + NO_2 \rightarrow \begin{array}{l} CH_2-C(Me)CO_2Me \\ O \quad\quad\quad\quad NO \\ CH_2-C(Me)CO_2Me \end{array} \qquad (I)$$

There are, of course, several more complicated possibilities involving more than two monomer molecules, but it is significant that stable nitroxide radicals have recently been reported in solutions of nitroso compounds[7] and have been attributed to dimers similar to the species postulated here[8].

If this formulation is correct, then the formation of radicals of types (II) and (III) might also have been expected:

$$\begin{array}{l} (Me)C(CO_2Me)-CH_2 \\ O \quad\quad\quad\quad NO \\ CH_2-C(Me)CO_2Me \end{array} \qquad (II)$$

$$\begin{array}{l} (Me)C(CO_2Me)-CH_2 \\ O \quad\quad\quad\quad NO \\ (Me)C(CO_2Me)-CH_2 \end{array} \qquad (III)$$

The three main lines of the former would be 1 : 2 : 1 triplets and those of the latter into 1 : 4 : 6 : 4 : 1 quintets. Under high gain, satellite lines flanking the main triplet have indeed been detected (Fig. 2), and if these are part of the expected 1 : 2 : 1 triplets due to radical (II), the proton hyperfine coupling constant is about 11·0 G. This value is close to that found for β-protons[5,6] in other nitroxides and is in good agreement with expectation for such radicals[5]. We were unable to detect any radicals of type (III) and conclude that such radicals, if formed, have too short a lifetime to be detected under our conditions.

We thank Dr. Golden of the Ministry of Aviation for supplying us with a sample of triacetonamine N-oxide. We also thank the Ministry of Aviation for financial support, and Dr. M. Ebert for helpful advice and assistance.

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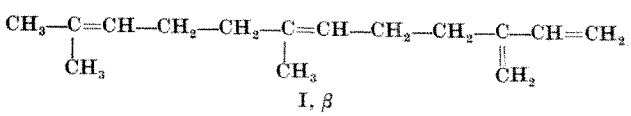
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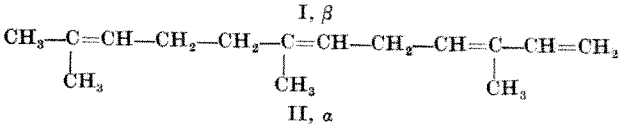
BIOCHEMISTRY

α-Farnesene in the Natural Coating of Apples

Earlier[1], we reported the presence in the natural coating of 'Granny Smith' apples of a hydrocarbon which we identified as the naturally occurring sesquiterpene β-farnesene (I)[2–4]. Identification was based on a comparison of its infra-red spectrum with that published for β-farnesene isolated from natural sources[4], together with the unequivocal identification of its fully hydrogenated product as farnesane (2,6,10-trimethyldodecane)[2,3] by comparison of its infra-red and mass spectra and gas chromatographic retention times with those of a pure authentic specimen. Additional evidence was, however, deemed necessary, and direct comparison with pure β-farnesene has now established that this identification was incorrect and that the farnesene present in apple coating is not the β- but the α-isomer (II), which has hitherto not been reported as naturally occurring or prepared in a pure state.



I, β



II, α

The evidence for the α-isomer embraces the comparison and interpretation of: (*a*) the nuclear magnetic resonance, mass, infra-red, and ultra-violet spectra of farnesene from apples; (*b*) the nuclear magnetic resonance, mass, infra-red and ultra-violet spectra of pure β-farnesene isolated from *Matricaria chamomilla* oil[4]; (*c*) the infra-red and ultra-violet spectra of β-farnesene prepared from farnesol by dehydration over potassium hydroxide[5]. It has been found impossible to synthesize α-farnesene by previously described methods[6]. However, the dehydration of nerolidol in dimethyl sulphoxide at 130° C yielded, besides β-farnesene and β-*bisa*bolene, a component the infra-red and ultra-violet spectra and gas chromato-

graphy retention times of which were identical with those of farnesene from apples.

A remarkable feature of the occurrence of α-farnesene in apple coating is that it is virtually the only sesquiterpene present. Simple column chromatography of the cold hexane extract of intact apples on highly active 'Florisil' or basic alumina yields α-farnesene in about 97 per cent purity.

α-Farnesene has also been found in the natural coatings of 'Crofton' and 'Delicious' apples, 'Packham' pears, and quinces. Its occurrence in these coatings, which have triterpene acids (mainly ursolic and oleanolic[7]) as major constituents, suggests a direct origin from a farnesyl or nerolidyl intermediate in the biosynthesis of these triterpene acids.

In 1919, Brooks, Cooley, and Fisher[8] obtained evidence that superficial scald, a serious storage disorder of apples, is due to volatile or gaseous substances (other than carbon dioxide) that are produced in the metabolism of the apple. The nature of such substances has hitherto remained obscure, but we now have evidence for a role of α-farnesene in scald. More farnesene was found in earlier picked apples, which are more liable to scald, and more in the scald-liable 'Granny Smith' than in the scald-resistant 'Crofton' variety. Oiled wraps, which reduce scald, have been found to absorb from the fruit more than half the farnesene that accumulates during storage. Diphenylamine, a more effective inhibitor of scald[9], delayed the oxidation of farnesene both in pure solution and in the natural coating of the fruit; this result suggests that scald may be caused by oxidation products. Farnesene has been detected in air which has passed over apples, and the effect of air movement on its loss from the fruit is at present being investigated.

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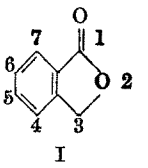
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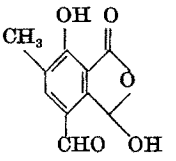
Isolation of 4-Hydroxyphthalide from Oat Grain

A NUMBER of substituted phthalide derivatives have been isolated from plant material[1] and most of these compounds carry either an alkyl substituent at position-3 (I) or an oxygen function at positions-5 and/or -7. Some of the latter group of compounds also carry a hydroxyl group at position-3 (for example, gladiolic acid (II)). It has been postulated[2] that all phthalides with an even number of carbon atoms or no alkyl group at position-3 have an oxygen function at position-7 and are derived from an even number of acetate units. On the other hand, phthalides with an uneven number of carbon atoms at position-3 (for example, eleutherol (III)) were considered to be related to 4-hydroxyphthalide by way of condensation of an odd number of acetate units. The isolation of 4-hydroxyphthalide from oat grain reported here offers an opportunity for further investigation of the origin of this group of compounds.

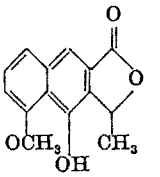
The isolation procedure used is outlined diagrammatically in Fig. 1. The petrol/benzene/water partition was carried out with a 5 per cent concentration of crude extract in the organic layer. Gradient elution was used to develop



I



II



III

the silica-gel column, the relevant fraction being eluted with about 50 per cent chloroform in benzene and partially solidified (vanillic acid). Bands on thin-layer chromatography plates were arbitrarily recognized by scanning the plate with ultra-violet light (350 mμ), bands (2) and (4) fluorescing blue and band (3) absorbing (vanillic acid) on the first plate, and band (2) fluorescing bright blue on the second plate. Material was recovered from the thin-layer chromatography plates by percolating ether through the adsorbent in a short chromatography column. The yield of solid material was very low (about 2·0 mg from 100 kg of grain), but proved reproducible with a second variety of grain (*Avena sativa* L., cultivar 'Blenda').

Mass spectrum analysis of the isolated material indicated a molecular weight of 150 and additional ions occurred at *m/e* 149, 121, 93 and 65. Examination of the metastable ions of the spectrum indicated that these represented losses of one mass unit followed by three successive losses of twenty-eight mass units, respectively. The formula $C_8H_6O_3$ (mol. wt., 150) would accommodate this cracking pattern with each of the oxygen atoms eliminated as carbon monoxide (ref. 3).

Ultra-violet (λ_{max}^{EtOH} 236 mμ and 293·5 mμ) and infra-red absorption spectra of the isolated material, when compared with those of authentic compounds, agreed most closely with those for 4-hydroxyphthalide (from G. T. Newbold via C. J. W. Brooks), the synthetic sample exhibiting one extra weak band at 1,285 cm^{-1} in the infra-red. Further confirmation of this comparison was obtained using gas–liquid chromatography on 7 per

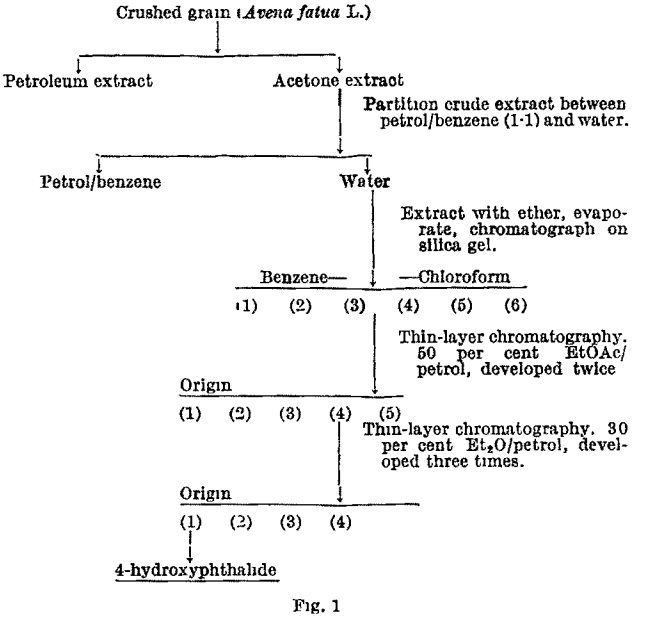


Fig. 1

cent *F*-60 + 1 per cent *EGSP-Z* at 175° C of the free phenols and the corresponding acetates, and using thin-layer chromatography of the acetates in 25 per cent ethyl acetate in petroleum ether. Respective mobilities of the compounds from the two sources were found to be identical.

While several other compounds were isolated during this work (for example, vanillic acid, ferulic acid), no other phthalides or closely related compounds have as yet been identified as present in oat grain and no evidence can be advanced concerning the origin of this compound. 4-Hydroxyl substituted phthalides are relatively a much less common group of compounds than the 7-hydroxyl substituted series, and the suggestion of a different biosynthetic pathway for the two series[2] cannot be ruled out without further investigation. It is hoped that this report will facilitate such an investigation.

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Cholestasis in Rats induced by Taurolithocholate

THE taurine conjugates of cholic and chenodeoxycholic acid[1] are preponderant bile acids of murine bile. The systematic names of the bile acids referred to in the text are: taurocholic acid, 3α,7α,12α-trihydroxy-5β-cholanyl taurine; taurochenodeoxycholic acid, 3α,7α-dihydroxy-5β-cholanyl taurine; taurolithocholic acid, 3α-hydroxy-5β-cholanyl taurine; lithocholic acid, 3α-hydroxy-5β-cholanic acid. Although these bile acids are excreted as the sodium salts, by convention they are often referred to in the acid form. In this communication the suffix, '-ate', is used to indicate the sodium salt. Intravenous administration of taurocholate to rats is known to result in increased bile flow and bile acid excretion[2].

The taurine conjugate of lithocholic acid has been identified as a minor constituent of rat[3] and human[4] bile. This communication describes the decrease in bile flow and bile acid excretion (cholestasis) that occurred with intravenous infusion of taurolithocholate.

Male Wistar rats (230–360 g) were anaesthetized with intraperitoneal pentobarbital. '*PE* No. 10' polyethylene cannulas were inserted into a leg vein, femoral artery and common bile duct. Each animal was placed in a restraining cage and maintained on an infusion of 5 per cent dextrose in 0·45 per cent sodium chloride.

Taurolithocholate (6 mM) and/or taurocholate (6 mM) were infused in a mixture consisting of 30 ml. of 25 per cent human serum albumin, 6 ml. of propylene glycol and 64 ml. of 5 per cent dextrose in 0·45 per cent saline. Propylene glycol aids solution of taurolithocholate and has very low toxicity[5]. The infusion mixture itself did not affect bile flow or bile acid excretion. The bile salt infusion mixtures did not cause visible haemolysis of washed erythrocytes *in vitro*.

Bile acids were quantitatively assayed enzymatically with 3α-hydroxysteroid dehydrogenase[6,7].

In ten animals, infusions of taurocholate for 30–60 min (0·07–0·48 μmole/min/100 g body-wt.) always caused an increase in bile acid excretion. In contrast, similar infusions of taurolithocholate always caused cholestasis. However, infusion of equimolar amounts of taurocholate together with taurolithocholate invariably increased bile acid excretion. The sequence of administration of the different bile acid infusion mixtures to the same animal did not alter the type of response.

Chromatographic analysis[8] of the bile samples showed taurocholate and taurochenodeoxycholate. In addition, taurolithocholate became detectable within 15 min of its infusion. Glycine conjugated and unconjugated bile acids were occasionally present in trace amounts. The decreases found in total bile acid excretion, therefore, are attributable to a decrease in the normal preponderant components.

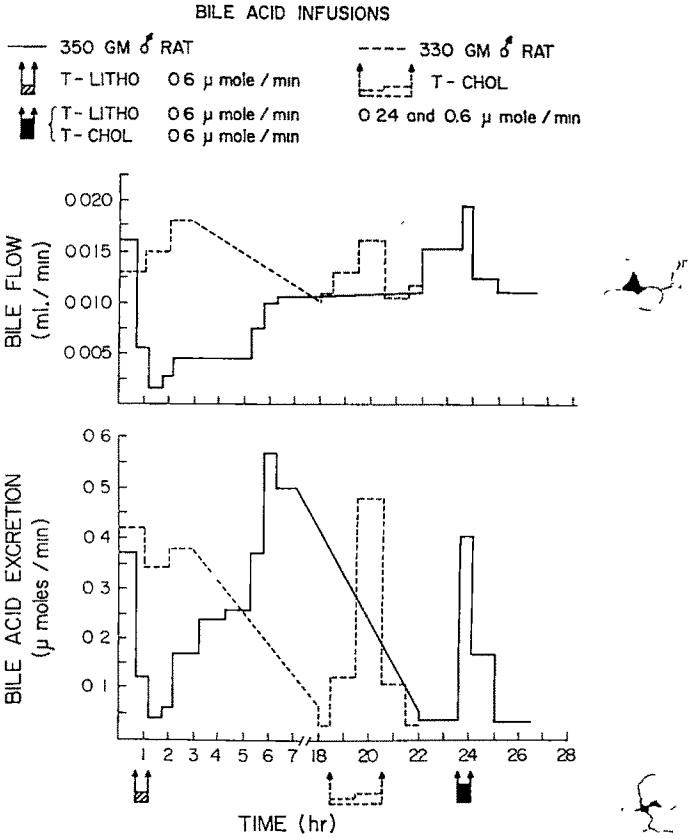


Fig. 1. Bile acid excretion and bile flow in rats beginning from time of bile duct cannulation. Bile acid excretion falls progressively (330 g rat) and is increased 18 h later by infusion of sodium taurocholate. Sodium taurolithocholate decreases bile acid excretion when given alone (350 g rat) but not when given together with sodium taurocholate

Fig. 1 shows the typical responses of the rats to bile acid infusions. The control rat (330 g) shows the progressive fall in bile acid excretion known to occur during the 24-h period following bile duct cannulation[9]. Replacement of the excreted preformed bile acids by taurocholate infusion restores bile acid excretion. In the experimental rat (350 g) the cholestatic effect of taurolithocholate is evident during an infusion given shortly after bile duct cannulation, before significant depletion of preformed bile acids has occurred. Following the period of cholestasis, bile acid excretion returns to the preinfusion levels before a progressive fall occurs. Simultaneous administration of taurocholate together with taurolithocholate to the same animal causes an increase in bile acid excretion.

Although the known direct relationship of bile flow to bile acid excretion was apparent during the infusion periods, a close correlation does not occur in the rat[10]. The lack of close parallelism has been attributed[11] to a significant biliary excretion of an inorganic electrolyte solution. The continuous infusion of saline in these experiments may have augmented electrolyte excretion

and lessened the relationship of bile flow to bile acid excretion.

During prolonged infusions of taurolithocholate, hyperbilirubinaemia occurred. Taurolithocholate was given at 0·2 μmole/min/100 g body-weight for 1 h, followed by one-tenth of this rate for 19 h. Average total serum bilirubin concentration[12] at the end of four experiments (in one experiment abdominal surgery and bile duct cannulation were omitted) was 5·6 mg/100 ml. Chromatographic analysis of the azo pigments[13] revealed azo *B* (glucuronide) and azo *A* (unconjugated). The occurrence of conjugated hyperbilirubinaemia was reflected in the development of deep yellow, diazopositive urine.

It is apparent from these results that the hepatic removal from plasma (uptake) and biliary excretion of taurocholate and taurolithocholate is rapid. Since both bile acids have a similar structure, it is probable that they share common pathways in their transfer from plasma to bile. In contrast to taurocholate, the excretion of taurolithocholate is relatively limited. Competition for either hepatic uptake or excretion, or both, could determine the occurrence of increased or decreased total bile acid excretion depending on the relative concentration of taurolithocholate. Thus, infusions of taurolithocholate sufficient to block excretion of endogenous taurocholate did not prevent the increase in bile acid excretion when larger amounts of taurocholate were given. The mechanism for these findings may be analogous to renal tubular transfer mechanisms, but a definitive explanation must await more precise knowledge of bile formation and of other metabolic effects of these bile acids. The solubilizing effect of taurocholate micelles[14] may have an important bearing on the excretion of taurolithocholate.

The small amounts of taurolithocholate normally present in bile are considered to be derived solely from bacterial dehydroxylation of chenodeoxycholate in the intestine[15]. In contrast to man, the rat can efficiently hydroxylate lithocholic acid brought to the liver by way of the enterohepatic circulation to form presumably less toxic derivatives. Investigations demonstrating morphological and metabolic effects of lithocholic acid have used greater amounts than are usually present in the intestine. Thus, lithocholic acid feeding to rats can cause bile duct proliferation with an inflammatory reaction[16] and also bile stone formation[17]. To these deleterious effects of lithocholic acid are added the cholestasis and jaundice that occur with infusion of taurolithocholate.

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A Method for extracting Mucoproteins from Epithelial Tissues

MOST of the connective tissue polysaccharides which have been isolated in the native state as their protein complexes have come from cartilage tissues[1,2]. On the basis of past experience, attempts have been made to use an isolation method that preserves the mucoproteins as far as possible unaltered and effects only mechanical breakdown of the tissue structure, while allowing the macromolecules to be isolated from the extracts.

We have applied a similar method in the extraction of polysaccharide protein complexes from the epithelial tissue of the stomach wall. Exhaustive homogenization of the tissue in a 'Virtis 45' homogenizer at 45,000 r.p.m. for up to 4·5 h liberated only a minor part of the acid sulphopolysaccharides of the tissue. Washing of the tissue residue with several portions of distilled water did not lead to any substantial increase in the amount extracted. The *p*H of the solution in the range from 2 to 8 had very little influence on the amount extracted. We also subjected the washed tissue homogenate to the action of highly purified collagenase[3], but no significant amounts of polysaccharides were liberated during 2-h incubation at 37° C. The polysaccharide contents of the various samples were estimated by a modification of a method based on hydrolysis with papain[4,5].

However, when the homogenization was carried out in molar sodium chloride, all the acid polysaccharides were liberated from the tissues after periods varying from 15 to 30 min. If the tissue was homogenized in distilled water and the residue washed with 0·15 M or more concentrated sodium chloride solution, all the polysaccharides were transferred to the supernatant.

The procedure that finally proved successful consisted of homogenization of the tissue in distilled water, followed by washing with sodium chloride solution. The extracted polysaccharides in the sodium chloride solution were precipitated with a threefold volume of ethanol and redissolved in water, whereupon the greater part of the co-precipitated tissue proteins remained undissolved. The clear supernatant was quantitatively transferred to a column packed with cellulose which had been treated with a 1 per cent solution of cetylpyridinium chloride in 0·05 M sodium chloride. The polysaccharides were eluted successively with 0·3, 0·6, 1·0, 1·2, 1·6 and 2·4 M sodium chloride solutions. The 0·6, 1·0, 1·2 and 1·6 molar sodium chloride solutions each eluted a fraction that was precipitated by cetylpyridinium chloride after dilution of the effluent with distilled water. Portions of the four precipitates were subjected to hydrolysis with papain and refractionated on the cellulose column pretreated with cetylpyridinium chloride as described earlier[4]. Other portions of the precipitates were subjected to disc electrophoresis on polyacrylamide.

All the fractions eluted from the cellulose column with 0·6 and 1·0 M sodium chloride solutions migrated slowly as one band on electrophoresis. The fractions eluted with 1·2 and 1·6 M sodium chloride solutions did not move from the point of application. It was found that all fractions were stained by alcian blue and amido black. Refractionation after hydrolysis with papain revealed that the fractions extracted by 0·6 and 1·0 M sodium chloride solutions consisted mainly of what we have previously identified as epithelial sulphopolysaccharides, while the fractions extracted by 1·2 and 1·6 molar sodium chloride solutions consisted of chondroitin sulphuric acid *B* (refs. 4 and 5). Hyaluronic acid from the stomach wall and the less-sulphonated epithelial polysaccharides were not present in these fractions, but had already been released from the tissue during the first homogenization with water.

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Vitamin B_{12} Concentrations in Human Tissues

THE development of microbiological technique for evaluating vitamin B_{12} concentration has made it possible to measure the wide variations in the quantity of vitamin B_{12} present in sera from humans in normal and various pathological states. By this means, the concentration of serum B_{12} has been found to be above normal in hepatitis[1], cirrhosis[2] and myelogenous leukaemia[3], but below normal in pernicious anaemia[4], total gastrectomy[5], sprue[6], old age[7] and pregnancy[8].

It has been demonstrated in patients that functional impairment of the ability to absorb B_{12} in the gastrointestinal tract results in a decreased level of serum B_{12} concentration. Because the B_{12} level is normally maintained at the expense of body stores, an evaluation of the magnitude of vitamin B_{12} storage in liver and other vital organs in disease may help to explain the mechanism of elevation of depression of serum B_{12} observed. This communication reports the measurement of B_{12} concentrations in various human organs, including the liver, kidneys, adrenals, heart, pancreas, spleen, lung and brain. The data obtained suggested that there is some association between chronological age and differences in liver stores of B_{12}. Certain diseases, including a hepatic tumour, also seemed to be related to differences that were observed in liver B_{12} concentration.

Tissue specimens were obtained from human organs during the course of examinations within 5 h of death and immediately stored at $-10°$ C. Within a week after sampling, the tissues were homogenized for a vitamin B_{12} assay. The procedure for the subsequent extraction of vitamin B_{12} from the organs and its quantification has been described elsewhere[9].

The vitamin B_{12} content was calculated in terms of μg/g of tissue wet weight on the basis of calibrated standard curves. Losses of B_{12} activity immediately after death and during the 7-day storage period after sampling were not estimated.

The distribution of vitamin B_{12} in various tissues is shown in Table 1. The tissues are ranked in order of diminishing concentration of the vitamin, which was greatest in liver. Considerable variations between replicate tissue sample concentrations are indicated by the magnitude of the standard deviations listed. The relatively high concentration of vitamin B_{12} in the adrenals in all the subjects examined suggests that this vitamin might in some way be involved in the metabolism of steroid compounds or the maintenance of the normal function of these glands.

Table 1. CONCENTRATION OF VITAMIN B_{12} IN HUMAN ORGANS

Tissues	No. of samples	Vitamin B_{12} (μg/g wet tissues)
Liver	6	0·653 ± 0·385*
Kidney	6	0·337 ± 0·338
Adrenal	6	0·161 ± 0·051
Heart	6	0·119 ± 0·041
Pancreas	3	0·116 ± 0·100
Spleen	5	0·055 ± 0·050
Lung	3	0·050 ± 0·053
Brain	2	0·027 ± 0·013

* Range estimate of standard deviation.

The data in Table 2 clearly show that there is an upward trend in hepatic B_{12} concentration associated with increased age of the subjects studied, although additional data would be required to establish this statistical pattern more definitely. Other related findings have also indicated a similar trend for liver B_{12} concentrations to increase progressively with advancing age[1,2].

Table 2. LIVER CONCENTRATIONS OF VITAMIN B_{12} IN HUMANS OF VARIOUS AGES

Age range	No. of cases	Vitamin B_{12} (μg/g wet tissue) Range	Average
Stillborn	5	0·026–0·090	0·056 ± 0·028*
4–48 (h)	3	0·096–0·123	0·108 ± 0·016
0·5–2 yr.	2	0·315–0·450	0·383 ± 0·120
20–40	5	0·240–0·497	0·310 ± 0·111
40–60	6	0·348–0·773	0·598 ± 0·168
60–85	6	0·270–1·562	0·775 ± 0·511

* Range estimate of standard deviation.

The weighted mean value of all the measurements observed for liver amounts to 0·41 μg/g. However, if the average hepatic concentration of B_{12} in the stillborn cases is excluded, the weighted mean value for the samples obtained from viable subjects is 0·49 μg/g. This adjusted value is in accordance with the mean value of 0·05 μg/g for hepatic B_{12} reported elsewhere[10,11]. Whether the markedly low concentrations of hepatic B_{12} that were found in stillborn infants (and in neonates) reflect some aspect of undeveloped liver function or a low requirement for the vitamin in these age groups remains to be investigated.

The effect of various diseases on hepatic vitamin B_{12} content is shown in Table 3. These results indicate that the vitamin B_{12} level of a human liver can vary to some degree with the type of disease encountered. In a disease where the liver is most directly concerned, as in cirrhosis, B_{12} concentration varied the most from the general level observed in corresponding age groups. However, considerable additional anamnestic data would be required in order to draw meaningful conclusions from the data shown for the other diseases in Table 3. The possibility that factors other than age may be important is emphasized by the hepatic B_{12} concentrations observed in two subjects who died accidentally. Their hepatic vitamin B_{12} concentrations of 0·245 μg/g were somewhat lower than might be expected from their chronological age, but by comparison they agree well with a recent investigation of twelve other individuals who suffered sudden, violent deaths, where the average hepatic B_{12} concentration was 0·280 μg/g (ref. 12).

Table 3. LIVER B_{12} CONCENTRATIONS IN DISEASE STATES

Cause of death	No. of cases	Average age	Vitamin B_{12} (μg/g wet tissues)
Pneumonia	2	1·5	0·383 ± 0·089*
Accident†	2	37	0·245 ± 0·003
Cerebral thrombosis	1	50	0·348
Coronary occlusion	3	53	0·542 ± 0·142
Myocardial infarction	2	55	0·698 ± 0·120
Leukaemia	2	61	0·885 ± 0·157
Cirrhosis	1	63	0·262
Carcinoma	4	63	0·481 ± 0·088
Atherosclerosis	3	68	0·936 ± 0·075

* Range estimate of standard deviation.
† One was killed in a car accident, the other died of a gunshot wound.

Table 4. AVERAGES OF VITAMIN B_{12} CONCENTRATIONS MEASURED IN REPRESENTATIVE LIVER AREAS

Cause of death	No. of samples/liver	Vitamin B_{12} (μg/g wet tissue)
Car accident	6	0·251 ± 0·006*
Coronary occlusion	6	0·481 ± 0·022
Cirrhosis	5	0·262 ± 0·071
Leukaemia	5	0·537 ± 0·039
Cancer of pancreas	5	0·506 ± 0·124

* Range estimate of standard deviation.

Table 4 compares the uniformity of concentrations of vitamin B_{12} localized in various lobes of single livers. The small standard deviations reflect the relatively small

interareal differences detected. However, concentration differences were greater in the cirrhotic liver studied than in all but the carcinomatous liver tissue, where areas that contained cancer cells had a higher B_{12} concentration than other adjoining, but normal, liver tissue obtained from the same subject. Without additional investigations, an exact meaning cannot be assigned to this observation. It is worth mentioning in this connexion, however, that it has been reported that mouse tumour cells are able to synthesize vitamin B_{12} (ref. 13).

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Effects of Thiols on *Escherichia coli* β-Galactosidases

IN the extensive experimentation that has involved the assay of β-galactosidase activity during the past 5 years it has been common practice to use thiols, and particularly β-mercaptoethanol. In our method of preparing this enzyme[1] we use thioglycolate for the primary purpose of reducing large aggregates to a minimum. The substitution of β-mercaptoethanol in the preparative procedure, using strain *ML*308 as a source of β-galactosidase, has resulted in a variety of effects, one of which is a progressive inactivation.

In exploring the action of β-mercaptoethanol on β-galactosidase we have verified the observation that, under assay conditions, the addition of this thiol (at concentrations greater than 10^{-3} M) increases the apparent activity by about 50 per cent. However, the thiol effect on our enzyme preparations is a function of the Mg^{++} (or Mn^{++}) concentration as well as *p*H. A typical example is shown in Fig. 1. At *p*H 7, both inactivation and activation by thiols may be seen, depending on the presence of Mg^{++} or Mn^{++}. At a *p*H value of 8·6 these effects are greatly diminished and the rates are reduced.

Highly purified preparations of β-galactosidase from *ML*308 lose activity readily but may be stabilized either by storing in 50 per cent glycerol or by removing Mg^{++} by dialysis against ethylenediamine tetraacetic acid (EDTA). The latter technique is successful only for dilute solutions of protein. However, changes occur in more concentrated solutions due to the fact that such a protein preparation cannot be eluted off DEAE columns even at high salt concentration.

We interpret these results as indicating that β-galactosidase has several conformational and association modes and that the most active form enzymatically is not the most stable thermodynamically. In the presence of β-mercaptoethanol and Mg^{++} the structure is permitted more possibilities to associate with the substrate. In the absence of Mg^{++} other associations occur which are enzymatically inactive. Numerous ultracentrifuge investigations have failed to show readily reproducible differences between active and thiol-inactivated enzyme. In gel-electrophoresis, the latter moves at about 0·9 times the rate of the former.

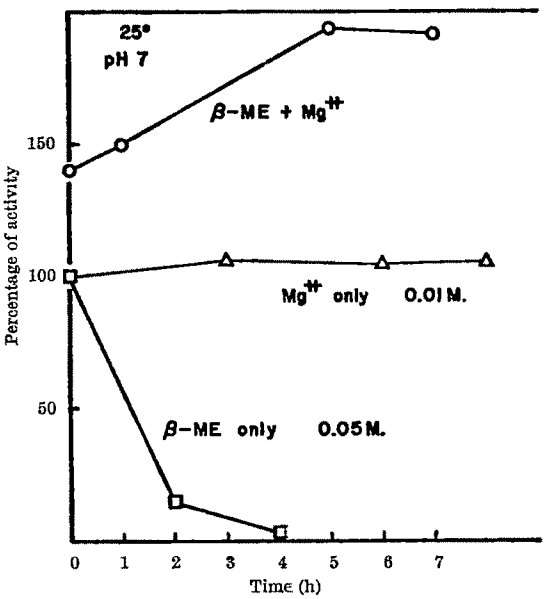


Fig. 1. The effect of β-mercaptoethanol on β-galactosidase activity when under assay conditions. Substrate solution. 9 ml. 0·05 M sodium phosphate buffer *p*H 7 plus 1 ml. *o*-nitrophenyl β-D-galactoside (30 mg in 10 ml.). The release of *o*-nitrophenol from the substrate by enzymatic action was plotted (420 mμ) by a 'Cary' spectrophotometer. Points on the graph correspond to slopes, linear under assay conditions

Beta-galactosidase isolated from strain *K*12 does not behave toward thiols as described here. Rather they seem necessary for retention of activity[2]. Presumably this is due to conformational and sequential differences in the two proteins.

Effects of β-mercaptoethanol such as we have observed in the enzyme isolation process have also been noted in the case of fatty acid synthetase[3]. The phenomenon of superactivity, or the marked enhancement of activity by β-mercaptoethanol, has been seen recently in experiments on lactate dehydrogenase[4]. The interrelation of divalent ions to β-mercaptoethanol activation has been recorded for histidinol dehydrogenase[5]. Whether or not all these effects have the same chemical basis remains to be determined.

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Progesterone Biosynthesis by Equine Granulosa Cells growing in Tissue Culture

OUR knowledge of the pathways of steroid biosynthesis in the ovary has been gained mainly by incubations of ovaries *in vitro*[1,2]. The tissues incubated have contained numerous cell types: granulosa cells, theca interna cells, stromal cells, interstitial cells, and sometimes luteal cells. Possibly such mixtures of two or more different cell types are able to secrete hormones that one cell type cannot secrete by itself[3–9]. Furthermore, during such incubations *in vitro* an exchange of precursors and products between different cell types may be facilitated because of breakdown of naturally occurring barriers, such as the basement membrane between the granulosa layer and the theca interna of the follicle.

Thus there is clearly a need for more information on the steroidogenic potential of individual cell types studied in isolation. The purpose of the present experiments was to explore the possibility of growing a corpus luteum in tissue culture from a pure cell line, namely the granulosa cells, and to determine whether or not such a tissue is capable of steroid synthesis.

Graafian follicles, 2–6 cm in diameter, were dissected out of equine ovaries which had been collected at various stages of the oestrous cycle, by a method previously described[3]. The granulosa cells were scraped out under aseptic conditions. Vital staining with eosin *Y* (ref. 10) and nigrosin[11] demonstrated that only 10–20 per cent of the granulosa cells were alive. The cells were grown at 37° C as stationary monolayers, either on glass coverslips placed in test-tubes or in 50-ml. glass bottles. Microscopically, the cultures appeared to consist of one cell-type only. The growth medium used consisted of 10 per cent horse serum, 30 per cent medium 199 (ref. 12), and 60 per cent Hanks's balanced salt solution[12]. After various incubation times the coverslip preparations were washed with warm Hanks's solution, fixed in Bouin's solution, and stained with haematoxylin and eosin. For some experiments, various radioactive steroid hormones in propylene glycol were added to the medium The old medium together with any dead cells was discarded before addition of fresh medium containing the steroids.

Regardless of the stage of the oestrous cycle of the mare, granulosa cells removed from vascular follicles were found to adhere to the glass forming monolayers, and to take on within 2–4 days many of the morphological features which are characteristic of luteal cells. The cytoplasm of these cells increased in amount and became more densely stained and granular in appearance; the nucleus increased in size, and the chromatin material became more distinct and abundant. Some mitoses were also to be seen. This luteinization process occurred irrespective of whether gonadotrophin was added to the medium.

It has been possible to show that these cultured granulosa cells are capable of steroid synthesis. Thirty-two vessels, sixteen with gonadotrophin added (5 I.U./ml. of a mixture of pregnant mares' serum and human chorionic gonadotrophin) and sixteen without gonadotrophin, were incubated for an average of 7 days. The media were pooled, a small amount of 7-^{3}H-progesterone added as an internal recovery standard, and a 224-ml. sample extracted with ether in the presence of alkali; the ether extract was chromatographed on paper in a ligroin/80 per cent methanol system[13].

The paper chromatogram revealed a large amount of progesterone, which was further characterized by the following criteria: (i) same R_F value on paper and same gas chromatographic retention time on 3·8 per cent *S.E.* 30 as authentic progesterone; (ii) absence of acetylable hydroxyl groups; (iii) infra-red spectrum identical to that of authentic progesterone; (iv) formation, on treatment with 20β-hydroxysteroid dehydrogenase[14], of a compound with paper and gas chromatographic properties identical with those of authentic 20β-hydroxypregn-4-en-3-one; the sulphuric acid: 80 per cent ethanol chromogen[15] of this compound showed the expected peak at 478 mμ; its acetylation product had the same paper and gas chromatographic properties as that of 20β-acetoxypregn-4-en-3-one. Control medium in which cells were not grown contained no detectable steroids.

It was calculated that the 340 μg of progesterone isolated from the medium had been formed by 20,000–40,000 live cells in the course of the 7-day culture period. In addition the medium also contained appreciable amounts of 20α-hydroxypregn-4-en-3-one (89 μg), 17α-hydroxyprogesterone (35 μg), androstenedione (35 μg), and a trace of oestradiol (> 2 μg). Contrary to Falck's observations in the rat[7,8] and Short's observations in the horse[3,4,6], it appears that equine granulosa cells by themselves are capable of some androgen and oestrogen biosynthesis in tissue culture when gonadotrophin is present.

When the 7- or 14-day-old cultures were incubated for 24 h with 10 μc. 16-^{3}H-pregnenolone (15 μc./μg), more than 90 per cent of the steroid was metabolized—the major product being progesterone. The specific activity of the progesterone so formed was only half that of the starting material, which can be taken as further evidence for the *de novo* synthesis of progesterone by these cells.

The pattern of pregnenolone metabolism by the luteinized granulosa cells was similar to that obtained with cultures of equine corpora lutea. Thus it seems as if equine granulosa cells really can develop into a corpus luteum in tissue culture. Surprisingly, they can do so regardless of the stage of the follicle from which they were obtained, and quite irrespective of the presence of gonadotrophin in the culture medium. It remains to be seen whether gonadotrophins can augment the secretory activity or prolong the life-span of such a test-tube corpus luteum.

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Trimethylamine-N-oxide Biosynthesis in the Bullfrog

THE comparative aspects of the mechanism of oxygenations dependent on molecular oxygen have recently been investigated using the biosynthesis of trimethylamine-N-oxide as a model[1]. That investigation raised a number of questions beyond the presence of the enzymatic activity in a given organism. In the case of the bullfrog, *Rana catesbeiana*, questions which remained were: the intra-

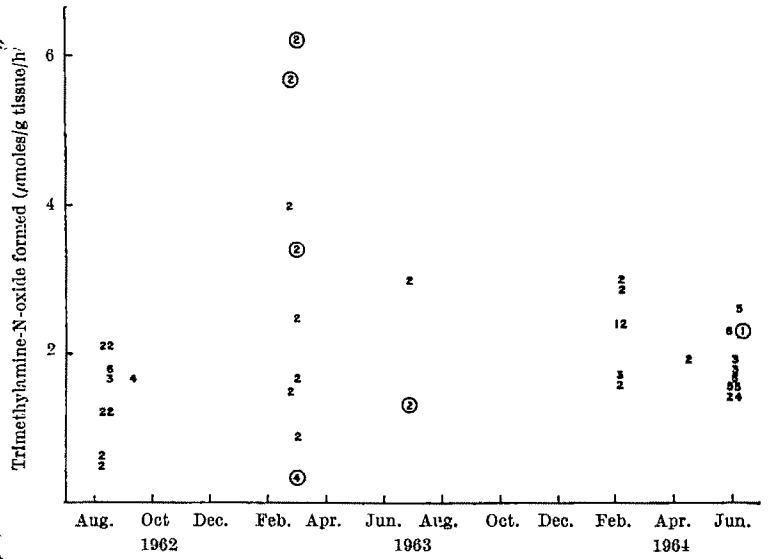


Fig. 1. Variation in trimethylamine oxidation activity with season and stage of tadpole development. The numbers on this figure refer to the stage of tadpole development. Circled numbers refer to tadpoles maintained in the laboratory for at least five months before assay. 1, Small tadpole, 2, large tadpole; 3, rudimentary hind legs; 4, well-developed hind legs; 5, rudimentary forelimbs, 6, well-developed forelimbs (tail not as yet resorbed)

cellular location of the enzyme, the reason for the apparent lack of trimethylamine-N-oxide synthesis in the tadpole and the nature of the developmental processes involved in the ultimate production in frog cells of high levels of activity. Continued investigation of trimethylamine-N-oxide synthesis in the bullfrog system has provided some answers to these questions.

In the present investigation assay conditions were the same as those adopted earlier[2]. Tadpole and frog liver microsomes were isolated according to the classical procedure of Hogeboom[3] and electron microscope techniques were essentially those of Palade[4].

In contrast to the lack of demonstrable trimethylamine oxidation in early experiments[1], in the most recent ones trimethylamine oxidation by tadpole livers was clearly detectable (0·3–6·2 μmole/g tissue/h). The activity was, however, lower than that exhibited by homogenates of adult bullfrog livers (8–11 μmoles/g tissue/h). Although no special importance was attributed to it at the time, the first tadpoles were taken in the later winter and were therefore in hibernation or had recently arisen from hibernation. In later experiments, tadpoles taken in the summer were used. The possibility then presented itself that there might be seasonal effects on the levels of activity, or that the levels of activity were a function of tadpole age. The latter was a possibility because bullfrog tadpoles very often do not undergo metamorphosis until one to three years after they hatch. Since all of the tadpoles used in these experiments were taken in nature, they varied considerably in age. The data presented in Fig. 1 clearly indicate that neither the season of the year nor the stage of development of the tadpoles used significantly affected the results obtained. Included in the group were small tadpoles (less than a year old); tadpoles at least a year old, which had been allowed to winter either in the laboratory or in their natural habitat; and tadpoles at all stages of metamorphosis, including those with forelimbs. In the period between the appearance of forelimbs and the completion of metamorphosis the animal's liver acquires the adult levels of trimethylamine-oxidation activity. These results are quite similar to those obtained by Cohen *et al.*[5], who found in the bullfrog that the liver enzymes associated with the biosynthesis of urea also show precipitous increases in activity during the late stages of metamorphosis. Thus the original tadpole data[1] were in error, and resulted from sampling at the lower end of the wide range of possible trimethylamine oxidation activities.

Table 1. TRIMETHYLAMINE OXIDATION BY SUBCELLULAR FRACTIONS

Subcellular fraction	μmoles/g liver (wet weight)/h Tadpole	Adult
Nuclei + cell debris	1·4	2 5
Mitochondria	< 0 2	1·1
Microsomes	1 4	3·7
Supernatant	0 2	2·6

Since, in hog liver, trimethylamine oxidation previously had proved to be a microsomal activity[6], it was of interest to verify that this activity in the bullfrog tadpole and adult was similarly located. The data presented in Table 1 verify that in the bullfrog trimethylamine oxidation occurs in the centrifugal fraction which in mammalian systems contains microsomes. That this is also a microsomal fraction in both the bullfrog tadpole and adult was verified by electron microscopy. The spread of activity among the various fractions was to be expected, due to the difficulty of preventing association of the microsomes, on the one hand, and excessive fragmentation during homogenization, on the other. It was for this reason that the specific activities of the various subcellular fractions of the adult liver were also determined. They were as follows (mμmoles/mg protein/h): nuclei mitochondria, 149; microsomes, 710; and supernatant, 48. Thus, in the bullfrog tadpole, trimethylamine oxidation appears to be a microsomal activity which undergoes the same metamorphic increase in activity as was previously shown to be true of a number of soluble enzyme activities[7].

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Triplet Nucleotide–Amino-acid Pairing; a Stereochemical Basis for the Division between Protein and Non-protein Amino-acids

Pelc and Welton have recently proposed[1] a stereochemical relationship between protein amino-acids and the codon triplet of nucleotides. I wish to propose a rather different possible relationship between nucleotide triplets and amino-acids which has emerged as a result of preliminary investigations aimed at understanding the usual exclusion and the occasional intrusion of the so-called non-protein amino-acids into protein.

It is unlikely that the non-protein amino-acids were excluded when life began to evolve because of lack of availability. A number of them, ornithine and homoserine, for example, are intermediates in the production of other amino-acids. Is there some stereochemical relationship between the four nucleotides and the protein amino-acids which favoured their aggregation—a relationship not involving enzymes which originally could not have been available? Theories concerning direct stereochemical relationships between DNA or RNA and amino-acids were popular some years ago in relation to the apparent

coding problem of sixty-four base permutations to twenty amino-acids, but a direct binding of amino-acids on to nucleotides implied an overlapping code which was shown to be unacceptable[2]. The discovery of transfer RNA appeared to solve the amino-acid–nucleic acid linkage problem but in reality shelved it. The existence in *t*RNA, however, of an isolated triplet allows a direct linkage of an amino-acid to the bases without requiring an overlapping code.

I propose that the anti-codon triplet for an amino-acid in a transfer RNA molecule provides a cage which will bind amino-acids (as their acyladenosine-monophosphates) in differing degrees so that they are made to lie in a position suitable for coupling to the non-specific-cytosine-cytosine-adenosine end of the *t*RNA chain. One must ask what incentive the amino-acid has to enter such a cage and what forces between the bases could create a precisely defined cage. Interaction between the free $-NH_+$ group of the amino acyl-AMP, ($>NH_2^+$ of proline) and some feature of the triplet common to all nucleotide triplets could provide the incentive and hydrogen bonding between the neighbouring bases the specific defining force for the cage. The importance of planar A—U and C—G base binding tends to overshadow the possibility of out-of-plane hydrogen bonding, but the existence of $-NH_2$ groups in three out of four bases allows great flexibility of such bonding. Out-of-plane hydrogen bonding between bases is expected to be weaker, but this is necessary, since the presence of messenger RNA must cause refolding of the anti-codon triplet to form planar bonds with its codon and release of the —CCA amino-acid end for peptide condensation.

I suggest that, just as chance causes the hydrogen bonding of C and G or U and A bases to be of a unique kind, so the fit between amino-acids and the triplet cages selects twenty chemically quite dissimilar amino-acids from the possible 100 found in Nature (there are more chemical species, but the 100 are biologically available).

First it is interesting to note that if the second base of the *t*RNA triplet is guanosine (anti-codons in Table 1)[3] the coding depends only on the first two bases. In this case, the last base acts only to seal off the end of the cage and its character is immaterial. Guanosine as the first base is almost as powerful in this respect, and cytosine as the first or second base is next to guanosine in its influence. Between them they control all the amino-acids which are stereochemically determined by only two bases. On the other hand, when the second base is uracil the first and last base are critical in determining the amino-acid which is bound. Cytosine and guanosine are the bases with both hydroxyl and amino groups projecting from their six-membered rings and are therefore better able to form hydrogen bonds than uracil with two hydroxyl groups (which can only hydrogen-bond with $-NH_2$ for stereochemical reasons) and adenosine with only one amino group. All other features of the six-membered rings are common to all bases. The possession by guanosine of two extra nitrogen atoms in its five-membered ring provides an explanation for dominance over cytosine

Table 1. THE GENETIC CODE

1st base	2nd base				3rd base
	U(A)	C(G)	A(U)	G(C)	
U	Phe	Ser	Tyr	Cys	A
(A)	Phe	Ser	Tyr	Cys	G
	Leu	Ser	'ochre'	?	U
	Leu	Ser	'amber'	Try	C
C	Leu	Pro	His	Arg	A
(G)	Leu	Pro	His	Arg	G
	?	Pro	GIN	Arg	U
	(Leu)	Pro	GIN	Arg	C
A	Ileu	Thr	AsN	Ser	A
(U)	Ileu	Thr	AsN	(Ser)	G
	Ileu	Thr	Lys	Arg	U
	Met	Thr	Lys	?	C
G	Val	Ala	Asp	Gly	A
(C)	Val	Ala	Asp	Gly	G
	Val	Ala	Glu	Gly	U
	Val	Ala	Glu	Gly	C

The anti-codons of the *t*RNA are shown in parentheses.

in hydrogen bonding and thereby in influence on the geometry of the cage.

Detailed testing with models of this hypothesis even of those amino-acids defined by two bases is a major undertaking. Proline was selected for examination since its side chain has no freedom of movement and its anti-codon is G.G.-3rd base, the most powerful hydrogen-bonding combination. Furthermore, several of its naturally occurring analogues have been studied in detail. A 'Courtauld' model of the triplet G.G.-3rd base was made and it was found possible to form three hydrogen bonds. C60-NH_2C2′,N1-N1′, C2NH_2-OC6′, between the guanosines. This left a narrow cleft into which the proline ring could fit and the NH_2^+ of the proline apparently could bond to the oxygen of the phosphorus between the second and third base. This is the only free phosphorus-bound oxygen (P=O); that of the phosphorus between the first and second base is on the outside of the cage. The naturally occurring ring-substituted proline molecules and pipecolic acid, a six-membered ring analogue, do not permit free access of the NH_2^+ group to the same point. The four-membered ring analogue azetidine-2-carboxylic acid does permit such access but makes fewer stabilizing van der Waals hydrogen contacts. The foregoing results are consistent with biochemical investigations which indicate that of the aforementioned analogues only azetidine-2-carboxylic acid is incorporated into protein[4,5]. The levels of azetidine-2-carboxylic acid used suggest that it reaches the amino-acid pool at a much higher level than that of the endogenous proline[4]. Experiments with isolated activating enzyme and *t*RNA are not conclusive, but an incorporation of azetidine-2-carboxylic acid at 3 per cent of the level of proline was indicated[5].

The present hypothesis differs from that of Pelc's in that it seeks to find some relationship between the anti-codon trinucleotides and amino-acids. With the limited examination so far made it would be premature to put this hypothesis forward as a general one; rather, it is claimed that it appears to provide an explanation in the case of a protein amino-acid and its non-protein analogues. The difficulty Pelc encountered with the many hydrocarbon side chains caused him to invoke bonds between methyl groups and bases, and hydrogen bonds between sulphur and bases, which are chemically rather unusual. These difficulties are removed if one maintains that it is hydrogen van der Waal's contacts which stabilize the amino-acids in the trinucleotide pocket. Certainly such contacts seem to be of particular importance in the stabilization of protein conformation[6]. By this approach the interaction of anti-codon and amino-acid is seen much more as a selection by steric restriction rather than as the formation of a chemical complex with the trinucleotide. At the present-day level of organization an aminoacyl-*t*RNA-synthetase attempts to put the amino-acid key into the anti-codon lock and if it succeeds binding to the —CCA terminal end occurs. Before the existence of enzymes the fit of lock and key would merely have been much slower.

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Significance of Sub-bands of Lactic Dehydrogenase Isozymes

THE five isozymes of lactic dehydrogenase (LDH) usually observed after electrophoresis of vertebrate tissue homogenates are generally considered to result from the five possible combinations of two basic sub-units, *A* and

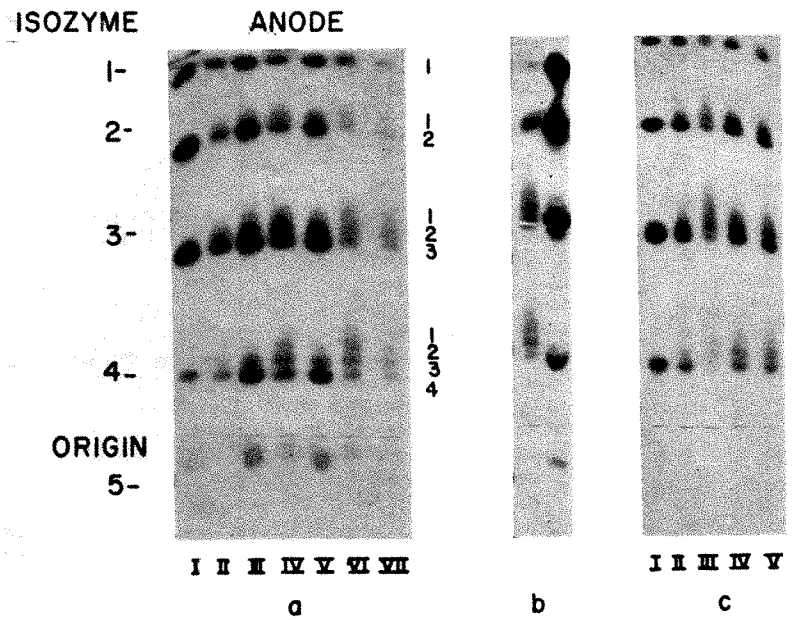


Fig. 1. Production of sub-bands by formaldehyde treatment. (*a*) Testis, I was not treated; the storage time between treatment and homogenization was 1 day for II and III, 3 days for IV and V, and 7 days for VI and VII. The concentration of formaldehyde used was 0·4 per cent for II, IV, and VI and 0·8 per cent for III, V, and VII. (*b*) A testis, exhibiting sub-bands without treatment, is on the left; kidney from the same cadaver is on the right. (*c*) Kidney, I was not treated; the storage time between treatment and homogenization was 1 day for II and III, and 7 days for IV and V. The formaldehyde concentration was 1·0 per cent for II and IV, and 2·5 per cent for III and V

B, combined into tetramer units[1,2]. The synthesis of each of the two sub-units is believed to be under the control of separate genes, which together account for the total isozyme composition[1,2].

Two sub-units arranged as tetramers cannot account for more than five isozymes. Some tissues, however, exhibit isozymes composed of a number of discrete sub-bands[3–5]. Conflicting hypotheses have been proposed in order to account for them.

This communication describes experiments that demonstrate that the isozymes of human testis, which originally show single isozyme bands, can be made to exhibit a number of distinct sub-bands after treatment with formaldehyde. Similar results have also been obtained with other human tissues investigated. The exhibition of LDH sub-bands, after appropriate treatment, may therefore be a general characteristic of human tissues.

Tissues were obtained at autopsy. The tissues were homogenized in distilled water (2 ml./g of tissue). Starch-gel electrophoresis and detection of LDH activity were carried out as previously described[6]. The electrophoretic buffer used was either the potassium phosphate–citric acid buffer, *p*H 7·0 (ref. 7), or a *tris*–citric acid buffer[7]. In the latter case, the gel buffer was prepared by adding 0·01 M citric acid to 0·01 M *tris* until the *p*H reached 7·0, and the electrode buffer was prepared by adding 0·03 M citric acid to 0·03 M *tris* until the *p*H reached 7·0. Both buffers produced comparable results.

Samples were treated with formaldehyde by dipping about 0·5 g of tissue, for 5 sec, in a solution of formaldehyde, which had been prepared by dilution with water. The tissue was then lightly blotted, wrapped in 'Saran Wrap', and stored at 3° C. The number of days the tissue was stored before being homogenized, and the concentration of formaldehyde used, are specified below.

Results obtained with a sample of testis, with and without formaldehyde treatment, are presented in Fig. 1*a*. The treated tissue had been divided into three portions, and each was stored for different periods of time before being homogenized. The isozymes of the untreated sample consisted of five single bands (the *X*-band is not easily discerned with the buffers used). The treated tissue, which had been stored for seven days after the formaldehyde treatment, before being homogenized, exhibited a single isozyme 1, two bands in the isozyme 2 position, three in the isozyme 3 position, and four bands in the isozyme 4 position. The portions of the tissues stored for shorter periods of time between the treatment and homogenization also exhibited sub-bands, but not always as many. In some cases, the number of sub-bands do not increase with longer periods of storage, but the amount of formaldehyde which penetrated different areas of the tissue may have varied, depending on the shape of the tissue and other possible factors. After the samples were homogenized, the isozyme patterns did not change over a period of several days (the samples were stored at 3° C).

Fig. 1*b* presents an isozyme pattern of a homogenate of testis which exhibited sub-bands without being treated with formaldehyde. It could not be determined with certainty whether or not this tissue had inadvertently been brought into contact with formaldehyde during the autopsy. The isozyme pattern of a homogenate of kidney tissue obtained from the same cadaver is also presented. Isozyme 3 exhibits some sub-bands, but the other isozymes are singlets.

A sample of kidney homogenate, with and without formaldehyde treatment, is shown in Fig. 1*c*. The kidney requires a higher concentration of formaldehyde for the demonstration of sub-bands than the testis does, but the distribution of the sub-bands is similar. The treatment of liver and heart tissues with formaldehyde also resulted in similar sub-bands.

The electrophoresis of treated samples of testis, conducted with the phosphate–citrate buffer, and with the discontinuous buffer described by Poulik[8], is compared in Fig. 2. The testis *X*-band is more sharp and distinct when the latter buffer is used. However, the regular increase in the number of sub-bands with decrease in isozyme mobility, after formaldehyde treatment, is not clearly demonstrated with this buffer. The formaldehyde causes the intensity of the *X*-band and isozyme 4 to decrease, and the intensity of isozymes 2 and 3 to increase.

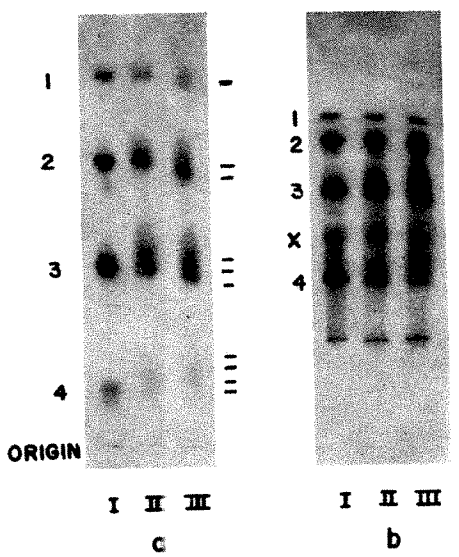


Fig. 2. Effect of buffer on separation of sub-bands. The buffer used was (*a*) phosphate-citrate, and (*b*) discontinuous buffer of Poulik. Testis samples were the same in each case: I was not treated. The formaldehyde concentration was 0·4 per cent for II and 0·8 per cent for III. Storage time between treatment and homogenization was 4 days.

This phenomenon is being investigated by treatment of purified isozymes with formaldehyde.

Costello and Kaplan demonstrated LDH sub-bands in untreated mouse tissue, which increase in number from isozymes 1 to 5 (ref. 9). They suggested that the sub-bands may be due to the synthesis of two types of *A* sub-units by separate genes. Two slightly different forms of *A* sub-units would account for sub-bands which correspond in number to the order of the isozymes. Koen and Shaw, who also investigated isozyme sub-bands in mouse tissues, have observed that in some cases the number of sub-bands is too great to be explained by this hypothesis[3].

Fritz and Jacobson found that sub-bands exhibited by homogenates of mouse muscle could be accentuated by the addition of β-mercaptoethanol to polyacrylamide gels[5]. They proposed, as a tentative explanation, that the mobility of the *A* sub-unit may be slightly different when it is combined with NAD, and that an equilibrium is produced by the mercaptoethanol between *A* sub-units with and without NAD. The validity of this explanation has been questioned by investigators, who could not confirm any difference in the isozyme patterns after the addition of NAD to the gels[3,10,11].

In addition to mouse tissues, regularly increasing sub-bands have been observed by various investigators in haemolysates of untreated human erythrocytes of small portions of populations sampled[4,10–13]. In some instances (ref. 4, for example), the sub-bands appear almost identical to those previously mentioned in testis and kidney tissues. The erythrocyte sub-bands have generally been explained by the hypothesis of mutant genes, which lead to the synthesis of two types of *A* or *B* sub-units.

The LDH sub-bands do not appear to be due to an equilibrium resulting from polymerization[3,13], and they retain their original mobility when isolated and submitted to a second electrophoretic separation[3].

The consistent production of regularly ordered, distinct sub-bands by formaldehyde treatment in various tissues suggests that the ability to exhibit such sub-bands under these conditions is a general characteristic of human tissues, rather than a result of genetic mutation. Two alternate explanations which would appear most likely are: (1) the sub-bands were originally present, but not demonstrated, until the formaldehyde treatment; (2) formaldehyde caused an alteration of some of the sub-units.

In the latter case, it is possible that detection of sub-bands in untreated tissues or haemolysates, already referred to, was due to sub-unit alterations, which occurred before or during processing, without formaldehyde treatment. Resistance of sub-units to alteration may be indirectly affected by other metabolic variations which are inherited.

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Effect of Age on the Composition of Bovine Nasal Cartilage

Malawista and Schubert[1] showed that 80 per cent of the chondroitin sulphate present in bovine nasal cartilage could be extracted from the tissue by homogenization in distilled water. Further purification of the extracted material indicated that 15 per cent of the dry weight was protein[2] which appeared to be covalently bound to the polysaccharide moiety. Small amounts of keratan sulphate have recently been found in the complex[3], and work in this laboratory has indicated that both mucopolysaccharides behave as though they are part of a single complex molecule[4].

Kaplan and Meyer[5] showed that the proportion of keratan sulphate in the total acid mucopolysaccharide fraction of human costal cartilage increased linearly with age. It seems possible that these changes reflect alterations in the chemical composition of the mucopolysaccharide protein complex in the tissue. In this communication the composition of the extracted mucopolysaccharide protein complex from bovine nasal cartilage is investigated as a function of the age of the animal.

Nasal cartilage was obtained from embryonic (23 in. crown to rump), young (2 years), and old (14 years) steers within 1 h of death. The loose fascia was dissected away and the cartilage minced at 0° C and then homogenized in distilled water as described by Malawista and Schubert[1]. The homogenates were first centrifuged for 60 min at 20,000 r.p.m. (Spinco, model *L* ultracentrifuge, No. 21 rotor) to remove collagen residues and then for 150 min at 45,000 r.p.m. (No. 50 rotor) to remove a heavy gel fraction[2]. The residues were extracted a further three times and the supernatants pooled and the mucopolysaccharide protein complex precipitated with cetyl trimethylammonium bromide (CTAB) and washed with distilled water. The quaternary ammonium ion was then removed by shaking the precipitate with a saturated solution of potassium thiocyanate in ethyl alcohol. After washing the precipitate with alcohol the crude preparation was dissolved in distilled water and passed through a jacketed column of 'Ecteola' ion exchange resin in the chloride form at 2° C. The eluate was again treated with CTAB and the quaternary ammonium ion removed from the precipitated complex as before. The passage of the crude preparation through the 'Ecteola' resin removed nucleic acid, free acid mucopolysaccharide and any unbound protein. The resin did not retain more than 2 mg of the protein polysaccharide complex per gram of dry resin.

An examination of the sedimentation velocity pattern in the analytical ultracentrifuge showed the presence of a single boundary in each preparation (Fig. 1).

The protein content of the preparations was between 15 per cent and 20 per cent of the dry weight and was not altered by reprecipitation with CTAB. This protein could be removed by digestion with papain and the acid mucopolysaccharide fraction isolated as described by

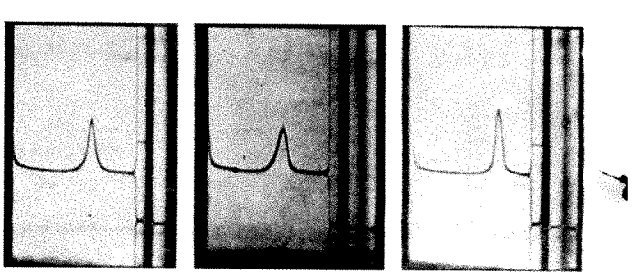


Fig. 1. Sedimentation velocity patterns of mucopolysaccharide protein complexes of cartilage isolated from: (1) embryonic steer; (2) young steer; (3) old steer. Sedimentation in 0·15 M KCl at a sample concentration of 0·3 g/100 ml.

Table 1. CHANGES WITH AGE IN THE POLYSACCHARIDE COMPOSITION OF THE MUCOPOLYSACCHARIDE–PROTEIN COMPLEXES EXTRACTED FROM BOVINE NASAL CARTILAGE

	Embryonic		Young		Old	
	g/100 g	Molar ratio*	g/100 g	Molar ratio*	g/100 g	Molar ratio*
Glucosamine } (1)	2·1	0·09	3·7	0·16	5·4	0·29
Hexose } (1)	2·8	0·12	4·3	0·19	6·1	0·33
Galactosamine } (2)	22·0	0·91	20·0	0·84	13·2	0·71
Uronic acid } (2)	21·9	0·84	19·8	0·77	14·3	0·71
Ratio of keratan sulphate (1)/chondroitin sulphate (2)	0·10		0·19		0·41	

* The values are calculated using total hexosamine as 1·00.

Scott[6]. Analysis of this fraction for uronic acid, hexosamine and hexose is shown in Table 1. The molar equivalence of (1) glucosamine and hexose and (2) of galactosamine and uronic acid indicated the presence of keratan sulphate and chondroitin sulphate in all three preparations. This was confirmed by the separation of the two mucopolysaccharides on cellulose columns as described by Gardell[7].

The ratio of keratan sulphate to chondroitin sulphate as indicated by the proportion of glucosamine to galactosamine in Table 1 varied according to the age of the animal, while the total acid mucopolysaccharide content of the complexes remained relatively constant. The keratan sulphate content of the total mucopolysaccharide increased from 9·0 per cent in the embryonic preparation to 29 per cent in that of the old steer. These results suggest that the changes in the ratio of keratan sulphate to total mucopolysaccharide observed by Kaplan and Meyer[5] in human costal cartilage with increasing age are probably due to changes in the composition of the acid mucopolysaccharide complex present in this tissue.

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Premature Birth in Rats induced by Ethionine Injection

THE injection of pregnant rats with certain antimetabolites, such as ethionine, in sub-lethal doses, may result in resorption or stillbirth[1–4]. We wish to report the effect of smaller, repeated doses of ethionine on the course and outcome of pregnancy in rats. Daily subcutaneous injections, in the doses listed in Table 1, were given from the twelfth day of pregnancy until delivery. Control animals received saline injections. All injections were in a volume of 1 ml. The date of conception was established by the first appearance of sperm on vaginal smears.

Most of the litters from females receiving ethionine were born prematurely. The degree of prematurity was, in general, directly related to dose. These findings are outlined in Table 1. The birth weight deficits of the pups were no longer evident after ninety days.

We subsequently injected a group of non-pregnant female rats with single doses of ethionine in the lethal range. When the survivors were mated with normal males fertility was not impaired and young were delivered after a normal gestation period. The birth weights and growth rates of the pups were normal.

It is interesting to contrast the premature pups born to ethionine-treated mothers with those born at term to mothers whose dietary intake was restricted during pregnancy. Both types of pups result from a nutritional stress and both are underweight at birth. Their subsequent progress is, however, quite different. The premature pups attain full adult weight; the pups of restricted mothers remain underweight throughout their lives and also show other evidence of permanent damage[5].

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Relationship between Age and Haemoglobin-splitting Activity in Chicken Muscle

CATHEPTIC activity towards proteins has been found in extracts of kidney, spleen, liver, lung, muscle, brain and erythrocytes[1–5]; therefore, the cathepsins may be considered to be part of the general enzymatic apparatus of animal cells. The action of the cathepsins is evident during the autolysis of animal tissues at acid *p*H. Recently, considerable interest has been shown in the catheptic activity of beef and chicken muscle because of their suggested role in meat tenderization, preservation and flavour[6–10].

The function of the cathepsins in living tissue is not clearly known. Sylven and Malmgren[11] have shown that the haemoglobin-splitting activity of young rapidly growing tumour cells is much greater than that of older tumour cell generations, and Ross and Ely[12] found higher activity in young rats than in adult and older rats. These investigations suggest that cathepsins may play a part in protein turnover and synthesis.

The investigation reported here was undertaken to determine whether haemoglobin-splitting activity (protease activity at *p*H 3·8) in broiler chicken breast and thigh muscles changes during maturation. Such information is important in elucidating the physiological role of the haemoglobin-splitting cathepsins and can be of value as a means of determining maturity.

Six sets of broilers, 4, 5, 8, 10, 12 and 14 weeks of age, were slaughtered by cutting the neck veins. The animals were bled and the breast and thigh muscles were removed. The tissue was immediately ground and then blended with distilled water. Haemoglobin-splitting activity was determined in tissue extracts by a modification[13] of Anson's[14] method. The amount of tyrosine and tyrosine-containing peptides liberated from denatured haemoglobin

Table 1

Dose of ethionine (mg/rat/day)	No. of pregnant rats	No. of rats showing resorption	No. of rats with live births	No. of rats with stillbirths	Length of gestation* (days) Mean	Length of gestation* (days) Range	Birth weight* (g) Mean	Birth weight* (g) Range
0	10	0	10	0	21·1	21–22	6·3	5·9–7·2
8·5	12	0	12	0	21·5	21–22	6·1	5·6–6·8
17	30	5	25	3	20·1	19–21	4·2	3·4–5·0
35	13	6	7	4	19·2	17–20	3·5	2·0–4·5
55	10	6	4	4	19·4	18–20	3·2	1·9–3·6
70	10	8	2	2	18·5	18–19	3·0	1·1–3·4

* Live births.

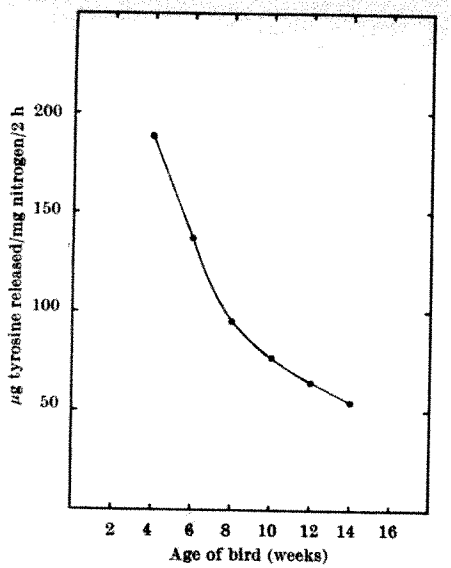


Fig. 1. Haemoglobin-splitting activity of breast muscle versus age of bird

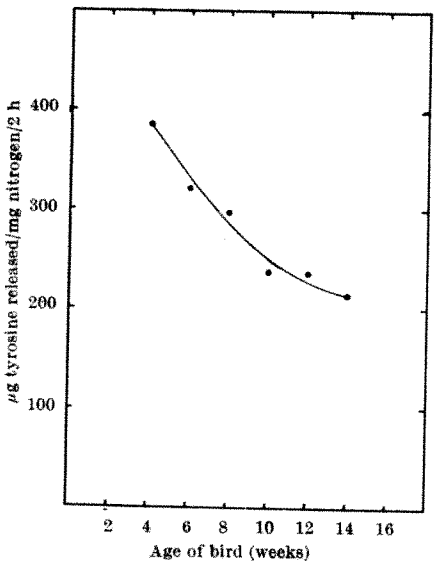


Fig. 2. Haemoglobin-splitting activity of thigh muscle versus age of bird

was used as a measure of haemoglobin-splitting activity Moisture and total nitrogen were determined as described in the *A.O.A.C. 1960 Official Methods of Analysis*[15].

Tables 1 and 2 show the average amount of tyrosine liberated from haemoglobin and the average moisture and nitrogen contents of six sets of breast muscle and three sets of thigh muscle. Moisture and nitrogen content changes little with age. The amount of tyrosine liberated decreased substantially with age in both breast and thigh muscle. Figs. 1 and 2 show the average tyrosine liberated (per mg of nitrogen) of six sets of breast muscle and three sets of thigh muscle as a function of age. The results show that haemoglobin-splitting activity was directly related to bird age.

Table 1. AVERAGE (6 BIRDS) BREAST MUSCLE COMPOSITION AND TYROSINE RELEASED FROM HAEMOGLOBIN (MG/G MEAT)

Age (weeks)	Moisture	Nitrogen	Tyrosine
4	750	37·8	7·13
6	744	39·3	5·41
8	744	39·9	3·81
10	739	39·9	3·08
12	735	40·4	2·58
14	735	40·5	2·21

Table 2. AVERAGE (3 BIRDS) THIGH MUSCLE COMPOSITION AND TYROSINE RELEASED FROM HAEMOGLOBIN (MG/G MEAT)

Age (weeks)	Moisture	Nitrogen	Tyrosine
4	762	32·6	12·55
6	760	32·9	10·58
8	758	32·8	9·72
10	765	32·7	7·75
12	763	33·3	7·87
14	761	33·9	7·26

We have undertaken to obtain information on the role of the haemoglobin-splitting cathepsin of chicken muscle. The results show that haemoglobin-splitting activity was high in young rapidly growing birds and decreased with maturity. This direct relationship is consistent with the proposal that the haemoglobin-splitting catheptic activity is related or involved in protein turnover and/or synthesis. However, the specific role of the cathepsins in muscle is unknown and remains a subject of considerable interest.

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Glycolytic and Dehydrogenase Activities of Dwarf Colony Mutants *Salmonella typhi* and *Salmonella typhimurium*

MUTANTS producing dwarf colonies occur in many species of micro-organisms. The delayed rate of multiplication of these mutants is sometimes related to disturbances in the metabolism of amino-acids and vitamins[1-3] and sometimes to respiratory deficiency[4,5].

Mutants of *Salmonella typhi* and *S. typhimurium* which produce dwarf colonies have been obtained by treatment with antibiotics and trypaflavine. (The mutant *Bc4M* is resistant to 200,000 U./ml. of streptomycin, the mutant *N20* is resistant to 800 μg/ml. of myecrine.) All strains used and their mutants have a typical antigenic structure —with the exception of the 'trypaflavine' mutant, which is without Vi-antigen. The idiosyncrasies of amino-acid metabolism, and of the oxidative and glycolytic processes of these mutants, were investigated. Two out of three mutants were auxotrophs (*Bc4M* and *N20*). The former required one of the amino-acids containing sulphur in addition to tryptophan, and the latter required isoleucine. The respiration of the mutants in the same phase of growth was as sensitive to cyanide in the presence of glucose as the respiration of the initial cultures, and thus our mutants differed from those described by Gause[4].

Mutants resistant to antibiotics were characterized by a disturbed Pasteur effect. They showed an intensive aerobic glycolysis twice as high as that of the initial forms (Table 1); the rate of lactate utilization by mutants and the parental strains was the same. Under anaerobic conditions (in the presence of cyanide and in an atmosphere of nitrogen) there was a similar accumulation of lactic acid by the mutants and the initial cultures. Such a disturbance of the Pasteur effect was not found with the third 'trypaflavine' mutant. There was a small decrease of glycolytic activity under anaerobic conditions as compared with the initial strain.

Table 1. GLYCOLYTIC AND DEHYDROGENASE ACTIVITIES OF MUTANTS OF *S. typhi* AND *S. typhimurium*

Strain	Lactic acid* (μg/mg of protein) Without KCN	Lactic acid* (μg/mg of protein) With KCN (10^{-2} M)	Formazan* (μg/mg of protein) in the presence of substrates: Glucose	Pyruvate	Lactate	Glutamate	Acetate	Succinate	Malate
S. typhi B (wild)	225	700	90	75	43	19	10	13	10
mutant *Bc4M*	500	800	62	33	24	10	2	0	0
S. typhimurium NI (wild)	159	654	75	72	75	58	19	38	42
mutant *N20*	319	806	34	28	38	20	4	16	22
S. typhi ty2 'trypaflavine' mutant	186	633	88	82	53	24	16	6	0
	155	479	54	41	38	14	14	10	6

* Lactic acid was estimated after the bacteria had been incubated for 1·5 h in the presence of glucose (25 μM). Formazan was estimated after the bacteria had been incubated for 45 min.

The dehydrogenase activity of the bacteria was also investigated, using for assay the reduction of triphenyl tetrazolium chloride to formazan. Dehydrogenase activity was far more intense in the presence of glucose, pyruvate and lactate than with succinate, malate, acetate, and glutamate as substrates.

Mutants resistant to antibiotics were characterized by a decreased dehydrogenase activity in the presence of all substances studied. The mutant cells (*Bc4M*) also lost their capacity to dehydrogenate in the presence of succinate and malate. The 'trypaflavine' mutant exhibited decreased dehydrogenation activity in the presence of glucose, pyruvate, lactate and glutamate, but it showed slightly increased dehydrogenation of succinate and malate (Table 1). As was demonstrated previously, the latter phenomenon is a typical feature of rough strains of bacteria[6]. Thus, in this communication, we are dealing with phenotypically similar mutants of *Salmonella* which produce dwarf colonies which differ only in the disturbances they effect in oxidative and glycolytic processes.

The common property shared by the mutants described here is a decreased dehydrogenase activity in the presence of certain substrates; however, the increase in aerobic glycolysis was not a feature common to the mutants studied. Two mutants were characterized by simultaneous disturbance of both amino-acid metabolism and oxidative and glycolytic processes.

From the data reported it can be seen that the morphological expression of the delayed multiplication of microorganisms (dwarf colonies) is probably a consequence of different metabolic processes.

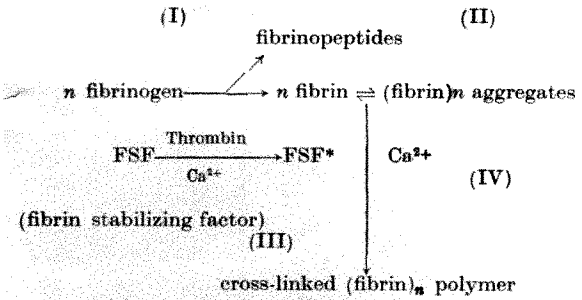
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HAEMATOLOGY

Inhibitors of Fibrin Cross-linking : Relevance for Thrombolysis

THE terminal stages of vertebrate blood clotting have been shown[1-4] to proceed by the following steps:



Reactions (I) and (II) can be seen when purified fibrinogen is clotted by thrombin. A gel forms which readily dissolves in a number of solvents such as 1 per cent monochloroacetic acid, 5 M urea or 1 M sodium bromide which would not disrupt covalent bonds. In whole blood or plasma reactions (III) and (IV) are also operating and, due to the functioning of the thrombin-activated fibrin stabilizing factor (FSF*), the aggregated fibrin molecules are immediately cross-linked. The gel thus becomes insoluble in the solvents mentioned and also acquires a greater mechanical strength. It was shown earlier that the chemistry of fibrin cross-linking is based on a transpeptidation (that is, transamidation) mechanism[1,3,5]. Amino groups of one fibrin molecule react with carbonyl functions of another to yield peptide-type of intermolecular bonds. As predicted from such a mechanism, certain amines[1,3-6] inhibit the cross-linking process by becoming incorporated into fibrin and thereby terminating the chain of polymerization. It is important to emphasize that these compounds do not interfere with the gelation (that is, aggregation) of fibrin; they specifically affect only the subsequent cross-linking step (that is, reaction IV). Accordingly, a very specific tool is available for modifying the chemical structure of clots and thrombi without affecting the 'clotting time' of blood itself. The extent of cross-linking will clearly depend on the concentration of inhibitor. Sufficiently high concentrations of the latter will reduce the size of cross-linked units so that they remain completely soluble in 1 per cent monochloroacetic acid. Nevertheless, even in low concentrations, the inhibitor might still be expected to loosen the chemical structure of clots (and thrombi) which, while perhaps no longer soluble in the acid, might be a more digestible substrate for fibrinolytic enzymes. In fact, it was shown[7] that whole human blood, clotted in the presence of 20 mM glycineamide, lysed far more rapidly and more completely than control blood which was clotted in saline of identical ionic strength. The plasmin system in these experiments was activated by urokinase added to the blood before clotting occurred.

This communication describes further experiments designed to extend these findings to the more complex system of using artificial thrombi made from human blood[8]. Glycine methyl ester and aminoacetonitrile, both of which mimic the end amino portions of fibrin molecules, were selected as cross-linking inhibitors for these initial studies.

Blood was obtained from healthy volunteers by venipuncture using 18-gauge needles and plastic syringes. It was immediately emptied into a plastic tube, cooled in ice and then distributed into 26 cm long 'Tygon' tubings (internal diameter 3/16 in.). In the experimental series, 1 ml. blood was mixed with 1 ml. of the inhibitor (that is glycine methyl ester or aminoacetonitrile at various concentrations). Stock solution of 0·1 M inhibitor was prepared in a *p*H 7·4, 0·05 M *tris* (hydroxymethylaminomethane)–hydrochloric acid buffer; in order to keep the ionic strength constant, further dilutions of the inhibitor were prepared with use of 0·05 M *tris* buffer containing 0·1 M saline. In the control series, 1 ml. of this *tris*-saline buffer alone was mixed with 1 ml. of blood. Each tube was bent to form a loop with the ends connected by a sleeve of tubing (internal diameter 5/16 in.). Thrombi were produced by rotating the loops on a turntable with a 40 degree tilt, at 33⅓ r.p.m. for 2 h at room temperature (about 24° C). At the end of this time, contents

of the loops were transferred to glass test-tubes and 1,100 CTA units of urokinase, dissolved in 0·3 ml. of 0·15 M saline, was admixed to each. (For definition of CTA units, see ref. 10; urokinase was a gift from Abbott Laboratories, North Chicago, Illinois.) Furthermore, to eliminate the possibility that glycine methyl ester or aminoacetonitrile might have acted by accelerating the action of the fibrinolytic enzymes *per se*, 1 ml. of 0·1 M inhibitor was now added to all tubes of the control series and 1 ml. of *tris*-saline buffer to those of the experimental one. Thus, final urokinase concentration was about 335 CTA units per ml. The test-tubes were gently shaken in a water bath at 37° C for 5 h. Their contents were then poured into sufficiently large volumes of 0·15 M saline to make visual examination of remnants of thrombi possible.

Some thrombi, not exposed to urokinase, were tested for solubility in 1 per cent monochloroacetic acid and were also selected for light microscopic studies.

The results (ten series of experiments involving five blood donors) were remarkably reproducible and may be summarized as follows.

Morphological characteristics of the thrombi, both in regard to consistency and histological appearance, were unaffected by the presence of cross-linking inhibitor. This was true even for as high as 50 mM glycine methyl ester which inhibited cross-linking to such an extent that the thrombus could be fully dissolved in 1 per cent monochloroacetic acid. Clearly, such inhibitors do not interfere with thrombus formation, and aggregation of fibrin without covalent cross-linking is apparently sufficient to hold the formed elements of blood together in a tight thrombus.

Thrombi formed in the presence of 25 and 12·5 mM glycine methyl ester were partially soluble in 1 per cent monochloroacetic acid, whereas the control ones were totally insoluble.

The main purpose of this work was to study the enzymatic lysis of experimental thrombi and, in particular, to see what effect chemical modification of their structure would have on this process. Thrombi produced in the presence of at least 1 mM glycine methyl ester were always completely lysed in 5 h following the addition of urokinase. Partial but appreciable lysis was observed down to 0·25 mM glycine methyl ester concentration: sometimes 0·5 mM of the latter enabled complete lysis to occur (Fig. 1). All thrombi formed in the absence of cross-linking inhibitor remained unaffected by the urokinase used in the experiments.

Aminoacetonitrile, another fibrin cross-linking inhibitor, gave results entirely analogous to those obtained with glycine methyl ester. In fact, it proved to be even more effective, for complete lysis ensued with thrombi formed in the presence of 0·2 mM aminoacetonitrile. The greater efficacy of this compound can probably be rationalized partly in terms of its rather low pK_a (ref. 9), which means that, in contrast to about a 10 per cent fraction of glycine methyl ester, almost all the added aminoacetonitrile would exist in the inhibitory unprotonated form. Furthermore, under the conditions of the experiments, aminoacetonitrile is chemically more stable than glycine methyl ester.

The experiments clearly demonstrate that the chemical structure of thrombi may be modified with the use of specific inhibitors of fibrin cross-linking so that they can be lysed much more rapidly. Possible pharmacological planning in this instance is an interesting and direct outgrowth of studies on the molecular mechanism of transpeptidation in the physiological polymerization of fibrin[1,3–6].

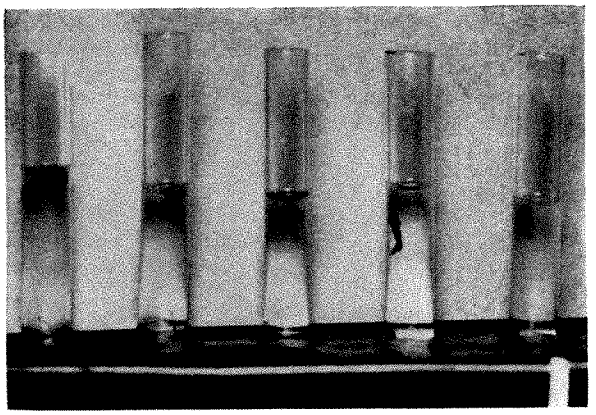


Fig. 1. Urokinase-induced lysis of thrombi. For experimental details see text. Approximate concentration of glycine methyl ester present during formation of thrombi (from left to right): 0; 0·06; 0·12; 0·25 and 0·5 mM

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New Genetic Variants of Transferrins and Haptoglobins in Pigs

POLYMORPHISM of transferrins in pigs was first shown by Ashton[1] and Kristjansson[2,3]. The alleles which control the synthesis of the three protein fractions were designated Tf^A and Tf^B; King[4] identified another allele Tf^C in animals of the Landrace breed. In the breeds so far investigated the frequency of this latter is very low[5,6], but transferrin *C* has been recently detected in the homozygous state in some Chinese pigs (*Sus vitatus*) inbred since 1958 at the Zoological Gardens, Prague[6] (Fig. 1).

By means of starch-gel electrophoresis[7] it was possible to detect a new variant of transferrins, controlled by the allele Tf^D, which has so far been found only in heterozygotes Tf^A/Tf^D and Tf^B/Tf^D of Bohemian large white pigs (Fig. 1). Proof of genetic control of the new transferrin was obtained by analysing the progeny of those animals in the serum of which Tf^D had been detected (Tables 1 and 2). The calculated gene frequency is $0{\cdot}004 \pm 0{\cdot}002$, $N = 457$.

Table 1. SEGREGATION OF THE Tf^D ALLELE IN THE LITTER OF SOW NO. 48

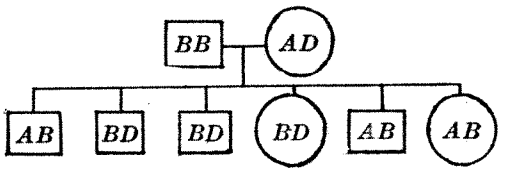


Table 2. OBSERVED AND EXPECTED DISTRIBUTION OF TRANSFERRIN TYPES IN OFFSPRING FROM MATINGS, INCLUDING THE ALLELE Tf^D

Matings	No. of Litters	No. of Offsprings	Transferrin type *AB*	*BB*	*BD*
$AD \times BB$	1	6	3/3·00		3/3·00
$BD \times BB$	2	7		2/3·50	5/3·50
$BB \times BD$	1	4		3/2·00	1/2·00
Total	4	17	3/3·00	5/5·50	9/8·50

Table 3. APPEARANCE OF PHENOTYPES WITH ALLELE Hp^1F IN THIRTEEN LITTERS FROM DIFFERENT MATINGS AND BREEDS—BOHEMIAN LARGE WHITE / BLACK AND WHITE PRESTICKÉ, CHINESE DOMESTIC (ZOO, PRAGUE) AND CROSS-BRED LARGE WHITE × CORNWALL

Matings	Number of Litters	Number of Offsprings	Haptoglobin type (observed/expected titles) 1F–1F	1F–1	3–1F	1–1	2–1	3–1	3–3
1F–1 × 1–1	3	11		4/5·50		7/5·50			
1–1 × 1F–1									
1F–1 × 3–1	3	21		4/5·25	5/5·25	4/5·25		8/5·25	
3–1 × 1F–1									
3–1F × 1–1	1	3		1/1·50				2/1·50	
3–1F × 3–1*	1	5		1/1·25	0/1·25			3/1·25	1/1·25
× 1F–1*	2	10	2/2·50	3/2·50	3/2·50			2/2·50	
× 1F–1F*	1	8	6/4·00		2/4·00				
1F–1 × 3–3	1	6			4/3·00			2/3·00	
2–1F × 1–1	1	7		5/3·50			2/3·50		
Total	13	71	8/6·50	18/19·50	14/16·00	11/10·75	2/3·50	17/13·50	1/1·25

* Litters of Chinese pigs.

The transferrin fractions *D* are easy to identify, and difficulties arise only during the simultaneous determination of transferrin and haptoglobin types from a serum sample to which haemoglobin has been added. The haptoglobin–haemoglobin complex then covers to a certain extent the transferrin fractions *D*.

In the haptoglobins of pigs[8], an unusual variant was also observed (Fig. 2). It is represented by a fraction, migrating a little faster than the zone controlled by the allele Hp^1, and is undoubtedly of genetic character (Table 3). It was also found in the aforementioned Chinese pigs and later also in other breeds.

Since a similar variant, but with a slightly slower migration than the Hp^1 zone, is known from literature, we suggest for the three fractions the nomenclature 1*F*, 1 and 1*S* in the order of decreasing electrophoretic motility and with a corresponding designation of alleles Hp^1F, Hp^1 and Hp^1S. The fraction 1 is identical with the present zone Hp^1. A reciprocal comparison was carried out with Canadian standard sera forwarded by F. K. Kristjansson. The type Hp^1S has not yet been observed in our laboratory.

It has lately been suggested that the haptoglobins in pigs[8] are not in reality true haptoglobins because they do not bind fresh haemoglobin, but only haemoglobin which has been stored and alkaline haematin or methaemoglobin[5,9,10]. That is why the above proteins are called haematin-binding proteins[9] or haem-binding proteins[5].

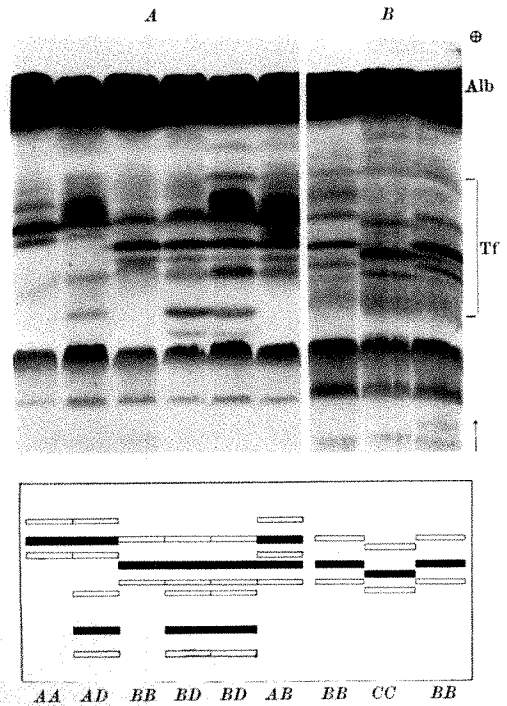


Fig. 1. Photograph and corresponding diagram of a starch gel with nine serum samples, showing (from left to right) the transferrin types in pigs: *AA*, *AD*, *BB*, *BD*, *BD*, *AB*, *BB*, *CC* and *BB*. Part *A* represents samples without haemoglobin, part *B* samples with an addition of chicken haemoglobin. Alb, albumin; Tf, transferrins. Buffer system: *tris*-citrate-borate (ref. 7), starch concentration 12·35 g/100 ml., stained in amido black

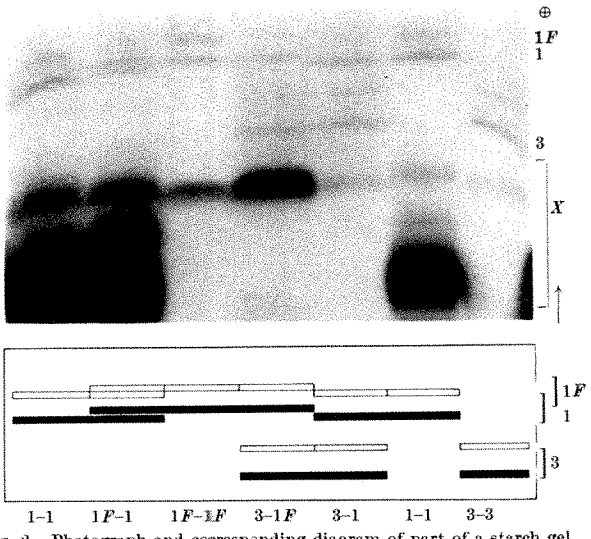


Fig. 2. Photograph and corresponding diagram of part of a starch gel with seven serum samples, showing (from left to right) haptoglobin types of pigs: 1–1, 1*F*–1, 1*F*–1*F*, 3–1*F*, 3–1, 1–1 and 3–3. Chicken haemoglobin (stored) 150–300 mg/100 ml. was added to the samples. Stained in benzidine. *X*, probably true haptoglobins of pigs

However, the acknowledged genetic hypothesis of control of their synthesis remains unchanged.

I thank Dr. F. K. Kristjansson, Animal Research Institute, Department of Agriculture, Ottawa, Canada, for sending standard sera, and the management of the Zoological Gardens, Prague, for the facilities given for collecting blood samples.

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HISTOCHEMISTRY

Ferritin-labelled Enzyme: a Tool for Electron Microscopy

THE labelling of immune globulins with electron-dense substances has been used, by various investigators, as a means of localizing sites of antibody–antigen interaction *in situ*[1,2]. Singer[3] conjugated ferritin and Pressman and Sternberg[4] iodine to gamma globulin with the view of subsequent interaction with antigen.

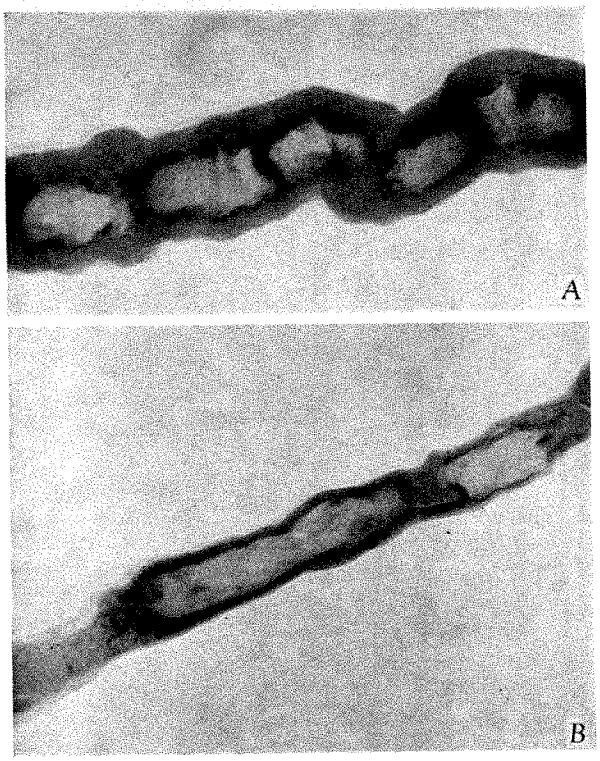


Fig. 1. Hypha of *Aspergillus niger*. *A*, Treated with ferritin-labelled chitinase. *B*, Treated with unconjugated ferritin. (× *c.* 82,750)

This led us to explore the possibility of using a similar technique in conjugating an enzyme rather than a globulin with ferritin. That enzymes such as lipase[5], lysozyme[6] and chitinase can be labelled with fluorochromes has been shown. In our investigation, chitinase was the enzyme chosen.

Chitinase, 20 mg/ml. (Nutritional Biochemicals, Cleveland, Ohio), was coupled with horse spleen ferritin (Pentex, Inc., Kankakee, Ill.) using the method outlined by Singer[3]. Following the final dialysis, a soluble and an insoluble fraction were recovered by centrifugation at 28,500*g* for 10 min. In our investigations, only the soluble fraction was used. In order to demonstrate that conjugation had actually occurred, chitin (Calbiochem, Los Angeles) was first reacted for 18 h with the soluble fraction buffered at *p*H 5·7 with 0·2 M phosphate buffer. Portions of the mixture (one drop) were placed on glass slides and heat fixed. The smears were then examined microscopically with tungsten illumination. The chitin particles appeared stippled with brown. Afterwards, the slide was thoroughly washed and treated with fluoroscein isothiocyanate-labelled chitinase[7]. Controls were prepared which had not previously been treated with the soluble fraction, but which were reacted with fluoroscein isothiocyanate-labelled chitinase alone. All preparations were examined with ultra-violet, bright field illumination (Zetopan microscope, Reichert, Vienna).

Control preparations showed uniform, bright, specific fluorescence. Conversely, those smears which had been previously treated with the soluble fraction revealed complete inhibition of fluorescence in the smaller chitin particles; while in the larger ones, fluorescence was limited to occasional areas. This indicated that the ferritin-labelled chitinase (FLC) had 'tied up' a larger percentage of the combining sites on the substrate.

Electron micrographs (RCA, *EMU* 2*A*) of chitin particles, both reacted with FLC and unreacted, revealed extensive deposition of electron-dense material in the former. That an enzyme-substrate reaction was occurring was demonstrated by a decrease in optical density in the FLC-chitin reaction mixtures over a period of 45 min. The rate of reaction is slow, which is characteristic of this enzyme system. The presence of a small amount of amino sugar (a product of the enzymatic hydrolysis of chitin) was detected by its characteristic colour reaction with ninhydrin. Later, FLC was tested against fungal material prepared by disruption with glass beads of a mycelial suspension of *Aspergillus niger* from which the lipids had been previously extracted.

Electron microscope investigations (RCA, *EMU* 3*H*) were carried out on FLC-treated and unconjugated ferritin-treated fungal preparations, both of which had been vigorously washed following a 2-h incubation period. Typical results are shown in Fig. 1. Note the heavy deposition of electron-dense material on the walls of the FLC-treated specimen. This deposition is evidently so heavy that structural detail is partially obscured.

We feel that the value of this method will become more evident as our work progresses and with the utilization of thin-sectioning techniques. Eventually, it should be possible to pinpoint the location of specific enzymatic substrates *in situ*. Additional investigative work is planned on all aspects of this technique with the view of extending it to other enzyme systems.

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Histochemical Demonstration of Oxytocinase in the Human Placenta

Serum of pregnant women inactivates oxytocin due to the presence of oxytocinase. Serum of men and non-pregnant women does not inactivate oxytocin. Oxytocinase is thought to be produced by the placenta. This enzyme (*E.C.* 3.4.1.2) hydrolyses L-cystine-di-β-naphthylamide and has been termed 'cystine aminopeptidase'.

The determination of serum oxytocinase activity is carried out by incubation in the presence of L-cystine-di-β-naphthylamide. The β-naphthylamine released is afterwards measured.

L-Cystine-di-β-naphthylamide may also be used as a histochemical substrate for the demonstration of oxytocinase activity. The use of this substrate in histochemistry has already been suggested[1]. No account seems to be available, however, of its use in the histochemical demonstration of oxytocinase activity. Incubation of tissue sections from placentae with L-cystine-di-β-naphthylamide results in the release of β-naphthylamine at the site of enzyme activity[2]. The β-naphthylamine undergoes a simultaneous coupling reaction with a suitable chromogen in the incubating medium which results in the deposition of a coloured final reaction product at the enzyme site. The only chromogen used so far is diazotized 3-chloro-4-nitro-aniline ('Echtrot 3*GL*' salt). The more familiar chromogens, fast blue *B* or garnet *GBC*, have not yet been used.

The following procedure has been evolved for the histochemical demonstration of oxytocinase activity: Frozen tissue sections are cut at 10μ from human placentae fixed in 70 per cent alcohol for 24 h. The sections were incubated in 40 ml. 0·15 M phosphate buffer to which 1 ml. of substrate solution and 20 mg 'Echtrot 3*GL*' salt have been added. The substrate solution is prepared by

dissolving 25 mg L-cystine-di-β-naphthylamide in 10 ml. hot 0·012 N hydrochloric acid and adding 10 ml. distilled water. The substrate is stable and may be stored at 4° C. The incubation period lasts 1 h at room temperature. The final reaction product is diffusely and evenly deposited along the outer margin of the syncytium. Background staining does not occur and sections incubated in the presence of trichloroacetic acid do not show enzyme activity.

The distribution of enzyme activity may reflect oxytocinase activity. Alternatively, another enzyme may be hydrolysing L-cystine-di-β-naphthylamide and the final reaction product represents the localization of this enzyme. Therefore, the trivial name cystine aminopeptidase should not be applied to histochemical activity observed with this substrate, as confusion may result. A similar situation already exists with leucine aminopeptidase (*E.C.* 3.4.1.1). Leucine aminopeptidase hydrolyses leucineamide more rapidly than L-leucine-di-β-naphthylamide. At least one other enzyme, however, hydrolyses L-leucine-β-naphthylamide more rapidly than leucineamide[3]. The histochemical activity with respect to leucine-β-naphthylamide represents a system of enzymes and does not reflect leucine aminopeptidase activity. In addition, the present biochemical nomenclature covering peptide hydrolases is inadequate due to overlapping specificities, and it has been suggested that the trivial names based on particular substrates should be abandoned[4]. The enzyme that hydrolyses the histochemical substrate L-cystine-di-β-naphthylamide is a cystine-di-β-naphthylamide hydrolase. If it is shown, however, that this enzyme specifically hydrolyses the cystine–tyrosine bond of oxytocin, then the term oxytocinase may be retained for histochemical use.

Thanks are due to Prof. J. Chassar Moir and Dr. A. H. T. Robb-Smith for the facilities provided for this work.

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Preferential Staining of Collagen by Acid Dyes in Methanol

THE effects of dye solvents on the selectivity of histological staining have not been extensively investigated. Robards and Purvis[1] have reported preferential staining of the gelatinous fibres of tension wood by chlorazol black *E* when dissolved in methyl cellosolve. Earlier the influence of solvents on the staining reactions of this dye was discussed by Cannon[2]. Puchtler and Sweat[3] reported a method for selectively staining haemoglobin in sections with amido black in a methanol solution, but not in water. Investigation of phospholipid staining by Luxol fast blue *G* revealed a changing selectivity dependent on the solvent used[4]. This dye in methanol solution was later found to stain only collagen and elastin[5].

When other sulphonated acid dyes were later used as methanolic solutions they preferentially stained connective tissue elements. This selective staining did not occur with aqueous solutions of the dyes. Among the dyes successfully used in this manner were acid fuchsin, aniline blue, methyl blue, fast green *FCF*, chlorazol black *E*, and pontamine fast turquoise 8*GLS*. Most of these dyes also selectively stain collagen when used as mixtures in saturated picric solutions, such as the Van Gieson stain.

Methylation, nitrosation and acetylation of tissue sections did not prevent the selective staining[5]. The staining was reduced by tanning with tannic acid, which affects the physical nature of collagen by the introduction of cross-linkages. Although the mechanism of this collagen staining has not been finally elucidated, it would seem to be of a physical nature rather than an affinity for specific chemical groupings in the collagen macromolecule.

It was considered that the most likely explanation for the preferential staining was a difference in the type of micelle or molecular arrangement of the dyes in methanol and water. The molecular form of the dye in methanol might conceivably have a greater affinity for collagen. However, as the molecular arrangement of the dyes in the two solutions appears to be comparable when examined in the analytical ultracentrifuge[6], this explanation is unlikely. It may be that methanol affects the permeability of the various proteins present in tissue sections, allowing only the collagen to stain. Collagen has a maximum solvation coefficient of 218 g/g, which is much higher than that for other non-globular protein polymers likely to be present in mammalian tissue sections, such as myosin (49 g/g) and tropomyosin (23 g/g)[7]; possibly this particular property of collagen is associated in some manner with its preferential staining.

As the collagen-staining reaction can be used after other histochemical and staining procedures in which the dyes used are not removable by methanol, it may be of value in histochemical investigations of connective tissue.

I thank Dr. Joel Kirschbaum of this Institute for examining the dye solutions in the analytical ultracentrifuge.

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Histochemical Distribution of Alcohol Dehydrogenases in Endocrine Tissue

HISTOCHEMICAL investigations of alcohol dehydrogenase in rodents using various alcohols[1] revealed no obvious differences in the sites of alcohol utilization. Primary and secondary alcohols in general tissues were utilized at the same sites; the present communication deals with the histochemical variations in sites of utilization of different alcohols in steroid-producing endocrine glands.

Human term placenta, obtained at Caesarean section, and rat (Royal Wistar) adrenal glands, testes and ovaries were frozen on solid carbon dioxide and the tissues were prepared and incubated as described by Ferguson[1], using the following alcohols (final concentration 2·5 per cent): methanol, ethanol, propanol, butanol, pentanol, hexanol, heptanol, octanol, nonanol, decanol, undecanol, dodecanol, tridecanol, phenol, hydroquinone, 2-ethoxyethanol, allyl alcohol, 2-methyl-butan-1-ol, 3-methyl-butan-1-ol, nonyl alcohol, tertiary butanol, tertiary butyl carbinol, *iso*-propanol, secondary butanol, pentan-2-ol, pentan-3-ol, octan-2-ol, *cyclo*hexanol, ethylene glycol, propylene glycol, glycerol, butan-1 : 3-diol, butan-1 : 4-diol, pinacol and furfuryl alcohol.

Control sections were concurrently incubated in the buffered medium containing no alcohol.

The results are summarized in Table 1.

Since Bonnichsen[2] isolated alcohol dehydrogenase from horse liver, the biochemical properties and mechanism of action of this enzyme have been widely described[3–14].

The reactions catalysed by alcohol dehydrogenase, namely, (I) primary alcohols:

$$R.CH_2OH + NAD^+ \rightleftharpoons R.CHO + NADH + H^+$$

and (II) secondary alcohols:

$$R.CHO.R + NAD^+ \rightleftharpoons R.CO.R + NADH + H^+$$

tend to move farther to the right as the *p*H is increased and to the left when the *p*H is decreased. *p*H 7·4 was chosen for the medium in this investigation, due to aqueous solubility of nitro B.T. at this value.

Utilization of alcohols by endocrine tissues can be classified according to the alcohol structure[1].

(1) Utilization of straight-chain alcohols (methanol-tridecanol) improved with increasing chain-length to an optimum of six carbon atoms, and thereafter the longer-chained alcohols were progressively less satisfactory substrates, perhaps because of their decreased solubility in the medium.

(2) Aromatic alcohols (phenol and hydroquinone). These two substrates are not usually dehydrogenated in tissues but are instead conjugated with glucuronic acid prior to excretion.

(3) 'Cellosolve' (2-ethoxy ethanol) was moderately used by the tissues; however, straight-chain alcohols of a corresponding length appeared to be more suitable substrates. Thus the ethereal oxygen atom appears to exert some effect on the terminal hydroxyl group, impeding its dehydrogenation.

(4) The unsaturated alcohol (allyl alcohol) was fairly well utilized, but the double bond seemed to impede dehydrogenation to a slight extent in comparison to straight-chain alcohols.

(5) Branched alcohols (isobutanol, 2-methyl-butan-1-ol, 3-methyl-butan-1-ol, nonyl alcohol, tertiary butanol, tertiary butyl-carbinol). The two tertiary alcohols were not used and the remaining branched alcohols were not as well utilized as were the corresponding straight-chain alcohols. 3-Methyl-butan-1-ol tended to be a more suitable substrate than 2-methyl-butan-1-ol, presumably due to the increased steric hindrance of the methyl group the nearer it is to the hydroxyl group.

Secondary alcohols (isopropanol, sec. butanol, pentan-2-ol, pentan-3-ol, octan-2-ol). Alcohol dehydrogenase has been shown to utilize various alcohols biochemically, but there do not as yet appear to be any references to different alcohol dehydrogenases. The present findings suggest that primary alcohol dehydrogenase and secondary alcohol dehydrogenase are two separate enzymes. With the primary alcohols, activity was demonstrable in all components of the ovary to an approximately equal extent but not at all in placenta. With secondary alcohols the corpora lutea were extremely active, whereas the rest of the ovarian tissues were only very slightly reactive. The placenta could also utilize some of the secondary alcohols although the primary alcohols were not suitable substrates.

(6) *Cyclo*hexanol was not utilized to any significant extent by the endocrine tissues examined under the conditions of the experiment.

(7) Polyhydric alcohols (ethylene glycol, propylene glycol, glycerol, butan-1 : 3-diol, butan-1 : 4-diol, pinacol) did not appear to be utilized as avidly as were the monohydric alcohols.

(8) Furfuryl alcohol. Pentose metabolism involves the formation of furfuryl alcohol as a by-product, and as the most notable metabolism of pentoses is connected with nucleic acids it is not surprising that furfuryl alcohol dehydrogenase was observed in all the tissues examined.

From this investigation it emerges that in any future histochemical investigations into alcohol dehydrogenases at least three substrates would be required: first, a straight-chain primary alcohol such as hexanol, in preference to ethanol, which is the substrate quoted for demonstration of this enzyme[15,16]; secondly, a secondary

Table 1

	Methanol	Ethanol	Propanol	Butanol	Pentanol	Hexanol	Heptanol	Octanol	Nonanol	Decanol	Undecanol	Dodecanol	Tridecanol	Hydroquinone	Phenol	2-Ethoxy-ethanol	Allyl alcohol	2-Methyl-butan-1-ol	3-Methyl-butan-1-ol	Nonyl alcohol	Tert. butanol	Tert. butyl carbinol	*Iso*propanol	Sec. butanol	Pentan-2-ol	Pentan-3-ol	Octan-2-ol	*Cyclo*hexanol	Ethylene glycol	Propylene glycol	Glycerol	Butan-1 : 3-diol	Butan-1 : 4-diol	Pinacol	Furfuryl alcohol
Placenta (human)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ovary (rat)																																			
Granulosa	–	–	+	+	+	+	+	+	Trace	+	–	–	–	–	–	Trace	+	–	Trace	Trace	–	–	Trace	Trace	Trace	–	Trace	–	–	Trace	–	–	–	–	+
Theca interna	–	–	+	+	+	+	+	+	Trace	+	–	–	–	–	–	Trace	+	–	Trace	Trace	–	–	Trace	Trace	Trace	–	Trace	–	–	Trace	–	–	–	–	+
Ova	–	–	+	+	+	+	+	+	Trace	+	–	–	–	–	–	Trace	+	–	Trace	Trace	–	–	Trace	+	Trace	–	Trace	–	–	Trace	–	–	–	–	+
Interstitial tissue	–	Trace	+	+	+	+	+	+	Trace	+	–	–	–	–	–	Trace	+	Trace	+	+	+	–	+	+	Trace	–	+	–	–	Trace	–	–	–	–	+
Corpora lutea	–	Trace	+	+	+	+	+	+	Trace	+	–	–	–	–	–	Trace	+	Trace	+	+	+	–	+	+	+	–	+	+	–	Trace	–	–	–	–	+
Germinal epithelium	–	–	+	+	+	+	+	+	–	+	–	–	–	–	–	Trace	+	Trace	Trace	Trace	Trace	–	+	+	Trace	–	+	Trace	–	Trace	–	–	–	–	+
Testis (rat)																																			
Leydig tissue	–	–	+	+	+	+	+	+	–	+	–	–	–	–	–	–	+	–	+	+	–	–	+	+	–	–	+	–	–	–	–	–	–	–	+
Seminiferous tubules	–	+	+	+	+	+	+	+	–	+	–	–	–	–	–	Trace	+	Trace	+	+	Trace	–	+	+	Trace	–	+	Trace	–	Trace	–	–	–	–	+
Adrenal (rat)																																			
Z. glomerulosa	–	–	Trace	+	+	+	Trace	+	–	+	–	–	–	–	–	Trace	Trace	–	+	+	–	–	Trace	Trace	–	–	Trace	–	–	Trace	–	–	–	–	+
Z. fasciculata	–	+	+	+	+	+	+	+	–	+	–	–	–	–	–	Trace	+	–	+	+	–	–	+	+	–	–	+	–	–	Trace	–	–	–	–	+
Z. reticularis	–	+	+	+	+	+	+	+	–	+	–	–	–	–	–	Trace	+	–	+	+	–	–	+	+	–	–	+	–	–	Trace	–	–	–	–	+

alcohol of a similar chain-length to demonstrate secondary alcohol dehydrogenase; and thirdly, furfuryl alcohol.

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IMMUNOLOGY

Mucolipids of the Brain in the Process of Formation of Post-vaccinal Antirabic Immunity

THE biochemical changes which develop in the brain during the process of forming post-vaccinal antirabic immunity are of considerable importance.

We have recently investigated the mucolipids of the brain. Neuraminic acid is a characteristic compound of these substances and, as has been shown by some investigators, can provide protection against certain infections[1-3].

White mice were used in the experiments. The animals were immunized with live rabies virus fixe ($L.D._{50}$ $10^{-6.5}$). The virus was injected intraperitoneally six times on alternate days (0·25 ml. suspension per injection). The concentration of the virus was gradually increased by decreasing the dilution factor from 10^{-6} to 10^{-3}. Groups of animals (with corresponding controls) were killed on the eighth, fourteenth, thirtieth and fortieth day after the beginning of immunization. Mucolipids in the brain were determined by Klenk's[4] and Svennerholm's[5] method using Promyslov's[6] modification. The antibodies present in the sera were determined by means of ordinary neutralization tests (Table 1).

The results presented in Table 1 show that on the eighth day after immunization the amount of mucolipids in the brain had not increased significantly. On the fourteenth day the quantity of mucolipids reached a maximal level. It remained high until the thirtieth day and then decreased, reaching normal levels by the fortieth day.

Table 1

Length of test (days)	Antibodies in serum (neutralization index)	Mucolipids in the brain of immunized animals (μg/g wet wt.)	Mucolipids in the brain of control animals (μg/g wet wt.)
8	0	177·71 ± 1·775	
14	0	236·46 ± 4·303	161·01 ± 5·423
30	1,047	218·89 ± 17·67	
40	4,571	159·84 ± 16·03	

It is of considerable interest that under the immunization scheme described here the antibodies in the serum were found comparatively late.

Thus, in the process of forming the post-vaccinal antirabic immunity, a stable increase in the contents of mucolipids in the brain was observed over a certain period, which preceded the accumulation of specific antibodies in the serum. This shows that the reaction of the organism after the inoculation of a vaccine preparation proceeds not only in the so-called 'organs of immunogenesis', but also in the systems mainly affected in the course of the disease.

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PATHOLOGY

Intracellular Plasma Protein: a Manifestation of Cell Injury in Myocardial Ischaemia

THE recognition of early cell injury poses an important problem in the study of disease, namely, the certain diagnosis of early myocardial infarcts. A variety of chemical and histochemical techniques have been tested for use in the identification of early myocardial ischaemia in experimental animals as well as in man[1,2]. The search has been only partially successful.

The interstitial fluid that surrounds cells *in vivo* contains plasma proteins of varying size and shape[3]. Thus the permeability of cell membranes is constantly being tested by a spectrum of protein molecules ranging from β_1-lipoproteins (molecular weight 1,300,000) to albumin (molecular weight 69,000). Under normal conditions the concentration of plasma proteins in interstitial fluid is low as compared with plasma. This is particularly true for large molecules such as β_1-lipoprotein and fibrinogen. Increased capillary permeability, which is common to many pathological conditions, including ischaemia, may result in a concentration of intercellular fluid protein comparable to that of plasma[3]. Cell injury associated with increased permeability of cell membranes may be marked by the intracellular diffusion of one or more types of plasma proteins. Labelled antibody to plasma proteins which are either commercially available or readily produced provides a sensitive way of specifically identifying these proteins in interstitial tissue and within cells.

The anterior descending branch of the left coronary artery of eight mongrel dogs was ligated as previously described[2]. The animals were killed from 1 to 24 h after the onset of ischaemia (Table 1). Blocks of myocardium were taken from the ischaemic anterior wall and from the normal myocardium of the posterior wall of the left ventricle. The tissue was fixed in 95 per cent ethanol at 4° C and processed for paraffin embedding as described by Sainte-Marie[4]. Serial sections were cut at 4μ. Slides of the ischaemic and control tissue from each animal were stained by the haematoxylin and eosin, periodic acid–Schiff (PAS) and diastase–periodic acid–Schiff (D-PAS) methods[5].

Table 1. INTRACELLULAR PLASMA PROTEINS AND D-PAS MATERIAL IN EXPERIMENTALLY PRODUCED MYOCARDIAL ISCHAEMIA IN THE DOG AS A FUNCTION OF TIME*

No. of animals	Period of ischaemia (h)	D-PAS material	Albumin	γ-Globulin	Fibrinogen
2	1	0	+ +	+	0
2	3	+	+ +	+	0
2	6	+ +	+ + +	+ + +	+ + +
1	12	+ + + +	+ + + +	+ + + +	+ + + +
1	24	+ + + +	+ + + +	+ + + +	+ + + +
8	0	0	0	0	0

* The maximum staining observed by the D-PAS and fluorescent antibody methods was designated + + + +. + + +, + + and + indicate approximately 75, 50 and 25 per cent of the maximum staining.

Other sections were incubated with fluorescein-labelled rabbit anti-dog albumin, rabbit anti-dog IgA globulin and rabbit anti-dog fibrinogen (obtained from Antibodies, Inc., Davis, California). These sections were examined with a large Zeiss fluorescence unit using a '*UG*1' excitor filter and a Kodak '2*B*' barrier filter. The specificity of the observed reactions was evaluated by exposing normal tissue from each animal to the labelled specific anti-sera and ischaemic tissue to labelled normal rabbit serum, labelled anti-sera absorbed with specific antigen and labelled anti-sera mixed with unlabelled anti-sera[6].

Cytoplasmic and nuclear changes sufficient to identify the ischaemic area were evident in the 12- and 24-h animals, but were equivocal in the 6-h animals and absent in the 1- and 3-h animals. The glycogen present normally in the dog myocardium was lost from the ischaemic areas of all animals (Table 1). By 3 h after the onset of ischaemia a D-PAS staining material was noted in the sarcoplasm but was very faint[1,2]. This reaction became more prominent as the infarcts developed (Table 1).

In normal portions of myocardium the fluorescent antibody technique showed albumin and IgA globulin in the blood vessels and interstitial spaces. Only auto-fluorescence was seen in the sarcoplasm. Fibrinogen was identified in the lumen of blood vessels but not in interstitial spaces or sarcoplasm.

Albumin and IgA globulin was seen in the ischaemic sarcoplasm of all animals. Although the ischaemic fibres in the 1- and 3-h animals could clearly be distinguished from adjacent normal fibres by the specific fluorescence for albumin or IgA globulin, the intensity of the specific fluorescence was greater in the 6-, 12- and 24-h animals (Figs. 1 and 2). In the 1- and 3-h animals the intensity of specific fluorescence for albumin was greater than for IgA globulin. In the 6-, 12- and 24-h animals the specific fluorescence for albumin and IgA globulin was about the same (Table 1). Fibrinogen was not found within muscle fibres until 6 h after the onset of ischaemia. The intensity of this specific fluorescence was greater in the 12- and 24-h animals.

The relationship between the intracellular plasma proteins and the D-PAS material was evaluated by comparing the distribution and intensity of staining of the plasma proteins by the fluorescent antibody technique with the

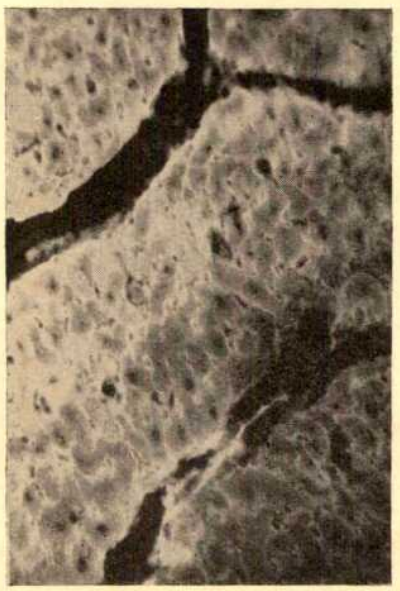


Fig. 1. Dog myocardium 1 h after ligation of a coronary artery incubated with fluorescein-labelled rabbit anti-dog albumin for 1 h. Adjacent normal muscle fibres are seen in the upper left. The sarcoplasm does not fluoresce. The sarcoplasm in the other areas was a bright yellow-green (bright area in the print) (×688)

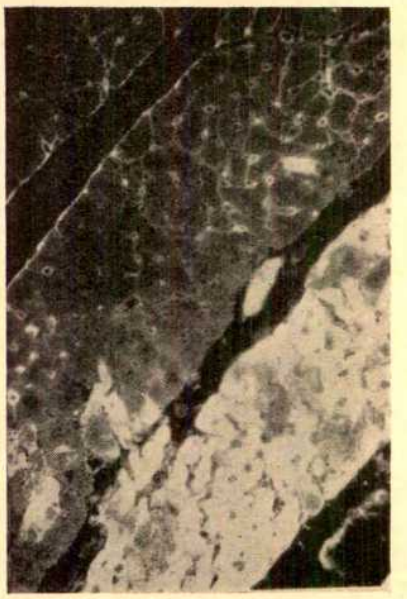


Fig. 2. Dog myocardium 12 h after ligation of a coronary artery incubated with labelled rabbit anti-dog IgA globulin for 1 h. The sarcoplasm of the normal muscle fibres (upper) does not show specific fluorescence in contrast to the sarcolemma and the ischaemic muscle fibres in the lower right (×688)

D-PAS material in alternate sections. Also, both reactions were performed on the same section. That is, a section was incubated with one of the labelled specific anti-sera and selected areas marked and photographed. Afterwards the section was digested with diastase and stained by the PAS method. The previously marked areas were photographed and compared with the staining by the fluorescent antibody technique. The distribution and intensity of staining by the two methods were quite comparable. That is, the muscle fibres showing D-PAS staining in the sarcoplasm also stained for plasma proteins; the greater the intensity of staining by the D-PAS method, the greater the specific fluorescence observed.

These data suggest that albumin and IgA globulin, normally present in intracelllular fluid, diffuse into muscle fibres quite soon after the onset of ischaemic injury. The albumin, presumably because of its smaller size and higher concentration in extracellular fluid, diffuses earlier than IgA globulin. Fibrinogen, being a much larger molecule, is not normally present in interstitial fluid in sufficient concentration to be demonstrated by the method used. However, by 6 h, the capillary permeability in the ischaemic area was such that fibrinogen had passed into the interstitial fluid and into the sarcoplasm of muscle fibres. Correlation between the distribution and intensity of staining by the D-PAS method and the influx of plasma protein indicates that the carbohydrate-rich (PAS positive) plasma proteins account for the D-PAS material found in ischaemic muscle fibres.

Myocardial ischaemia in man is accompanied by a similar intracellular diffusion of plasma proteins[7]. The possibility of using plasma proteins as an indicator of cell injury of a variety of types is supported by the finding that plasma proteins enter the cytoplasm of cells injured with arsenic[8].

Further, the finding of plasma proteins as described here, in cells injured by anoxia under circumstances which indicate that they are the result rather than the cause of the injury, necessitates a re-evaluation of the significance previously attached to the presence of certain plasma proteins in a variety of lesions. One of the criteria for the classification of disease as autoimmune has been the demonstration of immune globulins in the lesions[9]. The finding of an abundance of IgA globulin in ischaemic muscle fibres under conditions suggesting that the

globulin could not logically be considered an aetiological factor emphasizes the need for caution in using these criteria to designate a disease as autoimmune. The accumulation of fibrinogen in the renal glomerular cells of Masugi-type nephritis has been suggested as the cause of the observed cell injury[10]. The possibility that the fibrinogen accumulates as a result of the cell injury must be seriously considered.

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Carcinogenic Derivatives of *Cyclo*penta[a]phenanthrene

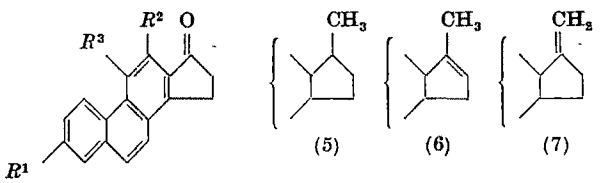
ALTHOUGH much is known concerning the relationship between carcinogenic activity and structure in hydrocarbons containing four or more condensed aromatic rings, comparatively little work has been reported on derivatives of 16,17-dihydro-15*H*-*cyclo*penta[*a*]phenanthrene (8) which have the same carbon ring structure as the natural steroid hormones. Butenandt and Dannenberg[1] have shown that of the methyl derivatives of this hydrocarbon, which is itself inactive, only the 7- and 11-monomethyl and 11,12-dimethyl compounds showed any carcinogenic activity, and even then the activity was very weak in skin painting experiments and non-existent by subcutaneous injection. It is noteworthy that 1,2,3,4-tetramethylphenanthrene exhibited activity of this order[2,3]. Later, in an investigation of the nature of the carcinogenic products formed from cholesterol during dehydrogenation with chloranil, Dannenberg[2] found that the *D*-ring unsaturated compounds (9) and (6) both possessed weak activity. Since these compounds were oxidized, by osmium tetroxide, at the *D*-ring double bond rather than at the "phenanthrene" double bond, the former was designated the *K*-region in these hydrocarbons.

With a view to preparing the phenanthrene analogue of 18-noroestrone, a new method for the synthesis of 15,16-dihydro-17-oxo*cyclo*penta[*a*]phenanthrenes was devised[4] in which the ketones (1)–(4) were readily accessible. These compounds were tested for carcinogenic activity together with the hydrocarbons (5)–(7).

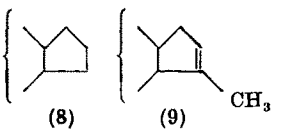
T.O. albino mice were randomly bred in these laboratories in a closed colony. They were fed on diet *GR*25 with water *ad libitum*, and were 3 months old at the start of the experiment. Ninety males and the same number of females were divided by a formal randomization process into nine groups each consisting of ten mice of each sex. The dorsal hair was removed from each animal by electric clippers. The chemicals were dissolved in redistilled 'AnalaR' benzene at a concentration of 0·5 per cent. One drop (6 μl.) of each solution was applied to the clipped skin of each mouse of the appropriate group twice a week for 52 weeks. One control group received the solvent (benzene) only, another was clipped but otherwise untreated. During the course of the experiment it was necessary to reclip the animals at regular intervals and when this was done the clipper blades were washed in acetone after clipping each group.

The size, appearance and position of all skin tumours were recorded at fortnightly intervals and those which appeared malignant were removed under ether anaesthesia, for histological examination. The mice which bore them were returned to the experiment. The results are shown in Tables 1 and 2. The histological classification refers to the first tumour removed from each animal only.

Of the hydrocarbons (5), (6), (7), Diel's hydrocarbon (5) was inactive as previously found by several groups of workers[5]. The production of one tumour after a long latent period by the dehydro-derivative (6) was consistent with the weak carcinogenic activity of this compound reported by Dannenberg[2]. In addition, the new exocyclic isomer (7) produced two tumours with similarly long latent periods.



	R^1	R^2	R^3
(1)	H	H	H
(2)	H	H	CH_3
(3)	H	CH_3	H
(4)	OCH_3	H	H



$R^1 = R^2 = R^3 = H$

Table 1. NUMBER OF TUMOURS PRODUCED BY COMPOUNDS UNDER TEST

Compound tested	No. of survivors at: 6 months	12 months	18 months	No. of animals with tumours at the site of application
1	18	9	6	0
2	19	4	1	15
3	18	7	6	0
4	20	15	4	0
5	20	13	7	0
6	19	13	10	1
7	17	11	5	2
Benzene control	17	13	10	0
Clip control	20	18	10	0

Table 2. TYPES OF TUMOURS PRODUCED BY THREE ACTIVE COMPOUNDS

Compounds	Histology	No. of mice with tumour	Latent period range (days)
2	Squamous carcinoma	10	130–305
	Spindle cell sarcoma	2	306–340
	Squamous papilloma	3	240–330
6	Squamous carcinoma	1	482
7	Squamous carcinoma	2	467–576

Marked carcinogenicity was shown by 11-methyl-15,16-dihydro-17-oxo*cyclo*penta[*a*]phenanthrene (2) which produced tumours in the majority of treated animals. The latent periods of these were shorter than those of the hydrocarbons (6) and (7). The carcinogenicity has been confirmed by two further skin painting experiments still in progress. The other ketones (1), (3), (4) were completely inactive, thus indicating the importance of a methyl substituent at the 11-position.

In view of the very weak carcinogenicity of 11-methyl-16,17-dihydro-15*H*-*cyclo*penta[*a*]phenanthrene [the deoxo derivative of (2)] found by Dannenberg, it appears that the presence of a carbonyl group at C-17 has greatly augmented the activity. This is surprising, because Shear *et al.*[6] found that the 15-oxo derivative of methylcholanthrene was much less carcinogenic than methylcholanthrene itself.

Further work with these and a number of related compounds is in progress and will be reported later. We thank

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RADIOBIOLOGY

Production of Carrier-free Iron-52 for Medical Use

IRON-52 has been used in this hospital since 1957[1]. As it is a positron emitter, coincidence counting techniques are used to investigate the distribution of erythropoietic tissue in patients with a variety of haematological disorders. In addition, its short half-life (8·2 h) has made it possible to investigate the effect of cytotoxic agents and ionizing radiation on marrow function by serial measurements at various intervals. It has also proved of value in the study of the kinetics of iron in animals. In some of the disorders studied, the iron-binding capacity of the serum may be low, and iron-52 of the highest specific activity is therefore desirable.

A recent paper by Yano and Anger[2] described a method for preparing samples of iron-52 for medical use, based, essentially, on the extraction of iron from a solution in hydrochloric acid of an alpha-bombarded chromium target. We have used a similar approach, but prepare "carrier-free" samples, and have developed the special technique described here to handle the initial stages of the separation, where the activity involved is at its height.

The Medical Research Council cyclotron has an α-beam of only 30 MeV, but targets can be placed inside the "dee-box" where beam currents of up to about 300 μamp are readily available. The targets consist of high-purity chromium, plated (by Fescol Ltd., London) to a thickness of 0·05 mm, over an area approximately 20 cm by 5 cm, on iron-free aluminium backing plates. They are placed at grazing incidence to the beam, which makes them effectively "infinitely thick" chromium targets.

After bombardment for 1 h, the chromium layer is dissolved by clamping the target plate in such a way that it becomes the base of a 'Perspex' etching chamber, into which is pumped 5 ml. of concentrated hydrochloric acid, microanalytical grade. The dissolution is completed within about 5 min, and carrier-free iron-52 is then extracted from the resulting solution, adjusted to about 8 M hydrochloric acid, into di-isopropyl ether. These steps are carried out by remote control inside a 4-in. lead-shielded enclosure.

The central feature of the apparatus used is the proportioning pump (supplied by Technicon Instruments Co, Ltd.) which transports the various aqueous and organic solutions as shown in the flow diagram (Fig. 1). Mixing of organic and aqueous phases takes place in a spiral of glass tubing, 125 cm long × 0·2 cm internal diameter, packed with glass beads of about 1·5 mm diameter, and is aided by the entrainment of air bubbles in the stream of liquid. The efficiency of this extraction procedure has been determined by tracer experiments, using iron-59, to be better than 85 per cent. The phases are afterwards separated in an open-ended U-tube, the organic phase being collected from the top of one limb and the aqueous from the top of the other; air bubbles, of course, escape at this point.

Note that the ethereal solution does not pass through the pump but is collected by displacement of air from the receiving vessel. This is because the carrier-free isotope is retained on the walls of the flexible pumping tubing (even fluorinated-silicone rubber tubing has been found unsatisfactory for this purpose), although it can be transported without loss through polythene tubing. Because of its rigidity, polythene tubing cannot be used in the proportioning pump itself.

When carrier iron can be tolerated in the final sample and, say, 100 μg are added at the start of the separation, further extraction steps can be carried out using the proportioning pump, as shown in Fig. 2. We normally take great care to exclude carrier iron, and all steps beyond the first extraction into di-isopropyl ether, together with the preparation of the iron-52 ferric citrate samples for clinical use, are carried out in more conventional apparatus by methods similar to those described by Yano and Anger. However, the use of the proportioning pump in the target etching and first extraction steps reduces the level of the activity which has afterwards to be handled from the equivalent of about 100 mc. of radium to about 150 μc. of iron-52.

After chemical separation we find that the yield of iron-52, corrected to the end of the bombardment, averages about 50 μc./100 μamp/h. Some iron-55, an X-ray emitting isotope with a half-life of 2·6 years, is also made during bombardment, and, in order to keep the iron-55/iron-52 ratio in the administered sample down to a minimum, the time elapsing between bombardment and clinical use is kept as short as possible: in fact, not more than 3 h are required for the chemical processing.

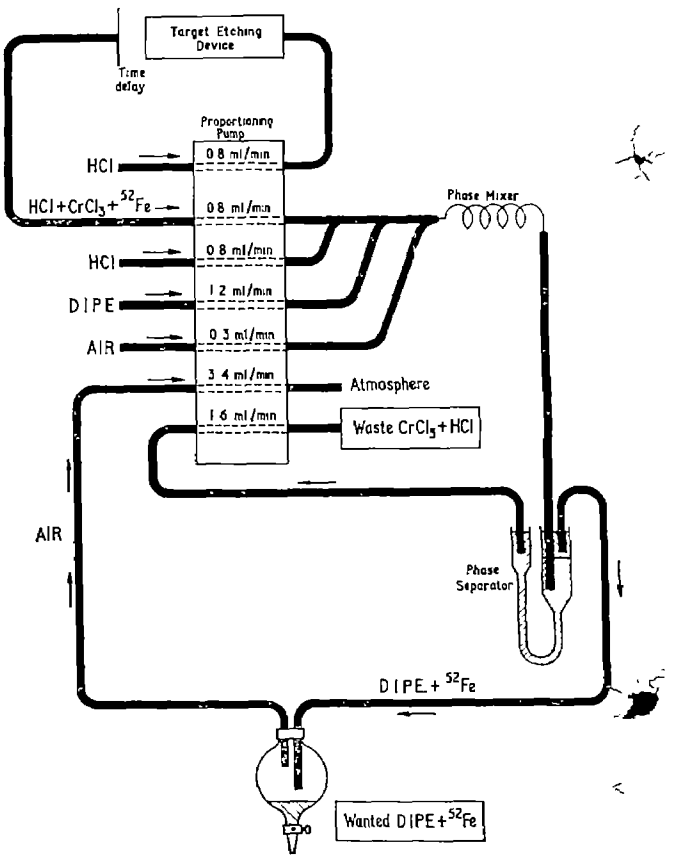


Fig 1. Flow diagram for the initial separation of carrier-free iron-52 from chromium targets

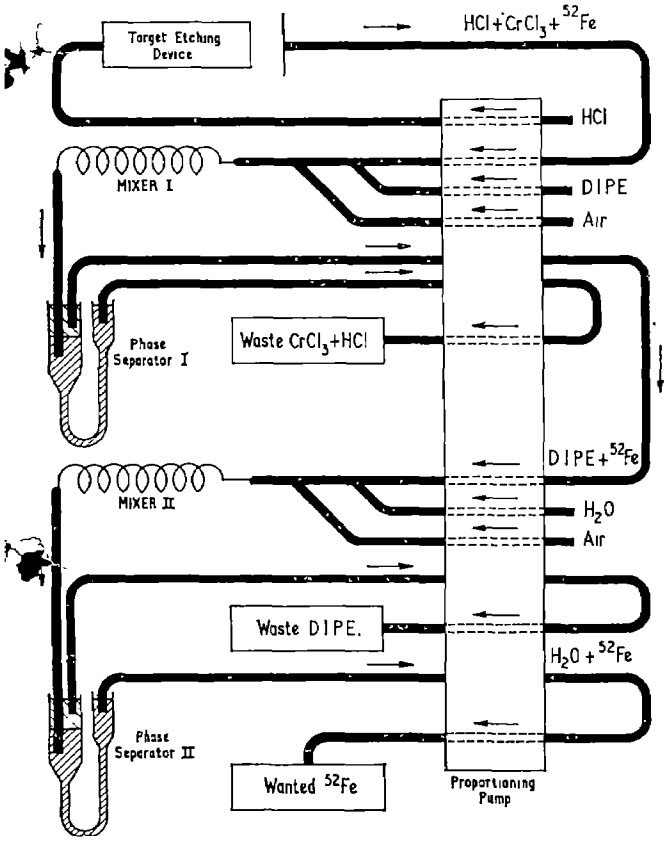


Fig 2. Flow diagram for the initial separation of iron-52 with carrier from chromium targets

The purity of a small fraction of the final product is checked while the main part is being sterilized by autoclaving; γ-spectrometry ensures that the sample is free from 5·7-d manganese-52 and other γ-emitting contamination (we find it unnecessary to add manganese carrier to achieve this), and microanalysis by the ring-oven technique shows that the content of inactive chromium, aluminium and iron is below the limit of detection —which for each element is equivalent to less than 5 μg in an entire 5-ml. sample.

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In vitro Distribution of Americium in Human Blood Serum Proteins

The distribution of plutonium in blood serum proteins has been investigated previously[1–3]. Normally, americium-241 is present in plutonium samples, and is produced there by the β-decay of plutonium-241. Accordingly it is of interest to compare the distribution of americium in blood serum proteins with that of plutonium.

Human blood was allowed to clot at room temperature for 2 h, stood overnight at 4° C, and then centrifuged. The supernate was dialysed for 16 h at 5°–8° C in Visking dialysis tubing against several changes of 0·1 M 2-amino 2-hydroxymethylpropane 1:3 diol (*tris*) and its hydrochloride in 1 M sodium chloride at *p*H 8·0. 10–20 μl. of a mixture of americium-241 and plutonium in 0·3 M nitric acid were added to about 6 ml. of the dialysed serum. The mixture of isotopes and serum was stored at about 8° C for 1 h and then resolved by gel-filtration through a column (93 cm × 2·5 cm) of 'Sephadex *G*-200' (ref. 4). The plutonium distribution in the various protein eluates was determined by measuring the delayed neutron emission following neutron irradiation[5]. The fission cross-section and delayed neutron yield for americium are much lower than the values for plutonium, thus the delayed neutron emission was specific for the plutonium present. The amount of americium-241 in equal portions was measured by γ-spectrometry. Plutonium and americium were found predominantly in the fraction containing albumin and transferrin. This fraction was further resolved by ion-exchange chromatography using diethylaminoethyl 'Sephadex *A*-50' (ref. 4).

The distribution of americium and plutonium in the 'Sephadex *G*-200' resolution of the proteins was very similar. However, in the ion-exchange stage, attempts to resolve the americium distribution failed to identify eluate americium radioactivity with any particular protein fraction. The difference between americium and plutonium in their distribution between albumin and transferrin is illustrated in Fig. 1. Since the methods of measurement of the plutonium and americium activities are quite different, no significance is attached to the relative peak heights. Two further experiments on the americium distribution in serum proteins showed similar qualitative behaviour but dissimilar quantitative behaviour. When americium species alone are subjected to elution through the ion-exchanger under conditions otherwise identical with the experiment depicted in Fig. 1, the americium elutes at a stage greater than the elution volume corresponding to the protein elution volume. This indicates that the americium in serum is chemically bound to protein—at least initially. It seems likely that americium forms protein complexes weaker than those of plutonium, and they do not survive the ion-exchange processes.

Filtration through 'Sephadex *G*-50' has been used for studies of the binding between proteins and iron[6]. Similar experiments were carried out to measure the distribution of americium and of plutonium between the protein fraction and the low molecular weight non-protein fraction. About 90–95 per cent of the americium and the plutonium were distributed in the protein fraction, and the remainder in the low molecular weight fraction. Experiments performed with added citrate ions produced the inverse effect; practically no actinide was found in the protein portions.

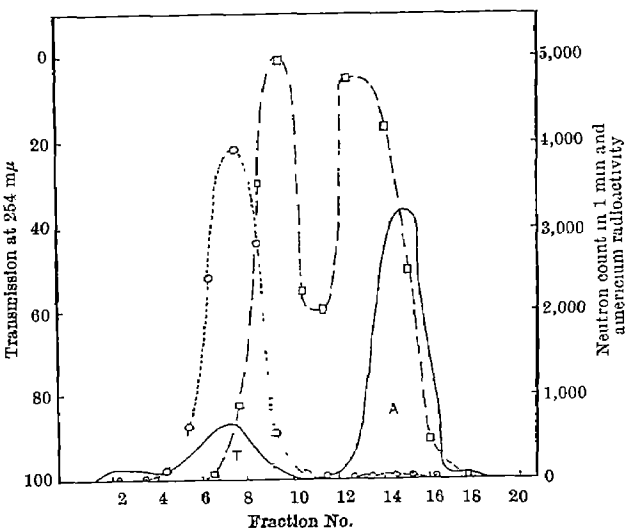


Fig 1. Resolution of the transferrin/albumin fraction by chromatography on DEAE-'Sephadex *A*-50'. Elution was started with 0 1 M *tris* and its hydrochloride in 0 1 M sodium chloride, followed by a gradient of 0 1 M–2 0 M sodium chloride in 0·1 M *tris*-hydrochloric acid. ——, Optical transmission; · · · ○ · · ·, radioactivity due to plutonium; — — □ — —, radioactivity due to americium-241; *T*, transferrin; *A*, albumin

From the point of view of physico-chemical equilibria, citrate appears to be very effective in removing americium and plutonium from their serum protein complexes.

Further investigations are being carried out to identify more precisely the americium complexes present in blood serum.

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Increased Proliferation of Transplanted Mouse Bone Marrow Cells by Pre-irradiation of the Recipient

THE transplantation of bone marrow cells from normal donor animals into heavily irradiated recipients rapidly restores haemopoiesis to normal. For example, we find that erythropoiesis in a heavily irradiated mouse is restored to normal within 7 days of the injection of 4×10^6 bone marrow cells, although this is only 1 per cent of the normal number of bone marrow cells in a mouse. This very considerable capacity for restoration of erythropoiesis suggests that before transplantation the progenitor cells responsible for recovery of erythropoiesis are proliferating slowly, either all the cells dividing slowly or a small proportion of them dividing more rapidly. This has recently been confirmed by Becker *et al.*[1] by incubating bone marrow cells *in vitro* with tritiated thymidine of sufficient activity so that all the cells synthesizing DNA and incorporating the isotope are killed by the subsequent β-irradiation. For bone marrow cells from normal animals, they found less than 10 per cent of the cells in DNA synthesis, while for rapidly dividing cells (6 days after 900 r. whole-body irradiation with bone marrow transplantation) 65 per cent were in DNA synthesis.

Because of the low proliferation rate of the progenitor cells in normal animals the stimulus for proliferation in the recipient animals will be low until some time after whole-body irradiation. It was therefore decided to determine whether pre-irradiation of the recipient animals would lead to the earlier stimulation of transplanted bone marrow cells.

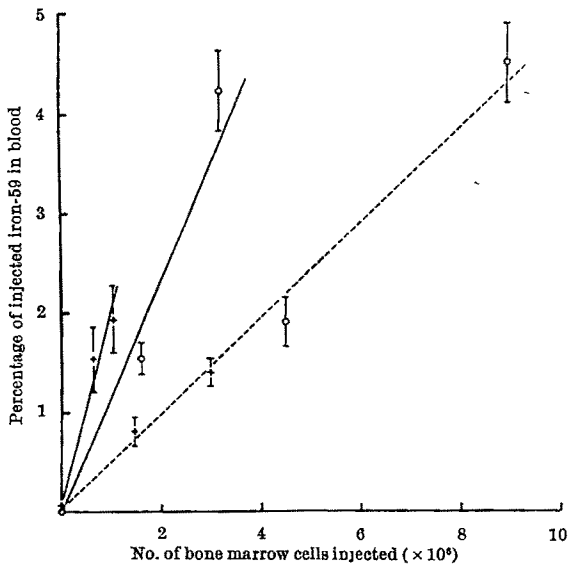


Fig. 1. Incorporation of iron-59 into red blood cells as a function of the number of bone marrow cells injected. ——, Bone marrow cells from unirradiated animals; – – – –, bone marrow cells from mice given 160 r whole-body irradiation 6 days previously, transplanted into: O, mice given 650 r. whole-body irradiation; and +, mice given 650 r. whole-body irradiation and 160 r. 6 days previously

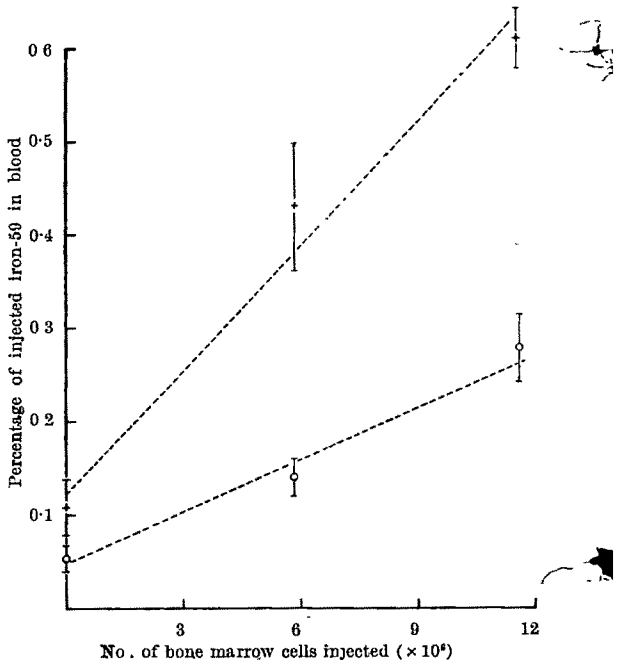


Fig. 2. Incorporation of iron-59 into red blood cells as a function of the number of injected bone marrow cells taken from donor animals directly after 160 r. whole-body irradiation. O, Mice given 650 r. whole-body irradiation; +, mice given 650 r whole-body irradiation and 160 r. 6 days previously

Bone marrow cells from unirradiated *C57BL* mice were injected into groups of recipient mice that had received 650 r. whole-body X-irradiation a few hours previously —some of these animals having also received 160 r. whole-body irradiation 6 days earlier. Five days after the 650 r. and transplantation, iron-59 was injected and 2 days later the activity of this isotope in the circulation was determined according to the method of Blackett *et al.*[2], to give a measure of the incorporation into the red blood cell precursors. Fig. 1 shows that to give the same incorporation of iron-59 only about half the number of normal bone marrow cells is required in the pre-irradiated animals than in the mice not pre-irradiated (change in slope significant at $P = 0{\cdot}05$ level).

To determine whether this increased proliferation also occurred with bone marrow cells from irradiated donors, cells were transplanted from animals that had received 160 r. 6 days previously (Fig. 1). No increased proliferation in the pre-irradiated recipients was observed. This absence of increased proliferation is not due to an inhibition of the effect by irradiation, since an increased response is observed if bone marrow cells are taken from animals directly after 160 r. irradiation, Fig. 2 (change in slope significant at $P = 0{\cdot}05$ level).

At present it is not known at what stage of erythropoiesis this increased proliferation occurs; whether it is an effect on the most primitive progenitor cell, or on the recognizable differentiating red blood cell precursors, or simply due to an increased transplantation efficiency of the cells.

It is suggested that these observations may be explained by the state of proliferation of the transplanted cells and the physiological state of the recipient at the time of the cell injection. The level of cell proliferation will be low in bone marrow cells taken from normal animals or from animals directly after 160 r. whole-body irradiation. On transplantation, the cells will start to proliferate earlier in the pre-irradiated recipients because they are still recovering from the 160 r. pre-irradiation, and so

already have a greater than normal stimulus for proliferation. The absence of any increased proliferation of bone marrow cells if they are taken from donor animals given 160 r. 6 days previously would be explained by the fact that these cells are already actively proliferating and continue to do so irrespective of whether the recipient animals have been pre-irradiated. The conclusion that cells responsible for transplantation are actively proliferating 6 days after 160 r. is based on the fact that the incorporation of iron-59 per 10^6 cells has not regained normal values at this time (Fig. 1). At later time intervals after 160 r., when recovery of the bone marrow is complete and cell division is again at the normal low level, it would be expected that the bone marrow cells would again show increased proliferation in pre-irradiated recipients.

Further studies are being undertaken to investigate this effect in more detail and to determine whether it provides a means for improving the grafting of bone marrow cells.

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RADIATION CHEMISTRY

A Correlation between Electron Affinity and Protection in the Radiation Chemistry of Hydrocarbons

It has been known for some time[1,2] that the presence of aromatic compounds in solutions of saturated hydrocarbons can protect the solvent against radiation-induced decomposition. Since the aromatic solute plays a mainly 'non-sacrificial' part, scavenging of chemical intermediates (atoms, radicals, or ions) cannot be the major process in the observed protection[3–5]. Rather, it has been proposed[2] that some sort of efficient energy transfer from the solvent, which absorbs the primary radiation, to the solute must operate. The recent observations of thermal electrons produced in relatively high yield in the radiolysis of aliphatic hydrocarbons in the glassy[6] and liquid[7] states indicated that it would be interesting to investigate whether there is a link between radiation protection by aromatic compounds and their electron affinity as it is known that these compounds are electron acceptors.

Lovelock *et al.*[8] have measured in the gas phase the electron absorption coefficients relative to chlorobenzene, χ, of a number of aromatic hydrocarbons. These electron absorption coefficients are related to the relative electron affinities, EA_{PhCl} by the expression $EA_{PhCl} = \log \chi$. Dilute solutions (5×10^{-3} M) of several of these aromatic compounds in *cyclo*hexane were deaerated and irradiated with cobalt-60 γ-rays (total dose $6{\cdot}0 \times 10^{18}$ eV ml.$^{-1}$), and the hydrogen yields, $G(H_2)$, were measured; the yield from pure *cyclo*hexane, $G(H_2)_0$, was 5·56 molecules per 100 eV. Because hydrogen is the major product of the radiolysis of *cyclo*hexane the $\Delta G(H_2)$ values, where $\Delta G(H_2) = 5{\cdot}56 - G(H_2)$, are a measure of the protective effect. In Fig. 1, $\Delta G(H_2)$ values have been plotted against the electron affinities, EA_{PhCl}. It can be seen that there is a satisfactory linear correlation between the protective effect of the aromatic compounds and their electron affinities. Only in the case of the halobenzenes is there a significant deviation.

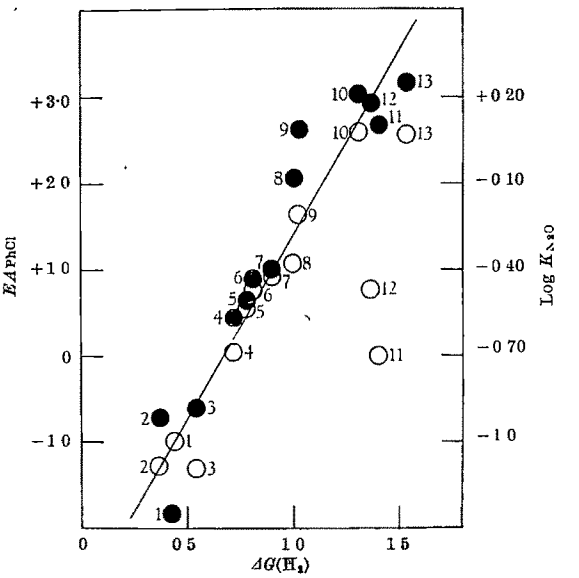


Fig. 1 Electron affinity, EA_{PhCl}, versus $\Delta G(H_2)$, open circles, log K_{N_2O} versus $\Delta G(H_2)$, closed circles (1) Aniline, (2) benzyl alcohol, (3) phenanthrene, (4) *cis*-stilbene; (5) *trans*-stilbene; (6) pyrene; (7) azobenzene, (8) anthracene, (9) benzaldehyde; (10) nitrobenzene; (11) chlorobenzene, (12) bromobenzene, (13) iodobenzene

It has been shown[7] that nitrous oxide scavenges thermal electrons produced in the radiolysis of *cyclo*hexane according to the stoichiometric equation:

$$e^- + N_2O \longrightarrow N_2 + O^- \qquad (1)$$

To test the foregoing contention that the aromatic solutes behave as electron acceptors in the radiolysis of *cyclo*hexane solutions, solutions containing both nitrous oxide and an aromatic compound were irradiated. The presence of the aromatic compound was found to depress the nitrogen yield, indicating competition for thermal electrons. From the depression in the nitrogen yield the relative electron affinities of the aromatic compounds, EA_{N_2O}, were calculated using the relation $EA_{N_2O} = \log K_{N_2O}$, where K_{N_2O} is the relative rate constant for the electron-capture process. The electron affinities obtained by this method have also been plotted against the $\Delta G(H_2)$ values in Fig. 1. Again, a satisfactory linear correlation exists.

From the foregoing it is evident that thermal electrons are a precursor of a significant part of the hydrogen yield in the radiolysis of *cyclo*hexane (reactions 2 and 3).

$$RH_2 \rightsquigarrow RH_2^+ + e^- \qquad (2)$$

$$e^- + RH_2^+ \longrightarrow R\dot{H} + H\cdot \qquad (3a)$$

and/or:

$$e^- + RH_2^+ \longrightarrow R + H_2 \qquad (3b)$$

Aromatic hydrocarbons present in low concentration provide an alternate path to the hydrogen-forming reaction of the thermal electrons (reaction 3) by a non-dissociative electron-capture process giving an aromatic anion (reaction 4). In an aliphatic hydrocarbon solvent these aromatic anions would be expected to be relatively unreactive and have a long life, possibly reacting finally with the primary cations, RH_2^+, of the solvent (reaction 5):

$$e^- + ArX \rightleftharpoons ArX^- \qquad (4)$$

$$ArX^- + RH_2^+ \longrightarrow ArX + RH_2 \qquad (5)$$

Thus, by the intermediacy of the aromatic solute a route is provided for the return of the electron formed in the primary process (2) to a solvent cation, RH_2^+, resulting in no net chemical change in the system.

Of the aromatic solutes investigated, only the halobenzenes are capable of dissociative electron capture (reaction 6) in addition to the non-dissociative process (reaction 4).

$$e^- + ArX \longrightarrow Ar + X^- \qquad (6)$$

Since the breaking of the Ar—X bond is very fast (the order of a molecular vibration, $\sim 10^{-13}$ sec) it would occur before the back reaction of the equilibrium (4). The observed deviation of the halobenzenes from the linear $\Delta G(H_a)$ versus EA_{PhCl} plot would indicate that the dissociation process (reaction 6) is more important in the liquid phase than under the gas-phase conditions[8] where the EA_{PhCl} values were obtained.

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Ultra-violet Absorption Spectrum of Thymine in Ice

WANG[1-3] has found that the production of photodimers is much greater when frozen aqueous solutions of thymine are irradiated with ultra-violet light than when liquid solutions of thymine are similarly irradiated. Adding alcohol to the solution before freezing or using thymidine, thymidylic acid, or uracil compounds instead of thymine all markedly reduce the formation of photodimer. He believes that the dimer formation in ice is facilitated by aggregation of the molecules. We have measured the absorption spectrum of various frozen solutions and have thus been able to obtain direct evidence of aggregation.

Samples were frozen on to a quartz plate to give a specimen about 1 mm thick and then immersed in liquid nitrogen in a quartz Dewar flask. The samples used were either pure water or an aqueous solution at a concentration of about 1 mg/ml. The apparent absorption of the pure ice was subtracted from that of each frozen solution. The absorption spectrum was measured with a Cary 14 spectrophotometer and a 2-in. diameter photo-multiplier placed 2 in. from the sample (model 1462 light scattering attachment). This apparatus detects a large fraction of the forward scattered light in addition to the transmitted light and thus more nearly gives an accurate absorption spectrum.

The absorption of thymine in ice is shown as the solid line in Fig. 1. A room temperature aqueous spectrum of

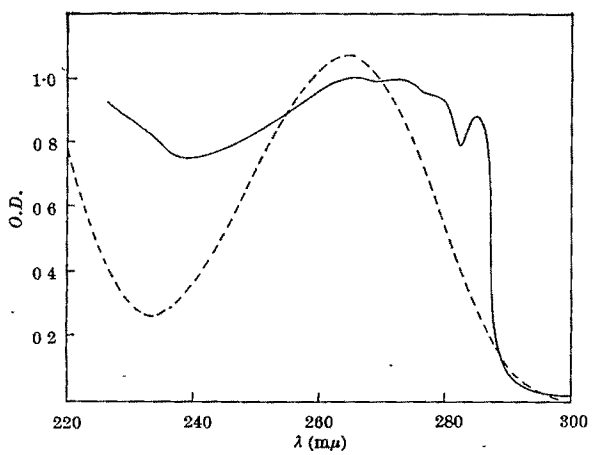


Fig. 1. Ultra-violet absorption spectrum of thymine frozen in ice at liquid nitrogen temperature (solid line) compared with a sample in liquid aqueous solution (dashed line)

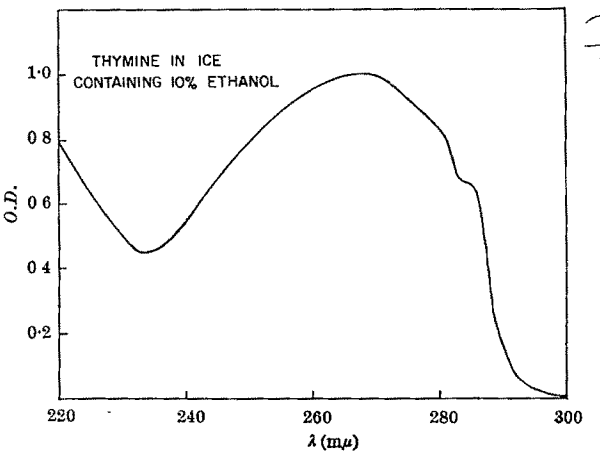


Fig. 2. Spectrum of thymine frozen in a 10 per cent (v/v) aqueous ethanol solution

thymine is also shown for comparison. The change in absorption is quite pronounced. In a frozen 10 per cent (v/v) solution of ethanol (Fig. 2) the thymine spectrum is more nearly like that in the liquid solution. The spectra of frozen solutions of thymidylic acid (5′), uracil, uridine and uridylic acid (5′) were also measured. Although they all gave broadened spectra in the frozen solutions relative to their liquid solution spectra, none showed the striking differences found in thymine.

The spectrum of the thymine in ice at liquid nitrogen temperature is essentially identical to that found by Sinsheimer *et al.*[4] for dry, sublimed films of thymine at this temperature. The four maxima seen in the ice spectrum agree with those in the dry film to ± 1 mμ. This is strong evidence that aggregates of thymine occur in the ice similar to those present in the sublimed film. Furthermore, the fact that alcohol partially restores the liquid solution spectrum strengthens the conclusion that the frozen solution spectrum indicates interaction between thymine molecules. Apparently, for thymine in ethanol or for thymidylic acid, uracil, uridine and uridylic acid, the solute–solute attraction is less (or the solute–solvent repulsion is less) than for thymine in water.

These conclusions are in accordance with Wang's photochemical experiments and his interpretation of them in terms of aggregation. However, the spectral evidence is a more direct measure of this aggregation.

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PHYSIOLOGY

Heparin-induced Hypocalcaemia in Rabbits

IT has been reported[1] that heparin contains certain mineral substances of the alkaline earth series, among them calcium. The amounts of these minerals vary with the batch of heparin and the quantities of these impurities are sufficient to cause error in the analysis of plasma calcium, barium and strontium if heparin is added *in vitro* to blood samples. We have found a much more serious

source of error in the estimation of plasma calcium when heparin is injected intravenously into rabbits.

Two groups of animals were used. In one group, thirty rabbits were anaesthetized with 25 per cent urethane at a rate of 6 ml./kg body wt Blood samples were removed via a jugular catheter. In the other group, forty rabbits were maintained conscious in a rabbit box, blood samples being collected from an ear vein. In both groups of animals 25 mg of heparin (Boots or Evans Medical Ltd.), dissolved in 5 ml. of physiological saline, was injected intravenously. Estimation of plasma calcium was carried out by titrating against EDTA with murexide as an indicator at a *p*H of 12, using an EEL titrator. Plasma samples were prepared by collecting blood into centrifuge tubes in ice and centrifuging at 1,500*g* in a refrigerated centrifuge.

In both groups of animals we found that the estimated plasma calcium fell rapidly when the samples were stored at 20° or 37° C, but not at 0° C (Fig. 1) (55 experiments out of 70). If heparin is added to blood after removal from the animal, no such fall occurs.

Fig. 2 indicates that the maximum rate of fall of plasma calcium occurs very rapidly after injection of heparin. However, 3 h after injection, while blood is still uncoagulable, the fall in plasma calcium on storage is very small. In this experiment, plasma calcium was determined within 20 min of sampling, and then determined again 24 h later, the samples being stored at room temperature (18–22° C).

In those samples which show the greatest fall, a precipitate forms which is considerably more dense than plasma.

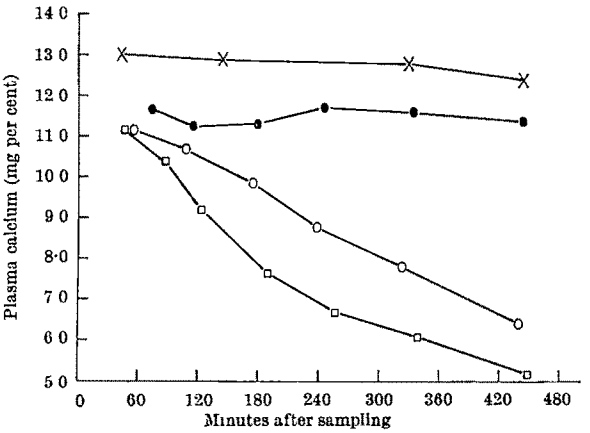


Fig. 1. The effect of temperature on the fall of plasma calcium *in vitro*. ×, Pre-heparin sample with heparin added *in vitro*, incubated at 37° C, ●, post-heparin plasma incubated at 0° C; ○, post-heparin plasma incubated at 20° C; □, post-heparin plasma incubated at 37° C. Each line represents the estimated plasma calcium from a single sample at a particular temperature, analysed at intervals

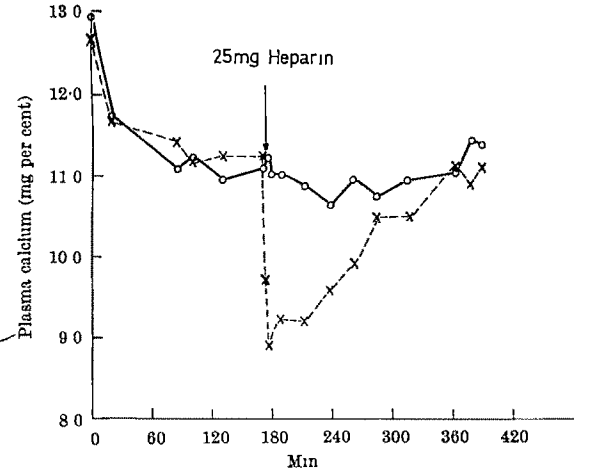


Fig. 2. Effect of intravenous heparin on plasma calcium over 24 h (rabbit). ○, Plasma calcium analysed 20 min after withdrawing the samples at different periods after the beginning of the experiment, ×, the same samples analysed 24 h later

Examination of this precipitate indicated that it is poorly soluble in saline and fat solvents, chloroform dissolving more than ether. Acid hydrolysis of the precipitate with boiling 8 N hydrochloric acid changes the nature of the precipitate so that it is now soluble in fat solvents. Analysis of this acid extract showed that calcium was now present, the ratio of calcium to total precipitate being 1·5 : 34 w/w.

The observation that heparin, injected intravenously, appears to cause an *in vitro* fall in plasma calcium on subsequent sampling of blood and storage, while *in vitro* addition of heparin to blood does not produce this phenomenon, suggests that the injection of heparin causes the release of some factor from the vascular bed. This factor subsequently appears to cause the build-up in the blood sample of a substance which can combine with calcium. We considered that the phenomenon is related to the release of lipoprotein lipase which follows the injection of heparin[2]. This releases free fatty acids from lipoproteins including chylomicra. In the animal these released free fatty acids are carried on proteins for eventual utilization by the animal's tissues. In plasma samples the enzyme will still be active, but the free fatty acids can no longer be cleared from plasma, and so accumulate to saturate plasma proteins. As this occurs the excess free fatty acids will form insoluble calcium soaps, which appear to be no longer capable of estimation by EDTA.

We have examined cats and rats to see if other species show the same phenomenon. We have been unable to detect it in cats, except in special circumstances involving the addition of chyle to plasma samples after heparin injection. We have found it in rats but less readily than in rabbits. Examination of the literature revealed that Heidland *et al.*[3] reported a heparin-induced hypocalcaemia in dogs. They attributed this to formation of a heparin–calcium complex. Other workers, Spitzer and Spitzer[4], French *et al.*[5] and Brown *et al.*[6], all mention the appearance of a precipitate when studying the clearing of chylomicra from plasma by heparin released lipoprotein lipase. However, they do not report having carried out calcium analyses on their samples as they were concerned principally with the action of lipoprotein lipase on the clearing of chylomicra.

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An Anovulatory Bilateral Hermaphroditic Rat

Any discussion of mechanisms in mammalian sex differentiation must consider two classes of chemical mediators: embryonic inductors and androgenic steroid hormones. The inductors, the chemical nature of which is unknown, play a vital part in differentiating the gonad and probably the secondary sex ducts[1], while the steroids are credited with influencing behaviour and brain–pituitary functions[2]. However, the definitive experiment obligating androgen as the organizer of the male type brain–pituitary axis has not been carried out. This communication describes a bilateral hermaphrodite occurring in Nature, which casts doubt on the indispensability of androgen for determining a male type of hypophysial-hypothalamic axis.

During a routine laparotomy of 30-day-old male Holtzman strain rats, an animal was found which had a scrotal testis of about normal size on the right side and a follicular

ovary on the left. A well-developed oviduct and uterus was present on the left along with a small rudimentary nephric (Wolffian) duct. On the right side a thread-like uterus was closely attached to an apparently normal deferens duct. Two small seminal vesicles were attached to the utero-vaginal rudiment, which had no external opening.

At 60 days of age the testis was removed and serially sectioned. Histological examination disclosed a small lateral area of ovarian tissue containing six large follicles, as well as a few smaller ones. The seminal tubules contained abundant sustentacular (Sertoli) cells, but very few germ cells. Interstitial cells were large and numerous.

Sixty days after removal of the testis (total age 120 days) the animal was killed. The body weight was 255 g, which is the normal weight of a female of this strain at 4 months of age. The reproductive tract was dissected *in toto*, fixed in Bouin's solution, and sectioned in paraffin. The adrenals and pituitary were large, weighing 74·7 mg and 20·0 mg respectively.

The ovary did not appear to undergo significant compensatory hypertrophy during the 60 days following removal of the testis. The follicles were of varying size, several being fully mature, but no corpora lutea were found. A few small seminal tubules surrounded by several interstitial cells were seen in the region of the hilus as well as in a portion of the cortex.

The uterus on the left side was filled with fluid and lined by tall columnar epithelium. The left uterus was atrophic. The utero-vaginal rudiment showed oestrogenic stimulation in the form of a heavily cornified epithelium. Small prostatic buds, with atrophic acinar epithelial linings, were found attached to the wall of the rudiment. The epithelium of the small seminal vesicles was also unstimulated.

While the testis was capable of causing differentiation of the nephric duct into a ductus deferens on both sides, it did not cause regression of the oviducts (Müllerian ducts). Furthermore, androgen production was insufficient to cause complete differentiation of prostatic lobes or to produce functional prostatic or seminal vesicle epithelium. In spite of this apparent lack of androgen, the hypothalamic-pituitary function resembled that of the androgenized female[3], that is, follicles in the ovary were well developed as were those in the small ovarian remnant attached to the testis, but failed to undergo luteinization. The well-developed theca and interstitial cells further contribute to the interpretation that gonadotropins were present, but no cyclic surge of luteinizing hormone, necessary for ovulation and formation of corpora lutea, occurred.

The original experiments involving alteration of pituitary function in female rats used testicular implants[4]. More recently, testosterone propionate has completely replaced this method of 'masculinizing' females[2] and is thought to duplicate the action of the testis. However, the lack of development of secondary sex organs seen in the above-described rat suggests a very low level of androgen during the so-called critical period for sex differentiation of the brain–pituitary system. Hollander *et al.*[5] reported that in mouse intersexes, in which the testes develop poorly, ovulation or follicular luteinization was always lacking. Therefore, the possibility that an embryonic inductor, responsible for testicular differentiation, also influences the brain–pituitary axis cannot be overlooked. Certainly, such a concept appears worthy of further examination.

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Disappearance of Iodine-131-labelled Human Growth Hormone from the Plasma of Diabetic, non-Diabetic and Acromegalic Subjects

THE disappearance of iodine-131-labelled human growth hormone (HGH) from plasma has been investigated as part of a study designed to measure the secretion rate of the hormone. This was undertaken because plasma levels alone may not be indicative of the secretion rate of the hormone. Twenty-six adult patients consented to the procedure. Seventeen were diabetics (six required insulin and seven had never been given insulin). Eight were acromegalic (four with diabetes), and five were normal. Thyroidal uptake of radio-iodine was blocked with Lugol's iodine. HGH prepared by the Raben[1] technique and purified[2] was iodinated by the method of Hunter and Greenwood[3]. Material of specific activity 30–150 μc./μg was used. It was diluted at once and stored in a diluent containing 0·3 per cent human serum albumin. Each batch was used within 14 days of preparation. The subjects were studied resting, fasting and after 12 h without food or drugs. Care was taken to ensure that where possible each member of the various groups of patients was studied with a different batch of ^{131}I-HGH, and this meant that each batch was used in several types of patients. A weighed dose of 2–5 μc. was given intravenously at about 9 a.m. Blood samples were collected at intervals during the next 2–4 h, cooled, and the plasma radioactivity counted in a well-type scintillation counter to give >95 per cent accuracy. The plasma proteins were then precipitated with cold 20 per cent trichloroacetic acid (TCA) and the supernatant counted. The protein-bound ^{131}I could then be calculated and was expressed as a percentage of the dose injected and plotted semi-logarithmically against time. In each of the five normals a disappearance curve similar to Fig. 1*A* was found. Equilibrium was reached in 10 min and a straight-line exponential curve thereafter gave half-times of from 23 to 45 min (see Fig. 2). All seven non-insulin-requiring diabetics gave curves similar to Fig. 1*B*, reaching equilibrium by 20–60 min and giving final straight-line curves with half-times of from 60 to 185 min (see Fig. 2).

All eight acromegalics showed a similar slow final phase in their disappearance curves (see Fig. 2). The six insulin-requiring diabetics gave curves the final straight-line portions of which gave half-times of from 23 to 185 min. Fractional turnover rates were calculated from these curves by the method of Matthews[4] (fractional turnover rate = fraction of intravascular HGH metabolized per minute). Table 1 shows that the fractional turnover rates follow the half-times approximately, being 0·03–0·06 in the normals, 0·01–0·028 in the diabetics not requiring insulin and 0·015–0·05 in those usually on insulin. The

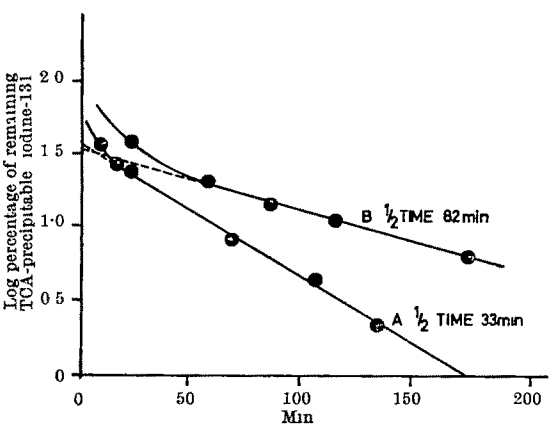


Fig. 1 Plasma disappearance curves of iodine-131-labelled human growth hormone in man. Protein-bound iodine-131 is plotted semi-logarithmically with time. *A*, Curve in normal subject W. R.; *B* curve in diabetic subject J. S

Table 1. RANGE OF THE CALCULATED FRACTIONAL TURNOVER RATES (F.T.R. = FRACTION OF INTRA-VASCULAR HGH REMOVED PER MINUTE) WITH THE MEAN AND THE STANDARD DEVIATION OF EACH GROUP

Group	Non-diabetics (5)	Diabetics on insulin (6)	Diabetics not on insulin (7)	Acromegalics + diabetes (4)	Acromegalics − diabetes (4)
F.T.R.	0·0322–0·0604 Mean = 0·0453 *S.D.* ± 0·01	0·0184–0·0474 Mean = 0·0346 *S.D.* ± 0·0115	0·007–0·0261 Mean = 0·0178 *S.D.* ± 0·007	0·009–0·019 Mean = 0·0157 *S.D* ± 0·0062	0·0133–0·0233 Mean = 0·0133 *S.D.* ± 0·0078

The non-diabetic values are significantly different from those of non-insulin requiring diabetics ($P < 0·01$) and from both groups of acromegalics ($0·001 > P < 0·01$), but not from the values for diabetics requiring insulin ($0·2 < P > 0·1$).

Table 2. BLOOD GLUCOSE-LEVELS AND FRACTIONAL TURNOVER RATES PER MINUTE OF HGH IN DIABETICS REQUIRING INSULIN

Blood glucose	50	116	270	336	380	554	660
Fractional turnover rate	0·0187	0·0211	0·038	0·0184	0·04	0·0368	0·0474

acromegalics all had fractional turnover rates of less than 0·03. It was noted that in the insulin-requiring diabetics the curves usually gave normal fractional turnover rates when the blood glucose was high (see Table 2), and these values show significant correlation ($r = 0·83$, $0·01 > P < 0·02$).

Laron *et al.*[5], using similar preparations of ^{131}I-HGH in adolescents, recently reported slow disappearance of their material which they suggest might be due to radiation damage to the HGH. In view of the absence of the slow phase in the normal subjects reported here it is believed that this is not the whole explanation in the present investigation. In addition, it was possible to precipitate almost identical amounts of iodine-131 by TCA and by anti-HGH sera from standard solutions and from the plasma of two normal and two diabetic subjects (both early and late in the procedure), so that immunological activity was not lost during the observations. Autoradiograms of electrophoreses in agar gel, of the labelled hormone, after incubation with various plasmas, have not as yet indicated that any differences occur in the 'binding' of the label between diabetics or acromegalics and normals. The patterns obtained from these investigations were similar to those reported by Hadden and Prout[6].

The explanation of the delayed disappearance of ^{131}I-HGH in acromegalics and in diabetics whose blood glucose is not greatly raised is uncertain. It could be accounted for by the presence of a large pool of HGH in the acromegalics—certainly it would be interesting to know if the adolescents studied by Laron *et al.*[5] had high plasma levels of HGH. The apparent correlation between the blood glucose-levels and the fractional turnover rates of HGH in the insulin-dependent diabetics may mean that there is some change in the peripheral uptake of the hormone with alteration of the blood glucose-level; alternatively it may mean that a high HGH turnover is associated with more severe diabetes. The fact that marked differences were found in the disappearance rates of ^{131}I-HGH between the normal and most other subjects in this study means that it may not be possible to deduce secretion rates from the blood-levels alone.

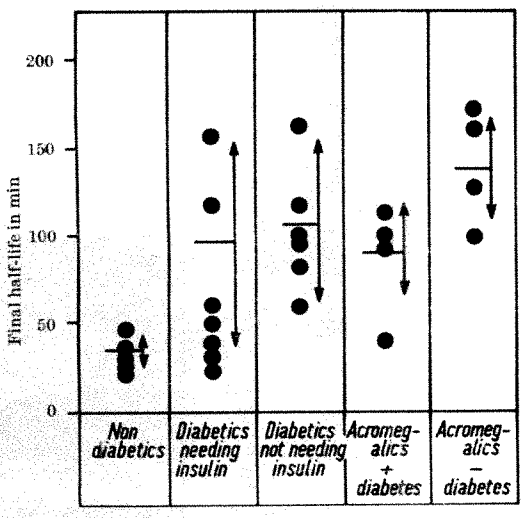


Fig. 2. Half-times of the final straight-line portions of the disappearance curves of ^{131}I-HGH in man. Both groups of diabetics give values significantly different from those of the normals ($P < 0·01$ and $P < 0·05$), as do both groups of acromegalics ($P < 0·01$ and $P < 0·001$). The means are shown by horizontal lines and ± one standard deviation by the arrowed vertical lines

The ^{131}I-HGH was kindly made available to me by Dr. F. C. Greenwood (Imperial Cancer Research Fund), whom I also thank for much helpful discussion. I also thank Dr. Bomford, Dr. Stuart Mason, Dr. Picton Thomas and Dr. A. Bloom for permission to study their patients.

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ANATOMY

Synaptic Contacts on Axons in the Cerebral Cortex

AXO-AXONAL contacts have been described in various areas of the nervous system, including the retina[1], spinal cord[2], lateral geniculate nucleus[3], olfactory bulb[4], superior colliculus[5] and the vertical lobes of *Octopus*[6]. In the spinal cord these junctions have been postulated as a basis for presynaptic inhibition[2,7]. In addition, axonal endings of basket cells have been described forming synapses with the initial segment of Purkinje cell axons[8,9], and physiological evidence for inhibition is found in this area[10]. Until now, axo-axonal contacts have not been seen in the cerebral cortex.

During the course of an ultrastructural investigation of the synaptic organization of the prepyriform cortex in the rat, I have seen presynaptic bags contacting unmyelinated axons. The stratified and uniform orientation of neurones in this area[11] of the cerebral cortex permits the investigation of different components of the neuropil with some degree of reliability, in contrast to the neocortex, for example, where the arrangement is much more complex.

The tissue was fixed by perfusion with a buffered paraformaldehyde solution[12] post-fixed in osmium tetroxide[13], and the preparations were double-stained with uranyl and lead solutions for electron microscopy[14]. A Siemens 'Elmiskop 1b' electron microscope was used for studying and photographing the preparations.

In the region immediately beneath the perikaryal or pyramidal layer, myelinated axons of 0·5–1·5μ diameter are seen, some of which are oriented towards the overlying somata. Occasionally an unmyelinated segment several micra long (Fig. 1) can be followed from a myelin sheath towards the overlying cell bodies. Such unmyelinated axons have not as yet been traced in continuity with the cell body so it cannot yet be decided whether they are initial segments of the overlying neurones or terminal unmyelinated portions which end on these neurones. The diameter of these unmyelinated axon segments is between 0·4 and 1·0μ and they contain filaments, mito-

chondria, multivesicular bodies, smooth endoplasmic reticulum, tubular structures and sometimes ribosomes. The occurrence of ribosomes in some of these processes suggests that they may be initial segments. All processes identified as unmyelinated axon segments could be directly traced in the same section, and seen to emerge from myelin sheaths. Such an arrangement has not been associated with dendritic processes in this area. Several of the unmyelinated segments, continuous with a myelinated portion, are seen to have one or more presynaptic bags contacting them (Fig. 1). These presynaptic bags are beween 0·5 and 1·2μ in diameter and contain predominantly ellipsoidal or polymorphic vesicles. The contacts are mostly of Gray's type II (ref. 15), but the "thickenings" are of variable density. The largest number of presynaptic bags counted thus far on any one such segment was five and in this case the unmyelinated segment was about 8μ in length. More commonly, short (1–3μ) segments were seen contacted by one or two presynaptic bags. Also, in the material examined so far, the unmyelinated segments of axons containing contacts have shown no branching.

The origin of the fibres which form the synaptic endings on these unmyelinated axons remains to be determined. Among the sources to be considered are the interneurones or inhibitory cells suggested from physiological investigations[16,17]. It is not known whether these endings produce excitation or inhibition of the axons with which they are in contact. Inhibitory phenomena have been described in the prepyriform cortex[18], and synaptic bags containing ellipsoidal vesicles have recently been shown in an inhibitory area of the cerebellar Purkinje cells[19]. If these endings are inhibitory in function their occurrence on the initial or terminal segment of axons (Fig. 2*A* or *B*) seems a more powerful "stop-valve" arrangement for "outgoing" or "incoming" impulses than, say, axo-somatic contacts.

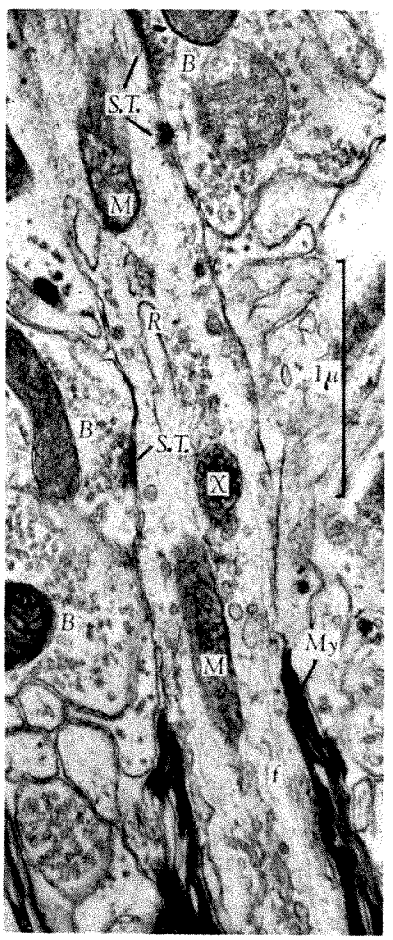


Fig. 1. Electron micrograph of an axon from the deep layer of the prepyriform cortex. Part of the myelin sheath (*My*) is seen below. The unmyelinated continuation is seen to have three presynaptic bags (*B*) contacting it, two of them forming synaptic thickenings (*S.T.*). This axon contains mitochondria (*M*), filaments (*f*), a multivesicular body (*X*) and smooth reticulum (*R*) with ribosomes to the right

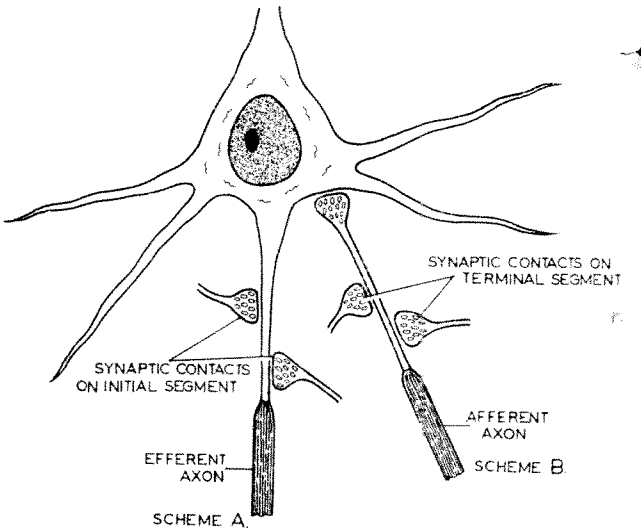


Fig. 2. A diagrammatic representation of a portion of a pyramidal cell from the prepyriform cortex showing possible axo-axonic relationships. The presynaptic bags contact unmyelinated axon segments which may be either the initial segment of the efferent axon (*A*) or the terminal segment of the afferent axon (*B*)

I thank Profs. J. Z. Young and R. Miledi and Drs. E. G. Gray and B. G. Cragg for advice and criticism. Valuable technical assistance was provided by Mrs. J. Astafiev, Miss C. Martin, and Mr. S. Waterman.

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PSYCHOLOGY

Tranquillizers in Pregnancy and Behavioural Effects on the Offspring

IN 1964 we[1] reported that we had repeated an experiment "exactly as described by Werboff and Kesner"[2] and had failed to confirm their reports that the administration of meprobamate to pregnant rats had deleterious effects on the offspring. Shortly afterwards, a report by Hoffeld and Webster[3] appeared which also denied that these offspring were deficient in learning ability or bodyweight. Werboff[4] has recently commented on these two papers, emphasizing that alterations in procedure were important in this area of research. In our case, the difference in results was attributed by Werboff to an

alteration in dosing procedure whereby our pregnant rats received far less meprobamate than did his own.

In order to eliminate any misunderstanding, we wish to emphasize that our pregnant rats received exactly the same amount of drug on exactly the same schedule as that described by Werboff, that is, 20 mg/kg per injection (three injections per day), or 60 mg per kg of body-weight per day for 4 consecutive days, a total of 240 mg of meprobamate per kg per rat. Therefore, our failure to reproduce his data cannot be due to any difference in drug doses or procedures used.

In the case of Hoffeld and Webster, who also used the same drug doses and procedures, Werboff attributes their failure to confirm his own report to two factors. Hoffeld and Webster's rats were pregnant at the time that they were shipped and probably suffered severe stress during this shipment. Secondly, the offspring of these rats were exposed to the stresses of colony housing and did not experience the handling and testing of the Werboff rats. But no explanation is offered as to why these rats should be healthier and learn better than Werboff's rats. Werboff's suggestion that the stresses listed above masked the deleterious effects of the drug is more puzzling than enlightening.

The fact remains that the results of Hoffeld and Webster agree completely with our findings. Both our group and theirs followed the procedures and schedules described by Dr. Werboff without difficulty, but failed to confirm his data.

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Threshold Regulation and Stimulus Wave-length

IN a repeat of earlier research[1,2] into the relationship between alpha abundance and visual thresholds for emotive words, red and green monochromatic lights were used as the visual stimuli. On the first presentation of each colour, results were in substantial agreement with those obtained in the previous work. Irrespective of stimulus wave-length, high thresholds for emotive words followed on sustained alpha abundance while low thresholds followed reduced alpha. The second presentation under a different colour, however, produced results which diverged somewhat from those previously reported. Irrespective of stimulus wave-length subjects tended to show higher thresholds for emotive than for neutral words, but those with higher thresholds for emotive words in red light showed reduced alpha abundance before reaching the threshold. This result is consistent with the finding[3,4] that a red stimulus in juxtaposition to a potentially emotive subliminal stimulus will evoke emotional disturbance, and the finding[5] that sympathetic activity decreases retinal sensitivity for light of long wave-length. All in all, the results suggest that while a given level of cortical activation may be necessary for awareness of a visual stimulus of low intensity, visual thresholds may also depend on a retinal variable under autonomic control.

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BIOLOGY

Allotypy and Eniotypy

INDIVIDUAL animals of a given species show well-defined, genetically controlled differences in the composition of certain isofunctional proteins. This molecular polymorphism is essentially of two types. The first type is one in which an isofunctional protein such as haemoglobin is present in all individuals of the species and the individuals differ from one another by the amino-acid sequence in limited regions of the molecule. The second type of polymorphism is characterized by the presence or absence of a particular protein in different animals of the same species. We wish to propose here a general nomenclature for these two polymorphic states.

Most instances of molecular polymorphism are of the first kind. When such polymorphism in the past has been revealed by the use of isologous antibodies, the phenomenon has been designated as allotypy (Oudin[1]). We propose now to extend this term to include all kinds of polymorphism of the first type, irrespective of the method used to reveal it. Medawar[2] has recently made the same suggestion. For the second type of polymorphism we wish to propose the term 'eniotypy' (that is "a type occurring in some", derived from the Greek ἐνίοτε, 'somewhere'). One can distinguish between these two types of polymorphism by the hetero-specific reactivity of isologous antibody.

Isologous antibodies to a molecule with allotypic polymorphism show a very narrow range of cross-reactivity for, usually, they react only with molecules from animals of the same species as the immunized animal. As an example of this we may quote the failure of isologous antibodies to mouse or rabbit immunoglobulin to react with the sera of other mammals belonging to the same order. Isologous antibodies to a molecule with eniotypic polymorphism, on the other hand, would be expected to show a wide cross-reactivity with the corresponding isofunctional molecules from animals of other species, as was observed in the investigations of murine antibodies to the murine complement factor MuB1 (refs. 3 and 4).

Such experimental findings can readily be accounted for in terms of tolerance to autologous determinants[3,5,6], because the animal completely lacking a particular protein molecule will not be tolerant to any of its determinants. This lack of tolerance provides the animal with the capability of synthesizing antibodies against all the determinants on the injected molecule, including some that are shared by animals of different species and even different natural orders. As a result, a typical eniotype antiserum, such as an antiserum to MuB1, reacts with the isofunctional proteins of a wide range of animal species and not just with proteins from other individuals within the species.

The foregoing classification of molecular polymorphism can be applied to an analysis of deficiency diseases such as various clotting defects (haemophilia, Christmas disease, absence of factors 5, 7, 8, and 10 (refs. 7 and 8) and metabolic deficiencies exemplified by total albinism (tyrosinase), Von Gierke's disease (glucose-6-phosphatase), familial goitrous cretinism (iodotyrosine deshalogenase), phenylketonuria (phenylalanine hydroxylase), Crigler-Najjar syndrome (glucuronyl-transferase)[9,10] and acatalasaemia[11,12]).

The inherited defects in these diseases may be due to a change in amino-acid composition (allotypy) which would affect the biological function of the molecule[13], or might be attributable to a complete deletion of the synthesis of the molecule (eniotypy). In none of the instances enumerated here has it been definitively established whether the deficiencies are due to total failure to synthesize a biologically active molecule (eniotypy), loss of function resulting from amino-acid substitution of the functionally

crucial portion of the molecule (allotypy), or reduction in the quantity of the molecule synthesized. It is even possible in some diseases that, in different individuals, different types of polymorphism might be involved, some allotypic and others eniotypic in nature.

We thank Dr. H. Parry of York University, Toronto, for suggesting the term 'eniotypy'. We also thank the following bodies for support in the experimental work on which the foregoing proposals are based: the Banting Research Foundation, the Canadian Arthritis and Rheumatism Society, the Medical Research Council of Canada, the National Cancer Institute of Canada, and the U.S. National Institutes of Health.

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Respiration in Yam Tuber Tissue

YAMS (*Dioscorea* spp.) are major food crops in many tropical countries. Global production of the edible tubers is approximately 20 million tons/year, the most important producer area being West Africa, where they often contribute up to half the total calorific intake of the population[1]. The tubers are frequently stored for periods of several months and, although they are generally said to 'store well', it has been shown[2–4] that appreciable losses in weight, of the order of 10 per cent after 3 months, and 25 per cent or more after 6 months, occur during the storage even of sound tubers. A number of storage rots are known[5], and attack by these organisms can further enhance storage losses. It has been estimated[1] that around a million tons of yams are lost, annually, in storage.

One of us has shown[4] that the weight losses occurring in sound tubers can only partially be accounted for by loss of moisture associated with the desiccation of the tubers during storage, and it was suggested that the remaining dry matter loss is caused by the respiratory metabolism of the dormant tuber. Some preliminary experiments on the evolution of carbon dioxide by yam tubers[6] have yielded results in fair agreement with the weight losses observed in field trials.

The information available indicates that these losses are much greater than those observed in the case of temperate tuber crops, such as the potato[7]. This is partly due to the fact that storage temperatures in tropical countries where yams are grown are higher (25°–30° C) than are normal in potato stores in temperate climates (5°–10° C): but the differences in weight loss are greater than would be expected from this factor alone. Furthermore, the observed rates of weight loss in yams are greater than those corresponding to the respiration rates, measured even at comparable temperatures in potatoes. The present experiments were therefore initiated to investigate the respiratory processes in yam tuber tissue, as the subject is obviously of interest from both theoretical and economic points of view.

The experimental material was purchased in the main yam market in Accra. After preliminary investigations, to develop technique, the main series of experiments was conducted with a single parcel of *D. rotundata* Poir—a species which is widely cultivated in West Africa—of the local variety 'Pasajo'.

The investigations of respiration were undertaken by means of the Warburg technique[8], using a Braum–Colora refrigerated circular Warburg apparatus. The oxygen uptake of slices of tuber tissue was measured at temperatures at 5° intervals between 5° C and 30° C; some experiments were conducted in the presence of antibiotics (penicillin, streptomycin and tetracycline, either singly or in combination) and some without. The slices were prepared by a modification of a technique developed[9] for other root crops as follows. A section was cut across the middle of a sound tuber with a sharp knife. The section was held under running tap-water, slightly scrubbed to remove all surface dirt and mounted in a commercial bacon slicer set at gauge zero. Thin layers were cut under hand pressure to a number greater by two than the number of small slices required for each experimental flask, and stacks of small slices were stamped from these using a 1-cm diameter cork borer. Before adding each stack of slices to the flask, the top and bottom disks were rejected. They served to reduce dehydration, further bacterial contamination and oxidation while the experiment was being assembled. In certain of these experiments, the slices were cooled to 5° C or 10° C and oxygen uptake was measured over a long period, for example, up to 44 h, before raising the temperature to near ambient (25° C) and continuing the measurements. A stack, usually of five slices, was placed in each Warburg flask in a phosphate buffer isotonic with yam tuber tissue and of the same *p*H value, 6·1; this buffer solution was 0·1 molar in potassium dihydrogen phosphate and 0·01 molar in sodium hydrogen phosphate. Some comparative measurements on slices of potato tubers were also made using a similar technique.

Under asterile conditions, rapid uptake of oxygen occurred at 30° C, and only slightly less rapid uptake at 25° C and at 20° C. At 15° C, however, the rate of oxygen uptake was only about a sixth of the 20° C rate, while at 10° C and 5° C, only very slow uptake occurred. At all temperatures, the rate of uptake of oxygen increased during the course of the experiment (5–6 h) by a factor of about two. A representation of the trend of the results is given in Fig. 1.

The increase in uptake of oxygen with time, after a partial time lag, suggested possible infection with some kind of micro-organism. Further experiments were therefore conducted in the presence of the antibiotics mentioned: penicillin had comparatively little effect, streptomycin rather more, while tetracycline was almost as effective as a combination of the three in reducing the rate of uptake of oxygen. The initial level of respiration was not greatly affected by antibiotics, but the increase with time previously observed was largely eliminated by their use.

After oxygen uptake by slices of tissue had been observed for up to 44 h at 5° C, and the temperature then raised to the near-ambient temperature of 25° C, a rapid increase in uptake of oxygen occurred in the absence of antibiotics; in their presence, the rate of uptake remained

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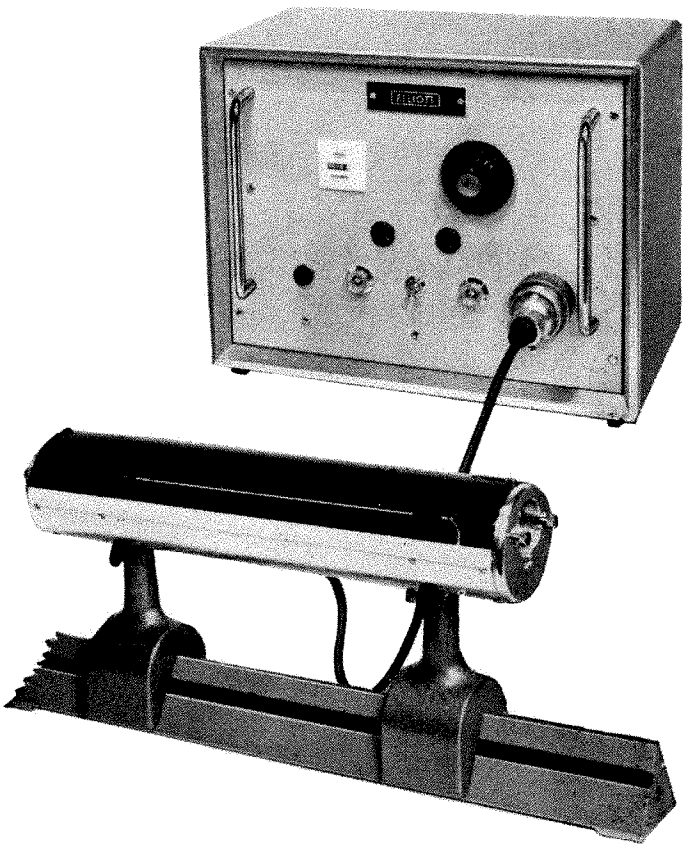


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constant for a short period, and then declined; after a similar chilling period at 10° C, and return to 25° C, the uptake of oxygen at the higher temperature was similar to that observed at the latter temperature, without previous chilling, whether in the presence or absence of antibiotics.

These results may be interpreted as follows: at temperatures near the normal tropical ambient, the destruction of the dry matter of yam tubers during storage (that is, during the period of dormancy) is caused, in part by the natural respiratory processes, and in part by the activity of micro-organisms within the tissue. This is in accordance with the observation that yam tubers, which become rotten towards the end of the storage period, lose weight rapidly, even while still apparently sound, in contrast to those tubers of the same origin that remain free from rotting for longer periods[3]. It would seem that apparently healthy yam tuber tissue is normally infected with biodeteriogens. These organisms, judging by the relative effects of the various antibiotics used, would appear to be Gram-negative bacteria. Reduction of the temperature to 15° C—well below normal tropical ambients reduces the rate of dry matter destruction (as measured by the uptake of oxygen), whether due to the enzymatic respiration of the tuber tissue or to microbial activity. Cooling to still lower temperatures reduces the rate further. This observation could well have a practical application to the storage of yams at reduced temperatures as a means of reducing storage losses. However, when the tissue is chilled to 5° C, permanent damage of the tissue is observed and rapid destruction by microbiological attack would be expected as soon as the temperature is raised to one at which normal tropical micro-organisms can rapidly proliferate. Chilling to only 10° C produces no such effect. It would thus appear that the lower critical temperature for safe storage lies between these limits (5°–10° C), as it does for several other tropical vegetables, such as sweet potatoes[10]. It is known[3] that chilling of yams to 1° C results in complete breakdown by decay on return to ambient conditions. No effect attributable to such chilling damage was observed in the potato slices.

Further investigations are being carried out on the temperature of the chilling damage, on varietal differences and on the micro-organisms involved in deterioration. Attempts are being made to relate these observations to storage trials on whole yam tubers.

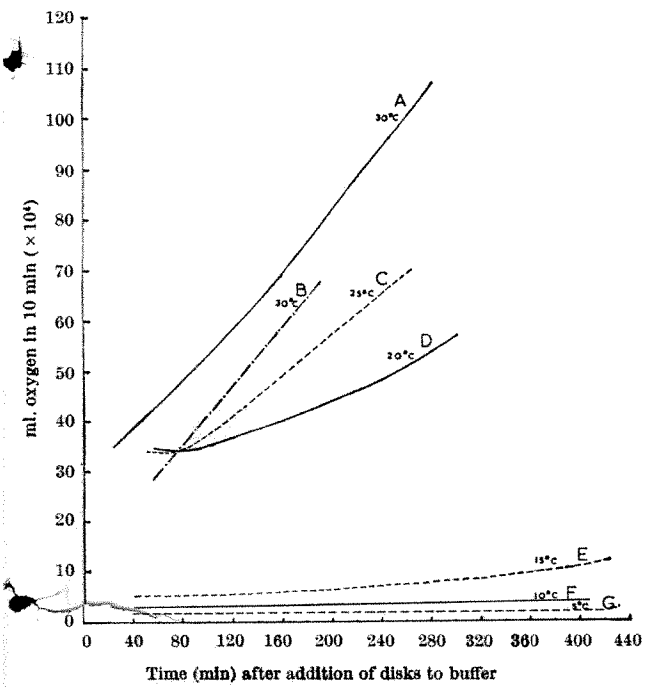


Fig. 1. Rate of respiration of yam (*D. rotundata* Poir; *A* and *B* different cultivars) tissue slices at different temperatures (asterile conditions). Ordinate, average ml. oxygen uptake/0·5 g fresh weight per 10 min (× 10⁴)—taken as an average between successive readings. Abscissa, time after addition of yam disks to the buffer (min). Temperature of Warburg bath: *A*, 30° C; *B*, 30° C; *C*, 25° C; *D*, 20° C; *E*, 15° C; *F*, 10° C; *G*, 5° C

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ENTOMOLOGY

Malic Enzyme Activity in the Fat Bodies of the Cockroach, *Leucophaea maderae* (F.)

Malate is reversibly converted to pyruvate through the agency of the malic enzyme. This reaction, which is effected in the presence of $NADP^+$ and Mg^{++} ions, constitutes the only known means whereby the synthesis of dicarboxylic acids is obtained from pyruvate and carbon dioxide. Simultaneous oxidation and decarboxylation are accomplished by the same enzyme, and although free oxalacetate does not appear in the reaction, this acid is decarboxylated. During equilibrium, there is a tendency for malate to become oxidized, and if $NADP^+$ is removed by coupling action, the process is reversed with the resulting fixation of carbon dioxide.

Faulkner[1] first discovered the malic enzyme in the haemolymph of insects. Fenwick[2,3] also discovered it in the fat bodies of the desert locust *Schistocerca*: and Prota[4] demonstrated its presence in the haemolymph of the mealworm, *Tenebrio molitor*. Thus, the purpose of this investigation was to determine whether or not the presence of malic enzyme in the fat bodies of the cockroach, *Leucophaea maderae*, is due to the activity of these tissues, the symbionts, or both.

Cockroaches were reared according to the method of Pierre[5] and the symbionts were isolated and cultivated according to the methods of Begg and Sang[6], Pant, Nayar and Gupta[7] and Pierre[5,8].

Homogenates were prepared from 1 g of the symbionts (48-h-old cultures cultivated in *Neurospora* agar on Petri dishes) and from similar weights of the fat bodies of normal adult and nymphal cockroaches, as well as corresponding aposymbiotic insects. The ages of the nymphs ranged from sixth to eighth instars. Materials were homogenized for 5 min with a hand homogenizer in 2 ml. of 0·03 M phosphate buffer at *p*H 7·4. The homogenates were then diluted with 2 ml. of 1 per cent $NADP^+$; sufficient of the same buffer was added to make a 3·3 per cent concentration. Homogenates were then incubated in a constant-

temperature water bath at 30° C for 30 min to oxidize the endogenous substrates. At the end of incubation, the homogenates were assayed for the enzyme activity.

Spectrophotometric determination of the malic enzyme activity was performed according to the methods of Faulkner[1] and Prota[4]. Assays were conducted with a Beckman *DU* instrument at a wave-length of 340 mμ. The instrument was set at zero optical density with 3 ml. of 0·03 M phosphate buffer at *p*H 7·4, and the reaction cuvettes consisted of the following: 0·2 ml. of substrate (0·25 M sodium malate); 0·25 ml. of 1 per cent $NADP^+$; 0·25 ml. of 0·033 M magnesium sulphate; 0·1 ml. homogenate; and 2 ml. of 0·03 M phosphate buffer at *p*H 7·4. Recordings of the optical density of reaction mixtures were performed at zero time, and then at 1-min intervals until the end of the experiment. The enzyme activity was determined by the change in optical density which occurred between readings at zero time and the end of the experiment.

Assays of the enzyme activity performed according to the method of Thunberg[9] were conducted as follows: 1 ml. of the homogenate was placed in the side arm of the tube; and 0·5 ml. of methylene blue (1 : 10,000), 1 ml. of 0·25 M sodium malate, 0·5 ml. of 1 per cent $NADP^+$, 0·5 ml. of 0·033 M magnesium sulphate, and 0·5 ml. of 0·03 M phosphate buffer at *p*H 7·4 were placed in the main portion of the tube. Two standards were prepared: (1) a standard which contained all the ingredients of the reaction tube except the substrate (sodium malate); (2) a similar standard the homogenate of which was inactivated by boiling, and in which 0·05 ml. of the methylene blue was used instead of 0·5 ml. All the tubes were sealed and evacuated for 5 min after which the homogenates in the side arms were tipped over into the main portions of the tubes. The latter were then immersed in a constant-temperature water bath at 30° C and the time for 90 per cent decoloration of the methylene blue was determined visually. Accordingly, the enzyme activity was expressed as the reciprocal of the time in minutes required for 90 per cent decoloration of the indicator. In all cases, twenty determinations of the enzyme activity were performed by each method used. This necessitated the use of different insects at each trial, and fresh cultures of the symbionts.

Spectrophotometric determination of the activity of the malic enzyme in materials of the symbionts and those of the normal adult and nymphal cockroaches of *L. maderae* showed the following results: 0·013; 0·343; 0·350, respectively. Corresponding values for the aposymbiotic adult and nymphal fat bodies were 0·340 and 0·327, respectively. Graphic representations of these activities are shown in Fig. 1, which shows the change in optical density of malate as the latter is converted to pyruvate in the presence of the enzyme. Except for the materials of the symbionts (the activity of which was negligible), results of the enzyme activity determined by the Thunberg technique[9] were 0·034 and 0·028 for the normal adult and nymphal fat bodies, and 0·028 and 0·030 for those of corresponding aposymbiotic insects, respectively.

As the results of the activity of the malic enzyme obtained in all the materials (except those of the symbionts) are comparable, it would seem that the symbionts play a negligible part in the activity of the enzyme. This observation seems obvious for the following reasons: (1) Tarver[10] demonstrated high activity for malic dehydrogenase in the symbionts and normal fat bodies of the cockroach, *L. maderae*, but found little activity in the aposymbiotic fat bodies; (2) Epstein[11] also demonstrated low activity of lactic dehydrogenase in the fat bodies of the same insect, but high activity was obtained in the materials of the symbionts; (3) Faulkner[1] obtained high activity for the malic enzyme in the fat bodies of insects, but saw no trace of lactate in the tissues. Thus, the latter worker attributed this to the reversible conversion of pyruvate to malate by the enzyme; (4) Zebe and McShan[12]

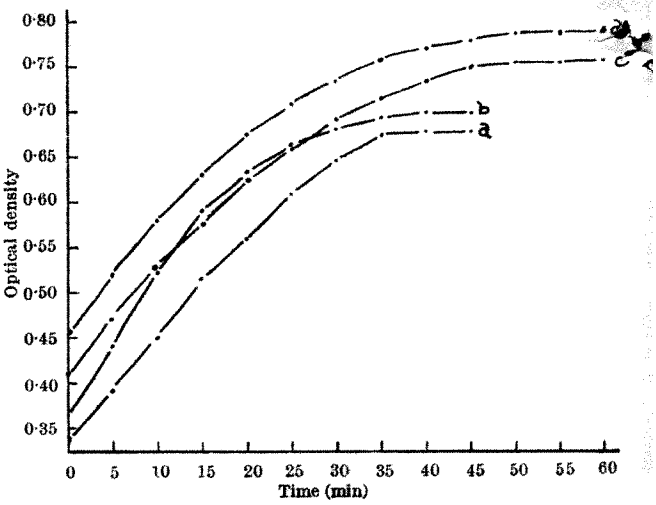


Fig. 1. Showing the activity of malic enzyme in the fat bodies of: *a*, aposymbiotic adult; *b*, normal adult; *c*, normal nymph; *d*, aposymbiotic nymph

reported that the absence of lactate in insect tissues is due to the continual presence of oxygen. It would thus seem that the fat bodies of insects convert malate to pyruvate through the agency of malic enzyme; and the subsequent conversion of malate to oxalacetate is effected through the agency of the symbionts which instead contain malic dehydrogenase.

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GENETICS

Induction of Cross-overs, Autosomal Recessive Lethal Mutations, and Reciprocal Translocations in *Drosophila* after Treatment with Diethyl Sulphate

ALTHOUGH crossing-over does not normally occur in the male *Drosophila*, it has been shown[1,2] that chemical induction of crossing-over in the male *Drosophila* is possible using chemical mutagenic agents, such as formaldehyde, dihydroxymethyl-peroxide, and mustard gas.

The present communication reports our findings on the induction of crossing-over in the male *Drosophila* after larval feeding of the mutagen[3], diethyl sulphate, and describes a further experiment designed to detect a possible association between the induction of crossing-over and the induction of autosomal recessive lethal mutations after larval feeding of diethyl sulphate. Tests are also included which show that diethyl sulphate does not induce reciprocal translocations between the second and third chromosome after feeding diethyl sulphate to

either male or female larvae, and after feeding diethyl sulphate to adult male *Drosophila*.

Larvae were cultured on an aseptic yeast–glucose medium[4] of the following composition:

Glucose	10 g
Dried killed yeast (Distillers Co., Ltd.)	10 g
Agar	3 g
Distilled water to 100 ml.	

After autoclaving, 0·5 per cent diethyl sulphate (British Drug Houses 1·177–1·18 g/ml. at 20° C) was added at 60° C. At 55° C the medium was dispensed as 25-ml. portions into four sterile bottles. Eggs, sterilized by Sang's[5] method, were transferred as newly emerged larvae to the treatment medium at a density of one hundred larvae per 25 ml. of medium, and treated over the entire larval life. Emerging males were individually mated to two virgin females of the appropriate test stock for 3 days.

For detection of induced cross-overs on the second chromosome larvae of the genotype *+ + +/dp cn bw* (dumpy, cinnabar, brown) were raised on a diethyl sulphate treatment medium, and cross-overs scored in the F_1 after mating the emergent males to female homozygous for *dp cn bw*.

Table 1. INDUCED CROSS-OVERS IN *Drosophila* MALES TREATED THROUGHOUT LARVAL LIFE WITH DIETHYL SULPHATE

Concentration of diethyl sulphate (per cent)	No. of males treated	No. of gametes tested	Cross-overs Single	Cross-overs Double	Total No. of cross-overs	Cross-overs (per cent)	Treated males which yielded cross-overs (per cent)
Control	70	3,004	None	None	None	0·00	0·00
0·5	47	2,581	* { 1 *dp* 1 *dp cn* 2 *dp cn* 1 *dp*	3 *cn*	8	0·31	14·9

* Recombinants in the progeny of the same male are bracketed together, all other recombinants occurred independently of one another in the progeny of different males.

Table 1 illustrates the cross-over types detected after larval feeding of male *Drosophila* with 0·5 per cent diethyl sulphate; 14·9 per cent of the males examined were found to yield a recombinant class(es).

Table 2. INDUCED CROSS-OVERS IN *Drosophila* MALES ARISING IN THE F_1 PROGENY OF THE AUTOSOMAL RECESSIVE LETHAL TEST

Concentration of diethyl sulphate	No. of males treated	No. of flies examined	Cross-overs	Total No. of cross-overs	Cross-overs (per cent)	Treated males which yielded cross-overs (per cent)
Control	82	5·024	None	None	0·00	0·00
0·5	131	4·149	3 *cn bw*	11	0·27	7·63
			* { 3 *dp* 2 *dp cn* 3 *dp cn*			

* Recombinants in the progeny of the same male are bracketed together; all other recombinants occurred independently of one another on the progeny of different males.

Table 2 illustrates a further larval feeding experiment, where diethyl-sulphate-induced cross-overs were detected by mating males of the genotype *+ + +/dp cn bw* to *CyL*[4]*/dp cn bw* virgin females to detect cross-overs in the F_1 and autosomal recessive lethal mutations[6] in the F_3. The results show that 7·6 per cent of treated males yielded cross-overs. The frequency of autosomal recessive lethal mutations was 22 per cent (total number of chromosomes 464, lethal chromosomes 102, percentage of treated males which yielded lethals 53·1).

To test for an association in the origin of induced cross-overs and autosomal recessive lethal mutations, a male from each F_3 lethal culture was crossed to females homozygous for the *dp cn bw* chromosome to detect which lethal chromosomes were cross-overs; Table 3 shows that three out of ninety-six lethals were associated with a cross-over event.

Table 2 shows an induced cross-over frequency of 0·27 per cent (11/4149), and a corresponding autosomal recessive lethal frequency of 22 per cent.

Assuming that the cross-overs and autosomal recessive lethals arose by independent events, a probability of $0{\cdot}22 \times 0{\cdot}0027 \times 100 = 0{\cdot}59$ per cent is expected for the association of a cross-over and a lethal mutation. However, the observed frequency is three in ninety-six, which is twelve times greater than the number expected if the events were independent ($96 \times 0{\cdot}27 = 0{\cdot}26$).

Table 3. TEST FOR THE ASSOCIATION OF DIETHYL-SULPHATE-INDUCED CROSS-OVERS WITH DIETHYL-SULPHATE-INDUCED AUTOSOMAL RECESSIVE LETHALS IN *Drosophila* MALES

Lethals tested	Cross-overs associated with a lethal	Total No. of cross-overs detected	Lethals which were associated with a cross-over (per cent)
96	1 *dp cn* 1 *dp* 1 *cn bw*	3	3·12

Thus, the data suggest there may be an association between the origin of induced cross-overs and autosomal recessive lethal mutations. However, this association, even if real, might be nothing more than a reflexion of the fact that certain males received a larger amount of diethyl sulphate and therefore responded more to both recombination and mutation induction. Alternatively, the chromosomes used may have been capable of generating synthetic lethals by recombination.

To test for the induction of reciprocal translocations by diethyl sulphate (0·5 per cent) between the second and third chromosome, treated male and female larvae, and male adults treated by an adult feeding method[3], were mated to flies homozygous for the second and third chromosome markers *bw* (brown) and *st* (scarlet), respectively.

The male and female larval treatments yielded no translocations in samples of 838 and 902 gametes, respectively, and the adult males yielded no translocations in a sample of 775 gametes.

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SOIL SCIENCE

Organic Matter as a Measure of Bulk Density of Soil

THE compactness of a soil is known to be related to its content of water and air, and to the temperature and the supply of nutrients[1,2]. Although a rough estimate of compactness is sometimes obtained with penetrometers, bulk density provides a more correct overall picture of the physical conditions of a soil. Loosely packed soils, which have a lower bulk density, will usually have better aeration, better drainage, will be warm and will have a longer growing season.

The bulk density of soil is dependent on the specific gravity of the particles it contains and their arrangement. Since the specific gravity of the mineral portion of soils is more or less constant, soil structure is chiefly responsible for fluctuations of bulk density. The structural properties, in turn, are determined largely by the interacting influences of texture and degree of aggregation—especially that due to organic matter. Thus, there is a relationship between bulk density and content of organic matter in soils rich in organic matter. It has been reported[3] that determinations of organic matter in forest soils may be used to estimate the bulk density, the exact measurement of which is time-consuming.

With such an end in view, data collected in the Ohio Soil Survey have been subjected to statistical analysis to establish the relationship between organic matter, determined by the rapid method of Walkley and Black[4], and bulk density. The soils were classified into three groups—well-drained, imperfectly drained and humic-gley soils. Simple correlation coefficients between content of organic matter and bulk density are significant (Table 1).

Table 1. CORRELATION COEFFICIENTS AND REGRESSIONS BETWEEN ORGANIC MATTER AND BULK DENSITY IN SOME OHIO SOILS

Soil group	No. of samples N	Organic matter mean X	Bulk density mean y	Correlation coefficient r	Regression equation
Humic-gley	30	6·13	1·24	−0·858*	$Y = 1{\cdot}62 - 0{\cdot}06X$
Imperfectly drained	40	2·98	1·37	−0·805*	$Y = 1{\cdot}53 - 0{\cdot}05X$
Well drained	40	2·05	1·40	−0·633*	$Y = 1{\cdot}52 - 0{\cdot}06X$

* Significant at 1 per cent level.

From the regression equations, it is plain that the contribution of the organic matter content to the bulk density is not very different for soils of these three categories. In humic-gley and well-drained soils, its contribution is −0·06 g/cm³ for each 1 per cent of organic matter. In imperfectly drained soils it is −0·05 g/cm³ for each 1 per cent of organic matter.

These studies suggest that organic matter in Ohio soils has a definite relationship to the bulk density of surface soils, and an estimate of the latter can be made from organic matter determined by Walkley and Black's rapid titration method.

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Trace Element Content of Organic Accumulations

A STUDY of the distribution of trace elements in rocks, soils and plants in Snowdonia[1], to be reported in detail elsewhere, has led to a realization of the relative importance of material borne in the atmosphere as a source for the trace elements in certain organic accumulations. Relevant analytical data are provided from two sources—first, from an investigation into the role of saxicolous lichens in the initial stages of pedogenesis and, second, from a routine survey of the trace element status of mountain topsoils.

As part of the investigations into the relationships between saxicolous lichens and their substrata, trace element analyses were made of a number of lichen species together with the weathered and fresh zones of a variety of subjacent rocks. Detailed examination of the results reveals a tendency for the concentrations of individual elements in a particular lichen species to converge to a 'preferred value'; composition is apparently dominated by some constant factor. For this reason, the correlation of the trace element content of a lichen with that of its rock substratum is normally poor, and the effect of the substratum is only dominant in the case of crustaceous species on rock types that weather rapidly, such as calcareous basic tuffs and ultrabasic rocks. The correlation of the 'preferred values' between different lichen species within one region (*Parmelia omphalodes* and *Lecanora gangaloides* in Snowdonia) is better than that obtained between samples of the same species in different regions

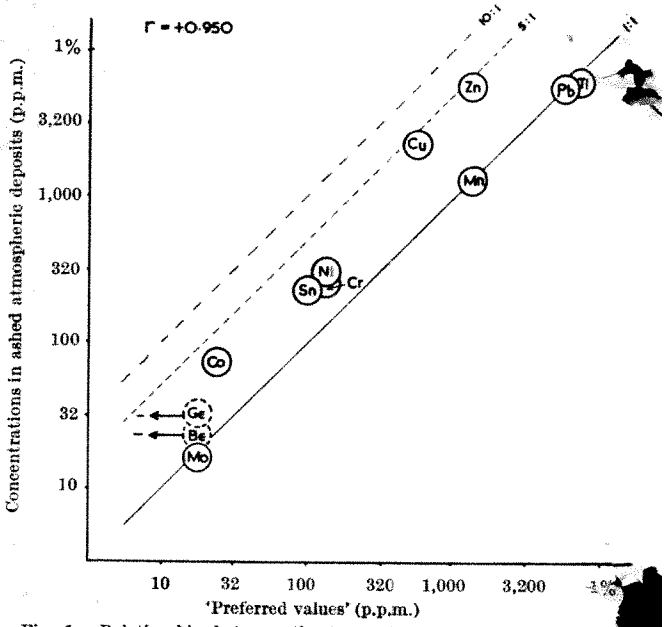


Fig. 1. Relationship between the trace element contents of ashed atmospheric deposits (average of four rural sites, 1947, supplied by Warren Spring Laboratory) and the "preferred values" in the ash of *Parmelia omphalodes* from Snowdonia

(*P. omphalodes* in Snowdonia and Finland[2]). The constant factor involved would therefore appear to be external and regional in nature rather than internal or physiological. In this study, a close correlation has also been demonstrated between values in lichen ashes and values for the ash of material deposited from the atmosphere[3] (see Fig. 1).

The 127 topsoils analysed during a survey of Nature Conservancy experimental sites in Snowdonia included rankers, peats, and peaty varieties of podsols and gleys. The distribution patterns observed in the concentrations of trace elements can be interpreted in terms of such factors as mineralogy, parentage, soil-type, organic matter content, and *p*H. The relationships between the amounts of individual elements in the ignited soils and the amounts of organic matter, adequately determined here as percentage loss on ignition[4], were evaluated in the form of the partial correlation coefficients with soil *p*H (r) in Table 1. By inspection, interference from soil parentage is inferred to be insignificant. Detectable concentrations of tin, molybdenum and silver also show a distinct association with soils of high organic matter content, but are too few to justify the evaluation of correlation coefficients.

From a comparison of the available data on the average composition of the lithosphere[5] with that of atmospheric material[3], it is possible to obtain an order of enrichment of trace elements to be expected in an organic soil due to atmospheric contamination:

Bi, Pb, Cu > Sn, Zn > Ni > Mo, Be, Ge, Co, Cr > V, Mn

This series has similarities with the sequence of correlation coefficients already given. On the basis of their concentrations relative to lead and copper in atmospheric material, the absence or rarity of detectable concentra-

Table 1. PARTIAL CORRELATION COEFFICIENTS OF ELEMENT CONCENTRATION AND *p*H

	r	
Tin	+0·69	*
Lead	+0·59	*
Copper	+0·50	*
Nickel	+0·17	†
Strontium	+0·15	†
Yttrium, barium, cobalt, gallium, chromium, manganese, zirconium, vanadium, rubidium and lithium	+0·05– −0·38	

* Significant at the 1 per cent level.
† Significant at the 5 per cent level if arbitrarily referred to zirconium (r = −0·19) as an "inert standard" to compensate for dilution by metabolically accumulated organic ash.

tions of bismuth, molybdenum, beryllium and germanium in the soil samples analysed is consistent with an atmospheric origin. Zinc should, however, be detectable in more soils than is the case, although further investigations[1] have shown that, in several of the organic-rich soils concerned, zinc is decidedly more mobile than lead or copper in terms of extractability by 0·5 N acetic acid. Further evidence of this similarity of trace element compositions is provided by the lead : copper concentrations in the ignited soils (Fig. 2). Here it can be seen that the dispersed ratios at low lead and copper concentrations converge at higher concentrations in more organic soils to a value of 2·4 which equals that recorded for the ash of matter deposited from the atmosphere[3].

It is therefore apparent that the contents of trace elements in both the saxicolous lichens and the organic topsoils follow a pattern similar to that of ashed atmospheric deposits. Correlation is, in fact, surprisingly good considering both the variable composition of atmospheric material between different size grades and within time and space, and also the possibility of differential mobilization and absorption or rejection of trace elements by the organic matter. It is not easy, however, to assess how far the trace element composition of organic accumulations is in fact controlled by atmospheric contamination. In the case of the lichens, which have particularly slow growth rates[6] and are ectohydrates[7], the evidence for the dominance of atmospheric sources in their trace element composition is strong. This is in accord with early views on their nutrition[8], but conflicts with the emphasis placed on the substratum as a source of nutrients by later investigators[2,9,10].

For organic soils, however, it is probable that the contribution of pedological and metabolic processes to the accumulation of specific trace elements is more important. Atmospheric contamination achieves greatest significance in deposits of particularly low mineral content (for example, 'acid' peats) and of elements such as copper which are known to be readily immobilized in organic soils. Concentration of lead, copper, zinc, tin, molybdenum and silver in the organic horizons of soils has been noted by numerous soil analysts[11], although it has usually been ascribed to accumulation by organic processes[12,13], and only rarely to atmospheric contamination[14]. The importance of the latter, as implied by the present studies, could be confirmed by comparisons with analyses of soils, lichens and atmospheric deposits from areas affected to a lesser degree by industrial contamination, or with analyses of buried organic soil horizons the formation of which pre-dated the industrial revolution.

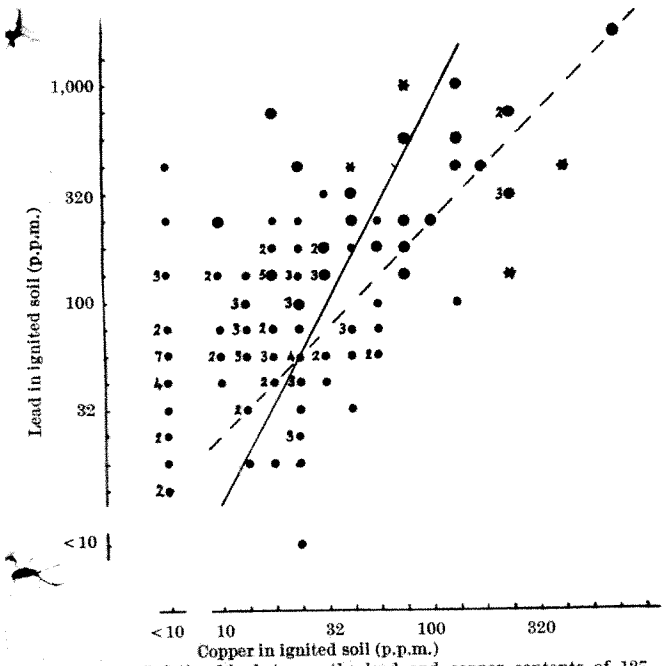


Fig. 2. Relationship between the lead and copper contents of 127 ignited topsoils from Nature Conservancy experimental plots in Snowdonia. *, Samples associated with ore deposits; •, loss-on-ignition <40 per cent; ●, loss-on-ignition >40 per cent; - - - -, ratio of lead : copper in ashed atmospheric deposits; ——, regression line of lead on copper (Pb = 11Cu − 97)

There are interesting corollaries to these conclusions. In the case of lichens, there is the possibility of using inorganic composition to monitor atmospheric contamination or, in particularly long-lived crustaceous species with an age measured in centuries[6], of tracing changes in the composition of atmospheric material. In soils, the contamination that can be detected in slowly accumulating organic topsoils ('mor' formation) must obviously also apply to soils where the organic matter is redistributed and decomposed within the profile ('mull' formation). Although obscured, it may still be of importance in trace element nutrition.

The composition of atmospheric material itself is at present presumably determined mainly by the selective volatilization of coal, supplemented in the case of lead by exhaust fumes from petrol engines and possibly, in the case of nickel, by extra-terrestrial material[15]. Coal is itself a lithified organic accumulation in which the concentration of such trace elements as bismuth, beryllium, germanium, silver, molybdenum, zinc and lead has been recorded. In some cases this concentration may be due to the low redox potential generated or to diagenetic processes, but it has also been ascribed[16] to accumulation in the original plant tissues by physical and metabolic processes. In organic matter accumulating at the present day processes of the latter type still operate[16,17], but in addition selected elements, concentrated organically some 250 million years ago, are now being returned by industrial atmospheric contamination. This complicates the interpretation of the trace element content of recent organic accumulations and poses many interesting problems.

Some of the results reported here form part of a survey carried out on behalf of the Bangor Research Station of the Nature Conservancy, and we acknowledge with gratitude the assistance and interest of the staff of that Station.

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Pyrophosphate in Soil; some Physico-chemical Aspects

As part of a series of experiments in this laboratory to evaluate *pyro*phosphate as a source of phosphorus for plants[1,2], attention has been given to the comparative rates of movement of *ortho*phosphate and *pyro*phosphate in soil and to their adsorption isotherms.

The adsorption isotherms were examined by shaking 5-g samples of finely ground soil overnight with 100 ml. of phosphate solutions which ranged from 0 to $1{\cdot}2 \times 10^{-3}$ molar. The quantity of *ortho*phosphate adsorbed was determined from the change in phosphate concentration in solution. To determine the true adsorption of *pyro*-phosphate without bias from the change in phosphate concentration in solution resulting from exchange of *pyro*phosphate with soil *ortho*phosphate, labelled *pyro*-phosphate was used. The quantity of *pyro*phosphate adsorbed was determined by the relative radioactivities of the solution after shaking with soil and of an appropriate standard. The soils were autoclaved before the experiment to prevent biological hydrolysis of *pyro*-phosphate. Phosphate adsorption maxima for eight soils, including those later used in the phosphate movement experiments, were calculated by adopting the Langmuir concept used by Olsen and Watanabe[3] (Table 1). The phosphate adsorption isotherms for two of them are shown in Fig. 1. The *pyro*phosphate sorption maxima range from 0·6 to 9 times those of *ortho*phosphate, implying that sites exist in soil which adsorb one form of phosphate but not the other.

Table 1. SOIL PHOSPHATE SORPTION MAXIMA
(m.moles phosphate per kg soil)

Soil	*Ortho*phosphate	*Pyro*phosphate	*Pyro* maximum / *Ortho* maximum
2	7·1	12·3	1·7
3	3·7	8·9	2·4
5	7·9	16·4	2·1
7	3·7	8·5	2·3
11	3·7	5·8	1·6
340	45·6	27·2	0·6
482	13·5	10·6	0·8
526	2·4	21·6	9·0

The relative rates of movement of NaH_2PO_4 and $Na_2H_2P_2O_7$ were examined in two experiments using soils which had been found to have contrasting adsorption characteristics (Table 1). In the first experiment the relative rates of diffusion into crumbs were followed using a technique previously described[4]. The soil crumbs were immersed at laboratory temperature in $1{\cdot}27 \times 10^{-3}$ molar labelled phosphate solutions for one week. The distribution of labelled phosphate across the face of sections of three crumbs of each soil can be seen in Fig. 2. In the soils examined it is apparent that *pyro*phosphate diffused very much more slowly than *ortho*phosphate. Similar differences were also observed using $6{\cdot}4 \times 10^{-4}$ molar solutions.

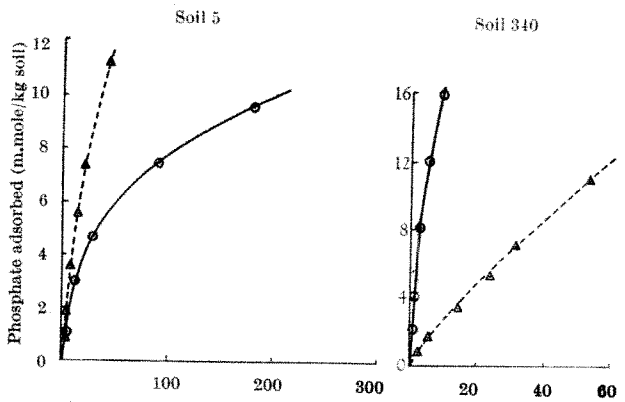


Fig. 1. Phosphate adsorption isotherms in soil 5 (*p*H 5·9, moderate *ortho*phosphate sorption capacity) and soil 340 (*p*H 5·3, high *ortho*-phosphate sorption capacity). ——, *Ortho*phosphate; - - - - -, *pyro*-phosphate

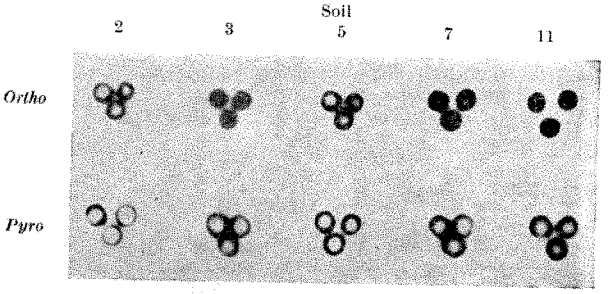


Fig. 2. Autoradiograph showing the distribution of phosphorus-32 across the face of soil crumbs after immersion for 1 week in $1{\cdot}27 \times 10^{-3}$ M phosphate solutions

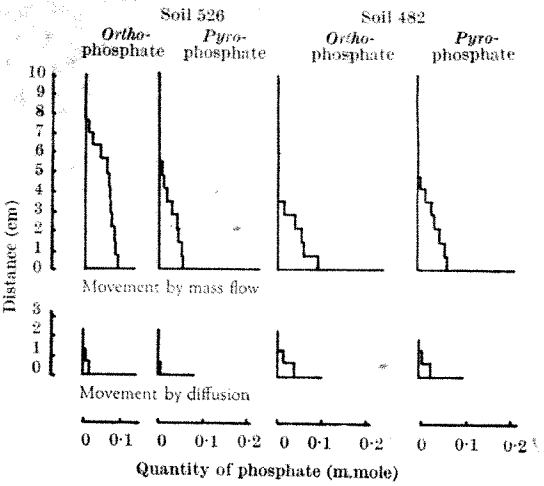


Fig. 3. Upward movement of phosphate in soil columns

In the second experiment a comparison was made between movement of 1×10^{-2} molar solutions of the two labelled phosphates up a column both by mass flow and diffusion. A 'Perspex' column similar to that used by Bodman and Coleman[5] was filled with < 2 mm soil. For mass flow, air-dry soil was used and the solution moved up the soil by capillarity. For diffusion, the soil was previously moistened and subsequent water movement was avoided by covering the soil column with a bell jar to prevent evaporation. After 5·5 h of mass flow and 18 h of diffusion the column was drained and sectioned, and the radioactivity in each 6·5 mm thick section was determined. The quantity of phosphate that was transported up the column and the distance which it moved were very much greater when movement was by mass flow (Fig. 3). On soil 526 both the total quantity of phosphate that moved beyond the basal section (Table 2) and the distance that it moved were greater with *ortho*phosphate than with *pyro*phosphate whether movement was by mass flow or diffusion. On soil 482, however, although diffusion of *ortho*phosphate was more rapid than *pyro*phosphate, mass flow rates were similar for the two forms of phosphate.

Table 2. PHOSPHATE DIFFUSED BEYOND THE BASE SECTION (m.mole)

		*Ortho*phosphate	*Pyro*phosphate
Soil 526	Mass flow	0·507	0·226
	Diffusion	0·014	0·004
Soil 482	Mass flow	0·248	0·252
	Diffusion	0·049	0·026

Thus these experiments, which are of an exploratory nature, show that *ortho*phosphate and *pyro*phosphate anions behave differently in soil and that the extent and direction of these differences vary from soil to soil. The physico-chemical significance of this in relation to the phosphate sorbing components of soil will provide a subject for further examination.

D. GUNARY

Levington Research Station,
Ipswich.

[1] Sutton, C. D., and Larsen, S., *Soil Sci.*, **97**, 196 (1964).
[2] Sutton, C. D., Gunary, D., and Larsen, S., *Soil Sci.*, **101**, 199 (1966).
[3] Olsen, S. R., and Watanabe, F. S., *Soil Sci. Soc. Amer. Proc.*, **21**, 144 (1957).
[4] Gunary, D., Hallsworth, E. G., and Crawford, D. V., *Exp. Pedol. Proc. Univ. Nottingham Easter School Agric. Sci.*, **1964**, 149 (1965).
[5] Bodman, G. B., and Coleman, E. A., *Soil Sci. Soc. Amer. Proc.*, **8**, 116 (1943).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Tuesday, June 21

UNIVERSITY OF LONDON (at Charing Cross Hospital Medical School, London, W.C.2), at 6 p.m.—Prof. S. G. Hershey. "The Microcirculation".*

Tuesday, June 21—Thursday, June 23

BRITISH VETERINARY ASSOCIATION, and BRITISH SMALL ANIMAL VETERINARY ASSOCIATION (at the Zoological Society of London, Regent's Park, London, N.W.1), at 9 a.m. daily—Symposium on "Some Diseases of Animals Communicable to Man in Britain".

Wednesday, June 22

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. R. Stoneley: "The Structural Development of the Gulf of Alaska Sedimentary Province in Southern Alaska".

CHALLENGER SOCIETY (in the rooms of the Linnean Society, Burlington House, Piccadilly, London, W.1), at 5.45 p.m.—Mr. D. J. Shearman and Dr. G. Evans: "On the Recent Sediments of the Persian Gulf".

Friday, June 24

MEDICAL RESEARCH COUNCIL (at the National Institute for Medical Research, Mill Hill, London, N.W.7), at 4.30 p.m.—Prof. Frank J. Fenner (Australian National University). "Some Recent Studies in the Genetics of Animal Viruses".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

TEMPORARY ASSISTANT LECTURER (preferably with interests in marine biology) IN ZOOLOGY—The Registrar, The University of Hull, Hull (June 23).

LECTURER IN THEORETICAL PHYSICS—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (June 24).

RESEARCH FELLOW IN THEORETICAL PHYSICS—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (June 24).

ASSISTANT LECTURER (preferably with research experience in fields other than soils or dairying, for example, food or taxonomy) IN THE SECTION OF FOOD AND AGRICULTURAL BACTERIOLOGY (situated in the Department of Agriculture)—The Registrar, The University, Leeds, 2 (June 25).

FELLOW IN THE DEPARTMENT OF MATHEMATICS to undertake research in elasticity or a related field—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (June 25).

LECTURER and an ASSISTANT LECTURER (with qualifications and experience in the fields of the strength of materials and/or structures) IN ENGINEERING (Civil) IN THE FACULTY OF SCIENCE—The Registrar, The University, Manchester 13, quoting Ref. 144/66 (June 25).

RESEARCH DEMONSTRATOR (with an honours degree in zoology and interested in working towards a higher degree) IN ZOOLOGY—The Chairman, Natural Sciences Committee, Magee University, Londonderry, Northern Ireland (June 25).

TUTORIAL STUDENT IN THE DEPARTMENT OF BOTANY—The Secretary, The University, Aberdeen (June 25).

CHAIR OF VIROLOGY—The Registrar, The University, Manchester, 13 quoting Ref. 137/66 (June 27).

SENIOR RESEARCH FELLOW (with a structural background and a particular interest in industrialized building) IN THE DEPARTMENT OF CIVIL ENGINEERING to work with Dr. E. R. Bryan on the stiffening effects of light cladding in buildings—The Registrar, The University, Manchester 13, quoting Ref. 138/66 (June 29).

DEMONSTRATOR (with a special interest in plant pathology, crop production or genetics, and preferably some research experience) IN THE DEPARTMENT OF AGRICULTURAL BOTANY; and RESEARCH ASSISTANTS (with, or expecting to obtain, a good honours degree in agricultural botany, botany or genetics) to carry out research in plant pathology, crop production or genetics—The Registrar, University College of Wales, Aberystwyth (June 30).

EXPERIMENTAL OFFICER (with a university degree or equivalent qualification, and preferably a knowledge of chemistry) IN THE DEPARTMENT OF PHYSICS OF THE FACULTY OF SCIENCE, to run an evaporation and target construction laboratory in the Nuclear Physics section of the Department—The Registrar, The University, Manchester 13, quoting Ref. 142/66 (June 30).

FELLOW (plant biochemist or microbiologist) IN BIOLOGY to study the biochemical self-sufficiency of chloroplasts—The Registrar, University of York, Heslington, York (June 30).

LECTURER IN CERAMICS—The Registrar, University of Manchester Institute of Science and Technology, Sackville Street, Manchester, 1 (June 30).

LECTURER IN PHARMACOLOGY at the Institute of Basic Medical Sciences (Royal College of Surgeons of England and University of London)—The Secretary, Institute of Basic Medical Sciences, Royal College of Surgeons, Lincoln's Inn Fields, London, W.C.2 (June 30).

LECTURER (with a higher degree in chemistry and/or biochemistry) IN PHYSIOLOGICAL CHEMISTRY at the University of the Witwatersrand, Johannesburg, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, June 30).

POSTDOCTORAL RESEARCH ASSISTANT IN PLANT BIOCHEMISTRY to work with Dr. H. Smith on the phytochrome regulation of phenolic biosynthesis, with special reference to hydroxylation mechanisms—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (June 30).

RESEARCH FELLOW (with good teaching experience and qualifications in biology and preferably an interest in, and knowledge of, measurement techniques) AT THE INSTITUTE OF EDUCATION, to assist in the work on the evaluation of the effects of science teaching at the G.C.E. Ordinary level—The Deputy Secretary, The University, Southampton (June 30).

TEACHING FELLOW IN PURE MATHEMATICS at Rhodes University, Grahamstown, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or The Registrar of the University (June 30).

CHAIR OF SENIOR LECTURER IN MATHEMATICS; and a LECTURER OR ASSISTANT LECTURER IN CHEMISTRY at the University of Malawi—The Inter-University Council, 38 Bedford Place, London, W.C.1 (July 1).

DEMONSTRATOR (with an honours degree in animal physiology, physiology, zoology or agricultural sciences) IN ANIMAL SCIENCE in the School of Agriculture—The Registrar, The University, Nottingham (July 1).

LECTURER IN VIROLOGY IN THE DEPARTMENT OF BIOLOGICAL AND HEALTH STUDIES—The Academic Registrar, Battersea College of Technology, Battersea Park Road, London, S.W.11 (July 1).

SENIOR LECTURER (suitably qualified person with research experience in either some aspect of computer science, such as system programming, or in numerical analysis) IN COMPUTING SCIENCE—The Secretary of the University Court, The University, Glasgow (July 1).

POSTDOCTORAL RESEARCH ASSISTANT (preferably with a strong interest in biochemistry) IN ORGANIC CHEMISTRY to pursue research upon the chemistry of the catabolism of porphyrins and related compounds, in collaboration with Dr. R. Bonnett—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (July 7).

GRADUATE PROFESSIONAL FORESTERS (preferably with colonial forest service and under the age of 40); and NON-GRADUATE FORESTERS (with at least 10 years experience in forestry) WITH THE WEST AUSTRALIAN FOREST SERVICE—The Official Secretary, Agent General for Western Australia, Western Australia House, 115 Strand, London, W.C.2 (July 8).

LECTURER IN THE TEACHING OF MATHEMATICS AND PHYSICS in the Institute of Education, University of Ibadan, Nigeria—The Inter-University Council, 38 Bedford Place, London, W.C.1 (July 8).

SCIENTIFIC OFFICER (chemist, with a good honours degree and interested in the practical aspects of soil fertility and crop nutrition)—The Secretary, Rothamsted Experimental Station, Harpenden, Herts, quoting Ref. 1051/74 (July 8).

CHAIR OF GEOGRAPHY—The Registrar, University of Strathclyde, George Street, Glasgow, C.1 (July 9).

CHAIR OF SOCIOLOGY—The Registrar, University of Strathclyde, George Street, Glasgow, C.1 (July 9).

GRADUATE for insect sampling in connexion with pest forecasting systems for the International Biological Programme—The Secretary, Rothamsted Experimental Station, Harpenden, Hertfordshire, quoting Ref. 1052/87 (July 9).

JUNIOR RESEARCH FELLOW IN GEOGRAPHY—The Registrar, The University, Sheffield (July 9).

LEVERHULME RESEARCH FELLOW (physical or inorganic chemist with interest in the kinetics and photochemistry of inorganic compounds) IN THE CHEMISTRY DEPARTMENT, University of the West Indies, Jamaica—The Inter-University Council, 38 Bedford Place, London, W.C.1 (July 10).

SENIOR LECTURERS or LECTURERS (with an interest in either (*a*) theoretical chemistry and/or molecular spectroscopy or (*b*) physical chemistry with an interest in gas kinetics) IN CHEMISTRY at the University of Ibadan, Nigeria—The Inter-University Council, 38 Bedford Place, London, W.C.1 (July 11).

RESEARCH ASSISTANT (university graduate or person of equivalent status) IN THE SCHOOL OF CHEMISTRY, to be responsible for the maintenance of a mass spectrometer, type MS9, and for recording and interpreting spectra—The Registrar, University Senate House, Bristol, 2 (July 15).

ASSISTANT LECTURER (with a degree in veterinary science, agricultural biochemistry with nutrition or agricultural science) IN THE DEPARTMENT OF ANIMAL HUSBANDRY AND VETERINARY HYGIENE—The Secretary, The Royal Veterinary College (University of London), Royal College Street, London, N.W.1 (July 16).

RESEARCH SCIENTIST or SENIOR RESEARCH SCIENTIST (Combustion or Chemical Engineer) (with a Ph.D. degree in an appropriate discipline or postgraduate research experience of equivalent standard and duration supported by satisfactory evidence of research ability in chemical engineering, combustion science or fuel technology) IN THE DIVISION OF COAL RESEARCH, Commonwealth Scientific and Industrial Research Organization, North Ryde, Sydney, N.S.W., Australia—The Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 480/504 (July 16).

JUNIOR RESEARCH OFFICER (with, or about to obtain, an honours degree in botany) IN THE DEPARTMENT OF BOTANY, to work on root competition and its relation to productivity—The Registrar, University College of Wales, Aberystwyth (July 20).

LECTURER (with a good honours degree and preferably interests in one or more of the following fields: philosophy of science, ethics or Greek philosophy) IN PHILOSOPHY at the University of Lagos, Nigeria—The Inter-University Council, 38 Bedford Place, London, W.C.1 (July 22).

LECTURER (with teaching experience, good academic qualifications in biology, and preferably a higher degree in education) IN EDUCATION—The Registrar, The University, Keele, Staffs (July 30).

SENIOR LECTURER or LECTURER IN PSYCHOLOGY at the University of Cape Town, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (July 30).

SCIENTIFIC OFFICER or SENIOR SCIENTIFIC OFFICER (Agricultural Meteorologist) for research on irrigation and associated micro-meteorology—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (August 15).

CHAIR OF MATHEMATICS and HEAD OF THE DEPARTMENT—The Academic Registrar, The City University, St. John Street, London, E.C.1.

ENTOMOLOGIST (young, British) for technical development work in the Sudan—The Manager, Agrochemical Group, CIBA Laboratories Limited, Horsham, Sussex.

RESEARCH ASSISTANT (suitably qualified chemist) for isotopic tracer studies of combustion mechanisms—Dr. C. F. Cullis, Chemical Engineering and Chemical Technology Department, Imperial College of Science and Technology, London, S.W.7.

RESEARCH ASSISTANT (preferably graduate with research experience); and a POSTGRADUATE RESEARCH STUDENT (with, or expecting to receive this year, an honours degree) IN THE DEPARTMENT OF PURE AND APPLIED CHEMISTRY, to join a team working on the characterization and properties of highly branched and network polymers—Prof. M. Gordon, Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, C.1.

RESEARCH ASSISTANT (with a good honours degree in mathematics or similar qualification, and preferably some experience of computing) IN THE DEPARTMENT OF MATHEMATICS, to study methods of stress analysis of engineering structures under the direction of Prof. J. N. Hunt—The Assistant Bursar (Personnel), University of Reading, Reading, Berkshire.

RESEARCH ASSISTANT (with a good honours degree or equivalent in botany, microbiology, biochemistry or related subjects) IN MICROBIAL GENETICS IN THE MICROBIOLOGY DEPARTMENT—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8.

RESEARCH ASSISTANTS (with good honours degrees in physics and prepared to work for higher degrees as full-time internal students of the University of

London) IN THE DEPARTMENT OF PHYSICS, to join teams working in the following fields: cosmic ray physics and high energy particle counting techniques; electrical discharges in gases and spectroscopy—Head of the Department of Physics, Northern Polytechnic, Holloway, London, N.7.

RESEARCH ASSOCIATE (serologist/immunologist of Ph.D. level)—Mason Research Institute, Harvard Street, Worcester, Massachusetts, U.S.A.

RESEARCH FELLOW or SENIOR RESEARCH FELLOW IN APPLIED MATHEMATICS, to join a group studying electrochemical topics, including nerve conduction, by the methods of mathematical physics—The Registrar, University of Queensland, Brisbane, Queensland, Australia.

RESEARCH OFFICER (with a degree or other qualification, and preferably a leaning towards physical chemistry of instrumentation) IN THE BIOCHEMISTRY DEPARTMENT, for a cancer-oriented project entailing the study of liver components (including nucleic acids) by various biochemical techniques—Dr E. Reid, College Annexe, Battersea College of Technology, 14 Falcon Road, London, S.W.11.

RESEARCH PATHOLOGIST (medically qualified) to take charge of mouse skin painting experiments, histological and bacteriological services, tissue culture work and animal husbandry—The Secretary, Tobacco Research Council, Glen House, Stag Place, London, S.W.1, marking envelope 'Personal'.

SENIOR LECTURER or LECTURER (physical or inorganic chemist) IN THE DEPARTMENT OF CHEMISTRY INCLUDING BIOCHEMISTRY—The Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa.

SENIOR LECTURER or LECTURER (preferably with interests in physiology, but other interests not excluded) IN THE DEPARTMENT OF ZOOLOGY, University of Ibadan, Nigeria—The Inter-University Council, 33 Bedford Place, London, W.C.1.

STATISTICIAN (with a first- or upper second-class honours degree in statistics or in mathematics with statistics, and preferably a knowledge of Mercury autocode or Algol) IN THE MATHEMATICS SECTION which is concerned with the solution of a wide range of problems arising from the field of electrical engineering—The Personnel Officer, Electrical Research Association, Cleeve Road, Leatherhead, Surrey.

TECHNICIAN IN CHEMICAL PATHOLOGY for the recently modernized and re-equipped laboratories—Hospital Secretary, Queen Mary's Hospital, Roehampton, London, S.W.15.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Source Book for Agricultural Education. By Robert T. Wright. (Moray House Publications, No. 4.) Pp. vii+132. (Edinburgh: Moray House College of Education, 1966. Distributed by Oliver and Boyd, Ltd., Edinburgh and London.) 5*s.* [224

Northern Ireland: Ministry of Agriculture. Leaflet No. 123. Marek's Disease (Fowl Paralysis) and Leucosis. Pp. 3. Leaflet No. 136. Courses of Study in Agriculture. Pp. 11. (Belfast: Ministry of Agriculture, 1966.) [224

Advancing the Great Society. Presidential Messages to Congress, 1964–65. Pp. 36. (London: United States Information Service, American Embassy, 1966.) [224

The University of Sussex. Sixth Annual Report, 1964–65. Pp. 55+xviii. (Brighton: The University of Sussex, 1966.) [224

BBC–2. By David Attenborough. (BBC Lunch-Time Lectures, Fourth Series—6.) Pp. 14. (London: British Broadcasting Corporation, 1966.) [224

Guide for the Identification and Reporting of Stranded Whales, Dolphins and Porpoises on the British Coasts. By F. C. Fraser. Third edition. Pp. ix+34. (London: British Museum (Natural History), 1966.) 2*s.* 6*d.* [224

The Carnegie Trust for the Universities of Scotland. Sixty-fourth Annual Report, 1964–65. Pp. 66. (Edinburgh: The Carnegie Trust for the Universities of Scotland, 1966.)

Joint Conference of the Animal Feeding Stuffs Trade Associations and the British Veterinary Association, University of Keele, Staffordshire, 15–17 April, 1966—Full Texts of Papers. Pp. 60. (London: Animal Feeding Stuffs Industry—British Veterinary Association Liaison Committee, 1966.) [224

Royal Observatory Bulletins. No. 109: Pivot Errors and Axis Flexure in the 7-inch Cooke Transit Circle. By R. d'E. Atkinson, L. S. T. Symms and K. C. Blackwell. Pp. A105–A121. (London: H.M. Stationery Office, 1966.) 3*s.* net [224

The University of Liverpool. Annual Report of the Tidal Institute and Observatory, 1965. Pp. 23. (Liverpool: The University, 1966.) [224

Other Countries

Texas Instruments, Inc. 1965 Annual Report. Pp. 26. (Dallas, Texas Instruments, Inc., 1966.) [293

Cimbebasia—SWA Research No. 12. A New Scincid Genus; and a New Record from South West Africa. By W. Steyn and A. J. L. Mitchell. *Gerrhosaurus flavigularis flavigularis* Wiegmann in South West Africa (Reptilia, Cordylidae). By A. J. L. Mitchell and W. Steyn. *Pterygopus garrulus* (Smith) from the Brandberg. By W. Giess. Pp. 19. No. 13: Preliminary Report of Two Stone-Working Ova-Tjimba Groups in the Northern Kaokoveld of South West Africa. By H. R. MacCalman and B. J. Grobbelaar. Pp. 39. No. 14: Fossil Eggshell Fragments of a Giant Struthious Bird (*Struthio oshanai*, sp. nov.) from Etosha Pan, South West Africa. By E. G. Franz Sauer. Pp. 52. (Windhoek, S.W.A.: Staatsmuseum, Posbus 1203, 1965.) [293

United States Department of the Interior: Geological Survey. Water-Supply Paper 1499–J. Water Resources of the Waterbury–Bristol Area, Connecticut. By R. V. Cushman, F. H. Paiszek, A. D. Randall and M. P. Thomas. Revised by H. L. Baldwin. Pp. v+86+plate 1. Water-Supply Paper 1801: Geology and Availability of Ground Water in the Northern Part of the White Sands Missile Range and Vicinity, New Mexico. By James E. Weir, Jr. Pp. v+78+plate 1. Professional Paper 478: Geology of the Coeur d'Alene District, Shoshone County, Idaho. By S. Warren Hobbs, Allan B. Griggs, Robert E. Wallace and Arthur B. Campbell. Pp. v+139+plates 1–10. Professional Paper 524–A: Pancho Rico Formation, Salinas Valley, California. By David L. Durham and Warren O. Addicott. Pp. v+231. 2 dollars. (Washington, D.C.: Government Printing Office, 1965.) [293

Washington State University. College of Engineering: Research Division. Bulletin 294: Some Studies on the Combustion of Small Wooden Dowels. By William L. Galligan, Patricia S. Gold, David A. Ward and E. Roy Tinney. Pp. viii+112. (Pullman, Washington: Technical Extension Service, Washington State University, 1965.) [293

Geological Survey of Tanzania. Quarter Degree Sheet 224, 224W a 241W: Kasanga. (Dodoma: Geological Survey Division, 1964.) [303

Institut Royal des Sciences Naturelles de Belgique. Bulletin. Tome 41, No. 2: Le Genre Canadien *Greenockia* Brown, R. A. C., 1952, Synonyme du Genre Russe *Hemiplethorhynchus* von Peetz, H., 1898 (Rhynchonelloidea). Par Paul Sartenaer. Pp. 9+2 planches. Tome 41, No. 3: Trois Nouveaux Genres de Brachiopodes Rhynchonellides du Famennien. Par Paul Sartenaer. Pp. 12+2 planches. Tome 41, No. 4: La Phylogenese des Siphonophores Siphonanthes. Par Eugène Leloup. Pp. 9. Tome 41, No. 5: Contributions à l'Etude des Bactéries Marines du Littoral Belge. 1: Un Appareil Simple pour Détacher et Obtenir en Suspension les Bactéries Contaminant des Surfaces. Par Guido Persoone. Pp. 6. Tome 41, No. 6: Zur Kenntnis des Genus *Phyllogomphus* (Odonata: Gomphidae). Von Karl F. Buchholz. Pp. 5. Tome 41, No. 7: Crustacean Parasites of African Freshwater Fishes Mostly Collected during the Expeditions to Lake Tanganyika, and to Lakes Kivu, Edward and Albert by the Institut Royal des Sciences Naturelles de Belgique. By Geoffrey Fryer. Pp. 22. (Bruxelles: Institut Royal des Sciences Naturelles de Belgique, 1965.) [303

Institut Royal des Sciences Naturelles de Belgique. Bulletin. Tome 41, No. 8: Les Lepidopteres Heteroceres Africains de la Collection Abel Dufrance. Par Serge G. Kiriakoff. Pp. 28. Tome 41, No. 9: Chaetognathes de Quelques Recoltes dans la Mer des Antilles et l'Atlantique Quest Tropical. Par Marie-Louise Furnestin. Pp. 15. Tome 41, No. 10: Notes sur les Mammiferes. 51: Remarques au sujet des Antilopes Tragelaphus et Limnotragus. Par Serge Frechkop. Pp. 11. Tome 41, No. 11: Contribution à l'Etude des Dryopoidea. 13: Notes sur le Genre *Helminthopsis* Grouvelle et Descriptions d'Espèces Nouvelles (Coleoptera Elmithidae). Par J. Delève. Pp. 31. Tome 41, No. 12: Contributions à l'Etude des Bactéries Marines du Littoral Belge. 2: Comparaison de Plusieurs Méthodes pour Détacher et Obtenir en Suspension les Bactéries Contaminant des Surfaces. Par G. Persoone. Pp. 12. Tome 41, No. 13: Notes sur les Microlepidoptères de Belgique. 9: Révision des Exemplaires Belges du Genre *Choreutis* Hübner (Glyphipterygidae). Par Edouard Janmoulle. Pp. 4. Tome 41, No. 14: Notes sur les Erigonides. 31: Acartauchenius Justus (O.P., Cambridge) Araignée Nouvelle pour la Faune de Belgique. Par Jacques Denis. Pp. 5. Tome 41, No. 15: Un Condylarthre de Hoogbutsel. Par G. E. Quinet. Pp. 5+1 planche. (Bruxelles: Institut Royal des Sciences Naturelles de Belgique, 1965.) [303

Transvaal Museum, Pretoria. Report for the year ending 31st March, 1965. Pp. 32. (Pretoria: Transvaal Museum, 1966.) [204

Annals of the New York Academy of Sciences. Vol. 135, Article 1: Myasthenia Gravis. By M. M. Adner and 94 other authors. Pp. 1–680. (New York: New York Academy of Sciences, 1966.) [204

Australia: Commonwealth Scientific and Industrial Research Organization. C.S.I.R.O. Divisions and Sections 1966. Pp. 23. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1966.) [204

Bulletin of the Museum of Comparative Zoology, Harvard University. Vol. 134, No. 7 (November 30, 1965). New Oceanic Cheilodipterid Fishes from the Indian Ocean. By Giles W. Mead and J. E. De Falla. Pp. 261–274. Vol. 134, No. 8 (February 25, 1966): Italian Wolf Spiders of the Genus *Pardosa* (Araneae: Lycosidae). By Paolo Tongiorgi. Pp. 275–334. Vol. 134, No. 9 (February 25, 1966). Wolf Spiders of the *Pardosa monticola* Group (Araneae, Lycosidae). By Paolo Tongiorgi. Pp. 335–359. (Cambridge, Mass.: Museum of Comparative Zoology, Harvard University, 1965 and 1966.) [204

U.S. Department of Commerce: National Bureau of Standards. Technical Notes 18–24. Quarterly Radio Noise Data, September, October, November, 1965. By W. Q. Crichlow, R. T. Disney and M. A. Jenkins. Pp. vi+84. (Washington, D.C.: Government Printing Office, 1966.) 50 cents. [204

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GAMBLING ON INNOVATION

THE view that one should not do things for the first time has, of course, a great deal to commend it. Nothing ventured, nothing lost, is as good a proverb as the more familiar version taught to schoolchildren still innocent of disappointment. The difficulty is that caution, however prudent, is the enemy of innovation. A person, a company, or a nation wishing to do something for the first time must be prepared to take a gamble. Good management may limit some of the risks, while zeal, enterprise and hard work can improve the chances of success. Yet all technical innovation entails the possibility of failure and financial loss. Sometimes, ironically, technical success may not ensure financial success. Fortune is not always kind to the innovators.

Considerations like these were not very deeply hidden last week when the British Hovercraft Development Corporation and its associated companies displayed the tiny collection of air cushion vehicles so far produced by the investment of some £10 million in the development of hovercraft. Superficially, of course, the mood was optimistic. For every design in production, there were half a dozen on the drawing boards. By the end of a week of excited talk and speculation, engineers were speaking of trans-Atlantic hoverships almost as if they were accomplished facts. Southampton Water seemed closer to the centre of technological change than it has been since the huge flying boats began to land there in the decade before the Second World War.

In reality, however, hovercraft are still a gamble. Technical problems, of construction as well as operation, remain to be solved before hovercraft large enough to be economically competitive can be put into service. Recent experience has been encouraging, but the venture is not yet certain of success. And there is always the danger that even successful hovercraft will become white elephants because of some more successful but competitive innovation over whose progress the developers of hovercraft have no control. What then should be the strategy of a nation such as Britain towards further investment in risky undertakings of this kind ?

The first thing to be said is that the problem of the hovercraft is a valuable example of the economic dilemma of innovation as well as a nasty conundrum for the United Kingdom. On what scale should development now be supported ? How quickly should the programme be made to move ahead ? Is it best to attack on a narrow front, with the hope that at least some of the investment will return quickly, or is it wiser to look for more distant benefits from a broader programme ?

Questions like these should be in the minds of all who support the development of innovations on whatever scale. A pharmaceutical company looking for a drug to serve some chosen purpose, or a nation wanting to put a man on the Moon, must take a view on most of them. In the nature of things it is no more possible to find a sure recipe for avoiding disappointment than it is to find an algorithm for picking horserace winners. As with horse-racing, however, it is best to know the rules.

The first need is for a hard-headed assessment of the potential benefits and the potential risks of embarking on each particular innovation. Unfortunately the innovators, who share some of the sentimentality of the racecourse, are not always as ruthless as they should be. There is no other way of understanding how, for example, the British and French Governments have decided to invest more than £300 million in the development of the *Concord* supersonic aircraft. The costs are certain to be high, and almost certain to be higher than any estimate prepared in advance. But the chances of recovering them, which depend on selling enough aircraft at a price which airlines think is economic, are bound to be uncomfortably small, if only because of the enormous strength and flexibility of the aircraft industry in the United States. The chances of recovering the development cost several times over, which should be the objective of well directed innovation, is almost negligible.

In less speculative fields it is possible, but unfortunately not habitual, to make much more precise estimates of the balance between risk and reward. Companies planning to develop a new kind of motor-car can usually estimate the demand for their product with some degree of accuracy. The same could and should be done with, for example, developments in machine tools or chemical processes. If technical innovation remains a gamble, it is still possible to estimate the odds and, thus to ensure that neither money nor the efforts of skilled people are rashly put at risk. Doing this should be more commonly regarded as an essential part of the management of modern research and development.

As well as estimating the odds, it is also sometimes possible to take steps to reduce them. In the language of the race-course, it is possible to lay off some bets. Much may be done by diversifying a programme of technical development so as to ensure that the product is not merely a working prototype of a particular design but an assurance of technological skill in a broad field. The guiding principle here is that the costs of a successful development project must be expected steadily to increase as it moves from the laboratory to the workshop and finally into production. This means that it is frequently economic, and certainly wise, to follow several lines of development at the beginning of a programme on the understanding that the less promising alternatives will be weeded out as time goes on.

From this point of view the U.K. Atomic Energy Authority took a great risk when it decided, more than a decade ago, to base all its reactor development on gas-cooled designs. It is lucky that water-cooled reactors did not turn out to be decisively more economic. Now, with hovercraft, there is a case for arguing that the British development should invest modestly in versions that move on rails as well as in versions which move on the sea, for, at this stage, it is not possible to know how the economics of the whole enterprise will work out. Penny-pinching could be a false economy.

The risks of false economy are especially acute when technical developments are competitive. If two organizations are embarked on the same development, the one who first succeeds with it is likely to win a commanding commercial advantage. To come second often brings only meagre rewards. Those engaged in the British hover-

craft development are all too acutely aware of the danger. The British aircraft industry has a long record of this kind of disappointment. The difficulty, of course, is very real. With limited money and limited manpower, it is tempting to cover as much ground as possible by spreading the available effort thinly. The consequence is often to ensure second place—or worse—in everything. In reality there is no escape from hard decisions to invest heavily in some projects and to ignore others.

How are decisions like these to be arrived at sensibly? The first need, of course, is that those responsible for making such decisions should know how complicated is the task. It is now a long time since a man with imagination and a good eye could design an aircraft or, for that matter, a tea-cup. The close interaction between the process of design and miscellaneous activities such as market research and production engineering is a necessary feature of modern technology. Industry is not always aware of its problems—and of the opportunities which their solution may present. Yet it may frequently be too much to ask that particular industries in isolation should make sensible decisions about their own affairs. In the last resort there is no substitute for a well-informed technical community within which there can be a vigorous appraisal of technical problems and opportunities. It is hard not to think that the decision about hovercraft in Britain would be much easier if it had been more widely argued.

AVIATION NO MORE

THE disbandment of the Ministry of Aviation has been talked of for so long that the actual announcement a week ago came as something of a surprise. With Mr. Fred Mulley, the Minister of Aviation, embattled with the other European members of ELDO, and with familiar anxiety about the costs of development projects such as *Concord*, it had begun to seem as if the Ministry's survival was assured for all time. For quite apart from the suspicion that the survival of the Ministry was a part of natural law, there was also the possibility that other ministers would be reluctant to take under the wings the troubles (and the capacity for inviting troubles) which have beset the Ministry of Aviation almost since its formation out of the old Ministry of Supply. But in any event the Ministry is to be dismembered in the summer. Most of it will go to the Ministry of Technology but responsibility for civil aviation, previously a dependant of the Ministry of Transport, will go to the Board of Trade.

The sequence of changes is important because the research establishments of the Ministry of Aviation are the most powerful research and development organizations in the United Kingdom. Though they are by no means exclusively harnessed to the needs of defence research, but on the contrary anxious to diversify their interests as much as possible, the establishments may now be able to make a greater contribution to civil research and development in the United Kingdom. That, at least, is what the Government is hoping. Fortunately the Ministry of Technology has by now lived through its early brashness, and so is unlikely to expect too much too quickly: that could be disastrous for morale at the establishments. The most urgent need is somehow to create the impression that this third major upheaval within three years of the administration of public science will, for the time being, be the last. If that can somehow be accomplished, the Government's hopes may easily be justified.

DECODER MISLED?

Stonehenge Decoded

By Gerald S. Hawkins in collaboration with John B. White. Pp. xiv+202+23 plates. (London: Souvenir Press, 1966.) 30*s*. net.

THIS book is an expansion of two articles in *Nature* (200, 306; 1963, and 202, 1258; 1964). It is tendentious, arrogant, slipshod and unconvincing, and does little to advance our understanding of Stonehenge.

The first five chapters, on the legendary and archaeological background, have been uncritically compiled, and contain a number of bizarre interpretations and errors. The rest of the book is an unsuccessful attempt to substantiate the author's claim that "Stonehenge was an observatory; the impartial mathematics of probability and the celestial sphere are on my side". Of his two main contentions, the first concerns alignments between pairs of stones and other features, calculated with a computer from small-scale plans ill-adapted for this purpose. Their directions were compared with the azimuths of the rising and setting Sun and Moon, at the solstices and equinoxes, calculated for 1500 B.C. Any alignment falling within $\pm 2°$ of one of these eighteen azimuths is accepted, arbitrarily, as "significant". The limit of error adopted is unrealistically large, since experiment shows that such alignments can be laid out with a pair of sighting-sticks to a repeatable accuracy of ± 5 min. Moreover, the assumption that the builders aimed at the full orb of the Sun and Moon, tangent to the horizon, instead of at the first or last flash, is not confirmed by an analysis of the author's (unpublished) tables of vertical errors.

In claiming to have found thirty-two "significant" alignments, Hawkins has in five cases exceeded his own limits of "significance". Furthermore, he has failed to show that his results are due to anything but chance. Even with the most favourable choice of data, no more than six of his alignments can be shown to be significant in the statistical sense. Before these can be accepted, they need to be measured on the site, and not from plans.

Hawkins's second contention is that the fifty-six Aubrey Holes were used as a "computer" (that is, as tally-marks) for predicting movements of the Moon and eclipses, for which he claims to have established a hitherto unrecognized 56-year cycle with 15 per cent irregularity; and that the rising of the full moon nearest the winter solstice over the Heel Stone always successfully predicted an eclipse. This, if substantiated, will provide the best explanation so far of this stone, though it should be noted that no more than half these eclipses were visible from Stonehenge. As for the suggested use of the Aubrey Holes, it may be remarked that a ring of pits, filled up as soon as they had been dug, is not a very practicable form of permanent tally. Moreover, it is questionable whether a barbarous and illiterate community (for this is Stonehenge I, not Stonehenge III), which has left us no other evidence of numeracy, could successfully have recorded the data needed to establish a cycle which exceeded the contemporary life-span, and could not even have been recognized as a cycle until several generations had elapsed.

The fundamental objection to this and to other equally unconvincing suggestions in this book arises from the false logic of Prof. Hawkins's working hypothesis, declared in the preface. "If I can see any alignment, general relationship or use for the various parts of Stonehenge," he says, "then these facts were also known to the builders." As he so truly adds in the next sentence, "Such a hypothesis has carried me along over many incredible steps".

R. J. C. ATKINSON

BARE BONES OF HEREDITY

Towards an Understanding of the Mechanism of Heredity

By H. L. K. Whitehouse. Pp. xii+372+4 plates. (London: Edward Arnold (Publishers), Ltd., 1965.) 55*s*. net.

THE problem of presenting genetics has radically changed in the past 10 years. Formerly, the subject having become the connecting framework of biology, the most obvious requirement was to explain its connectedness. This task was fairly straightforward. A common chemical basis for heredity, certain universal principles of differentiation, processes of evolution experimentally demonstrated, a mechanism known to control differentiation and evolution and also to be subject to them, these together offered us a wide choice of connected treatments.

To-day the task is more complicated. New mechanisms of heredity have been discovered which are understood at different levels and with different degrees of exactness. Their evolutionary connexion is not yet fully clear. Their chemical relationship is uncertain. Their physiological scope, owing to the range of size between bacteria and the higher organisms, is sharply contrasted. The experimental evidence in the new fields is fragmentary and sporadic. We may give the name of chromosomes to the structures responsible for heredity in all organisms. And we may agree that they are all based on DNA. But we also know that the organization of the DNA is of different kinds and the mechanisms which they operate may therefore vary in character.

In these circumstances an attempt to present the whole story in one volume is bound to seem hazardous and even heroic. Dr. Whitehouse has understood this and has attempted to get round the difficulties by a radically new approach. Let us, he says in effect, put crossing over in the centre of the field and let us try to separate the mechanism of heredity from its biological background. Let us in this way cut out the evolutionary and physiological trimmings and show the bare bones of the subject.

How does the method work? Undoubtedly the result provides a review and an interpretation of the whole subject, unified, documented and illustrated, in the way a research worker needs. The account proceeds in historical order of study, beginning mainly with the higher organisms. Here, outside the field of crossing over, Whitehouse refers to gene action, nuclear-cytoplasmic reactions, plastid inheritance, sex determination, and the induction and analysis of mutation. Within the field of crossing over he discusses some of the consequences of structural hybridity. He sacrifices genotypic control and its relation to the breeding system, which is a pity as they throw light on several aspects of the strictly mechanical problem. Also at this point he makes one commonly repeated mistake, derived probably from Morgan. He refers to Janssens as supposing that the structure of a chiasma and the event of crossing over had one character, one origin and one connexion. In fact Janssens' theory was not this at all. He assumed every conceivable kind of character, origin and connexion for them; and one of his assumptions for the connexion we now believe to have been correct.

It is in the fields of micro-organisms, however, that Whitehouse comes to grips with the problem of crossing over on the chemical level and makes his own original interpretations. Here he meets the difficulty of reconciling his views of micro-organisms with the properties of chromosomes in the higher organisms. He overcomes it by supposing that the chromosomes of the higher organisms are composed of single DNA molecules. The known variations in the size of chromosomes and of their DNA contents, however, do not allow this view.

They are usually multiples; they are also variable multiples; and the degree and timing of their multiplication, as recent observations have shown, vary under genotypic control. The multiple (or polynemic) DNA molecule creates several theoretical difficulties. It not only excludes Dr. Whitehouse's attempt to reconcile chromosome structure in different classes of organisms; it also, as was realized 30 years ago, introduces problems for the theory of gene mutation. But we must not think we can reach quick conclusions by ignoring a part of the evidence.

These are examples of the kind of questions, new and old, that will arise from reading Dr. Whitehouse's book. And his book will have to be read by those who want to answer them.

C. D. DARLINGTON

TWO VIEWS ON INSECTS

Basic Arthropodan Stock with Special Reference to Insects

By A. G. Sharov. (International Series of Monographs in Pure and Applied Biology, Division: Zoology, Vol. 30.) Pp. xii+271. (London and New York: Pergamon Press, Ltd., 1966.) 80*s*. net.

A LACK of distinction between fact and fiction characterizes this book. The author's views, for which there is frequently no evidence, are put down as if they were accepted facts. The uninformed reader, seeking information, can have no clue as to what is factual and reliable and what is not. For example, we are told that the Onychophora are a side branch of the Annelida, with no evidence given, and no consideration of the now considerable body of evidence derived from studies on functional morphology. Then we are told what happened to the ctenophore-like ancestor of the Articulata when it became a creeper, as if this was an established fact. Further, the Spintheridae are stated to be the most primitive of present-day Polychaeta, but in fact they are highly specialized Amphinomidae, entirely lacking in the attributes described by Sharov which form the keystone for his view on the origin of the Arthropoda. *Spinther* possesses polychaete parapodia of a specialized nature, not lobopodia. The anterior segments do not become preoral; the "cyclometry" of the adult has nothing to do with ctenophore ancestors and is entirely absent in the young worm which has normal worm-like proportions of its body and an almost terminal mouth. Turning to the Arachnida, we find many statements which are unacceptable to workers on this group. That the Pedipalpida originated from the Eurypterida is pure conjecture. Errors of facts, often several per page, occur throughout the book.

Conclusions of others, based on good evidence, are set aside or altered to mean something contrary. For example, it is said that Tiegs and Manton consider that the Myriapoda were derived from the Onychophora. Neither of these authors has ever at any time expressed such a view. Their suggestion that the Myriapoda and Onychophora may have had a common origin, and that they stand closer to each other than to any other group of invertebrates, is an entirely different statement, and it is based on facts. Similarly, an extra segment (labial) is pushed into the head of Crustacea by Sharov, in defiance of the abundant good work on crustacean embryology published since 1928. An extra segment is also planted into the onychophoran head; no distinction is made between a statement by Pflugfelder[1] concerning an embryo of *Paraperipatus* and the close series of stages belonging to four species of *Peripatopsis*[2,3]. Ten years of work on hundreds of embryos of *Peripatopsis* was summarized by a selected series of accurate reconstructions. It happens to be usual for the cavities of the anterior somites of these Onychophora to arise from several initial spaces which then rapidly fuse. This is no evidence for the existence of multiple anterior segments, and the super-

structure of classification and so on based on this erroneous concept of head segmentation is valueless.

The basic organization of the Arthropoda is scarcely considered. Instead we have a series of topics. But a dissertation on primary and secondary segmentation puts back the clock on those that have preceded it. Havoc is made of the known facts concerning the composition of the head in Arthropoda. The error of Snodgrass[4] concerning his suggested protocephalon of the Anostraca, erected on false premises[5], is built on. The confines of the acron are not precisely appreciated, nor the fact that anterior segments are frequently not cylindrical, the dorsal part of the head being derived from unsegmented embryonic tissue[3,6,7].

The account of trunk segmentation and of leg structure is equally fallacious. What Sharov calls paratergal lobes in Crustacea, most persons would regard as pleural. We are now beginning to have accurate information concerning the significance of the structure of the pleuron, leg-base and leg structure in arthropods[8–10]. A first essential is accurate information, both of structure and of usage. Sharov's whole discourse on legs is perverted in support of the monophyletic idea of arthropod evolution.

A short text-book account of the Monura and Thysanura precedes a consideration of the derivation of the Pterygota from Thysanura-like animals.

Only at page 218 do we come to brief reference to the internal structure of arthropods. But at once we are confronted not with essential anatomy, but with speculations put down as facts. Non-speculative data and sounder ideas based on facts are passed by.

The "new principles of classification" of the lower Arthropoda emerging from this welter of distortions and inaccuracies are without value. For example, the Arachnida are not composed of the five listed classes but of at least sixteen; the Merostomata comprise the Xiphosura *and* the Eurypterida; the simplification of the Crustacea is not helpful here, although Dahl was at liberty to discuss the possible relationships between the recognized classes. The structure of the proboscis and its mode of action in *Spinther* and in the Pyconogonida are entirely different. The proboscis of *Spinther* is not even typical of the Polychaeta, but is unique. The extrusion mechanism is dependent on septa, septal muscles and coelomic pressure. The evolution of an arthropodan haemocoel in a *Spinther*-like animal would render the proboscis mechanism inoperative. Functionally, therefore, there is no basis whatever for Sharov's suggested Proboscifera exhibiting an early arthropodan stage of evolution. Then, the categories Dignatha and Trignatha are used as taxa for which there is now no justification; these terms indicate grades of advancement, not relatedness of the constituent animals[5]. The weighty evidence against the validity of the Mandibulata is not considered, although the data are included in the references. Neither is evidence mentioned which is against the supposed crustacean origin of myriapods or the proto-symphylan origin of insects. Even the Scutigeromorpha among centipedes are claimed to be the most primitive members of this class although there is abundant evidence to the contrary[9].

The whole book purports to prove the accuracy of the ideas of Snodgrass[11] concerning the monophyletic evolution of the Arthropoda, and fails most thoroughly. Snodgrass himself, had he lived, would have been broadminded enough to have reorientated his ideas in the face of the newer evidence

S. M. MANTON

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BRITISH ANIMAL MEDICARE

Animal Health

A Centenary 1865–1965—a Century of Endeavour to Control Diseases of Animals. (Ministry of Agriculture, Fisheries and Food.) Pp. xvi + 396 + 30 plates. (London: H.M.S.O., 1965.) 80*s*. net.

OCTOBER 1965 was the centenary of the Government veterinary service in Britain and to mark the occasion the Ministry of Agriculture, Fisheries and Food published a historical survey of official veterinary activities in Britain which had started in 1865 with the formation of a Veterinary Department of the Privy Council to deal with a disastrous outbreak of cattle plague, and which had been carried on since then without a break by various bodies, culminating in the Ministry's Animal Health Division of the present day.

During that period, the State Veterinary Service has achieved many notable successes in the control and eradication of the more serious diseases of farm animals; cattle plague, contagious bovine pleuro-pneumonia, epizootic lymphangitis, rabies, glanders, psoroptic and sarcoptic infestation of horses and sheep have all been eliminated, bovine tuberculosis virtually so, and swine fever is apparently well on the way to final eradication; increasing success is being attained in keeping foot-and-mouth disease from these shores while the sporadic cases of anthrax which occur are kept well under control. This is a record of which the Division can well be proud and it is to be congratulated on setting down the story of its vicissitudes in such detail, as this could provide a useful guide, both to further veterinary activities in Britain and to developing countries which have still to undertake the tasks tackled by the British veterinary service over the past century.

Animal Health is divided into three main sections. The first is a general historical review describing the evolution of the Animal Health Division; then a long section dealing with the activities of the Division, including a description of the main animal diseases with which it has to deal; and finally a brief description of the international veterinary scene followed by a discussion of the principles and practice of veterinary preventive medicine.

The historical survey, divided into two main sections, namely, 1865–1914 and 1915–1965, concentrates on the most important events in disease control in Britain during the past 100 years. Taken presumably largely from official reports which it extensively quotes, this is probably the least readable, though possibly the most important section of the book and it is one to which future veterinary historians will undoubtedly find themselves indebted. By contrast, the section on animal health responsibilities, dealing as it does *in extenso* with individual scheduled diseases and various others with which the Division has to deal, will be of more general interest. The various diseases are well described along with the measures taken for their control and eradication, the difficulties—technical and administrative—that were encountered and the steps taken to overcome them. The effects of these measures in relation to the notifiable diseases are graphically shown in a series of charts illustrating fluctuations in disease incidence over the past 100 years.

The second part closes with a chapter outlining the research and investigation services of the Division. This deals at some length with the development and activities of the Central Veterinary Laboratory at Weybridge and its subsidiary at Lasswade near Edinburgh. There is also a short account of the Veterinary Investigation Service which acts in a consultative capacity assisting veterinary surgeons in practice to deal with unusual flock and herd problems and carries out investigations into animal disease problems of local concern..

Part 3 describes the activities of various international organizations concerned with animal health and disease

such as the World Veterinary Association, the Office International des Epizooties and the Food and Agriculture Organization of the United Nations. The Ministry's Animal Health Division has played an important part in building up and supporting such bodies, the importance of which has grown with the enormous increase in international traffic in livestock and their products and with the need to devote ever increasing attention to animal disease problems of developing countries in the war against want. To such countries in particular the final chapter dealing with the principles and practice of preventive-medicine will be of special significance. A. ROBERTSON

NEW INTERNATIONAL REFERENCE ATMOSPHERE

CIRA 1965

Cospar International Reference Atmosphere 1965. Compiled by the Members of Cospar Working Group IV. (Committee on Space Research established by The International Council of Scientific Unions.) Pp. xi+313. (Amsterdam: North-Holland Publishing Company, 1965.) 54*s*.

SINCE the advent of the first artificial satellite in 1957 a great deal has been learnt about the Earth's upper atmosphere. On the basis of the earliest results, the Committee on Space Research started work on a reference atmosphere in 1960 and the first tables were published towards the end of the following year under the title *CIRA 1961*. Over the next few years many new results were obtained for different levels of solar activity. A new set of tables has, therefore, been compiled by members of Cospar Working Group IV, and is known as *CIRA 1965*, although much of the tabulation was complete by May 1964.

The reference atmosphere is presented in three parts, each of which is preceded by a description of both the observational data and the methods used in the construction of the tables. Part 1 was prepared by K. S. W. Champion and gives the mean atmospheric structure for heights between 30 and 300 km. The maximum and minimum departures of the atmospheric properties from their mean values are also indicated. Part 2 is concerned with the atmospheric structure and its variations in the region from 30 to 80 km. This section was prepared by G. V. Groves using data obtained from a large number of rocket firings; temperature, pressure, density and west-to-east wind components are all given as functions of latitude and season.

The largest section, Part 3, was prepared by I. Harris and W. Priester and contains tables of density, temperature, pressure, scale height, mean molecular weight and number densities for heights between 120 and 800 km. This region of the atmosphere is under strong solar control and exhibits several different types of variation. The atmospheric properties are tabulated at two-hourly intervals of local time for ten levels of solar activity, the radiation flux on a wave-length of 10·7 cm being taken as the index of long-term solar activity. Although the procedure for interpolating the tables to give the atmospheric properties at any particular time appears complicated at first sight, it is not difficult provided one has ready access to geomagnetic indices and solar flux data. There are two deficiencies in Part 3: first, the reference atmosphere applies only to the equatorial and temperate zones of the Earth and, secondly, there is now evidence that the magnitude of the diurnal variation in density is under-estimated in the region below 200 km for conditions of low solar activity. An additional parameter, which could usefully have been tabulated in this part, is the density scale height.

Two review papers are included as appendixes. In the first, K. S. W. Champion discusses the structure of the lower thermosphere, which comprises the region between 100 and 200 km. It is a pity that, in an otherwise admirable paper, the author should have perpetuated Izakov's error in believing that King-Hele's values of density can be improved by juggling with values of drag coefficient and density scale height. In the second review, L. G. Jacchia gives an extremely lucid account of the structure and variations of the atmosphere above 200 km. Recent results have shown, however, that he was too pessimistic when discussing the possibilities of evaluating density above 600 km at solar minimum.

CIRA 1965 represents the most detailed tabulation of upper atmospheric properties that has ever been attempted, and Cospar Working Group IV is to be congratulated on the result. The discussion in each part and the two review articles ensure that the reader is fully aware of the limitations in the data which were used to compile the model. G. E. COOK

LAWYERS AND MONOPOLIES

Competition and the Law

By Alex Hunter. (University of Glasgow Social and Economic Studies.) Pp. 328. (London: George Allen and Unwin, Ltd., 1966.) 45*s*. net.

THE modern British economy, at least in its large private sector, is theoretically one of private enterprises operating in competition. But it has been well known for years that that competition has not been free or unfettered. The period since 1945 has seen significant investigations into the degree of competition, and the increased realization of the limits on competition imposed by the enterprises themselves has provoked Parliamentary attempts, by legal means, to loosen these fetters and to restore greater freedom of competition.

Competition and the Law is an economist's examination of what has become a field of interest to both economists and lawyers, as well as to industry and commerce themselves, and made, moreover, by a scholar who has had personal experience of the systems of investigation and control, having assisted and advised on some of the early cases prepared for the Restrictive Practices Court. It is naturally, therefore, on the economic side that this book is strongest and most interesting; on the legal side it is at times rather shallow and cursory.

The first part comprises three chapters dealing with the background, the relevant economic theory and the measurement of monopoly power. They bring out the increasing concentration of industry and economic power in Britain, and the steady movement towards mergers which have tended to produce oligopolistic groupings in which the few (Table 1 takes as its basis the three) largest enterprises in given industries have a predominant share in net output and in percentage employment within that industry. Thus in 1958 in the man-made fibres industry the three largest enterprises had 89 per cent of the net output and 81 per cent of the employment; but equally in three-quarters of industries in that year the three largest enterprises had less than 50 per cent of either net output or of employment. The tendency to oligopoly production in the large-scale process industries is clear, and is even more clear in the cases of some sub-industries such as transmission chains and cotton thread.

The second, and much the longer, part of the book is an examination and criticism of the body of legislation in force in the United Kingdom as at mid-1965. (It is the author's, and the reader's, misfortune that the Monopolies and Mergers Act, 1965, was passed too late to be taken into account in the book.) In discussing the legal doctrines which existed before 1948 (and still exist, so far as not superseded by legislation) there might have been mention of much earlier legislative attempts to grapple with substantially the same problems, such as the Scots Acts

of 1535, 1540, 1579 and 1592 against forestalling and regrating, and the English Acts of 1266, 1353, 1533 and particularly of 1549 and 1552 on the same subjects.

Prof. Hunter discusses the legislation setting up the Monopolies Commission and its work, particularly the general Report on Collective Discrimination of 1955, which led to the Restrictive Trade Practices Act, 1956. This Act is in turn examined very thoroughly, particularly the fundamental Section 21, which established the rule that a restriction in pursuance of any agreement under review was to be deemed contrary to the public interest, unless the Court were satisfied of at least one of seven stated sets of circumstances, and also that the restriction was 'not unreasonable'. The author devotes two chapters to the operation of judicial review, as exemplified by the cases hitherto contested, considering the criteria established by the Act, and various defects in the processes.

Prof. Hunter then turns to resale price maintenance, which is a newer subject of judicial control and on which we have as yet no judicial guidance and where the operation of the legal control has yet to become apparent, and concludes by discussing monopoly, oligopoly and merger. In general Prof. Hunter finds the present system satisfactory; indeed, he considers the Monopolies Commission "an investigatory instrument of great flexibility and potential range", but he has a number of recommendations for change in the present legislative structure, most of which appear to have substantial merit.

The book is very readable, despite an unfortunate number of phrases masquerading as sentences, and not overburdened with technicality, and its carefully analysed exposition of the present situation presents a clear and fair picture.

DAVID M. WALKER

MAGNETISM DIGEST

Magnetism and Magnetic Materials

1965 Digest—a Survey of the Technical Literature of the Preceding Year. Edited by R. L. White and K. A. Wickersheim. Pp. xiii+260. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 88*s*.

THE problem of keeping abreast of the scientific literature in any field of research is immense. As a partial solution to this problem, for several years the American Institute of Physics and Institute of Electrical and Electronic Engineers, on the occasion of their annual conferences on magnetism and magnetic materials in the United States, have sponsored the production of a *Magnetic Materials Digest*. These volumes contain references, drawn from a large number of sources, to work in the field of magnetism and magnetic materials published in the preceding year. They therefore provide a very convenient way of surveying the work done in a particular field of magnetism over the previous years.

The 1965 *Digest* differs slightly from its predecessor. There has been a change in title to emphasize that it is not a manufacturer's source book and the volume has a new, improved format following a change in publisher. Those readers familiar with previous volumes in the series will find the usual changes in chapter and section headings reflecting the changes of interest and emphasis in magnetism research. The Bell Telephone Laboratories *Index to the Literature of Magnetism* has again been the principal source of references, more than sixteen hundred of which are listed, the great majority having been published in 1964.

If the number of publications is taken as a measure of interest in a topic, then for 1964, non-metals, theory, soft magnetic materials and thin films head the list in that order. However, these numbers may be misleading because the contents of some papers are such that they are, or could have been, referred to in more than one chapter. An outstanding example is that of the chapter on superconductivity which in *Magnetism and Magnetic Materials* has nineteen references compared with 208 in the *Digest* for 1964. Many of the papers on superconducting phenomena now appear in their more natural places rather than in a special chapter of their own.

J. H. PHILLIPS

SCIENTIFIC BIOGRAPHIES

Geschichte der Mikroskopie

Leben und Werk Grosser Forscher. Herausgegeben von Hugo Freund und Alexander Berg. Unter Mitarbeit Namhafter Autoren. Band 1: Biologie. Pp. xv+375. Band 2: Medizin. Pp. xv+506. (Frankfurt am Main: Umschau Verlag, 1963, 1964.) n.p.

VOLUMES 1 and 2 of *Geschichte der Mikroskopie* form the first in a collection of short biographies of scientists written mostly by German authors. A third volume dealing with the physical sciences and technology is in preparation. Despite the title, most of the subjects are people who have used the microscope as a tool and only a minority have made any contribution to microscopy as such. Judging from modern experience, one might comment wryly that possibly many of them had little knowledge of either the instrument itself or of the proper way to use it. A sceptic might answer that this is unimportant because optics is such a poor way of forming images in any event; after all, even a perfect lens trying to form an image of a point source only manages to get about 84 per cent of the light anywhere near the right place! All the same, one might still wish to see fewer stopped down or lowered condensers, badly tilted mirrors and uncentred lamps at allegedly scientific demonstrations.

Volume 1 begins with a general review by the editors covering the history and the development and applications of microscopy. The treatment is inevitably sketchy and occasionally irritating, as when the name of C. R. Burch is omitted from the discussion of reflecting microscopes. The bibliography, however, is quite extensive and useful. In general the bibliographies are one of the most valuable features of the book; each chapter is followed by a selection of the papers of the subject himself as well as related references. Another pleasant feature is the inclusion of a good photograph or portrait of each subject.

It is only to be expected that major prominence is given to German scientists. In Volume 1, for example, no British names appear and only four American (Chambers, McClung, Minot and Wilson) out of a total of thirty articles. One might have thought that at least one out of Brown, Bowman, Hardy, Sharpey-Schäfer or Strangeways merited inclusion. Histochemistry is poorly represented and Raspail, one of the founders of the subject, is not mentioned. W. Flemming, the great German cytologist, and the Swede, Retzius, who produced such a fantastic volume of illustrated work with the microscope, are other surprising omissions.

Volume 2, dealing with medicine, contains forty-six articles, of which three are on American and three on British workers. The latter include Alexander Fleming and Grafton Elliott-Smith; truly the word 'microscopy' is liberally interpreted!

When all this is said, however, these books do make pleasant reading and several of the great names in microscopy such as Abbé, Harting, Köhler, Naegeli, Schwendener, Strugger, Ambronn, Purkinje and Valentin are to be found, as well as a host of others distinguished in the use of the microscope as an essential tool in their biological or medical research. Collections of this sort can always be criticized for their choice of material but on the whole this is a welcome addition to the literature of scientific biography.

R. BARER

Kempe's Engineers Year-Book for 1966

Edited by C. E. Prockter under the direction of B. W. Pendred. 71st edition. Vol. 1: Pp. xiv+1356. Vol. 2: Pp. 1399. (London: Morgan Brothers (Publishers), Ltd., 1966.) 95*s*. net the two volumes.

A NEW edition of *Kempe's Engineers Year-Book* is an annual event, though recent editions differ only slightly one from another in their content of data and descriptive matter relating to most branches of engineering, with references to original sources.

This seventy-first edition has one new chapter on rubber as an engineering material. The chapters on legal notes for engineers, nuclear energy and grinding and abrasive processes, respectively, have been rewritten; otherwise there are additions and minor modifications to a number of chapters. Noteworthy among the additions are rocket engines, including theory and fuels; details of positive displacement rotary flow meters and density metering; theory of detonation and explosive forming and electric instruments for linear measurement (surveying). Mention of the design criterion afforded by the plastic theory of ultimate load-carrying capacity of structures, in the chapter on design of steel structures, is a significant though minor improvement. Further revision of the chapters on theory and design of structures in respect of modern criteria and data, including flexibility and stiffness coefficients of structural members, would improve future editions.

The chapter on mining engineering will presumably require revision soon to take account of advances in automation in coal-mining and the re-organization of the National Coal Board which is taking place this year.

Useful as they are, these volumes could, it is believed, be improved substantially in respect of design charts and data. For example, tables of hyperbolic functions would improve the mathematical data provided, and water hammer and surge charts would be useful additions to the chapter on water engineering. Such additions could be made at the expense of descriptive material relating to obsolescent plant and equipment and theoretical matter which is readily available in elementary textbooks.

T. M. CHARLTON

Physics of Nuclear Fission

Edited by N. A. Perfilov and V. P. Eismont. Translated from the Russian. Pp. vi+210. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1964.) 63*s*.

THIS book consists of twenty chapters on various aspects of fission, varying from general topics like "Asymmetry in the Mass Distribution of Fission Fragments" to highly specialized ones like "Radiochemical Study of the Isotopes of the Rare-earth Elements formed in the Photofission of Uranium". Some chapters are short review articles, while others are accounts of single experiments. However, the original Russian edition, of which this book is a translation, appeared in 1962, and the latest references are (inevitably) to work published in 1961.

If this work had appeared in translation in a review journal in 1962 or 1963 it could have been recommended, but its present appearance in hard covers seems entirely pointless.

B. ROSE

Mechanisms of Hormone Action

Edited by P. Karlson. (A NATO Advanced Study Institute, Meersburg/Bodensee, 20–26 Mai, 1964.) Pp. xii+275. (Stuttgart: Georg Thieme Verlag; New York and London: Academic Press, 1965.) 49.80 D.M.

MECHANISMS of Hormone Action records the proceedings of the North Atlantic Treaty Organization advanced study institute held in Meersburg, Bodensee, in May 1964. At this conference there were twenty-five participants from Germany and thirty-one from other European countries and the United States. The intention of the conference was to invite experienced researchers to present results and discuss their ideas in detail and so to stimulate their younger colleagues in the development of new and independent research. Altogether twenty-one papers were presented, in English, French and German, under three main headings: "Hormone Action by Control of Enzyme Activity"; "Enzyme Action by Changes in Permeability"; and "Hormone Action by Gene Regulation and Enzyme Induction". A synopsis of the discussion is also included.

The idea of considering the mechanism of action of hormones under a number of headings is a useful one. It is perhaps not only a reflexion of the interests of those devising the programme of the conference but also an indication of the volume of published work and of general tendencies of thought that forty pages of the text are concerned with hormone action by control of enzyme activity, while ninety-eight pages are devoted to the subject of hormone action by changes in permeability; hormone action by gene regulation and enzyme induction takes up 137 pages of text. Of course some overlap between the subject-matter in these three sections is inevitable, but the relative weight of the sections is almost certainly significant.

To me, the absence of an index to this book is a definite disadvantage. Although the argument might be put forward that the preparation of an index could delay seriously the production of the volume, the extra time involved is surely less serious than the diminished usefulness of the book for purposes of reference which inevitably results from the absence of an index. If a scientific book is worth printing and publishing at all it is, in my view, worth indexing even at the expense of a little delay in publication. Apart from this blemish the book is well produced and the diagrams and photographs (numbering 115 altogether) are excellent.

F. G. YOUNG

Libraries in the Modern World

By George Chandler. (The Commonwealth and International Library of Science, Technology, Engineering and Liberal Studies: Library and Technical Information Division.) Pp. vii+164. (London and New York: Pergamon Press, Ltd., 1965.) 17*s*. 6*d*. net.

IT is a valid criticism of some books that the author has never made up his mind as to the type of reader he is addressing. When an author frankly admits that he is aiming at several levels of readership and disclaims satisfying any single level, the critic can be disarmed. Nevertheless, Dr. Chandler is fully justified in his hope that the various levels of readership will be sufficiently repaid for purchasing *Libraries in the Modern World*. The skill with which he contrives to display a balanced but accurate picture of all types of libraries, their activities and interrelations matches his candour, and the chief criticism that can be made of his effort is that he includes no reference to the various, more detailed accounts of particular classes of libraries which would enable the reader to pursue a particular interest further.

Dr. Chandler describes first the international function of libraries. This chapter alone contributes something towards the formation of the informed public opinion on the whole subject of the library service which is needed in Britain. Chapters follow on local public libraries, on academic libraries, private libraries, industrial libraries and on national special libraries, while finally there are two chapters on international libraries and documentation services and on international and national library associations. The chapters are not all of equal merit and that on industrial libraries is rather patchy and has some surprising omissions. None the less, if due regard is paid to such limitations, Dr. Chandler has provided what could be a useful introduction to a wide range of readers interested in one aspect or another of the use and management of libraries.

R. BRIGHTMAN

A POSSIBLE GENETIC BASIS FOR SPECIFIC PATTERN IN ANTIBODY

By SIR MACFARLANE BURNET
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IN the original formulation of the clonal selection theory of immunity[1], it was suggested that the necessary range of immune patterns might arise if "at the appropriate stage of development a limited genetic determinant carrying the coding responsible for globulin pattern releases control in such a fashion that purely random arrangments are allowed" (ref. 1, p. 60). Since 1958–59 there have been great advances in the understanding of protein structure and particularly of the structure of immunoglobulins (see, for example, ref. 2). In addition, there has grown up in the past 10 years an intense interest in the evolution of chemical pattern in proteins which may well have a very important bearing on the antibody problem. It seems appropriate, therefore, at this time to attempt to rephrase the above quotation in the light of recent developments.

Structure of Immunoglobulin G Antibodies

It is generally accepted that an IgG antibody molecule is an elongated flexible structure of dimensions, according to Edelman and Gally[3], of 240 × 54 × 19 Å. It is symmetrical in character with specific combining sites at each end. The physical character of the combining sites is unknown, but it is accepted that they represent a configuration of parts of the peptide chains making up the macromolecule.

Appropriate treatment with proteolytic enzymes, reducing agents capable of splitting disulphide bonds, and detergents has provided a series of large fractions the interrelations of which are conventionally shown in Porter's well-known diagram[4] (Fig. 1). This shows four chains held together by three (or five) disulphide linkages. The two light chains have a molecular weight of about 25,000 with 212 amino-acid residues[5]; the heavy chains with a molecular weight of 55,000 ± would have ± 450 residues. Attached to the heavy chains is an oligosaccharide unit containing galactose, mannose, fucose, acetylhexosamine and sialic acid[6].

In man, the light chains have a common character in all types of normal immunoglobulins and are found in most types of myeloma proteins. The Bence-Jones proteins found in the urine of myelomatosis patients appear to be identical with light chains[7]. There are two serological types of light chain characterized by antigens κ and λ.

Recent work from several laboratories is consistent with the finding first announced by Hilschmann and Craig[5] that the light chains of myeloma proteins have two halves each comprising 106 residues which are strikingly different in character[8,9]. Most work has been done with proteins the light chains of which are of κ type, but it seems clear that, apart from differences in the sequence

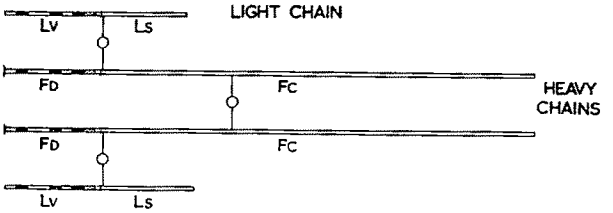


Fig. 1. A modification of the Porter diagram to illustrate the hypothesis of four "variable" sub-chains all of the same pattern

of the C-terminal region related to the L–K antigenic difference[10], all light chains are made up in essentially the same fashion. The C-terminal half of κ-type light chains is identical in the three that have been investigated except for single residue replacements at three points. One of these, No. 189, is of special interest because replacement of leucine by valine is associated with a change in allotype from INVa to INVb (refs. 8 and 11). The N-terminal half, residues 1–106, differs strikingly from one myeloma κ protein to another. Milstein finds an indication in one that even the cysteine residues can be displaced. It is clear (*a*) that the C-terminal half is a typical polypeptide chain controlled by a stable cistron and subject only to rare mutational replacement of single residues, and (*b*) that the N-terminal half must have an entirely different type of genetic control. Yet, because this half is uniform in all myeloma protein molecules produced by the single clone of neoplastic cells, it is evident that there is firm genetic control during at least the productive phase of the disease. It is scarcely conceivable that the controlling sites in the genome are in the same cistron, and for ease of discussion we may speak of Ls (stable, C-terminal) and Lv (variable, N-terminal) sub-chains.

The heavy chain is not so amenable to investigation and no amino-acid residue sequence has been published. It is evident from the work of Meltzer *et al.*[12], Franklin *et al.*[13], and Franklin[14] that the crystallizable fraction Fc is of uniform structure apart from the presence or absence of two peptides which are correlated with the Gma+ or Gmb+ allotype antigens. Tryptic peptide maps of myeloma protein heavy chains show additional spots individual for each protein and therefore presumably carried on the Fd segment. In the comparable position in similar tests on normal IgG heavy chain there is much weak 'background', and this indicates that there is great heterogeneity in these peptides[15]. It seems certain, therefore, that the heavy chain, like the light chain, is composed of a variable Fd and a stable Fc segment. So far as I know, there is nothing to exclude the possibility that Fd and Lv are identical.

Nature of the Combining Site

Appropriate manipulation of fragments and recombined fragments of IgG antibody has shown:

(1) That half molecules containing one light and one heavy chain can bind specifically to antigen but have no precipitating power[16,17]. They can be recombined from two antibodies to produce hybrid antibody molecules never found in Nature[18].

(2) Separated light chains have only very little or no binding power for antigen (hapten). Separated heavy chains usually have definite binding power, but this is greatly increased when homologous light chain is recombined with it[19,20].

(3) Light chains recombine specifically with homologous heavy chains to re-create full biological activity[21]. According to Metzger and Mannik[22], this is more striking if the corresponding antigen is included in the system.

(4) It is possible to investigate the combining site directly by using hapten either to shield the site from iodination, etc., "pair labelling"[23,24], or when appropriately modified to transfer tritium label to amino-acids in the site ("affinity labelling")[25]. Results are consistent in showing

that both light and heavy chains are involved[24,25]. The first group using pair labelling found most of the shielded residues in the light chain, while with affinity labelling both are involved.

(5) In a report which is in the course of being published, Doolittle and Singer[26] present evidence that similar peptides are labelled in both light and heavy chains. The peptides from the two chains concur in the average size of the labelled peptides, their hydrophobic quality and the range of heterogeneity.

There is a relatively simple hypothesis which satisfactorily covers all these findings and which could be tested experimentally. This is that the binding site is composed of a loop in which two identical chains Fd and Lv lie congruently, residue to residue, either parallel or in spiral relationship. This chain is controlled by a specific cistron *V* (variable) and is represented four times in the IgG molecule.

Fig. 2 suggests the disposition of such a duplex chain at one end of the IgG molecule. The primary requirement of a combining site is that it should present a specific adsorptive pattern that is constant in character and uniquely differentiable from the essentially random configurations of portions of other polypeptide chains which make up the surface of the macromolecule. The simplest unique quality that could be applicable to a wide variety of random patterns is that two (or more) strands of identical character should lie in parallel or helical contact with each residue matched with its equivalent residue on the other chain. Many more complex arrangements could be imagined, but the one suggested has the advantage that it can in principle be tested simply by establishing the sequences at the N-terminal end of the light chain and of the Fd section of the heavy chain with due regard to the fact that the N-terminal amino-acid is blocked on this chain[27].

In addition to the findings already noted, this hypothesis accounts for the symmetry and equivalence of the two combining sites, and for the similarity, sometimes very striking[28], of the specificity and avidity of IgM and IgG antibody when the change over from M to G occurs. It also requires a much smaller degree of phenotypic restriction than if the immune pattern is controlled by a number of separate genetic segments and leaves open an approach to the sub-cellular transfer of genetic (immunological) information if future experimental results should establish the occurrence of such processes.

Somatic Mutation as a Source of Diversity

The obvious implication of the hypothesis by which each combining site is composed of two identical chains varying widely from one IgG to another is that they are controlled by a single cistron of quite unusually labile character.

A rough calculation suggests that at birth a child may possess of the order of 3×10^{10} stem cells, thymocytes and lymphocytes which have undergone 32+ cell generations from the fertilized ovum. To ensure that at birth at least one amino-acid replacement from the initial pattern had occurred in all cells would require a mutation rate of $1 : 10^{4}$ per nucleotide per cell generation. This is the same order of frequency as the phage mutation *T2r*$^{+}$ to *T2r* (ref. 29). Even more relevant is the existence of 'hot spots' of high mutability in the phage *T4* (ref. 30), and the fact that with bromuracil the mutability of a given locus may be raised to 10^{4} times the spontaneous rate. The possibility of an unduly high mutation rate in a mammalian cistron cannot therefore be excluded *a priori*.

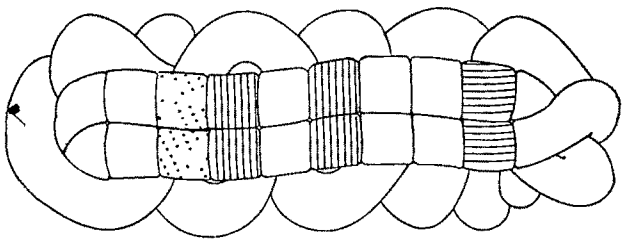


Fig. 2. A diagram to indicate the possible arrangement of a duplex polypeptide as combining site at the end of an IgG antibody. The cross-section of the molecule has been taken as 54 Å × 19 Å and the average effective size of an amino-acid residue as 5 Å.

From an examination of the first accounts of amino-acid sequence in the Lv chain of three myeloma proteins, the results quite closely resemble what is found in comparative investigations of the structure of haemoglobin—including the relation of myoglobin and the individual globin chains (reviewed by Zuckerkandl and Pauling[31]) and of cytochrome *c* (reviewed by Margoliash and Smith[32]). Perhaps the most important impression one gains from this work on species differences in proteins is the random character of the changes that have occurred in the course of evolution. The number of invariant groups in the globin chains, that is, residues constant in position through the whole of the available material, has now fallen to 11 in 150–160, and Jukes[33] has published a table indicating that the number of alternatives known for each site gave a distribution very close to a Poisson one with a mean of 2·1 alternatives per site. Similarly, the lengths of sequences which are invariant in the cytochrome *c* material from yeast to man also show a close approach to a Poisson distribution. In evolutionary changes the important matter is not the fact of mutation but the ability of a given mutation (*a*) to allow adequate function of the cells involved, and (*b*) to replace the previously existent sequence as that characteristic of the species. Only an infinitesimally small number of the mutations of protein sequence that must occur will ever find opportunity, through selective survival, genetic drift and other processes, to become a new standard for the species. Even so, it appears from the pattern of results that random processes predominate.

If it is legitimate to look for somatic mutation at an accelerated rate in cistron V for the source of immune patterns, one would expect, despite the fantastic difference in the time-scales involved, to find essentially similar random changes of amino-acid sequence occurring. Such random changes produced in one way or another are vital to any theory of clonal selection.

The present hypothesis of the origin of immune pattern has at least the virtue of demanding experimental tests. It should be found that the Fd and Lv chains from a given myeloma protein are identical or have considerable segments in common. Available information does not exclude the possibility that the variable portions of light and heavy chains are derived from distinct cistrons and, if there is no identity in pattern found experimentally, a more complex hypothesis will have to be sought. One would prefer, however, not to abandon the simple hypothesis solely on evidence from myeloma proteins, until it is more clearly established that they are structurally equivalent to monoclonal antibodies.

Should the results show a consistent pattern in the variable segments it will become of first-rate importance to look for evidence as to how the variable pattern arose. There have been reports of myeloma proteins showing two spikes on electrophoresis, presumably indicating either a secondary mutation in the primary myeloma clone or a second primary mutation to neoplastic activity. The difference between the two patterns, both presumably derived from the pattern(s) characteristic of the fertilized ovum, should be illuminating. In any such investigation, the existence and extent of phenotypic restriction would be highly relevant. Since the Lyon phenomenon by which, in the female, one of the two *X*-chromosomes becomes non-functional in every cell is the only fully established example of phenotypic restriction at the somatic level, it is perhaps worth considering the possibility that the 'variable' cistron is carried on the *X*-chromosome. For

this reason, a search for diclonal 'gammopathies' in males might be more rewarding than in females.

There seem to be at least three possible origins of diversity: (1) accelerated replacement of single nucleotide pairs as suggested here; (2) intra-cistronic inversions, duplications or deletions as suggested by Smithies; (3) a process equivalent to transformation in bacteria in which the replacing segment of DNA may come either from within the cell or from an active adjacent cell. When further variable chains have been examined for sequence it should become possible seriously to consider these alternatives.

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INTENSITY OF THE GEOMAGNETIC FIELD IN INDIA OVER THE PAST 4,000 YEARS

By R. N. ATHAVALE

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BAKED bricks and pots acquire magnetization as they cool from the high temperatures prevailing inside kilns and hearths to normal temperatures. This effect can be detected later, when it is termed 'thermoremanent magnetization'. The magnetization is in the direction and proportional to the ambient magnetic field of the Earth at the time of firing. Folgheraiter[1] and Koenigsberger[2] suggested that such materials obtained from ancient (archaeological) sites could be used for studying the Earth's past magnetic field. Such an application had to await the development of a satisfactory technique for studying detailed variation in field-strength itself. This was achieved by Thellier *et al.*[3], who compared ancient remanent magnetization in brick samples with that acquired when these samples were heated and cooled in the present magnetic field. This pioneering work led to the interesting observation that 2,000 years ago, the Earth's magnetic field in France was about 60 per cent stronger than it is at present.

In view of the importance of this result to geomagnetic studies and other allied fields, it was considered desirable to conduct similar studies in other areas. India has a geomagnetic latitude spread of 0°–25° N. and also has a large number of archaeological sites, many of which have recently been dated by the Radiocarbon Laboratory at this Institute. In view of these considerations, the present study was undertaken.

The method used was, in principle, the same as that of Thellier *et al.*[3], who mainly used bricks for the purpose. The materials most commonly obtained from archaeological excavations are, however, pots and potsherds. In order to make use of these, a suitable technique had to be devised. Small pieces of pottery or brick were firmly embedded in plaster of Paris inside individual cylindrical porcelain containers (height, 4 cm; diameter, 4 cm). With their orientation thus fixed, the magnetic moment of the sample was measured by means of an astatic magnetometer (sensitivity, 10^{-5} gauss). Each sample was first rotated below the magnetometer along the axis of the cylinder (Z component) and the directions of maximum (X-direction) and minimum (Y-direction) deflexion determined. Proper reference marks were then made and the deflexions along the X, Y and Z co-ordinates measured by the standard procedure which involved making a correction for sample inhomogeneity. The sample concerned was then heated to 300°–400° C with the X-direction aligned with the meridian, cooled and its magnetization remeasured. The sample was then reheated to the same temperature in reverse orientation, cooled and the magnetization measured again. These measurements enable us to obtain partial thermoremanent magnetization in ancient and laboratory fields by addition and subtraction of the vector co-ordinates X, Y and Z in the manner suggested by Thellier[3]. The sample was further heated to 675° C (which is the Curie point for haematite), cooled and its magnetization remeasured. This enabled us to deduce another value for the ratio of the ancient field strength to the present one.

The samples were heated in a non-inductive portable furnace. The heating elements were constructed from 22 gauge platinum wire, wound around quartz tubes with the lead wire of each element passing through the centre of the tube in a direction opposite to that of winding[4]. Ten such elements were mounted symmetrically inside a cement pipe (diameter, 22·5 cm; height, 13 cm). A thick copper vessel (diameter, 16·5 cm; height, 7·5 cm) was placed co-axially inside the furnace. Four samples were heated simultaneously inside this vessel in nitrogen atmosphere. A duplicate assembly made it possible to use the furnace to heat one set of samples while the other set cooled.

The precision of the experimental procedure was determined in the following way. A single pot was heated to 1,000° C in an oxidizing atmosphere for 2 h in order to destroy initial magnetization and to ensure that the constituent minerals remained stable during subsequent heatings, and then allowed to cool. In this way it acquired

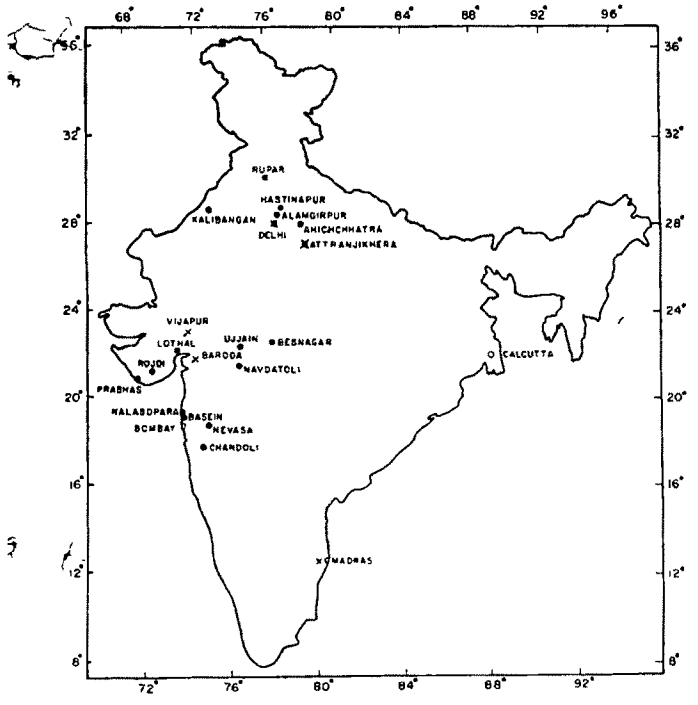


Fig. 1. Map of India showing sample collection sites. ×, Modern samples, ●, archaeological samples

a magnetization corresponding to the laboratory field. Four pieces of this pot were then run through the experimental cycle already described. The ratio of the two magnetizations in the laboratory field thus determined should theoretically be 1·00; the average of eight values deduced from the experiment was 1·03. The scatter in the values corresponded to a standard deviation of 9 per cent, which may be taken as an indication of the magnitude of the overall error of the measurements.

Results on Modern Samples

The basic assumption made in all such archaeomagnetic intensity studies is that the thermoremanent magnetization acquired by baked bricks and pots is proportional to the strength of the Earth's ambient field at the time of firing and that it does not change afterwards. In order to verify the first part of this assumption, we collected samples of modern pottery from five different localities and ran them through the experimental procedure already described. Fig. 1 shows the localities whence the modern samples were collected. Table 1 gives the field strength at the various sites as determined experimentally from measurements on modern pottery and also the value derived from the co-ordinates of the sites from data reported by Vestine *et al.*[5] for 1945 and extrapolating them to 1963. Bearing in mind that the actual field at the site of the hearth could be different from the extrapolated value by a few per cent, agreement between the extrapolated values and the experimental ones (within a standard deviation) is quite satisfactory. This indicates that the method is effective for modern pottery. Our procedure involved the determination of intensity ratios at only two temperatures; however, the elaborate method adopted by Thellier *et al.*[3] involved measurement of ratios at a series of temperatures, thereby making possible more accurate determinations. The precision of our procedure is, however, quite adequate for detecting any variation of large magnitude such as has been observed by other workers[3,6].

Table 1. Results on Modern Samples

Site	Geographical lat. N.	Co-ordinates long. E.	No. of samples	Experimental value of field (G)	Calculated value of field (G)
Delhi	28° 35′	77° 12′	8	0·458 ± 0·045	0·478
Attranjikhera	27° 42′	78° 44′	4	0 461 ± 0 086	0·474
Vijapur	23° 30′	72° 40′	4	0·435 ± 0·020	0·447
Baroda	22° 10′	73° 37′	4	0·437 ± 0 030	0·445
Madras	13°	80°	8	0·428 ± 0 031	0·413

Criterion for Stability of Magnetization of Samples

Only perfectly baked red ware giving a good metallic sound was chosen for the work. Samples showing grey core or giving a dull note when struck were not used, as it was considered that these would most likely contain unstable magnetic minerals. The baking temperature for material thus selected is generally above 700° C and it can be assumed, therefore, that samples chosen on this basis had acquired their thermoremanent magnetization over the entire range of temperature from 675° C (the highest Curie temperature—that of haematite) to the environmental temperature to which they cooled.

For each sample, we obtained two values for the ratio of ancient to laboratory field expressed in terms of the magnetization acquired by the sample; one for an intermediate temperature (partial thermoremanent magnetization) and one for the Curie temperature. In order to ensure that the magnetic minerals in the ancient samples had not undergone any chemical change during the reheating process in the laboratory, a comparison was made between the two ratios. Any archaeological sample the ratio of which differed by more than 30 per cent was considered to contain an excessive amount of unstable minerals and was rejected. Such samples formed only about 15 per cent of the measured samples.

Results on Archaeological Samples

Fig. 1 shows the location of archaeological sites whence the samples were obtained. Table 2 gives the results of magnetic measurements and other pertinent data for these samples. The "ages" of the samples are based on radiocarbon dates and archaeological evidence.

The ratios of the ancient to present fields at the sites, averaged from a set of samples, are given in the final column; the errors quoted are one standard deviation, which indicates the magnitude of scatter in the results. The present field at the sites was estimated by first making use of tables compiled by Vestine *et al.*[5] for 1945 and then correcting the value given to the 1963 one by a factor obtained from 1963 and 1945 values at the Bombay Magnetic Observatory. In Fig. 2 the values deduced for the intensity (normalized to Bombay) are plotted. A considerable random scatter in the data is obvious and no

Table 2. Results on Archaeological Samples

Site	Geographical co-ordinates Lat. N.	Long. E.	No of samples	Age (before present)	Ancient/present fields at site
(1) Alamgirpur	28° 45′	77° 50′	8	350 ± 200	0·82 ± 0·06
(2) Basein Fort	19° 30′	72° 45′	8	420 ± 10	1·00 ± 0 06
(3) Hastinapur	29° 09′	78° 03′	5	700 ± 50	0 96 ± 0·12
(4) Attranjikhera	27° 42′	78° 44′	2	900 ± 150	0·85 ± 0 07
(5) Ahichchhtra	28° 22′	79° 07′	4	975 ± 125	0·87 ± 0 08
(6) Ahichchhtra	28° 22′	79° 07′	4	1150 ± 50	0·87 ± 0·04
(7) Rupar	30° 58′	76° 32′	3	1500 ± 150	0·81 ± 0 07
(8) Ahichchhtra	28° 22′	79° 07′	4	1550 ± 50	0·78 ± 0·08
(9) Ahichchhtra	28° 22′	79° 07′	4	1725 ± 125	0·76 ± 0·04
(10) Ahichchhtra	28° 22′	79° 07′	4	1850 ± 100	0·76 ± 0·08
(11) Prabhas	21°	70° 30′	4	1800 ± 150	0·97 ± 0·12
(12) Nevasa	19° 34′	74° 54′	8	2000 ± 100	0·91 ± 0·08
(13) Attranjikhera	27° 42′	78° 44′	7	2000 ± 150	0·77 ± 0 11
(14) Ujjain	23° 11′	75° 46′	4	2150 ± 100	0·88 ± 0·04
(15) Nalasopara	19° 30′	72° 45′	8	2200 ± 25	1·16 ± 0·11
(16) Hastinapur	29° 09′	78° 03′	2	2200 ± 100	0 86 ± 0·07
(17) Hastinapur	29° 09′	78° 03′	3	2300 ± 100	1 00 ± 0 09
(18) Besnagar	23° 32′	77° 48′	4	2300 ± 100	0·90 ± 0·04
(19) Ahichchhtra	28° 22′	79° 07′	4	2300 ± 100	0·80 ± 0·08
(20) Hastinapur	29° 09′	78° 03′	2	2400 ± 100	0·88 ± 0·07
(21) Hastinapur	29° 09′	78° 03′	2	2500 ± 100	0·79 ± 0·07
(22) Attranjikhera	27° 42′	78° 44′	6	2975 ± 100	0·99 ± 0·24
(23) Chandoli	19°	74°	8	3000 ± 100	0·89 ± 0·20
(24) Prabhas	21°	70° 30′	2	3350 ± 100	0·87 ± 0·07
(25) Navadatoli	22° 11′	75° 36′	12	3600 ± 100	0·91 ± 0 10
(26) Lothal	22° 31′	72° 15′	10	3800 ± 100	0·93 ± 0·13
(27) Kalibangan	29° 25′	74° 05′	6	3800 ± 100	0·75 ± 0·05
(28) Rojdi	21° 51′	70° 54′	6	3900 ± 100	0·70 ± 0 12
(29) Kalibangan Locus-1	29° 25′	74° 05′	3	4200 ± 100	0·80 ± 0 07
(30) Kalibangan Locus-2	29° 25′	74° 05′	2	4200 ± 100	0·67 ± 0·06

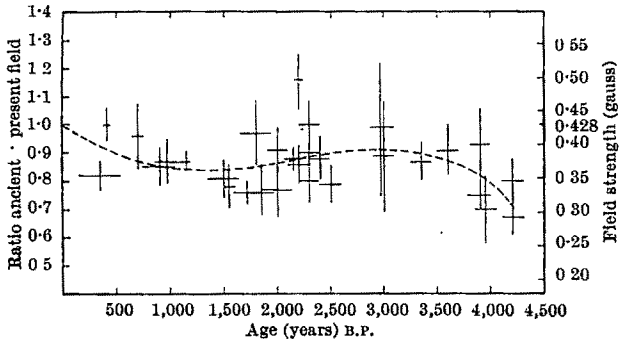


Fig. 2

marked secular variation is indicated. The data show, however, that in the past the field intensity was smaller by about 15 per cent.

We have also drawn a continuous curve through the points based on a least square analysis. If this is seriously considered it may be taken to indicate that the ancient mean field strength over India was about 15 per cent lower than the present 1,300 years ago, about 10 per cent lower 3,000 years ago, and about 30 per cent lower 4,000 years ago.

In determining the intensity ratios we have not taken into account any viscous magnetization which the samples might have acquired since they were first baked. It is known, however, from previous investigations that the magnitude of the error arising from this effect is quite small. Thellier *et al.*[3] have reported that out of the nine different sites selected by them, samples of baked earth from eight had secondary magnetization which was between 0·2 and 2 per cent of the initial magnetic moment. In samples from only one site the contribution due to this factor was 4·6 per cent. It is, therefore, safe to assume that accounting for the effect of viscous magnetization would not significantly alter the results presented here as they were obtained with a technique which had a precision of ± 9 per cent.

Discussion

Results of archaeomagnetic intensity obtained so far can be divided into three categories:

(1) The French[3] and Russian[6] results on baked bricks and clay; these show a gradual increase in the field intensity in the past with a peak value of 1·6 times the present value for 2,000-year-old samples and before this a decline to a value almost the same as at present for 4,600-year-old samples.

The measurements of Thellier *et al.*[3] were made on oriented samples so that the inclination of the field at the time the bricks were fired could also be measured, and a correction made for the measured intensity in order to allow for any variation in inclination with respect to the present one. The application of such a correction changes the absolute value of field-strength by as much as 10 per cent in some of their results.

(2) The Japanese results[6] on basalts and baked pottery; these show the present intensity for 1,000-year-old samples, a sharp increase by a factor of 1·5 for approximately 2,000-year-old basalt samples, and an equally sharp decline to present value for 3,000-year-old samples.

The results on basalts, which contain complex mineralogical constituents, are usually considered less reliable for estimating ambient field strength. Furthermore, these results were for unoriented samples, and were not corrected for any changes in intensity brought about by any secular variation in inclination of the field. Watanabe[7] has carried out extensive work on this aspect in Japan on samples as old as 6,000 years and finds the range of secular variation of inclination to be between 40° and 60° around a mean value of 50°. DuBois[8] reports a similar spread of values between 40° and 60° for the first millennium of the Christian era, for samples from the south-western United States. Using the formula $F = \frac{2M}{R^3(1 + 3\cos^2 I)^{\frac{1}{2}}}$ (where F is the field intensity at the site, I is the inclination, and M and R are the magnetic moment and radius of the Earth), and taking the extreme values of 40° and 60° and average value of 50° for the inclination in Japan, it can be seen that the uncertainty in the estimation of intensity values due to this cause could be as much as ± 12 per cent.

(3) Indian results on baked pottery and brick samples over the past 4,000 years show that the average field was not much different from the present—in fact lower only by about 15 per cent. These results are also for unoriented samples, and for reasons already discussed they may show variation in field intensity to the extent of ± 8 per cent due to possible variation in inclination (the present inclination in this region is about 25°).

There are obvious discrepancies between the three groups of data if the variation is considered to be global in scale. All three results can, however, be reconciled if they depict regional phenomena. Magnetic observatory records over the past 100 years tend to support this contention. They show that the intensity at the Indian and other South East Asian stations has been increasing while that at the European stations has been decreasing. It seems, therefore, that definite conclusions regarding the absolute variation of the dipole moment, averaged over the world, can be made only after data from many other regions are available.

On the other hand, recent measurements of the variation in the activity of carbon-14 produced by cosmic rays over the past few thousand years indicate that some changes in the dipole moment have probably occurred. Damon *et al.*[9] measured the activity of carbon-14 in a number of tree-rings and historically dated wood samples. They found the variation, $\Delta\ ^{14}C$, small (± 1–2 per cent) as far back as 2,000 years B.P., and a systematic increase in the carbon-14 content in the more distant past with a gradient of about 0·4 per cent per century. They give a $\Delta\ ^{14}C$ value of about + 7 per cent for a 4,600-year-old (historical date) Egyptian sample. Recently, Schell[10] has made similar observations based on studies on tree-rings going back 3,100 years. He has also summarized other available data in carbon-14 variation and has shown that they follow the same general trend as already discussed here. If we assume that the variation in carbon-14 activity is mainly caused by changes in the dipole moment, then the increase in radiocarbon production before 2,000 years B.P. would require the average strength of the Earth's magnetic field to be lower than its present value. This is in qualitative agreement with our magnetic data, if they are considered on a global scale.

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OBITUARIES

Prof. F. Debenham

PROF. FRANK DEBENHAM had been a colleague, friend and helper to so many that his death on November 23, 1965, at the age of eighty-one brings sadness to all. His was a life of vigour and indeed of vision, for he constantly looked ahead to new organizations and new methods which should be improvements on those already in being. Into old age he retained his enthusiasms, his friendships and his continued interest and writing on a variety of small but always practical topics in the realms of cartography and survey. Not least he remained fascinated by those frozen fishes which appeared so mysteriously on the surface of the Ross Ice Shelf and which excited such interesting hypotheses about the nourishment of ice shelves by the freezing of sea-water.

Born in Australia in 1883, Debenham was of the right age and training to become geologist to Scott's second Antarctic Expedition between 1910 and 1913. There he did much valuable work and forged the links of friendship with Sir Raymond Priestley and Sir Charles Wright, which later bore such excellent fruit in the foundation of the Scott Polar Research Institute in Cambridge. Intervening war service and wounding in Salonika preceded his return to Cambridge and his appointment in 1919 as Royal Geographical Society lecturer in cartography. There he soon succeeded Philip Lake as reader in geography, and not long afterwards had the distinction of being appointed the first holder of the new chair of geography in the University of Cambridge. His activities were such that by 1936 the new Department had been built and was well on the way towards that distinction which it possessed by the time of his retirement from the chair in 1949.

Yet many will think of 'Deb' primarily as the 'architect' of the Scott Polar Research Institute, of which he was part-founder and director from 1925 until 1946. The death of Scott and his companions returning from the South Pole in 1912 had stirred Britons perhaps more than any other peacetime event in centuries. They rallied nobly to Scott's last appeal to "see that those who are dependent on us are properly provided for". The subsequent Mansion House Fund reached a total of £76 500, a large sum at that time.

When the Mansion House Fund had fulfilled its dual task of providing for dependants and of publishing the scientific reports, there still remained a sum of £15.000 set aside as a 'Polar Research Fund'. It was in the early 'twenties that Debenham, before ever he was professor, put forward proposals which sprang from discussions a decade before in Antarctica with Raymond Priestley and Charles Wright. Their practical idealism was now to bear fruit. Debenham was able to show convincingly that often hard-won experience of polar expeditions in the past had been lost when their members dispersed, and that the techniques of life and travel in cold regions had been inadequately recorded. To ensure that future expeditions might fully profit from the past, on the sure basis of recorded experience, Deb proposed the foundation of an institute for polar research. That was the origin of the Scott Polar Research Institute in Cambridge—first an idea; then a tiny beginning in an attic room in the Sedgwick Museum of Geology in 1920; later, the construction, with the residue of the Mansion House Fund, of a particularly beautiful small building opened in 1934; and gradually the growth of the Institute to its present position of international repute. Deb happily lived to know of the recent munificent bequest from the Ford Foundation which will now allow the Institute, which was so close to his heart, to expand anew some 40 years after he had laid the first foundation.

G. C. L. BERTRAM

Prof. José María Albareda

PROF. JOSÉ MARÍA ALBAREDA died in Madrid on March 27, within a few days of his sixty-fourth birthday. Albareda was born in Caspe. He studied at the University of Madrid, where he graduated in pharmacy, obtaining his doctorate in 1927. He then studied chemistry in the Faculty of Science in Zaragoza and obtained his doctorate in chemistry in 1931. He immediately embarked on his career of soil research, at first under Prof. Rocasolano and Rius Miró in the University of Zaragoza. Between 1935 and 1936 he developed a course in soil science while holding the Conde de Cartagena chair of the Royal Academy of Science of Spain.

He was the first director of the Institute Ramiro de Maeztu of Madrid, and in 1940 was appointed to the chair of geology and zoology in the Faculty of Pharmacy in the University of Madrid, where he remained until his retirement in 1959. He helped towards the development of the new University of Navarra at Pamplona, and was appointed rector in 1960, a position he held at the time of his death. In 1959 he was ordained a priest.

Albareda was a leader in the field of pure and applied research, and with considerable foresight he, in collaboration with various university professors, planned the development of research in Spain, particularly through his service to the Spanish Research Council. After the foundation of the Consejo Superior de Investigaciones Científicas in 1939 he was appointed general secretary, and remained so until his death. Albareda directed much of his attention towards the development of soil science and to the problem of development of agriculture in Spain. He was in charge of the Instituto de Edafología from its formation in 1942 until his death. During this period the work performed by Albareda and his colleagues covered a wide field of soil science and had important practical applications. During the 26 years in which Albareda was general secretary in the Council he built up an extensive research programme with a great number of centres in Madrid and other cities entirely dedicated to research, and he established the foundation of a new era in the history of Spain.

The work of Prof. Albareda received international acclaim and he was elected corresponding, foreign or honorary member of a number of European societies. He was awarded a great number of distinctions, including his election as a member of the Spanish Royal Academy of Science in 1941, of Pharmacy in 1941, and of Medicine in 1952, of the Pontifical Academy of Sciences of the Vatican in 1948, and also of the Royal Academies of Science of Barcelona and Zaragoza.

Prof. George Humphrey

THE death of George Humphrey at the age of seventy-six on April 24 has removed one of the last surviving links in Great Britain with the pioneer psychological laboratory of Wilhelm Wundt at Leipzig. He acquired at Leipzig a

profound knowledge of the methods and the content of the German psychology of his day, which was to serve him as the basis for much of his later work. From Germany he moved to Harvard, where he completed his postgraduate studies under another eminent pioneer in experimental psychology, Raymond Dodge. Humphrey spent most of the next thirty years in North America, settling in 1924 in Canada as professor at Queen's University at Kingston, Ontario. He helped with many of the applications of psychology during the Second World War, and from 1938 to 1944 was successively secretary and president of the Canadian Psychological Association. At Queen's, too, he wrote one of his most important works, *The Nature of Learning* (1933). The experimental demonstration of the phenomenon of habituation in snails, and the discussion of the fundamental importance in all organisms of learning not to respond to stimuli, was a notable contribution to the psychology of learning.

In 1947 Humphrey returned to Britain as first professor of psychology in the University of Oxford. He was then fifty-eight years of age, and it was a bold decision to undertake to establish a school of psychology in a university that had been conspicuously resistant to it. The present size and standing of the Oxford school is a tribute to the skill with which Humphrey laid the foundations. While at Oxford he completed his second major book, *Thinking: Its Experimental Psychology* (1951).

Humphrey retired in 1956 and moved to Cambridge, where he continued to write almost to the end of his life, his last book on *The Chemistry of Thinking*, written in collaboration with C. R. Coxon, appearing in 1963. Right up to the end he campaigned for the need to experiment in psychology. "When any branch of knowledge can experiment, it must experiment, or it will die a dreary death" he maintained in one of his last essays. And this applied, he believed, not only to the conventional field of experimental psychology, but to the more dangerous areas of social psychology. L. S. HEARNSHAW

Mr. H. W. Simmonds

MR. H. W. SIMMONDS died in Auckland, New Zealand, on March 16, 1966, at the age of eighty-nine.

He was born in Kent, but left England in 1902 to go to New Zealand. Throughout his life he was a devoted lover and observer of Nature, his interests covering a wide field of natural history, though the study of insects was his main concern, particularly the biological control of insect pests.

In 1920 he joined the Colonial Service as Government Entomologist in Fiji, and it was during his period of Government service, from 1920 to 1937, that the greatest successes in biological control of insect pests and weeds in Fiji occurred. A team of entomologists—Tothill, Taylor and Paine—successfully controlled three serious pests which threatened the coconut-growing industry of Fiji. Simmonds worked in association with the team throughout, and, in the course of this work, travelled extensively in the South Pacific and the East Indies. One result of his travels was the publication of his bulletin on insect pests and diseases of the coconut palm in Melanesia and Southern Polynesia.

Undoubtedly the two successes in biological control for which Simmonds will be chiefly remembered by the people of Fiji are those which brought about control of the weed Koster's Curse and the insect housefly. In 1930 Simmonds brought back from Trinidad a thrips which was successful in controlling the weed. In 1938 he brought in a predatory beetle from Java which is said to have reduced formidable housefly populations to the reasonable level which exists to-day.

After retirement, Simmonds continued to work in the field of entomology, and in 1938 and 1945 made overseas trips on behalf of the Government of Western Samoa, seeking parasites of the coconut rhinoceros beetle, and in 1945 he introduced the parasite *Scolia ruficornis* to Samoa, where it is now well established. In 1963, when the coconut rhinoceros beetle was first discovered in Fiji, Simmonds joined the Coconut Pests and Diseases Board, with which organization he was still working at the time of his death, most of his time having been occupied with work on *Scolia ruficornis*, which is now established in Fiji.

Simmonds was a talented illustrator of insects, a student of mimicry in insects, an enthusiastic worker for Nature conservation and a devoted and gifted gardener and orchid-grower. He was awarded the O.B.E. for his services while employed by the Government and was made an Honorary Life Member of the Fiji Society in recognition of his work for Nature conservation and the establishment of nature reserves. B. A. O'CONNOR

NEWS AND VIEWS

Theoretical Physics in the University of London: Prof. R. K. Eisenschitz

PROF. R. K. EISENSCHITZ retires this year from the chair of theoretical physics in the University of London at Queen Mary College. He is well known for his contributions to statistical mechanics and the theory of liquids, made over a number of years at the Royal Institution under Sir William Bragg, at University College, London, under Prof. Andrade, and at Queen Mary College. His early career in Germany at the Kaiser Wilhelm Institute was notable for his work with F. London on the quantum-mechanical calculation of van der Waals forces but, like many of the theoretical physicists who moved to Britain, he had begun as an experimenter and worked for a time in industry. In his theoretical studies Prof. Eisenschitz moved naturally from general problems in statistical mechanics and the theory of elastico-viscous properties of matter to the fundamental problems of liquid theory. In recent years he has paid special attention to Kirkwood's approach and to approaches implying ideas of collective motion. More recently he has studied properties of electrons in liquid metals. Many of Prof. Eisenschitz's research students are now in important positions and owe much to his thorough and rigorous training. Before his appointment to Queen Mary College there was no theoretical research school in physics and no theoretical option for final-year undergraduate physicists; the new professor will find about a dozen students in each. Prof. Eisenschitz's many friends and colleagues at the College and in the University will miss his kindly interest and judgment.

Conservation Unchanged

THE British Nature Conservancy, now incorporated into the newly formed National Environment Research Council, seems not to be suffering from the association. The Research Council for the past year has been responsible for the Conservancy, the Geological Survey, the Institute of Oceanography and for research in fisheries and hydrology. Dr. Duncan Poore, the new director of the Conservancy, calls them "uneven bedfellows", but expects to gain from the association through co-operation on research with the other similarly oriented organiza-

tions. Dr. Poore thinks that more paper work may be involved, but noted that the responsibilities of the Conservancy remain the same—to acquire and manage the nature reserves, conduct research and publish information. The Nature Conservancy now has to share its cake with a number of other organizations, but the cake is larger, so Dr. Poore feels "there will be more give and take".

In the year ahead Dr. Poore hopes to place greater emphasis on the interaction of the Conservancy's research with its management of the reserves. The most important projects include research in Scotland on the management of grouse and deer, in Wales on the most productive uses of sheep, and a number of projects on productivity and conservation in conjunction with the seven-year International Biological Programme of the International Council of Scientific Unions. In the past the staff may have been too small to apply satisfactorily the results of research, but Dr. Poore hopes that the Nature Conservancy will improve this aspect of its work, chiefly by acting as a catalyst and stimulating and co-ordinating various bodies to produce guide-lines for future action. One example of such activity is the recent *Report on Broadlands* (Nature Conservancy, 1965).

Industry at Warwick

THE new University of Warwick is doing its best not to be an ivory tower above the car works of Coventry. In an attempt to bring industry into close contact with the University, five associate professors from industrial research have joined the teaching staff on a part-time basis, and further appointments are planned. By bringing men from industry into its teaching, Warwick is following a practice long established in such countries as The Netherlands; in the United Kingdom the practice has spread to the Manchester Institute of Science and Technology, the University of Birmingham and the Imperial College of Science and Technology, but Warwick plans to extend this concept to disciplines other than science, including economics, humanities and the social sciences.

Three appointments have been made in the school of molecular science and two in engineering science. The industrialists will give advice, direct research, and take part in postgraduate teaching through lectures and supervision of research. No courses are being given as yet, but a number are planned for the autumn; they will probably take the form of a series of twelve or fourteen lectures.

Graduate students have been using industrial laboratories, such as those at Bristol Siddeley, since the University opened in 1964 (though undergraduates were not admitted until October 1965), but by establishing a more personal contact the University hopes to increase the use of such facilities. Prof. J. A. Shercliff, head of the School of Engineering Science, plans to involve appropriate industrial research organizations as various divisions in his School are set up. For example, Dr. R. H. Macmillan, the director of the Motor Industry Research Association and one of the new associate professors, will be concerned largely with the automotive engineering aspect of the School of Engineering.

In another field, Mr. Peter Hall, director of the Royal Shakespeare Theatre in Stratford, has been appointed associate professor of drama, and the University hopes to start a drama department working in close collaboration with the nearby Royal Shakespeare Company.

Radiobiology at Harwell

AFTER nearly two decades of existence, the Radiobiological Research Unit of the Medical Research Council at Harwell has grown to be the largest but one of the Council's specialist research units. Yet a recent visit showed that there are still opportunities for the Unit to spread its wings. The facilities, of course, are excellent. The graphite moderated reactor *Beppo* has, for example, made possible a long-term study of the production of mutations in mice by radiation. The Unit is now reaping a rich harvest of results from this work. Accumulated evidence on the overall effects of genetic mutation suggests that the mouse is less mutable than had been thought. One intriguing finding has been that the frequency of specific mutations produced by neutron radiation can differ between males and females.

Goats are being used by the Experimental Pathology Group to simulate something like the reactions which human beings might show to acute whole-body neutron irradiation. Apart from lethal dose experiments, the degree of injury to the gut and bone marrow is being investigated. Death within the first few days after irradiation can usually be attributed to injury of the gut, and later deaths, up to 30 days after irradiation, are considered to be the consequence of injury to bone marrow and other tissues. Death from gut damage seems to result from the release of bacteria in the gut into the general circulation.

Adaptations of Moorhead's method for culturing human lymphocytes have proved a great boon to those studying the effects of X-rays and reactor radiation on human chromosomes. The group under Dr. J. Loutit, director of the Unit, is embarking on a study of the chromosome damage produced in these cells by various types of radiation. Dose response and dose-rate effects seem to be linear for neutron radiation, but curvilinear for γ-radiation.

In the effort of the Radiochemical Group to decrease the uptake of radioactive strontium by dietary supplementation, a number of compounds have been tested. Among the most efficacious are calcium phytate and sodium alginate—the latter reducing the strontium-90 uptake by as much as 75 per cent when given as 5 and 10 per cent additives.

Non-symbiotic Nitrogen Fixation

THERE are so many ways for nitrogen to enter and leave the soil that the difficulties of drawing up a nitrogen balance are formidable. The numerous types of microbial action that may be involved, the variations in the experimental data from different parts of the world, and the serious lack of knowledge of the quantitative aspects of many of the processes, make it a complex problem. This applies particularly to non-symbiotic fixation on which a valuable review entitled "Non-symbiotic Nitrogen Fixation in Soil and Soil–Plant Systems", by A. W. Moore, is presented in *Soils and Fertilizers* (29, 113; 1966). Reliable figures on the rate of accumulation of nitrogen per unit area are still required. However, there is overwhelming evidence that nitrogen is fixed by blue-green algae in rice paddy fields and that the fixation is increased by the presence of the rice plant, a fact of agronomic importance. It is also probable that there is a net gain of nitrogen in other soil–plant systems, such as under forest conditions, but it has not yet been possible to obtain evidence by growing plants satisfactorily in a gas-tight closed system using atmospheres labelled with nitrogen-15. A great many species of micro-organisms have nitrogen-fixing power, and it seems that biological nitrogen fixation is an exothermic reaction, so that a supply of energy is needed only for the growth of the organism. The organism also requires mineral nutrients, adequate moisture, heat and aeration, and probably a suitable *p*H range. It is also probable that 'growth factors' are involved and that there are interactions between nitrogen fixing and non-fixing organisms. There remain the possibilities of nitrogen fixation by higher plant tissues and by litter, and of the part played by plant roots.

Malayan Soil Survey Manual

THE development of agriculture has been accorded a high priority by the Government of the States of Malaya,

and a survey of soil resources is being actively pursued. To standardize techniques and nomenclature and to assist the surveyor, a compact guide to the description and mapping of soils has been produced: *Soil Survey Manual for Malayan Conditions*, by M. L. Leamy and W. P. Panton (Pp. x+226. Ministry of Agriculture and Co-operatives, Malaysia. Division of Agriculture, Bull. No. 119. Kuala Lumpur, 1966. 11*s*. 8*d*.). There are brief descriptions of the geography, parent material, vegetation and physical features of the country, followed by instructions on examining and describing the soil profile. There follow descriptions of mapping units and classification with emphasis on the special difficulties in Malaya presented by inaccessible forest regions, swamps and steep hills. Aerial photographs have been of limited use, but a geological map (1 in. to 12 miles), a rainfall map, and good quality topographic maps are available. The mapping scale of 2 in.–2½ in. to 1 mile has been chosen for reconnaissance survey purposes. About 100 soil series have been accepted as a basis of a register, but classification will remain fluid until more data have been collected. Many of the main series are in the great soil groups: reddish-brown latosols and yellow latosols, ground water laterite, yellow podsolic, organic and alluvial.

Protection for Tuna

ATLANTIC tunas will share with whales some immunity from hunting as a result of a convention on conservation drafted, by the representatives of seventeen nations, at what is called the Conference of Plenipotentiaries on the Conservation of Atlantic Tunas held at Rio de Janeiro in May this year. The convention has been prompted by evidence in recent years that more boats are chasing fewer fish. It applies to the whole of the Atlantic Ocean and adjacent seas, and the hope is that it will maintain the population of tuna and tuna-like fish, defined as the Scombriformes with the exception of the families Trichiuridae and Gempylidae and the genus *Scomber*, at those levels which permit the greatest sustainable catch. The commission that will be responsible for administering the convention when this has been ratified by at least seven countries will be empowered to collect information and to recommend research programmes. It may also, on the basis of the evidence it collects, recommend limits on the rate of catch in the convention area.

Parliament in Britain

ANSWERING questions in the House of Commons on June 16, the Prime Minister, Mr. H. Wilson, announced the Government's decision that responsibility for the aircraft industry should, in due course, go to the Ministry of Technology, together with most of the research and development functions: responsibility for civil aviation would go to the Board of Trade. Responsibility for the shipbuilding industry would also be transferred from the Board of Trade to the Ministry of Technology, thus placing both the aircraft and the shipbuilding industry ministerially with the rest of the engineering industry. Mr. Wilson said the decision was also in line with the Government's declared policy to transfer more and more of its research from defence to civil industry. Responsibility for the supervision of the air corporations and independent airlines would remain with the present Minister of Aviation until decisions had been taken regarding future purchasing programmes of the airline corporations, but all other aspects of civil aviation would be transferred immediately to the Board of Trade.

While it was intended to transfer most of the research and development work conducted by the Ministry of Aviation to the Ministry of Technology, a comprehensive examination was being made to determine to what extent procurement responsibilities of the Ministry of Aviation might more appropriately be transferred to the Ministry of Defence, and this examination could be completed before the Ministry of Technology assumed their new responsibilities. Questioned further as to the future of the Royal Radar Establishment and the other Royal Establishments such as Farnborough, Mr. Wilson said that their importance was growing in the civil field and particularly in electronic developments for export. There was no question of fragmenting the Royal Radar Establishment and he expected that responsibility for it would pass, together with responsibility for the electronics industry, to the Ministry of Technology.

IN a written answer in the House of Commons on June 16, the Minister of State for Education and Science, Mr. G. Roberts, stated that the Patent Office building was transferred from the Board of Trade to the British Museum on April 1, to form, with the Museum's own collection of scientific literature, the nucleus of the National Reference Library of Science and Invention. Temporary accommodation in London has been found for that part of the total collection which could not be housed in the existing Patent Office building, and the Ministry of Public Building and Works was negotiating a site for a permanent building. The library will offer its users an information retrieval service, but it is too early to say what part the library will play in the mechanization of storage and retrieval of information.

IN answer to a question in the House of Lords on June 16, Lord Champion said that the Selective Employment Payments Bill contains a provision that will bring into entitlement for the selective employment premium those establishments certified by the Minister of Technology to be engaged in scientific research relevant to manufacturing activities. This includes independent laboratories working for manufacturing industry. Establishments of this kind do not conform to any set pattern and every case will have to be judged on its merits. The clause is intended to ensure that such organizations receive equitable treatment.

University News: London

DR. A. B. FOSTER, reader in organic chemistry at the University of Birmingham, has been appointed to the chair of chemistry in the Institute of Cancer Research, Royal Cancer Hospital. Dr. N. Lichfield has been appointed to the chair of economics of town planning, tenable at University College. The title of professor has been conferred on the following: Dr. L. S. Bosanquet (mathematics, in respect of his post at University College); Dr. F. F. Heymann (physics, in respect of his post at University College); Dr. J. L. Mongar (pharmacology, in respect of his post at University College).

Massachusetts Institute of Technology

DR. J. P. RUINA, professor of electrical engineering at the Massachusetts Institute of Technology, who has been on leave since September 1964 while serving as president of the Institute for Defense Analyses in Washington, D.C., will return to M.I.T. on July 1, as vice-president for special laboratories. He will be the Institute's senior officer on matters relating to the two national service laboratories the Institute administers for the Federal Government—Lincoln Laboratory and Instrumentation Laboratory. Dr. Thomas K. Sherwood has been appointed as the first to occupy the Lammot du Pont professorship of chemical engineering. Prof. Sherwood is one of the world's leading authorities on fluid flow and mass transfer, and wrote the first significant text in this field, *Absorption and Extraction*, in 1937.

Pennsylvania

DR. CHARLES C. PRICE has been appointed University professor of chemistry in the University of Pennsylvania. University professorships were established at Pennsylvania in 1961 to honour those faculty members who are

the higher frequencies (Table 1) we see that there is excellent agreement over an 80 : 1 range of frequency, thus providing very strong evidence that the radiation has a black-body spectrum. The values from Table 1 are shown plotted in Fig. 1 compared with the spectrum of a black-body at 3° K. Also indicated are the minimum brightness temperatures measured at lower frequencies where the galactic radiation is dominant.

One obvious possibility to account for the excess brightness at 7·35 cm and 3·2 cm was the integrated radiation from a population of hitherto undetected radio sources

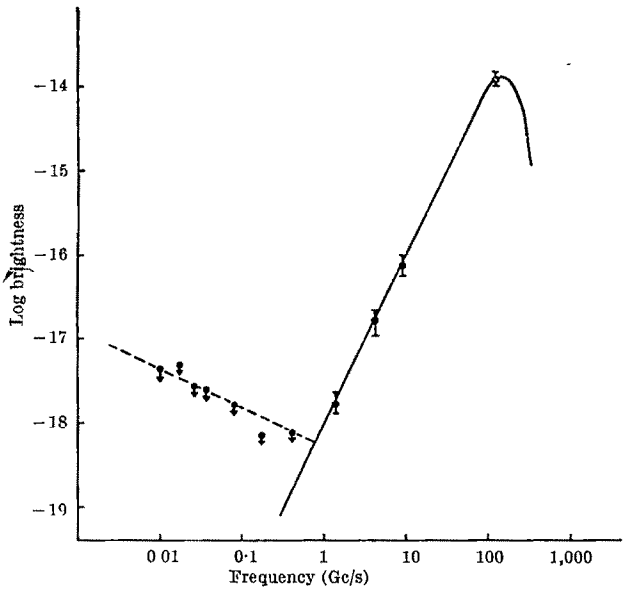


Fig 1. The excess radio brightness, in units of ergs sec^{-1} cm^{-2} (c/s)$^{-1}$ ster^{-1}, is shown plotted against frequency. The solid line is the brightness expected from a black-body at a temperature of 3° K. At frequencies less than about 0·8 Gc/s radiation from the Galaxy becomes dominant

Table 1

Wave-length (cm)	Excess brightness temperature (° K)	Ref.
0·26	2·84 − 3·34	3
3·2	3·0 ± 0·5	2
7·35	3·1 ± 1	2
20·7	2·8 ± 0·6	Present paper

with spectra peaking at centimetric wave-lengths. This is most unlikely to be the explanation of the 20·7 cm excess since Kellermann and Read[19] have carried out a survey of radio sources at 21 cm wave-length in an area of 1 steradian and found no such population. The integrated emission from all the radio sources at this wave-length is thought to be less than 0·1° K.

We thank Prof. M. Ryle, Dr. R. G. Gordon and Mr. F. Sadler for their advice and assistance. One of us (T. F. H.) also thanks the Science Research Council for a maintenance grant.

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NUCLEAR RIBONUCLEOPROTEIN PARTICLES CONTAINING MESSENGER RIBONUCLEIC ACID

By O. P. SAMARINA, A. A. KRICHEVSKAYA and Dr. G. P. GEORGIEV
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IT has been shown that the chromosomes of animal cells contain significant quantities of D-RNA—RNA with DNA-like base composition[1,2]. Chromosomal D-RNA is actively hybridized with homologous DNA, and stimulates the incorporation of amino-acids in a cell-free system[4] and the biosynthesis of specific proteins in non-damaged cells[5]. It would seem, therefore, that chromosomal D-RNA contains the newly formed cellular *m*RNA. In hybridization experiments, cytoplasmic RNA competes with chromosomal D-RNA[6]. Thus at least part of the latter is transferred after the synthesis into the cytoplasm.

We endeavoured to obtain information about the nuclear structures containing D-RNA as it was felt that this might help to establish the state of nascent *m*RNA. Moreover, such information would throw some light on mechanisms of the removal of *m*RNA from DNA-matrix and its transport to centres of protein synthesis.

Nuclei were isolated from Ehrlich carcinoma cells with the aid of osmotic shock and from rat livers by the sucrose-glycerophosphate method. A 'nuclear pellet' was obtained from five rat livers, or 50 ml. of ascites tumour, and was successively extracted by 2–3 ml. portions of 0·14 M sodium chloride–0·01 M *tris*–0·001 M magnesium chloride (STM) at *p*H 7·0 and 7·8–8·0. The first extract obtained at *p*H 7·0 contained a mixture of *r*RNA (nuclear sap ribosomes), *s*RNA and traces of *m*RNA. On the other hand, the newly formed high molecular weight RNA of the second and third extracts, obtained at *p*H 7·8–8·0, was similar to DNA (*m*RNA).

These extracts were layered over sucrose gradients and ultracentrifuged. Two peaks of ultra-violet absorbing material were obtained which had sedimentation coefficients of 30*S* and 4*S*. The main part of radioactive *m*RNA was found in the 30*S* peak (Figs. 1 and 2). Using this method about two-thirds of the newly formed chromosomal *m*RNA could be obtained as the 30*S*-peak.

The data obtained indicate that the 30*S*-peak is an *m*RNA-protein complex. The presence of protein was evidenced by: (1) a relatively low D_{260}/D_{280} ratio of peak material—it is equal to 1·45–1·5; (2) the decrease in the sedimentation constant of *m*RNA after deproteinization of extract by SDS-phenol treatment: 12–18*S* instead of 30*S* (Fig. 1); (3) when amino-acid incorporation

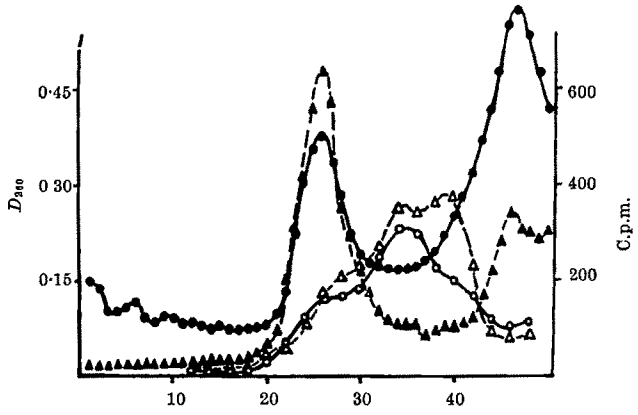


Fig. 1. Sedimentation profiles of nuclear extracts containing *m*RNA and of RNA isolated from these extracts. $Na_2H^{32}PO_4$ was injected intraperitoneally into mice with Ehrlich ascites carcinoma (50 μc per animal) 2 h before they were killed. Carcinoma nuclei were isolated by osmotic shock and extracted once by STM, *p*H 7·0, and twice by STM, *p*H 7·8. The second and third extracts were combined. Half of it was deproteinized by phenol–0·5 per cent sodium dodecylsulphate and RNA was isolated. Nuclear extract and isolated RNA (2 ml. portions) were layered over 25 ml of 15–30 per cent sucrose gradients (in STM, *p*H 7·0) and ultracentrifuged during 12 h at 25,000 r.p.m. Seven or fourteen drop-fractions were collected and analysed by their ultra-violet absorption and radioactivity. ●——●, Ultra-violet absorption of extract; ▲ - - - ▲, radioactivity of extract; ○——○, ultra-violet absorption of isolated RNA, △- - - - △, radioactivity of isolated RNA

into protein was investigated, the radioactive protein was observed in the same 30*S*-peak (Fig. 2). Thus the significant part of chromosomal *m*RNA is revealed as part of special ribonucleoprotein particles.

To elucidate the nature of particles observed, the following experiments were performed. The unfractionated[1,2] chromosomal *m*RNA or the main 18*S*-peak of chromosomal *m*RNA (obtained by means of gradient ultracentrifugation) were mixed with nuclear extracts in STM, *p*H 7·0. The mixtures were layered over a sucrose gradient and ultracentrifuged. In both cases some part of *m*RNA was degraded, but a considerable quantity of labelled *m*RNA, sometimes about 70–80 per cent, was revealed in the 30*S*-peak. If the RNA of the nuclear extract was labelled with adenine-^{14}C and free *m*RNA with phosphorus-32, both labels coincided (Fig. 3). Thus it is possible *in vitro*

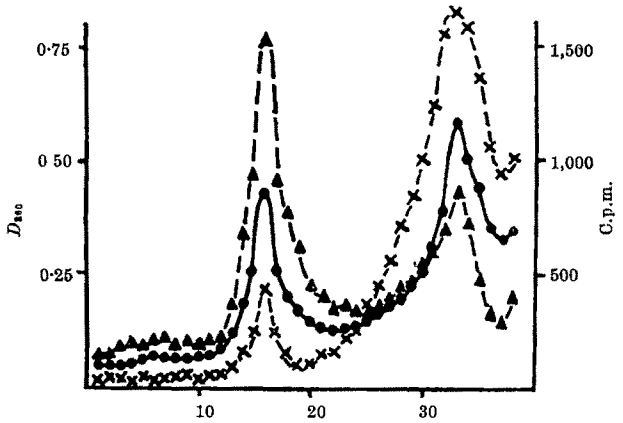


Fig. 2. Demonstration of labelled protein in 30*S*-peak. Rats were treated with $Na_2H^{32}PO_4$ (1 mc. per animal) and carbon-14 labelled protein hydrolysate (200 μc. per animal), after 2 h the animals were killed and liver nuclei isolated by the sucrose-glycerophosphate method (see ref. 2). Combined second and third extracts of nuclei by STM, *p*H 7·8, were ultracentrifuged in sucrose gradients (conditions as in Fig. 1). After determination of the ultra-violet absorption, each fraction collected was divided into two portions. One was treated with 5 per cent trichloroacetic acid for 30 min at 90° C in the presence of a carrier protein and then used for the determination of carbon-14 labelled protein in a scintillation counter. Both portions were washed on 'Millipore' filters and counted. ●——●, Ultra-violet absorption, × - - - - ×, carbon-14 counts (labelled protein); ▲ - - - - ▲, phosphorus-32 counts (labelled RNA)

to obtain 30*S* *m*RNA protein typical for nuclear extracts. It is interesting that nuclear extracts can bind the relatively large quantity of *m*RNA added (equal to about one-third to one-half the RNA already present in the 30*S*-peak.

A number of questions follow these findings:

(1) Is this reaction specific to *m*RNA? To answer this, the interaction of 18*S*-*m*RNA and 18*S*-ribosomal RNA with nuclear extracts was compared. In both cases some part of the radioactive material was displaced into the heavier zone, but only in the case of 18*S* *m*RNA was the radioactive 30*S*-peak observed (Fig. 3). It can be concluded that the type of interaction described is in fact specific for *m*RNA.

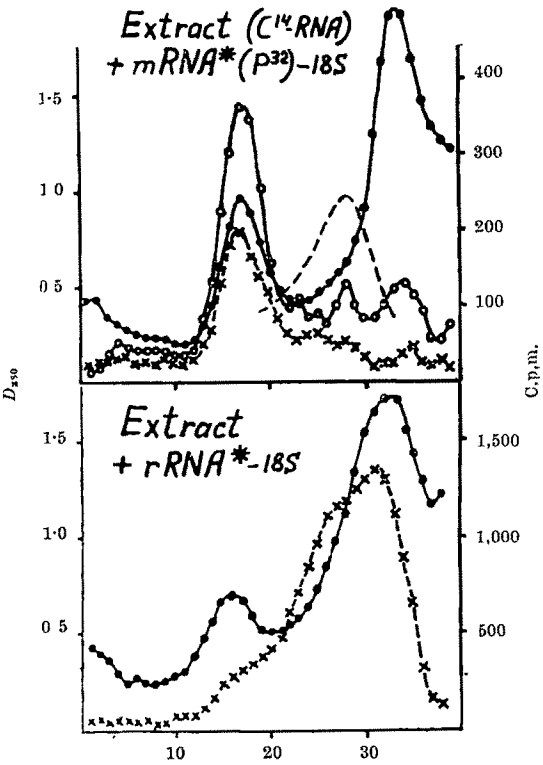


Fig. 3 Interaction of free RNAs with nuclear extracts. Rat liver chromosomal *m*RNA and cytoplasmic *r*RNA labelled with phosphorus-32 (over 12 and 36 h) were isolated and fractionated by sucrose gradient centrifugation. 18*S*-components of both fractions were collected, dissolved in STM and added (50 μg) to nuclear extracts obtained from rat livers, of animals administered with adenine-^{14}C (0·3 mc. per animal) 2 h before killing. The mixtures were ultracentrifuged in sucrose gradient (conditions—see Fig 1). Carbon-14 and phosphorus-32 counts were differentiated with the aid of filters ●——●, Ultra-violet absorption; ○———○, radioactivity of extract (^{14}C-RNA). × - - - ×, radioactivity of added RNA (*m*RNA-^{32}P or *r*RNA-^{32}P); - - - - , sedimentation profile of free 18*S*-*m*RNA (obtained in separate experiment) The profile of 18*S*-*r*RNA is similar

(2) What is the mechanism of this interaction? Are *m*RNA molecules bound to already existing 30*S*-particles or is the formation of *m*RNA protein due to the combination of *m*RNA and proteins of lower molecular weight? In one experiment the excess of *m*RNA-^{32}P was mixed with nuclear extracts labelled with ^{14}C-amino-acids. The appearance of phosphorus-32 in the 30*S*-peak indicated the formation of *m*RNA-protein complex. The quantity of bound RNA was equal to ~60 per cent of the RNA which already existed in the 30*S*-peak. At the same time there was no enlargement of labelled protein in the 30*S*-peak (Fig. 4). In the second experiment, nuclear extracts were ultracentrifuged; fractions coinciding with the 30*S*-peak, 4*S*-peak and 6–25*S* zone were collected and dialysed. To each of the fractions was added the labelled free *m*RNA 18*S*-peak fraction. The

mixtures were recentrifuged and analysed (Fig. 5). Only with the 30*S*-peak was the labelled *m*RNA revealed as 30*S*-material. In other cases the sedimentation constant of *m*RNA was not significantly changed. Thus both types of experiment indicate that *m*RNA is bound to already existing 30*S*-particles and does not react with smaller protein molecules.

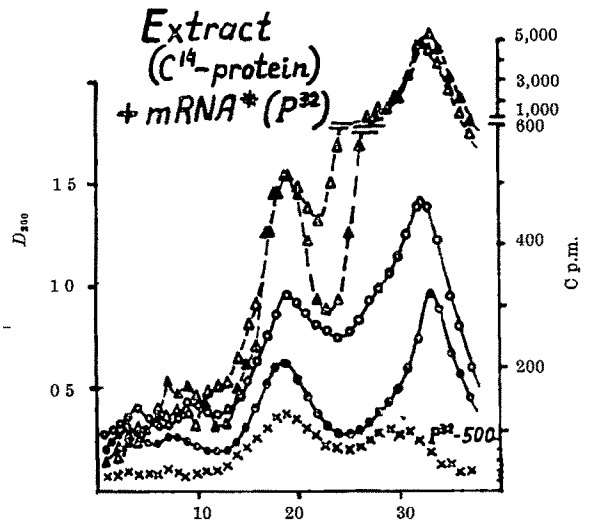


Fig 4. Interaction of *m*RNA with carbon-14 labelled proteins of nuclear extracts. Nuclear extracts of rat livers labelled with carbon-14 protein hydrolysate (200 μc per animal for 12 h) were mixed with excess of chromosomal *m*RNA (500 μg), labelled with phosphorus-32, and ultracentrifuged in sucrose gradient. The control extracts, containing the same quantity of material but without extra *m*RNA. were also investigated in separate tubes. Radioactivity of phosphorus-32 and carbon-14 was determined in separate portions, treated by cold (^{32}P-RNA) and hot (^{14}C-protein) trichloroacetic acid correspondingly. Carbon-14 was counted in a scintillation counter. ●——●, Ultra-violet absorption of control extract; ▲ - - - - ▲, radioactivity of control extract (carbon-14); ○——○, ultra-violet absorption of extract, mixed with *m*RNA; △ - - - - △, radioactivity of extract mixed with *m*RNA (carbon-14); × × ×, radioactivity of *m*RNA (phosphorus-32) added

(3) What is the significance of 30*S* *m*RNA-protein? Is it an already existing structure or an artificial one? Just as in the other cases concerning cellular nucleoproteins, these questions are not easy to answer. However, some indirect evidence may be mentioned in support of the theory of the pre-existence of the nuclear *m*RNA proteins observed. As has already been shown, actinomycin inhibited not only RNA synthesis but also *m*RNA transport. If the cells were incubated with phosphorus-32 and then treated with actinomycin, the main part of newly formed *m*RNA was retained in the chromosomes, and was not transferred into the cytoplasm[2]. In these conditions 30*S*-*m*RNA-protein is completely absent in nuclear extracts (Fig. 6). On the other hand, significant quantities of *m*RNA were observed in the nuclear residues obtained by exhaustive extraction of nuclei by STM-solutions. It is possible that 30*S*-*m*RNA protein is a type of nucleoprotein in which *m*RNA is transported from the nucleus into the cytoplasms—the inhibition of the latter process can be correlated with the disappearance of 30*S*-particles. It is interesting to note that the significant part of cytoplasmic *m*RNA is also not bound to ribosomes and is sedimented under similar centrifuge forces[7–9].

Our experiments indicate that *m*RNA newly formed in the cell nucleus is an integral part of special nucleoprotein particles with a sedimentation constant of about 30*S*. The mode of complex formation is specific binding of *m*RNA to already existing 30*S*-particles. Some indirect data make it possible to suggest that these *m*RNA-proteins are a transport form of *m*RNA. A further question concerns the nature of 30*S*-particles. They are not complexes of DNA, RNA polymerase and nascent RNA, since treatment of the extract with DNase does not

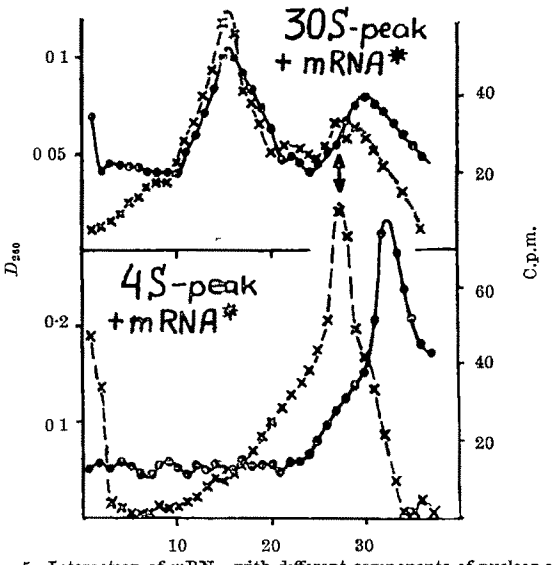


Fig 5. Interaction of *m*RNA with different components of nuclear extracts Non-labelled nuclear extracts were ultracentrifuged and analysed. 30*S* peak and low-molecular material (4*S*) were collected, dialysed against STM, *p*H 7·0, mixed with 18*S* *m*RNA (25 μg), labelled with phosphorus-32 and repeatedly ultracentrifuged in sucrose gradient. ●——●, Ultra-violet absorption; × - - - - ×, radioactivity. Arrow indicates the position of peak of free *m*RNA

change the properties of the 30*S*-peak fraction. One possibility is that 30*S*-particles are small ribosomal sub-units or the precursors of small ribosomal sub-units with a somewhat lower sedimentation constant. It was postulated that ribosomes may play a part in the liberation of *m*RNA from the enzyme-matrix-product complex of chromosome and transport of *m*RNA into the cytoplasm[10]. Lerman *et al.* suggested that whole ribosomes do not participate in this process, but instead their smaller sub-units[11].

30*S*-sub-units may bind chromosomal *m*RNA, transfer it to the cytoplasm and there, after addition of 50*S*-ribosomal sub-units, initiate protein synthesis. But as yet there are no conclusive data to prove this hypothesis.

Another possibility is that the 30*S*-particles observed are the nucleoprotein particles of a new, as yet unknown, type and that they are concerned with the transport of *m*RNA from the chromosomes to ribosomes.

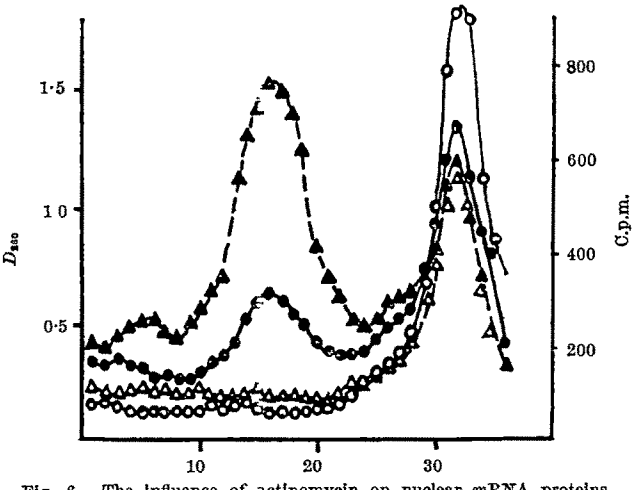


Fig. 6. The influence of actinomycin on nuclear *m*RNA proteins. Rats were treated with phosphorus-32 (1 mc. per animal) and after 1·5 h half the animals were killed (control) and the other half treated with actinomycin ('Aurantin', 1·5 mg per animal). The latter group was killed 3 h after the actinomycin had been administered. Nuclear extracts were obtained from rat livers of both groups and ultracentrifuged in sucrose gradient ●——●, Ultra-violet absorption of control extract; ▲ - - - - ▲, radioactivity of control extract; ○——○, ultra-violet absorption of extracts from actinomycin-treated animals; △ - - - - △, radioactivity of extracts from actinomycin-treated animals

Further experiments, needed to clear up this question, are now in progress.

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BIOSYNTHESIS OF THE PHYTOSTEROL SIDE CHAIN

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PHYTOSTEROL biosynthesis has become increasingly investigated during recent years[1–11], and one aspect which is at present receiving much attention is the introduction of the supernumerary group at C-24 of the sterol side chain. Several years ago the additional methyl group in ergosterol was shown to arise from methionine[11], and it has recently been established that the extra ethyl group of β-sitosterol[8] and α-spinasterol[9] and the ethylidene group of fucosterol[10] also arises from methionine by a double transmethylation. A transfer of an intact ethyl group from a donor such as ethionine does not occur[7,8]. A mechanism for the introduction of the methyl groups into phytosterols proposed by Castle, Blondin and Nes[8] is shown in Fig. 1.

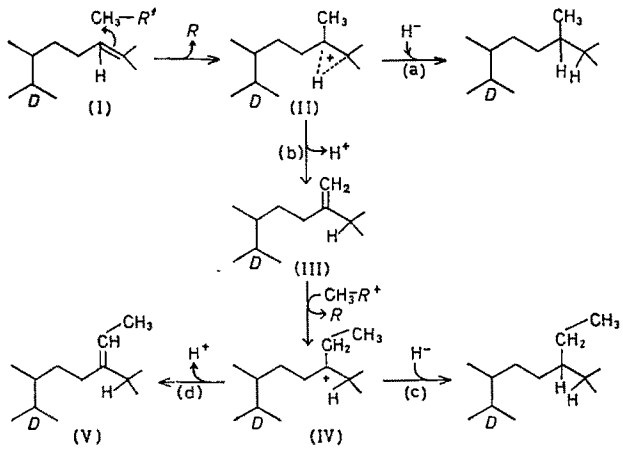


Fig. 1

Results obtained with [^{14}C,^{3}H-methyl]methionine[11] were considered to indicate the intact transfer of the methyl group as previously observed during methyl transfer to oxygen[12] and nitrogen[13]; this would be in accord with route (*a*) in Fig. 1. A recent re-examination of the problem has shown this conclusion to be in error[14]. Ergosterol biosynthesized by *Neurospora crassa* in the presence of [D_3-methyl]methionine was found by mass spectrometry to contain only two deuterium atoms. The production of a C-24 methylene steroid as intermediate (III, route *b*, Fig. 1) followed by reduction to a C-24 methyl steroid would account for this result, but a number of other possible mechanisms have also been proposed[15].

No experimental information is available to indicate the mechanism by which the ethyl group found in sterols such as β-sitosterol is introduced. Circumstantial evidence for the involvement of a C-24 ethylidene steroid is provided by the occurrence of 4α-methyl-$\Delta^{7,24(28)}$stigmastadien-3β-ol (citrostadienol, 24-ethylidene-lophenol) together with β-sitosterol, the major sterol, in citrus fruit[16] and potato leaves[17] and also by the presence of both fucosterol and β-sitosterol in pollen[15]. To provide experimental evidence that C-24 ethylidene steroids are intermediates in C-24 ethyl steroid biosynthesis we used [^{14}C,^{3}H-methyl]methionine. An isotope effect with tritium has been reported in chemical and biochemical reactions[18–20] so caution must be exercised in the design and interpretation of experiments which involve tritium. The results just discussed with [^{14}C,^{3}H-methyl]methionine indicated that 86–91 per cent of the tritium was retained in ergosterol biosynthesis[11]. However, if one hydrogen atom is lost, as indicated by investigations with [D_3-methyl]-methionine[14], then only 67 per cent of the tritium should be retained. The discrepancy between the two results has been ascribed to an isotope effect[15] in which carbon–tritium bonds are less readily broken than carbon–hydrogen bonds, with a consequent enrichment of the ergosterol in tritium. In an attempt to eliminate this source of error we adopted a comparative approach. [^{14}C,^{3}H-methyl]-methionine (prepared by mixing [^{14}C-methyl] and [^{3}H-methyl]methionine supplied by the Radiochemical Centre, Amersham) was incorporated biosynthetically into different steroids containing respectively a C-24 methylene, methyl, ethylidene or ethyl group. The radioactivity of the isolated steroids was determined in a Packard 'Tri-carb' liquid scintillation spectrometer and the tritium: carbon-14 ratio calculated[21].

The C-24 methylene steroid chosen was eburicoic acid[22], which was isolated from a culture of *Polyporus sulphureus* following incubation for 8 days with [^{14}C,^{3}H methyl]methionine. The tritium : carbon-14 ratio of the eburicoic acid was compared with that of ergosterol biosynthesized by *Blakeslea trispora* grown for 4 days in the presence of a sample of the same batch of [^{14}C,^{3}H-methyl]methionine. The tritium : carbon-14 ratios obtained were in good agreement (Table 1), which means that in this case the comparative approach is sound; it is known that one methyl hydrogen is lost in the biosynthesis of ergosterol[14], and because of its structure one methyl hydrogen must be lost from eburicoic acid. The major sterols of excised maize leaves incubated with a sample of the same methionine also had a tritium : carbon-14 ratio close to that observed for eburicoic acid and ergosterol (Table 1). Gas liquid chromatography (3 per cent *SE*-30) revealed that the maize sterols were a mixture of β-sitosterol (45 per cent), stigmasterol (30 per cent) and campesterol (25 per cent), which could not be separated on thin-layer chromatography. Accordingly, the mixture was subjected to ozonolysis and the ethyl isopropyl acetaldehyde derived from the side chain of

Table 1. TRITIUM : CARBON-14 RATIOS OF STEROIDS BIOSYNTHESIZED IN THE PRESENCE OF THE SAME SAMPLE OF [^{14}C,^{3}H METHYL]METHIONINE

Steroid	Source	Observed tritium. carbon-14 ratio
Eburicoic acid	*Polyporus sulphureus*	3·93 : 1
Ergosterol	*Blakeslea trispora*	3·75 : 1
β-Sitosterol, stigmasterol and campesterol mixture	Maize	3·73 : 1
Ethylisopropylacetaldehyde	Ozonolysis of the maize sterol mixture	3·78 : 1

stigmasterol isolated by steam distillation. This had the same tritium : carbon-14 ratio as the original sterol mixture and eburicoic acid and ergosterol (Table 1). It was important to examine a C-24 ethyl sterol if possible and to compare it with a C-24 ethylidene sterol, such as fucosterol. A survey of plant sterols by gas liquid chromatography revealed that about 95 per cent of the total sterols in leaves of larch (*Larix decidua*) collected shortly after their opening in the spring was β-sitosterol; minor amounts of campesterol and a sterol with a relative retention time the same as fucosterol on 'Hi-EFF 8*B*'[23] were detected, but no stigmasterol was observed. A recent report has also indicated the general predominance of β-sitosterol in conifers[24]. The same type of experiment was, therefore, carried out on larch leaves. Portions (1·0 g) of chopped 10–20 day-old leaves were incubated in conical flasks with 1·0 ml. of a solution containing [^{14}C,^{3}H-methyl]methionine. The flasks were incubated at 25° C with illumination provided by two 'Cryselco' daylight emission lamps (2 ft., 20 W) placed about 25 cm above the flasks. This method gave good incorporation of radioactivity into the non-saponifiable lipid. Samples of the same batch of methionine, which was different from that used in the previous experiments, were incubated with *Fucus spiralis*. Portions of chopped *Fucus spiralis* (2·0 g) were moistened with 2·0 ml. of the [^{14}C,^{3}H-methyl]-methionine solution, to which 2·0 ml. of "double strength" sea water (obtained by boiling a sample of sea water to half its original volume) were added. Incubations were at 25° C as for larch. Fucosterol was isolated as the main sterol and counted. To complete the comparison, samples of the same batch of [^{14}C,^{3}H-methyl]-methionine were incorporated into ergosterol by yeast (*Saccharomyces cerevisiae* N.C.Y.C.667) and into maize sterols under the same experimental conditions as described for larch. At the end of the incubations the non-saponifiable material was extracted from each incubate; the appropriate authentic sterol (5·0 mg) added as carrier (β-sitosterol in the case of maize) and the sterols precipitated as their digitonides. After regeneration the sterols were isolated by chromatography on alumina and assayed for radioactivity. Samples of the sterols were further purified by thin-layer chromatography and by recrystallization (four times) from chloroform-methanol after the addition of carrier sterol. The results obtained (Table 2) show good agreement for the tritium : carbon-14 ratios in the various sterols, irrespective of the method of purification.

The similarity of the tritium : carbon-14 ratios of fucosterol and ergosterol indicates that the four hydrogens of the ethylidene group of fucosterol are derived from the two methyl groups of methionine. Since the tritium : carbon-14 ratio of β-sitosterol was also in close agreement with the ratio observed for ergosterol and fucosterol this in turn suggests that only four of the five hydrogen atoms present in the ethyl group of β-sitosterol are derived from methionine. Thus, during alkylation in β-sitosterol biosynthesis, two hydrogen atoms appear to be lost from the introduced methionine methyl groups. These results are compatible with the intermediate production of C-24 methylene (III) and C-24 ethylidene (V) steroids by a mechanism envisaged in routes (*b*) and (*d*) of Fig. 1. The route shown in Fig. 2, while mechanistically acceptable, appears to be eliminated by the observed tritium : carbon-14 ratio of β-sitosterol; further, the loss of a methyl hydrogen atom during ergosterol biosynthesis[14] is not in accord with Fig. 2.

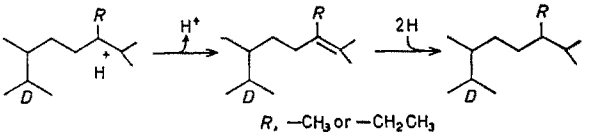


Fig. 2

Fig. 2 and route (*c*) of Fig. 1 would both require that five hydrogen atoms from methionine are retained in β-sitosterol. The present results tend to show that only four hydrogens are retained, but it is recognized that if a very considerable hydrogen isotope effect exists it might be difficult to differentiate between tritium : carbon-14 atomic ratios of 4 : 2 and 5 : 2, when the ratios of fucosterol and β-sitosterol are compared. Preliminary experiments designed to evaluate the extent of the hydrogen isotope effect in respect to the present problem indicate that it may not be so great as to invalidate our conclusions. Also it is hoped that the incorporation of [D_3-methyl]methionine into a C-24 ethyl steroid will further provide confirmation of our results.

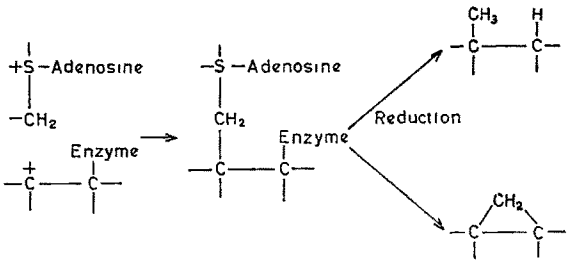


Fig. 3

Lederer[15] suggested that the introduction of the methyl group into ergosterol might involve an intermediate ylide derived by oxidation of S-adenosyl methionine (Fig. 3). This mechanism can be extended to explain the formation of ethyl and ethylidene derivatives; in this case the tritium : carbon-14 ratio would be the same as expected if Fig. 1 (routes *b* and *d*) were operative. Thus

Table 2. TRITIUM : CARBON-14 RATIOS OF STEROLS BIOSYNTHESIZED IN THE PRESENCE OF THE SAME SAMPLE OF [^{14}C,^{3}H METHYL]METHIONINE

Sterol	Source	Incubation time (h)	Observed tritium : carbon-14 ratios of sterols purified by: Digitonide precipitation	Thin-layer chromatography	Recrystallization
Ergosterol	Yeast	24	4·24 : 1	4·28 : 1	4·26 : 1
Fucosterol	*Fucus spiralis*	24	4·36 : 1	4·40 : 1	4·40 : 1
β-Sitosterol	Larch	24	4·36 : 1	4·30 : 1	4·30 : 1
		6	4·34 : 1	4·33 : 1	—
β-Sitosterol, stigmasterol and campesterol mixture	Maize	6	4·25 · 1	4·28 : 1	—

the experimental evidence available at present does not allow a definite choice to be made between these two mechanisms. However, the structure of *cyclo*laudenol could be cited as support for Fig. 1. As shown in Fig. 4 the carbonium ion, II, produced by addition of a methyl group to the double bond of I (for example, *cyclo*artenol) may be stabilized by loss of a proton not only as indicated to produce a C-24 methylene steroid III (for example, 24-methylene *cyclo*artenol) but also as shown to give a C-25 methylene steroid VI (for example, *cyclo*laudenol). This has an analogy in carotenoid biosynthesis in which a common carbonium ion can be stabilized by loss of a proton from either adjacent carbon atom to form a β-ionone or α-ionone residue[25]. Determination of the tritium : carbon-14 ratio of *cyclo*laudenol biosynthesized in the presence of [^{14}C,^{3}H-methyl]methionine or mass spectrometry of *cyclo*laudenol biosynthesized from [D_3-methyl]methionine would provide experimental evidence for the involvement or otherwise of an ylide derived from methionine.

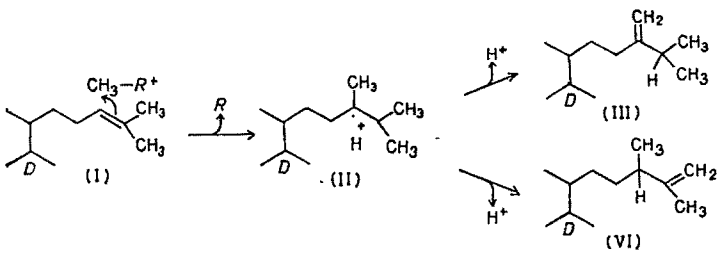


Fig. 4

The mechanism outlined in Fig. 1 requires that the introduction of the methyl group at C-24 will be accompanied by a migration of the hydrogen originally present at C-24 to C-25 in the C-24 methylene steroid produced. Moreover, during the second transmethylation step this hydrogen atom will remain at C-25 in both C-24 ethylidene and C-24 ethyl steroids. The synthesis of [4*R*-4-$^{3}H_1$-2-^{14}C]mevalonic acid has made it possible to test this experimentally. Cornforth, Popják and co-workers[26] showed that [4*R*-4-$^{3}H_1$-2-^{14}C]-mevalonate is incorporated by liver preparations into squalene without loss of tritium. Following squalene cyclization into lanosterol, one tritium atom from C-9 is eliminated to give a tritium : carbon-14 atomic ratio of 5 : 6. Subsequent modification of the lanosterol results in the loss of two more tritium atoms and one carbon-14 atom to give cholesterol with a tritium : carbon-14 atomic ratio of 3 : 5. As one of the tritium atoms retained in cholesterol is at C-24 of the side chain then, in phytosterols biosynthesized from [4*R*-4-$^{3}H_1$-2-^{14}C]mevalonate, the tritium: carbon-14 ratio would be the same as in cholesterol (3 : 5) or 2 : 5 depending on whether the hydrogen at C-24 is or is not retained during alkylation. Experiments with pea sterols (β-sitosterol, stigmasterol and campesterol)[27] and ergosterol in *Aspergillus fumigatus*[28] gave the same tritium : carbon-14 atomic ratio (3 : 5) as found in cholesterol[26], which showed that the tritium originally at C-24 before alkylation was retained. But these experiments provided no evidence for the possible migration of the hydrogen atom to C-25. Evidence for this movement has been provided by the biosynthesis of fucosterol from [4*R*-4-$^{3}H_1$-2-^{14}C]mevalonate by *Fucus spiralis*[29]. Chopped portions of *Fucus spiralis* were incubated with the doubly labelled mevalonate under conditions similar to those given above for the methionine experiment. Incubations were for 11 h at 25° C for experiment 1 and 24 h at 20° C for experiment 2. Fucosterol was isolated from the non-saponifiable lipid following the addition of 5 mg of authentic fucosterol by digitonide precipitation and alumina chromatography. Samples were further purified by silica gel thin-layer chromatography and crystallization from chloroform-methanol. Squalene was obtained by alumina chromatography and thin-layer chromatography. Determination of the tritium : carbon-14 ratios (Table 3) showed that no tritium was lost during squalene biosynthesis in *Fucus spiralis* as found previously in liver[26], peas[27] and *Aspergillus fumigatus* Fresnius[28]. The tritium : carbon-14 ratio observed in fucosterol indicated that three tritium atoms had been retained and was the same as that found in cholesterol[26], pea sterols[27], and ergosterol[28]. As no tritium can be present in fucosterol at C-24, a migration to C-25, or less likely to C-23, must have occurred.

Table 3. INCORPORATION OF [4*R*-4-$^{3}H_1$-2-^{14}C]MEVALONATE INTO SQUALENE AND FUCOSTEROL BY *Fucus spiralis*

	Observed tritium : carbon-14 ratio	Corrected tritium : carbon-14 ratio*
[4*R*-4-$^{3}H_1$-2-^{14}C]Mevalonate	5·33 : 1	1 : 1
Liver squalene†	5·34 : 1	6 6
Experiment 1.		
Squalene	5·37 : 1	6·05 . 6
Fucosterol (purified by thin layer chromatography)	3·28 : 1	3·08 : 5
Fucosterol (crystallized)	3·06 : 1	2·87 : 5
Experiment 2.		
Squalene	5·24 : 1	5·90 . 6
Fucosterol (purified by thin layer chromatography)	3 36 1	3·15 : 5
Fucosterol (crystallized)	3 36 1	3·15 : 5

* The corrected tritium: carbon-14 ratio was calculated assuming that the tritium: carbon-14 ratio of the original mevalonate was 1 : 1 (ref. 28).
† The sample of liver squalene was kindly provided by Dr. Popják for comparison purposes.

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ELECTRON MICROSCOPICAL DEMONSTRATION OF FERRITIN IN THE DENTAL EPITHELIAL CELLS OF URODELES

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THE teeth of urodeles have characteristic orange tips which are due to the incorporation of an iron compound into the outer layer of the tooth during development. Before coloration of the tooth, numerous orange-yellow granules can be seen in the cytoplasm of the inner dental epithelial cells which surround the developing tooth tip. Application of Prussian blue stain produces an intense positive reaction in these granules and a faint blue colour throughout the cytoplasm, indicating the presence of iron in the ferric state. This is indicative of both ferritin and haemosiderin[1] and not, as has commonly been believed, of haemosiderin only. A similar iron pigmentation occurs in the rodent incisor, in the dental epithelial cells of which the presence of ferritin has been demonstrated in the iron granules[2]. The purpose of the present investigation was to establish the presence of ferritin in the dental epithelial cells of urodeles.

Developing teeth of adult and larval forms of *Amblystoma mexicanum* and *Triturus vulgaris* were dissected from the jaws, fixed with glutaraldehyde followed by osmium tetroxide and embedded in 'Araldite'. They were afterwards sectioned for examination in the electron microscope.

Low-power electron micrographs of the inner dental epithelial cells surrounding the developing tooth tip revealed the presence of large granules of considerable electron density in positions within the cells corresponding to the yellow-orange granules seen in the light microscope.

At higher magnifications (Fig. 1) these granules, which ranged from 0·2 to 2·0μ in diameter, were seen to consist of aggregates of dense spherical particles, approximately 60 Å in diameter. Numerous identical 60 Å particles (*P*, Fig. 1) were dispersed throughout the surrounding cytoplasm. These presumably are responsible for the faint blue reaction in the cytoplasm with the Prussian blue stain. The 60 Å particles are of the same order of size as the ferritin molecules described by previous workers[3,4]. They did not appear to be associated with any of the organized cytoplasmic structures and certainly were not found within the mitochondria or the α-cytomembranes. They were, however, found within the nucleus in this tissue but never aggregated. Some of the cytoplasmic aggregates were enclosed within a membrane (*D*, Fig. 1) but in others no membrane was observed (*B*, Fig. 1). Some, but not all, consisted of a second, slightly less dense, component within which the 60 Å particles were embedded (*A*, Fig. 1). This may represent the protein moiety apoferritin. The distribution of the 60 Å particles within the aggregates was not generally uniform. However, a few have been found with very regular arrangement characteristic of crystalline order (Fig. 1). These features agree with those previously described for ferritin deposits in the liver[3–5].

The molecular structure of both isolated and intracellular ferritin has been described by Muir[5]. Only the central iron nucleus of the ferritin molecule can be seen in electron micrographs. The apoferritin protein coat surrounding the iron nucleus is indistinguishable from the cytoplasm and therefore measurement of the individual dense particles only gives a value for the central iron nucleus. It is this component which measures 60 Å. From the crystalline aggregates the total molecular diameter can be estimated by measuring the separation distances between the centres of the molecules in the crystal (*C*, Fig. 1). This was found to be 92 Å, which is in general agreement with a molecular diameter of 94 Å recorded by

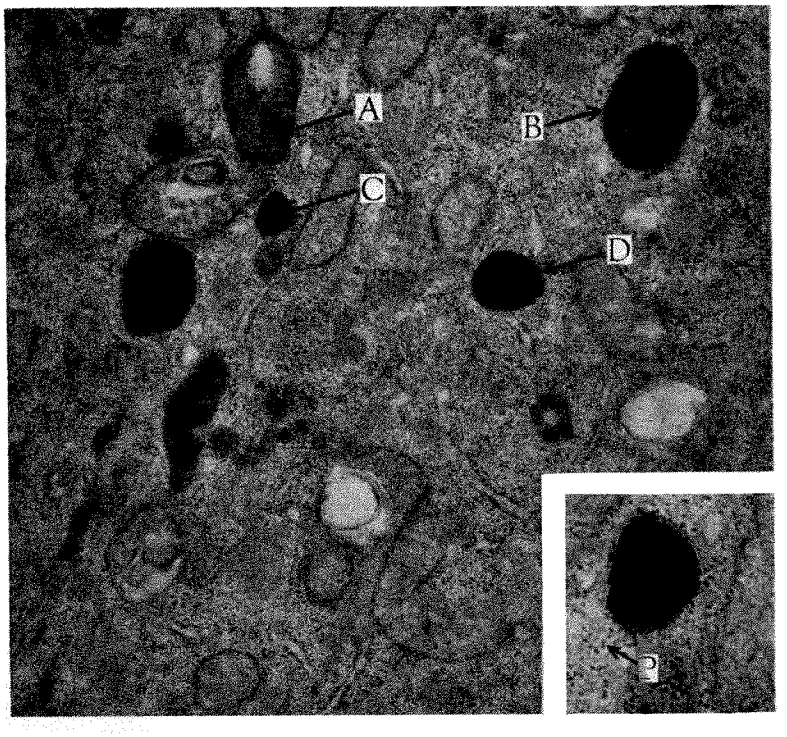


Fig. 1. Electron micrograph of part of an inner dental epithelial cell of *Amblystoma mexicanum* demonstrating ferritin aggregates in the cytoplasm. (×26,675.) Inset is a higher magnification of granule *C*. (×67,900)

Farrant[6] and Muir[5] from isolated horse spleen ferritin which had been shadow-cast.

Ferritin molecules also have a characteristic substructure which can be demonstrated in micrographs with a resolution of approximately 25 Å. The substructure of the molecule is due to the arrangement of the iron which is thought to be in the form of six micelles situated at the vertices of a regular octahedron and surrounded by a shell of protein. This molecular arrangement gives rise to five possible images in the electron microscope[5]. Examples of all these images have been resolved within the 60 Å particles in the present material both in the dispersed cytoplasmic particles and also in a few of the aggregated particles, the form most commonly observed being the paired parallel lines. In a number of the particles within the aggregates resolution of the characteristic substructure was not possible; there was also a variation in density and size of the particles. It has been claimed[3,7] that absence of any substructure, together with variation in density and size of the particles in the aggregates, is indicative of a different substance, namely haemosiderin, but this may not necessarily be the case. The increased density could be explained assuming that there is superimposition of one molecule of ferritin on the other; there are also likely to be many intermediate forms of substructure caused by random orientation of the molecules[5]. If these intermediate forms do represent incomplete micelles of iron hydroxide it would seem that they could be either ferritin which has been only partially synthesized from the apoferritin or, as suggested, ferritin molecules in various states of degradation into haemosiderin. Ultracentrifuge investigations[10] have demonstrated that crystalline ferritin is a heterogeneous mixture consisting of a complex of apoferritin-iron hydroxide and about 25 per cent free apoferritin. Fineberg and Greenberg[11] conclude that the iron-free apoferritin is the initial product in the synthesis of ferritin, but whether it is synthesized in the cytoplasm generally or within specific cytoplasmic organelles does not appear to be known. In addition, it has been shown by several workers[3,4,7] that ferritin is a component of haemosiderin and that it always precedes the formation of the latter[8]. The hypothesis has been suggested[9] that haemosiderin is formed by oxidation and proteolytic degradation of ferritin resulting in a rearrangement of the iron content and an altered protein structure. This would give rise to a different size and appearance of the iron micelles in the electron microscope indicative of haemosiderin and would explain the insolubility of haemosiderin in contrast to the soluble ferritin. The exact differences, in terms of electron microscopy, between ferritin and haemosiderin do not seem to have been conclusively demonstrated.

To obtain further information on the nature of the iron compound in this tissue, selected area electron diffraction was attempted on the aggregates of ferritin in the sections, and compared with the values obtained by Richter[7] for haemosiderin granules from human liver and for partly hydrated alpha ferric oxide ($Fe_2O_3.H_2O$). The results from this investigation (columns 1 and 2) and the values obtained by Richter (columns 3 and 4) are shown in Table 1.

Electron diffraction patterns were obtained from both the 'obviously ordered' (*C*, Table 1) and the 'apparently disordered' (*D*, Table 1) aggregates. The diffraction patterns are from the iron-containing core of the ferritin molecules only, which is presumably in the form of small crystallites. As can be seen from Table 1 the d_{hkl} values for both these types of granule are almost identical; this would suggest that they contain the same iron compounds. The agreement with Richter's values for $Fe_2O_3.xH_2O$ indicates the presence of hydrated Fe_2O_3 and the similarity of this material with liver haemosiderin. It is not possible to determine the degree of hydration of the ferric oxide using electron diffraction since the material has been previously dehydrated and may also undergo changes in the electron beam. It is also possible that the preparation procedures have altered the nature of the ferric oxide, which may not be in crystallite form *in vivo*.

Table 1. RESULTS OF ELECTRON DIFFRACTION ON INTRACELLULAR IRON GRANULES

d_{hkl} Å *C*	d_{hkl} Å *D*	d_{hkl} Å Haemosiderin	d_{hkl} Å $Fe_2O_3.xH_2O$
3·10	3·10	3·10	—
—	2·58	2·54	2·59
2·25	2·25	2·21	2·24
1·83	1·82	1·82	—
1·61	1·61	1·57	1·58
*1·30–1·17	1·30–1·17	2·35 1·30 1·20	1·36 1·36

The results given in the first two columns are from the present investigation; columns 3 and 4 are from those published by Richter (ref. 7).

* A range is given here since this outer ring is very diffuse, and may represent two or more d_{hkl} values.

This investigation has identified the iron present in the dental epithelial cells of urodeles as ferritin, by its molecular size, substructure, and electron diffraction pattern. It was seen to be present in considerable amounts in the cytoplasm and several of the molecular aggregates of ferritin appeared to be in crystal form. Ferritin molecules were also found dispersed within the nucleus. Ferritin has not previously been reported in amphibia except in a brief mention of its presence in the liver of the toad[5], and this was thought to be due to the diet of mammalian liver on which the animals were reared. It should be mentioned that most of the material in the present investigation was from animals feeding in their natural environment and is therefore an entirely normal occurrence. Most of the reports of the occurrence of ferritin in the liver have been on pathological material, or on experimental material in which excess storage of iron has been abnormally produced.

The reasons for the presence of this iron storage compound in what is regarded as a highly specialized and still metabolically active cell, and the subsequent secretion of iron into the developing tooth, are not known. The finding of the iron-hydroxide containing ferritin in the dental epithelial cells would seem to link up well with the suggestion by Schmidt[13] that the iron in the teeth is in the form of haematite (Fe_2O_3). In this connexion it is of considerable interest that ferritin has been demonstrated[12] in the epithelial cells which form the calcified denticles of the radula of a marine chiton and moreover that the iron here is in the form of the mineral magnetite ($Fe_2O_3.FeO$). The relationship between ferritin in the cells and the formation of the magnetite or haematite remains to be established.

In a very recent article[14], fractionated ferritin, of varying densities, has been examined with the electron microscope and it was found that the low-density fractions have molecules with a smaller iron nucleus than the higher density fractions. It is also suggested that the range of patterns of the iron-nucleus can be accounted for by a range of variable dense regions in which the number and geometrical arrangement of the iron-oxide crystallites are variable and are not necessarily in the form of six micelles as previously suggested, but randomly sited within the protein shell.

I thank Mr. R. W. Fearnhead for his assistance with the electron diffraction, and Prof. A. E. W. Miles, in whose department the work was undertaken during the tenure of a Medical Research Council grant.

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PHOTOSYNTHETIC NITRITE REDUCTASE AND THE SIGNIFICANCE OF HYDROXYLAMINE IN NITRITE REDUCTION IN PLANTS

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PREVIOUS work[1,2] showed that a nitrite reductase system in vegetable marrow (*Cucurbita pepo*) was able to reduce nitrite quantitatively to ammonia at rates exceeding the reduction of hydroxylamine when using identical preparations and conditions. The direct electron donor was the reduced benzyl viologen free radical ($BV^{\cdot}$). From kinetic considerations of rates, K_m values for both substrates and inhibitory effects of nitrite on hydroxylamine reduction, it was concluded that free hydroxylamine is not an intermediate product of nitrite reduction by plants. This view is consistent with results reported for *Escherichia coli*[3,4], where NADPH was an electron donor, and is contrary to the classical idea of nitrite reduction[5] which has been widely accepted on the basis of recent work carried out with fungi[5,7].

The conclusion reached with marrow[2] was, however, limited in validity because of the use of a non-physiological electron donor, especially as in a reaction involving six electrons the possibility of some non-physiological steps could not be excluded. Natural electron carriers found to function in nitrite and hydroxylamine reduction in both light and dark systems of bacteria and plants[8,9-11] are non-haem iron proteins now described by the generic name ferredoxin[12]. In published reports[9,10] giving comparative data for ferredoxin-dependent systems, the rate of hydroxylamine reduction always exceeded that of nitrite. We have now established that nitrite is reduced to ammonia by the chloroplast-ferredoxin system at rates substantially greater than those for hydroxylamine.

Chloroplast grana were obtained from whole chloroplasts of *Spinacia oleracea* which had been extracted and washed once in a solution consisting of 0·4 M sucrose; 0·01 M sodium chloride; 0·05 M *tris*, *p*H 7·5[12]. The washed plastids were ruptured by suspending them in a large volume of 0·05 M *tris*, *p*H 7·5. Grana were collected by centrifugation at 19,000*g* and normally washed three times by re-suspension in 0·05 M *tris* unless stated otherwise. Spinach ferredoxin was prepared from washed, frozen[12] lamina by a method involving acetone fractionation, dialysis, protamine precipitation and DEAE-cellulose chromatography[13,14], and was pure by spectrophotometric criteria[14-16]. The protamine step[13,15] was found to be essential to obtain a correct spectrum at 260–278 mμ relative to values at 330, 420 and 465 mμ when using frozen leaves. Crude nitrite and hydroxylamine reductase protein was extracted from spinach as described for marrow (*C. pepo*)[2]. The protein was precipitated by ammonium sulphate between 50 and 75 per cent saturation resuspended in 7 mM *tris*, *p*H 7·3, and dialysed against several large volumes of this buffer for periods up to 16 h to remove ammonium ions. The reaction mixture included chloroplast grana equal to approximately 650 μg chlorophyll, 600 μg ferredoxin, 150 μmoles *tris*/hydrochloride buffer, *p*H 7·5, and crude enzyme in 3 ml. The reaction was carried out in Thunberg tubes in an illuminated water bath at constant temperature for 20 min at 20° C with 45,000 lux from 150-W tungsten spot lamps. The gas phase was either air, or argon when anaerobic conditions were required. Anaerobiosis was assured by heating the grana at 55° C for 5 min to destroy oxygen evolution activity and substituting the physiologically second light reaction of the chloroplasts by electrons from an ascorbate-indophenol couple[17]. Nitrite and hydroxylamine were both determined by a method which used alkaline iodine to prevent interference by ascorbate[9] or benzyl viologen[2] where used by the method already described[2]. No losses occurred in dark controls and all changes were differences from zero time values.

Table 1 shows results obtained from simultaneous assays (as described earlier) of nitrite and hydroxylamine reductase activities carried out with identical replicate samples of separate spinach preparations obtained on different occasions. Results obtained with a preparation from *C. pepo* (kindly provided by D. P. Hucklesby) which had been subjected to further purification on hydroxyapatite and DEAE-cellulose[18] are also included. Nitrite was reduced more rapidly (in one case six times faster) than hydroxylamine at equal substrate concentrations, and ammonia production from nitrite always exceeded that from hydroxylamine. These results thus confirm and extend the conclusion reached in earlier work[2,9].

Table 1. REDUCTION OF NITRITE OR HYDROXYLAMINE BY ILLUMINATED SPINACH GRANA FERREDOXIN AND DIFFERENT ENZYME PREPARATIONS ASSAYED FOR THE TWO ACTIVITIES SIMULTANEOUSLY*

Enzyme† preparation	Protein (mg)	Grana system‡	Activity* Nitrite loss	Ammonia gain	Hydroxylamine loss	Ammonia gain
Spinach *A*	4·2	Anaerobic	1890	1920	998	1415
Spinach *B*	1·3	,,	1165	—	190	—
Spinach *C*	1·3	,,	645	—	163	—
Spinach *D*	(I)3·4	Aerobic	1660	1700	852	365
Spinach *D*	(II)1·7	,,	1700	2010	865	900
Marrow	0·27	Anaerobic	1680	1780	657	104

* Assay for 20 min at 20° C, 650 μg chlorophyll, 600 μg ferredoxin; 2 μmoles substrate, 45,000 lux *p*H 7·5, in 0·05 M *tris*/hydrochloride 3·0 ml. Activity as loss of substrate or gain of ammonia (mμmoles).

† Spinach enzyme was precipitated by ammonium sulphate and dialysed as described in the text. Preparations *A*, *B*, etc., made on separate occasions: I, II, etc., tested on separate occasions. Marrow enzyme was further purified by chromatography on DEAE cellulose and gradient elution with citrate buffers (ref. 18).

‡ Spinach grana either washed 3 times as described and used in air (aerobic) or after heating 5 min at 55° C and used in argon with $1·25 \times 10^{-3}$ M ascorbate and 7×10^{-5} M dichlorophenol-indophenol (anaerobic).

Using rate-limiting ratios of reductase enzyme to chlorophyll and the anaerobic systems with heated grana, the rates of substrate reduction were plotted against total ferredoxin concentration (salt free) as shown in Fig. 1, and yielded different curves for the two substrates. This suggested that considerations of saturation concerned the reductase enzyme side and not the light-grana reduction side of the ferredoxin reaction. Reciprocal plots indicated tentative values of 10^{-15} M as the apparent K_m for total ferredoxin for a molecular weight[14,15] of 14,000 when mediating nitrite reduction and 3×10^{-6} M when mediating hydroxylamine reduction.

The difference in these values therefore resembled that observed for $BV^{\cdot}$ in the *C. pepo* systems[2], but the affinity for ferredoxin appears to be appreciably greater. Thus, not only are the observed values smaller for ferredoxin, but the quantities present in the reduced (donating) form would be some fraction of the total amounts shown in Fig. 1. Values calculated for V_{max} suggest that the concentrations used in Table 1 and other experiments were sufficient to produce 62 per cent maximal rates for nitrite and 74 per cent for hydroxylamine reductase activities. Thus any degree of unsaturation would have depressed hydroxylamine reductase less than nitrite. At maximal velocity, nitrite reduction could be more than seven times greater than hydroxylamine in some of the preparations used in this work.

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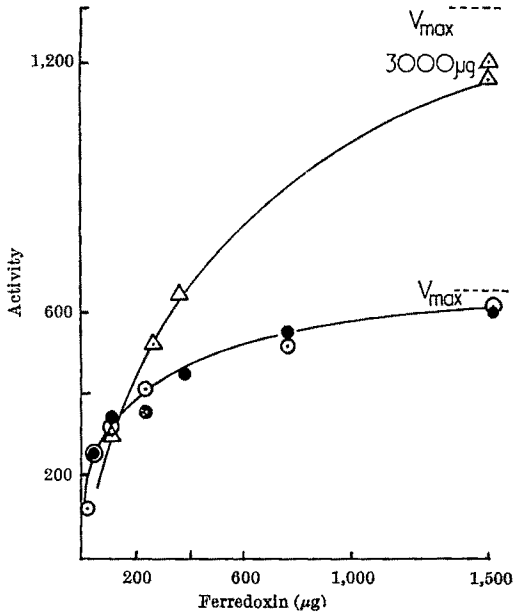


Fig 1. Effect of ferredoxin concentration on activity of nitrite or hydroxylamine reductase present in the same preparation assayed in presence of spinach grana equivalent to 650 µg chlorophyll illuminated with 45,000 lux, for 20 min at 20° C. Other conditions as described in text. Activity was measured as substrate loss (nitrite, triangles; hydroxylamine, circles); additional results for ammonia production from hydroxylamine (solid circles) are also presented. Values for V_{max} shown by horizontal lines were calculated from reciprocal plots

When the enzyme system was partially fractionated as shown in Table 2, there was a greater increase in specific activity for nitrite than for hydroxylamine reduction. Comparison of the activities obtained with ferredoxin and grana, or BV* (using the method already described[2]), showed the former to be the more effective for either substrate. The ratio between the two activities for nitrite was nearly constant and a less consistent but fair correspondence was found for hydroxylamine. In another experiment with enzyme which had been increased approximately fifty-fold in specific activity, the total reduction of nitrite (2 µmoles) produced 2 µmoles of ammonia. This showed that purification had not resulted in any lesion in the overall electron transfer reaction yielding ammonia when ferredoxin was the electron donor. In the corresponding 20-min period there was a loss of only 0·8 µmoles of hydroxylamine with quantitative ammonia formation. The inhibitory effect of nitrite on hydroxylamine reduction reported for *C. pepo* with BV as electron donor[2] was observed similarly when ferredoxin was substituted in a system utilizing glucose-6-phosphate and NADP as the electron donor system. These results justify the conclusion that benzyl viologen used strictly in the manner described in earlier work[2] is a valid reagent for the assay of nitrite and hydroxylamine reductase activities during fractionation and for certain kinetic studies.

Table 2 REDUCTION OF NITRITE OR HYDROXYLAMINE BY PARTIALLY FRACTIONATED SPINACH ENZYMES USING ILLUMINATED GRANA AND FERREDOXIN OR BV AS ELECTRON DONOR SYSTEMS*

System	Ferredoxin		BV*	
Activity†	Nitrite	Hydroxylamine	Nitrite	Hydroxylamine
Fraction used				
I Extract in 0 05 M *tris*/HCl, *p*H 7 5	53	37	15	14
II Ammonium sulphate-preparation 50–75 per cent	112	64	31	21
III 'Sephadex *G* 25'	88	45	25	13
IV Adsorption of DEAE-cellulose column, elution between 0·13 and 0·2 M sodium chloride, *p*H 7, re-adsorption and elution at 0·4 M sodium chloride	3050	670	810	144
IV Separate experiment‡				
Substrate loss:	1950	520	507	107
Ammonia gain:	2000	500	550	205

* Assay conditions were as for Table 1 in ferredoxin system, or as described for earlier work with *C. pepo* (ref. 2) in BV system using 34 per cent reduced dye.
† Activity as mµmoles substrate loss in 20 min/mg protein.
‡ All added nitrite (2,000 mµmoles) reduced in 20 min by 0·35 mg protein.

Table 3. EFFECT OF TREATMENT OF SPINACH GRANA ON REDUCTION OF NITRITE OR HYDROXYLAMINE IN RELATION TO ADDED ENZYME PROTEIN*

Treatment of grana and conditions	Substrate loss	
	Nitrite	Hydroxylamine
Grana washed once. No added enzyme	1028	672
Grana washed twice. ,, ,, ,,	417	183
Grana washed three times. No added enzyme	221	54
,, ,, ,, ,, plus 0 77 mg of added enzyme	817	508
,, ,, ,, ,, No added enzyme but ascorbate-indophenol couple included	133	27
Grana washed once. No added enzyme but grana heated 5 min at 55° C and ascorbate-indophenol couple included	178	156
Grana washed once, heated 5 min at 55° C plus 0·77 mg of added enzyme and ascorbate-indophenol couple included	1,135	1,245

* Assay conditions as for Table 1 under aerobic conditions but using 1,000 µg chlorophyll.

The possibility that nitrite and hydroxylamine reductase activities reside in the same protein has been raised[2-4]. During numerous attempts to fractionate the enzymes we have often observed a loss of hydroxylamine reductase relative to nitrite (see Table 2) without separation into different fractions. Very recently, however, we have obtained an apparent segregation into distinct proteins each of considerably increased specific activity using 'Sephadex *G* 200' when nitrite reductase was retarded less than hydroxylamine reductase.

Spinach chloroplasts washed once in the sucrose extracting medium, and once-washed grana, possess considerable nitrite and hydroxylamine reductase activity without added enzyme. Table 3 shows that successive washing of grana obtained after hypotonic rupture of chloroplasts caused a progressive loss of reductase activity and that the decrease was proportionately greater for hydroxylamine than for nitrite. Once-washed grana heated for 5 min at 55° C lost 75 per cent of endogenous nitrite and 50 per cent of hydroxylamine reductase activity, although they retained their capacity to reduce ferredoxin without added flavoprotein after prolonged washing[19] or after heating when they were also provided with the indophenol-ascorbate couple[20]. As can be seen from Table 3, the addition of enzyme restored the capacity of washed or heated grana to reduce both substrates in the ferredoxin system. There was no stimulation when the indophenol-ascorbate couple was provided for thrice-washed grana in the presence of added enzyme, under normal oxygen-evolution conditions. These results provide conclusive evidence for the presence of reductase activity in the chloroplasts but do not exclude the possibility of additional cytoplasmic sites.

Previous work[1,2] showed that when benzyl viologen is reduced more than about 75 per cent corresponding to a calculated redox potential of about −0·39 V a direct chemical reaction with nitrite occurs at *p*H 7·5 without a full yield of ammonia. Such a reaction also occurs at lower *p*H values with less reduced benzyl viologen[18]. The data of Table 3 show that no direct reaction is likely to be involved in the ferredoxin system under the conditions used, which provide substantial amounts of chlorophyll and saturating light intensity, and evidence for direct reaction has never been observed. The extent of net ferredoxin reduction during the reaction is at present unknown, but a redox potential of −0·39 V/−0·40 V would be expected for 30 per cent reduction[14]. The work of Chance and San Pietro[21] may, however, offer a guide as to the degree of reduction expected. In a steady-state system containing only 2·5 µg chlorophyll/ml. in equilibrium with dissolved atmospheric oxygen the reduction reached 28 per cent with a light intensity stated elsewhere to be saturating (see refs. 13 and 14 cited in ref. 21). In the presence of NADP as an acceptor the reduction decreased to about 0·4 of the above, namely 11 per cent. In our

work, anaerobic conditions have been used with more chlorophyll and in some cases (Fig. 1) rate-limiting amounts of reductase. The degree of ferredoxin reduction could therefore have exceeded 30 per cent even in the presence of the substrate, but typical Michaelis–Menton kinetics were nevertheless observed.

In previous work[9] with *C. pepo* grana, where notably lower light intensities were used, hydroxylamine reductase exceeded nitrite reductase activity. Suppression of oxygen evolution with chlorophenyl-dimethylurea (CMU) substantially increased both activities, but the disparity was much less because the increase was more than twice as great for nitrite as for hydroxylamine. In the present work with *S. oleracea* grana, which do not "age" quickly like those of *C. pepo*, inhibition of oxygen evolution by CMU in the presence of the indophenol-ascorbate couple has produced no advantage over an oxygen-evolving system although maximal rates so far obtained have been observed using heated grana with the couple under anaerobic conditions. These observations are consistent with the possible operation of a redox factor in the ferredoxin system similar to that thought to influence the BV system[2], and this point is under investigation.

Our results are interpreted as meaning that while nitrite and hydroxylamine are reduced photochemically by the same or closely related enzymes located in chloroplasts, hydroxylamine is not an intermediate in the commonly accepted sense in nitrite reduction in higher plants. This view is contrary to the situation envisaged for fungi[6,7], but is consistent with that proposed for *E. coli*[3,4].

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ASCORBIC ACID AND THE LUNG

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THERE have been no general investigations of the ascorbic acid levels in the tissues of guinea-pigs nor of their depletion on scorbutigenic diets since the comprehensive report of Penney and Zilva in 1946[1]. Reports on the effects of several substances such as ACTH and insulin on ascorbic acid levels[2] have appeared. Others have been concerned with the vagaries of precise determination[3,4] and the presence or absence of tissue binding[5].

In so far as we can ascertain, there have been no general investigations on the presence of ascorbic acid in the lung. Skirko and Grubina investigated the histochemical localization of vitamin C in lungs of rats exposed to silica particles[6]. The participation of pulmonary ascorbic acid in general depletion states in guinea-pigs has not been reported. This lack of information together with certain considerations of the vascular integrity of the lung prompted us to investigate two points: (*a*) whether ascorbic acid could be determined quantitatively in the lungs of guinea-pigs, and (*b*) whether these levels were responsive to depletion states induced by scorbutigenic diets. Both these questions were answered affirmatively. Evidence was also obtained which suggests that scorbutic depletion involves the mobilization of two possibly separate pools of ascorbic acid in guinea-pig tissues.

All the guinea-pigs used in these experiments were from mixed commercial stock obtained nearby. Two sets were used. The first consisted of twenty-seven adult females (391–613 g) obtained in November 1964. After several days on fresh stock diet (Rockland Guinea-pig Diet, Teklad Corp., Monmouth, Illinois) without supplementation, fifteen were set aside as follows: four were killed as zero-h controls and eleven were placed on a totally scorbutigenic diet (Nutritional Biochemicals, Cleveland, Ohio). A further three were killed at 72 h, four at 120 h, and four at 240 h. At the end of the first run, the remaining twelve animals, which had been fed continuously on stock diet, were treated similarly. Three were killed as zero-h controls, and then three each at 72, 120, and 240 h after starting on the scorbutigenic diet. The second set, obtained in February 1965, consisted of forty animals, four males (332–544 g) and thirty-six females (263–544 g). They were placed on stock diet for 2 weeks with water *ad libitum*. During this period, small amounts of fresh cabbage were added to the cages at irregular intervals. Four randomly selected animals were killed as zero-h controls. The remainder were placed on a scorbutigenic diet. Four more animals each were randomly selected from the cages at 24, 48, 72, 96, 120, 144, 240, and 336 h. The four remaining at the conclusion of the experiment were returned to stock diet.

All the animals were killed by a sharp blow on the head. They were dissected immediately. In the first set, lungs, liver and kidneys were removed for chemical analysis; these and the adrenals were removed in the second set. As appropriate, whole organs or portions of organs were blotted, weighed, and quickly frozen for later determination.

At the conclusion of each phase, after all the tissues had been frozen, they were quickly thawed in small batches. Quantitative determination of total ascorbic acid (TAA) was immediately undertaken according to the method of Roe and Kuether[7], as modified for solid tissues by Penney and Zilva[1].

The depletion curves from the first phase are shown in Fig. 1. The initial levels for the second half of the investigation accord with our general experience, namely, that higher levels are maintained by our stock diet and supplemental feedings of cabbage than are found on arrival from commercial sources. The levels for the lung are generally higher than those for either liver or kidney. Their decline on scorbutigenic diet is clearly indicated. Since we omitted the adrenal, we could not determine how the lung levels compared with those for other organs. This point, and the apparent biphasic nature of the

curves, especially those for the liver, led us to undertake the second and more detailed analysis.

The results of this experiment are shown in Table 1. The mean values with the calculated standard errors of the mean are given. These same figures are then plotted graphically on semi-logarithmic co-ordinates (Fig. 2).

Table 1. LEVELS OF ASCORBIC ACID, MG/100 G TISSUE

Hours of depletion	Lung	Adrenal	Liver	Kidney
0	7·37 ± 0·18	48·70 ± 3·10	4·86 ± 0·64	2·62 ± 0·38
24	8·40 ± 1·13	36·50 ± 2·50	3·15 ± 0·45	1·89 ± 0·06
48	5·40 ± 0·69	39·30 ± 4·63	2·76 ± 0·25	1·68 ± 0·17
72	4·18 ± 0·71	13·60 ± 1·60	1·13 ± 0·18	0·94 ± 0·19
96	2·04 ± 0·34	9·50 ± 0·81	1·26 ± 0·22	0·51 ± 0·11
120	2·21 ± 0·36	13·74 ± 1·75	1·28 ± 0·32	0·51 ± 0·05
144	1·49 ± 0·27	11·52 ± 1·75	1·07 ± 0·25	0·54 ± 0·13
240	1·83 ± 0·34	4·10 ± 1·73	0·46 ± 0·13	0·34 ± 0·05
336	0·49 ± 0·11	2·12 ± 0·42	0·19 ± 0·00	0·20 ± 0·10

* Mean ± standard error of mean.

All these investigations indicate substantial amounts of ascorbic acid in the lungs of guinea-pigs responsive to depletion states in a fashion generally similar to other organs. When comparable data from the work of Penney and Zilva are examined, the lung would appear to have levels similar to the small intestine, and possibly the spleen[1]. The lung is then a major site of ascorbic acid storage in guinea-pigs on a per 100 g weight basis. Because the lungs are a relatively light tissue (less than 1 per cent of body weight) they constitute only a small fraction of the total body stores of the vitamin.

That levels determined on a 24-h interval basis show a definite lack of uniform depletion is shown by the absolute levels in the tables and by the logarithmic plot. Definite plateaux between 72 and 144 h can be noted for adrenal, liver, and kidney, and a longer plateau is seen for the lung from 96 to 240 h. The points for the lung at zero, 72, 120 and 336 h, taken alone, would, if connected, yield an exponential plot. This would give rise to the possibly false impression of a single pool diffusing from the intracellular

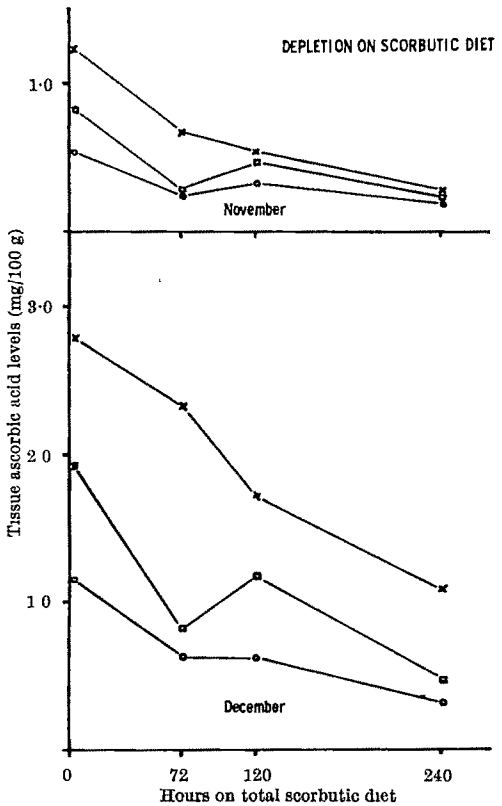


Fig. 1. Parallel linear plots of both halves of first experiment. The upper portion was determined in November, and the lower in December, 1964, after more preparation on stock diet. The 120-h lung point for December is questionable, only two samples being available; the third was lost in processing. ×, Lung; □, liver; ○, kidney

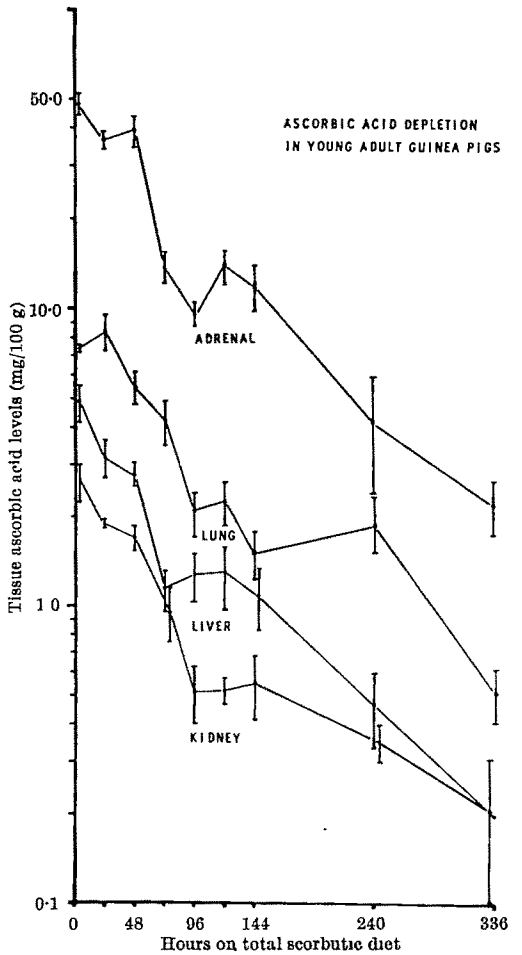


Fig. 2. Semi-logarithmic plot of the second, more extensive experiment. Four animals per point. All curves show non-exponential behaviour about 72 or 96 to 144 h; the "plateau" for lung extends to 240 h. Portions of curves before and after plateau are essentially exponential, but at slopes different one from the other. Standard error of the mean at 336 h for liver was 0·00. Higher zero h levels are attributable to greater duration of preparatory diet of good quality

and interstitial compartments. Similar plots can be constructed for the other organs investigated, yielding a picture essentially similar to that found by Penney and Zilva, who plotted TAA levels at 1, 3, 8, 15, and 23 days[1]. We cannot be sure whether the further variation between zero and 24 h represents a normal increase in the pulmonary TAA level through some mobilization and transport mechanism or simply a variation in the sampling and chemical assays. The specific values for the lung levels at zero h were: 7·15, 6·98, 7·65, and 7·68 mg/100 g; those at 24 h were: 5·45, 9·65, 10·50, and 8·00 mg/100 g.

The behaviour of the other tissue levels suggests that the phenomenon is a general one. Since the slope of the depletion curves is a reflexion of the initial levels, an effect of tissue 'flush' of ascorbic acid such as described by Penney and Zilva on loading, either by diet or by supplementation[1], must be considered an explanation for the biphasic slopes. The essential agreement between our three experiments, despite clearly different initial levels, mitigates against that possibility. The further differences between the several tissues in the duration of the apparent 'plateau' may indicate separate rates of turnover or utilization. Whether there is any order or sequence in depletion such as has been determined for the embryo during development[8] cannot be stated on the basis of this investigation.

It is tempting to consider that two forms of ascorbic acid exist in animal tissues, one in a rather loose state of association which depletes at a rapid rate, and the other in a high degree of association, or perhaps polymerization

the pool of which is entered only after the former is essentially depleted. Whether this is fully true, and whether a bound form is chemically similar to the bound fraction of plants, ascorbigen[9], remains to be established.

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RATE OF DISSOLUTION OF GRISEOFULVIN AND HEXOESTROL IN BILE SALT SOLUTIONS

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In order for a solid drug to be absorbed to any appreciable extent across the gastrointestinal barrier, it must be in solution[1]. Thus, for relatively water-insoluble drugs, the rate-limiting step in the absorption process is usually dissolution in the biological fluids of the gastrointestinal tract[2]. The importance of dissolution kinetics in the absorption process has been discussed in detail by numerous investigators[3–8].

Noyes and Whitney[9] derived the following equation for the dissolution rate of solid substances:

$$\frac{dc}{dt} = k(C_s - C) \qquad (1)$$

where dc/dt is the rate of dissolution, C_s is the equilibrium solubility of the solid and C is the concentration of solid in solution at time, t. The k in this equation is dependent on a number of physico-chemical factors, some of which are the surface area of the solid exposed to the dissolution media, the temperature, the intensity of agitation and the diffusion coefficient of the dissolved solid[2,10].

It can readily be seen from equation (1) that, for relatively water-insoluble drugs, the solubility term C_s is one of the more important factors governing the rate of dissolution of the drug. Since the rate of dissolution is directly proportional to C_s, any change in the nature of the dissolution media which will effectively increase C_s should, in theory, increase the rate of dissolution of the drug. One means by which this could be accomplished is by the addition of a surface-active agent to the dissolution media.

It is possible for surface-active agents to increase the dissolution rate of a relatively water-insoluble drug by either of two mechanisms: (i) by decreasing the interfacial tension between the drug and the dissolution medium, thus allowing the latter to wet the drug more completely[11]; or (ii) by means of micellar solubilization. The latter mechanism involves the incorporation of the water-insoluble material into micelles (that is, aggregates composed of monomers of the surfactant) formed above the critical micelle concentration (CMC) of the surfactant[11–13].

Very few reports have appeared in the literature concerning the influence of surfactants on the rate of dissolution of therapeutically significant drugs. The effect of the surfactant sodium lauryl sulphate on the dissolution characteristics of benzoic acid[14], salicylic acid[15] and certain crystalline forms of prednisolone[2] has been investigated. The results of these investigations show that the dissolution of these drugs is substantially increased in the presence of the surface-active agent. No reports have appeared in the literature on the effects of physiological surfactants on the dissolution characteristics of drugs.

Perhaps one of the most important groups of surfactants present in man, as well as in other animals, is the bile salts. These physiological surfactants have been shown to play an important part in both the digestion and absorption of dietary fats in the small intestine[16–19]. Their physico-chemical properties are similar to those of typical colloidal surfactants, including their ability to solubilize water-insoluble substances above their CMC (refs. 20–38).

In a previous communication[38], we have reported a portion of an extensive physico-chemical investigation, being conducted in our laboratory, to examine some of the factors which influence the micellar solubilization of relatively water-insoluble drugs in aqueous solutions of both conjugated and unconjugated bile salts. This investigation was initiated in connexion with the hypothesis that one of the steps in the gastrointestinal absorption of relatively insoluble drugs involves the preliminary solubilization of the drug by bile salt micelles normally present in the small intestine during fat absorption[38]. In view of the dynamics involved in the absorption process it was decided to consider the effect of bile salts on the rate of dissolution of poorly soluble drugs. The dissolution of both hexoestrol (obtained from Gallard-Schlesinger Chemical Manufacturing Co., New York) and griseofulvin (generously supplied by the Schering Co., Bloomfield, New Jersey) was investigated in 0·04 M solutions of the bile salts sodium cholate and sodium deoxycholate, as well as in distilled water. The bile salts, obtained enzymatically pure from Mann Research Laboratories, Inc., New York, were dried *in vacuo* for 36 h before use.

The apparatus in which the investigations of dissolution were conducted was similar to that used by Higuchi and Shefter[10]. It consisted chiefly of the following items: a 150-ml. beaker, a magnetic stirrer, a 2·3-cm fluorocarbon-covered stirring bar, and a timer.

The procedure used for the dissolution experiments was as follows: a weighed sample of the drug (20 mg griseofulvin and 100 mg hexoestrol), in excess of its predetermined equilibrium solubility in 0·04 M bile salt solution, was rapidly added to exactly 100 ml. of dissolution medium at 37° ± 1° C.

The solution was agitated by means of a magnetic stirrer. The intensity of agitation was high, but was maintained constant for all experiments performed with the individual drugs. At 2-, 5- and 10-min intervals a 5-ml. sample was withdrawn from the beaker and replaced by the same volume of dissolution medium. The sample was rapidly filtered through a 'Millipore' filter assembly (0·45μ pore size). An aliquot of the clear filtrate was diluted with anhydrous reagent methanol and the concentration of drug in solution determined spectrophotometrically from the absorbance values at 278 mμ for hexoestrol and 292 mμ for griseofulvin, using the Beer's law relationship[38]. All dissolution experiments were conducted in a constant-temperature room maintained at 37° ± 1° C. A cumulative correction was made for the previously removed samples in determining the total amount dissolved by using the formula:

$$C_n = C_{n,\,\text{meas.}} + \frac{5}{100} \cdot \sum_{s=1}^{n-1} (C_{s,\,\text{meas.}}) \qquad (2)$$

where $C_{n,\text{meas.}}$ denotes the spectrophotometrically measured concentration, and C_n is the concentration of the nth sampling expected in the medium if the previous samples had not been removed[39].

Fig. 1 shows the dissolution behaviour of griseofulvin in water, 0·04 M sodium cholate and 0·04 M deoxycholate at 37° C. A bile salt concentration of 0·04 M is considered to be the approximate molarity of the total bile salts present in the small intestine during fat absorption[40–42]. Each curve is drawn through points which represent an average of two dissolution runs. As can be seen from these curves the concentration of griseofulvin, at any time, is substantially greater than that in water. The dissolution rates in these media decrease in the following order: sodium deoxycholate solution > sodium cholate solution ≫ water. This parallels the order observed for the equilibrium solubility of griseofulvin in water and 0·04 M bile salt solutions at 37° C (ref. 38).

Table 1. RELATIVE DISSOLUTION RATES* FOR GRISEOFULVIN AND HEXOESTROL AT 37° C

Dissolution medium	Griseofulvin Time (min) 2	5	10	Hexoestrol Time (min) 2	5	10
Water	1·0	1 0	1·0	1 0	1 0	1 0
Sodium deoxycholate (0·04 M)	7 5	6·6	6·0	14·6	18·6	24 0
Sodium cholate (0 04 M)	7 2	6 1	5·5	20 3	30 4	36·0

* Ratio of the amount of drug solubilized in the bile salt medium divided by the amount of drug solubilized in the aqueous medium at the same time interval.

The dissolution curves for hexoestrol in each dissolution medium are shown in Fig. 2. The dissolution rates in these media decrease in the following order: sodium cholate solution > sodium deoxycholate solution ⋙ water. The same sequence was observed in equilibrium solubility experiments with hexoestrol in 0·04 M bile salts and water at 37° C (ref. 38).

The relative dissolution rates, that is, the amount of drug solubilized in the bile salt medium divided by the amount of drug solubilized in the aqueous medium at the same time-interval for both griseofulvin and hexoestrol, are shown in Table 1.

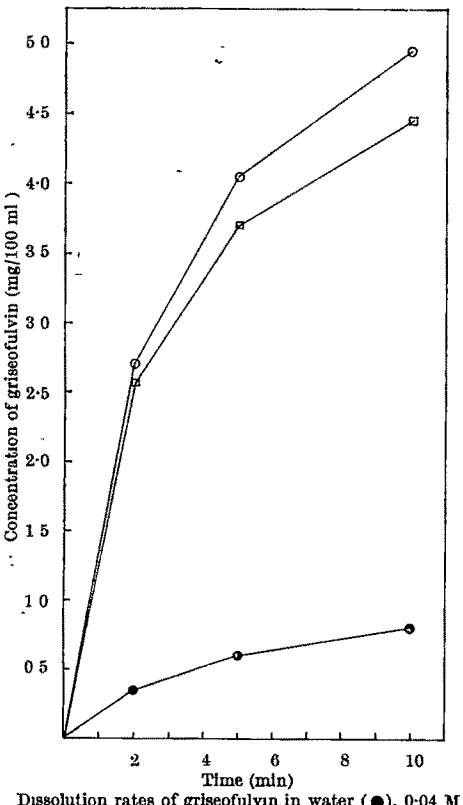


Fig. 1. Dissolution rates of griseofulvin in water (●), 0·04 M sodium deoxycholate (○), and 0·04 M sodium cholate (□) at 37° C

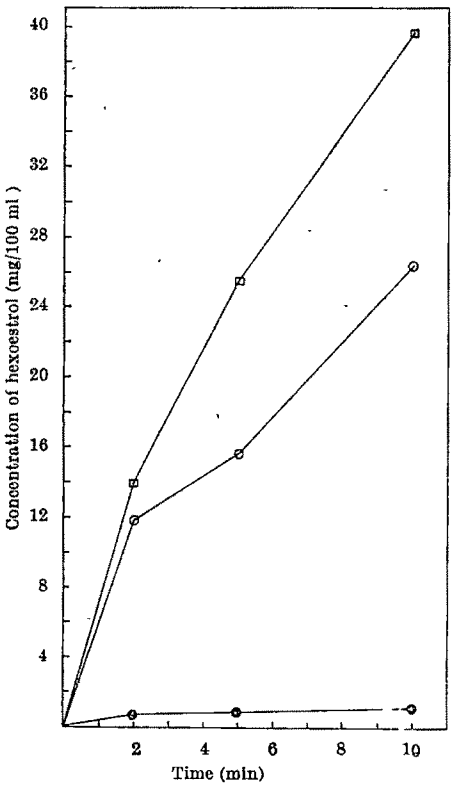


Fig. 2. Dissolution rates of hexoestrol in water (●), 0 04 M sodium deoxycholate (○), and 0 04 M sodium cholate (□) at 37° C

The enhancement in the dissolution rates of these two drugs in the presence of bile salts is probably due to a lowering of the interfacial tension between the drug and the dissolution medium as well as to micellar solubilization. The latter mechanism is probably more significant, since the bile salt concentration used in this investigation is approximately four times their CMC values.

The marked increase in the dissolution rates of the relatively water-insoluble drugs, hexoestrol and griseofulvin, in the presence of bile salts serves to strengthen the possibility that these physiological surfactants play an important part in the dissolution step of the absorption process.

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SEPARATION OF CHEMICALLY UNMODIFIED HISTOLOGICA LCOMPONENTS OF KERATIN FIBRES AND ANALYSES OF CUTICLES

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IT is well known that keratin fibres are particularly inert towards many chemicals and enzymes, and this has been ascribed to two main factors[1]: (1) The complex histological structure consisting mainly of cuticle, cell membranes and cortical cells, each of which tends to be complementary in its inertness towards chemicals (for example, formic acid, see later). (2) The high degree of cross-linking of the cuticle and cortical cells by disulphide bonds originating from cystine. The separation of the histological components of keratin fibres has thus been a major problem of keratin chemistry over the years, as shown by the many attempts to achieve separations by different methods (reviewed by Bradbury and Chapman[2]).

Probably the best method of producing essentially chemically unmodified components is ultrasonication or simply rapid shaking of the fibre in formic acid[2–5]. By this means the cell membrane complex which underlies the cuticle and surrounds each cortical cell is partly or completely dissolved in the 98 per cent formic acid and cuticle cells and afterwards cortical cells are liberated by the mechanical agitation. This mechanism, as previously reported[4], is confirmed by (i) electron microscopy which shows that the cell membrane structure is disrupted by immersion in acid, and (ii) analysis of the material dissolved by the formic acid. The latter, which amounts to about 2 per cent by weight of the fibre, consists mainly of lipid, and the amino-acid composition of the protein component is similar to that obtained by other workers[6]. In addition, it is known that immersion of proteins in formic acid at room temperature does not cause hydrolysis of peptide bonds[7–10] but only some formulation of hydroxyl groups of serine and threonine[10,11]; thus one is able to obtain samples of cuticle and cortical cells which are not appreciably modified by the method of preparation. Furthermore, the cell-membrane complex is also obtained separately as the material dissolved by the formic acid, but some possible complications are at present being investigated such as the possibility of dissolution of some protein from other parts of the fibre or lipid from the nuclear remnants of the cortical cells.

The technique of shaking in formic acid has been applied to Merino wool[4] and the amino-acid analysis of cuticle agrees nicely with that of cuticle produced by ultrasonication in various liquids[3]. In this article we show that the technique of shaking in formic acid is capable of disrupting a representative sample of keratin fibres. Furthermore, amino-acid analysis of the cuticle samples has shown the presence of the unusual amino-acid citrulline in every case and that, in general, the cuticle contains more of those amino-acids which cannot form α-helical homopolypeptides[12,13] than does the parent fibre.

The keratin fibres investigated were as follows: Merino 64's wool; bibrik, a Pakistani carpet wool; mohair; human hair; alpaca; the fur of the Australian marsupial 'brushtailed possum' (*Trichosurus vulpecula*) and rabbit fur. The fibres were cleaned by the method described previously[2], except that distilled water was substituted for the detergent solution of 'Gardinol BW', which recent work has shown cannot be completely removed from the fibres by washing with water. Samples of the cleaned, air-dried fibres (1·5 g) were cut into 0·5–1 cm lengths and agitated in 50 ml. of 98 per cent formic acid in a cylinder, 4 cm diameter, by means of a 'Vibromix' agitator (A. G. für Chemie-Apparateban, Zurich). The latter was operated with a 3 cm diameter-perforated plate oscillating at 100 c/s and at maximum amplitude. After an initial treatment for 5 min to disperse any skin flakes, the fibres were teased apart and subjected to 55 min of agitation in fresh formic acid at room temperature. The dispersed material from this second treatment was centrifuged, the formic acid solution removed and the residue washed three times with water and three times with ethanol. The cuticle cells were separated from cortical cells and residual fibre by the differential screening method of Bradbury and Chapman[2], applied to an ethanol suspension. The purity of the cuticle cells was checked by optical microscopy. Microscopic examination of the residual fibres showed that in no case was all the cuticle absent and that removal was very uneven because of partial felting. The material dissolved in the formic acid solution was recovered by lyophilization.

The yields of the components are shown in Table 1. Possum fur appears to be disrupted more rapidly than the fibres of the mammals, but in every case the liberation of cortical cells is slow. In order to obtain large quantities of the latter, it is preferable to use more vigorous mechanical agitation such as ultrasonication, by means of which 20 per cent of cortical cells can be produced in 1 h (ref. 2).

Amino-acid analyses of the cuticle samples were made using the Technicon system (column 0·63 cm diameter by 125 cm long) as described previously[3]. However, in the earlier work[3,4] the occurrence of citrulline was not noticed because of the relatively small amount present in wool cuticle and also because the peak occurs at the same position as that of proline. The neglect of this point does not make any appreciable difference to the proline figures previously reported[3,4].) Since proline has an absorption

Table 1. YIELDS FROM 'VIBROMIX' TREATMENT OF FIBRES IN FORMIC ACID

Fibre	Cuticle (per cent)	Cortical cells (per cent)	Cell membrane complex (per cent)
Merino 64's	1·0	0 1	2·5
Bibrik	2·2	0 1	3·7
Mohair	0 9	0 1	1·5
Human hair	0 8	0·1	2 8
Alpaca	4·0	0 1	3 2
Possum fur	7·0	2·8	6 0
Rabbit fur	0·7	0·1	3 2

maximum at 440 mμ and citrulline at 570 mμ, it is possible to estimate the amounts of each amino-acid present in the mixed peak from the areas under the 440 and 570 mμ peaks. Routinely, when the ratio of these areas becomes less than 6·0, there is obviously an appreciable amount of citrulline present. Under these conditions, the duplicate analysis is done in such a way that 20 min before the aspartic acid peak is recorded (as shown by the first chromatogram) the temperature of the column is reduced to 15° C. This is maintained until the proline peak has been recorded after which the temperature is returned to its normal value of 60° C. This procedure changes the order of elution; proline now appears before glutamic acid, while citrulline appears in the normal position after glutamic acid. We have also noted the occurrence of a peak between ammonia and lysine, which has been identified as ornithine. This amino-acid is known to be produced by decomposition of citrulline during hydrolysis in 6 M hydrochloric acid at 110° C (ref. 14), and preliminary results (which require confirmation) indicate that ornithine may not occur as such in the native material.

In Table 2 are given the citrulline and ornithine results for the various keratin derivatives; the complete amino-acid analyses will be reported later. In Merino wool citrulline and ornithine are not present in the cortical cells and this accounts for the negligibly small value in the whole fibre. This is probably also the case for Lincoln wool and mohair, but the other six keratins in Table 2 contain considerable amounts of medulla, in which citrulline is known to be present[14]. It is therefore clear that citrulline occurs in all the cuticle samples, but is absent from the cortical cells of Merino wool and probably also of the other keratins. Rogers has shown the presence of citrulline in the medulla and also in the inner root sheath protein of the hair follicle[14–16]. It seems likely that there is a common mechanism for the incorporation of citrulline into the protein produced by these three types of cells, but whether citrulline is incorporated as such or whether as arginine, which is later modified by an enzymatic reaction on the formed protein to citrulline, is not known[14].

There is naturally a considerable variation in the amino-acid composition of the various keratins and cuticles therefrom, but there is one regularity which has been noticed. There is very approximately the same difference between the amino-acid composition of the cuticle and the fibre from which it was derived for every keratin; the following figures represent average percentage differences over all the keratin samples. As well as the citrulline content already noted the cuticles contain more cystine 44, cysteic acid 233, proline 49, serine 31, valine 27, and glycine 26 and less aspartic acid 36, glutamic acid 19, threonine 21, isoleucine 26, methionine 29, leucine 19, tyrosine 28, phenylalanine 36 and arginine 32 than the respective whole fibres. The cuticle is deficient in acidic and basic groups and is therefore less polar than the keratin as a whole[3]. The cuticle contains more of those amino-acids which are unable to form α-helical homopolypeptides in the solid state or in solution[3,12,13]. It has been proposed that the wool fibre cuticle is amorphous, whereas the cortical cells have an α-helical component, because of the increased amount of these α-helix inhibiting amino-acids in the cuticle[3]. It is now clear that this difference in amino-acid composition between cuticle and whole fibre is of general occurrence and apparently causes the formation of an amorphous, highly cross-linked cuticle, which forms a suitably inert outer covering for the keratin fibre.

Table 2. CITRULLINE AND ORNITHINE CONTENT (μMOLES PER G DRY PROTEIN) OF HYDROLYSATES OF KERATINS AND CUTICLES THEREFROM

Type of keratin	Whole Keratin		Cuticle	
	Citrulline	Ornithine	Citrulline	Ornithine
Merino 64's wool*	0	0	30	13
Lincoln wool	0	0	15	0
Mohair	0	0	33	14
Bibrik	25	0	25	17
Human hair	11	0	45	5
Alpaca	34	0	78	41
Rabbit fur	61	9	170	78
Possum fur	8	44	297	161
Porcupine quill†	79	26	15	0

* There are negligible quantities of citrulline and ornithine in cortical cells, disrupted cortical cells (ref 3) and skin flakes (ref. 4) and a small amount in the cell membrane protein produced on immersion of Merino wool in formic acid (ref. 4)

† The cuticle was produced in this case by dissection.

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GLYCEROL AS A SUBSTRATE FOR BRAIN METABOLISM

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THERE is a considerable body of evidence which indicates that under ordinary conditions the brain utilizes glucose as its metabolic substrate and that other substances are not utilized to any significant extent. Since pyruvate is one of the intermediates in the oxidation of glucose, it would be expected that the cells of the brain can readily metabolize pyruvate. Brain slices and suspensions do readily oxidize pyruvate[1,2]. However, the brain of the intact animal is apparently unable to utilize pyruvate which is present in the blood circulating through it. In animals which are hypoglycaemic as a result of hepatectomy, the abnormal electrical activity of the brain and the coma and convulsions which result are relieved by administration of glucose but are not relieved by administration of pyruvate[3,4]. This lack of utilization of pyruvate by the brain *in vivo* is probably due to the limited access of pyruvate to the brain tissue (the 'blood-brain barrier'). This relative impermeability of the brain to systemically administered pyruvate has been demonstrated experimentally in cats[5]. The impermeability to pyruvate may be due in large part to the relative impermeability of the brain to negatively charged substances[6]. Glycerol is an electrically neutral substance which can be converted by mammalian tissues to glucose

and to pyruvate. As a component of the fat molecule, it is normally produced and metabolized in mammalian tissues. It can be administered orally or parenterally without noxious effect[7] and it has been reported that injection of glycerol can relieve the cerebral symptoms of insulin hypoglycaemia[8]. However, it was not shown whether this action was due to the direct utilization of glycerol by the brain or to the prior conversion of glycerol to glucose. This article presents the results of experiments performed *in vitro* and *in vivo* which indicate that the mammalian brain can utilize glycerol as a metabolic substrate.

The *in vitro* experiments were performed with slices of guinea-pig brain. A guinea-pig was decapitated, the brain was rapidly removed, chilled in ice and thin slices of cortex were prepared with a Stadie-Riggs microtome. The slices (about 200 mg wet weight) were incubated for 1 h at 37° in 2 ml. of an isotonic saline-phosphate buffer (*p*H 7·4) medium[9] in Warburg vessels with 0·10 ml. of 10 per cent potassium hydroxide in the centre well and oxygen as gas phase. Each experiment with slices from a single brain was composed of five vessels: a blank control and four experimental vessels. To the experimental vessels were added 0·1 μc. of glycerol-1-^{14}C (New England Nuclear Corp.) and carrier glycerol in amounts increasing progressively from 56 to 448 μmoles. The total volume of fluid in each vessel was 2·3 ml. At the end of the incubation period, 0·2 ml. of 5 per cent perchloric acid which had been placed in the side arm was tipped into the vessel. The centre well and the paper in it were carefully washed three times with water and the washings were evaporated in a planchet under an infra-red lamp. The tissue slices were removed from the reaction vessels, rinsed in water and dried to constant weight at 140° C in weighing bottles.

The dried planchets were allowed to cool in a desiccator and their radioactivity was measured in a windowless gas-flow counter. A small excess of dilute hydrochloric acid was then added to each planchet and the planchets were again dried and counted. This was done to ensure that the radioactivity in the centre well material was derived from absorbed $^{14}CO_2$ and that there had been no significant contamination by material from the incubation medium. In all cases, the second measurement of radioactivity was not significantly above the background value, indicating that contamination had not occurred. The self-absorption correction factor was determined by measuring the radioactivity of carbon-14 samples with and without the addition of the same amount of alkali that had been added to the centre well. From the corrected values of the counts per minute for each planchet and the known efficiency of the counter, the number of μc. of $^{14}CO_2$ in each planchet was calculated.

Since it can reasonably be assumed that the carbon-14-labelled glycerol is being oxidized at the same rate as the carrier glycerol, the total amount of carbon dioxide produced can be calculated. Each molecule of glycerol which is oxidized gives rise to three molecules of carbon dioxide, therefore:

$$\mu\text{moles of carbon dioxide produced} = 3 \times \mu\text{moles glycerol oxidized}$$

The fraction of the total glycerol oxidized is the same as the fraction of the carbon-14-labelled glycerol converted to $^{14}CO_2$, therefore:

$$\frac{\mu\text{moles glycerol oxidized}}{\mu\text{moles glycerol added}} = \frac{\mu\text{curies } ^{14}CO_2 \text{ produced}}{\mu\text{curies glycerol-}^{14}\text{C added}}$$

$$\mu\text{moles of } CO_2 \text{ produced} = 3 \times \mu\text{moles glycerol added} \times \frac{\mu\text{curies } ^{14}CO_2 \text{ produced}}{\mu\text{curies glycerol-}^{14}\text{C added}}$$

The experimental results and the calculated values for one experiment are shown in Table 1. The production of carbon dioxide as a function of the initial quantity of glycerol for this experiment is shown graphically in Fig. 1.

Table 1. PRODUCTION OF CARBON DIOXIDE FROM GLYCEROL BY GUINEA-PIG BRAIN SLICES (EXPERIMENT 4)

Brain slices, dry weight (mg)	μmoles glycerol	corrected c.p.m.	$^{14}CO_2$ (mμc./h)	Carbon dioxide (μmole/h)	Carbon dioxide (μmole/h/mg)
15·4	56	956	0·96	0·16	0 011
12 0	112	652	0·65	0·22	0·018
15 8	224	736	0·74	0 50	0 032
14·7	448	732	0·73	0·99	0 068

Table 2. PRODUCTION OF CARBON DIOXIDE FROM GLYCEROL BY GUINEA-PIG BRAIN SLICES

Expt. No.	μmoles of glycerol added	56	112	224	448
		Carbon dioxide (μmoles/h/mg dry weight)			
1		0 031	0 036	0·058	0·102
2		0 010	0 017	0·024	0·046
3		0 017	0·026	0·036	0·056
4		0 011	0 018	0·032	0 068

It is apparent that the rate of production of carbon dioxide is an approximately linear function of the initial glycerol concentration. The individual values for all the experiments are listed in Table 2.

McIlwain[10] reported that guinea-pig brain slices in a 2-mM glucose medium consume oxygen at the rate of 60 μmoles/h/g (wet weight). Assuming that the respiratory quotient was unity and that about 80 per cent of the wet weight is water, this means that, with glucose as substrate, carbon dioxide production is about 0·012 μmoles/h/mg (dry weight). In our experiments with glycerol as substrate, carbon dioxide production of the same order of magnitude was obtained (Table 1) when the glycerol concentration was about 25 mM (56 μmoles/2·3 ml.). Thus, glycerol is required at levels more than ten times that of glucose to produce a similar respiratory rate. In this respect, glycerol is similar to fructose as a substrate for brain slices[10].

The *in vivo* experiments were performed in adult male albino rabbits, weighing about 3 kg. Each rabbit bore a chronically implanted catheter through which injections were made directly into the right internal carotid artery. The implantation and testing of such catheters have been described[11]. Silver coil electrodes had been implanted into the skull in the frontal, parietal and occipital areas on both sides. Bipolar electroencephalograms (EEG) were recorded with a Grass 'Polygraph' recorder. The rabbits were fasted about 18 h before the experiment and were un-anaesthetized, unrestrained and quiet throughout the experiments. At the start of the experiment dihydroergotamine methanesulphonate (0·3 mg/kg) was injected to block the hyperglycaemic action of epinephrine which is released during insulin hypoglycaemia. 30 min later, regular insulin (1·5 U/kg) was injected intravenously. During the period of 30–60 min after the injection of insulin, the blood glucose level of the rabbit dropped from about 50 to 20 mg/100 ml. and the changes in the EEG which are characteristic of hypoglycaemia appeared. These changes consisted of a decrease in the amplitude of

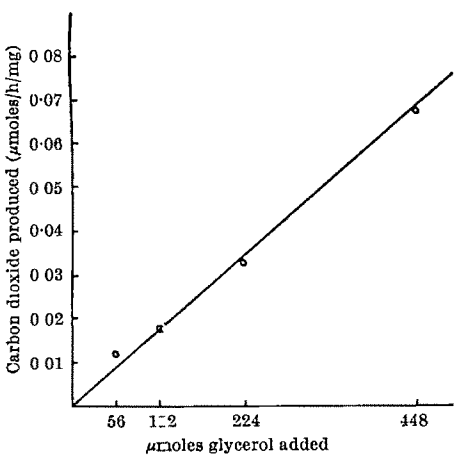


Fig. 1. The effect of glycerol concentration on the production of carbon dioxide from glycerol by guinea-pig brain slices

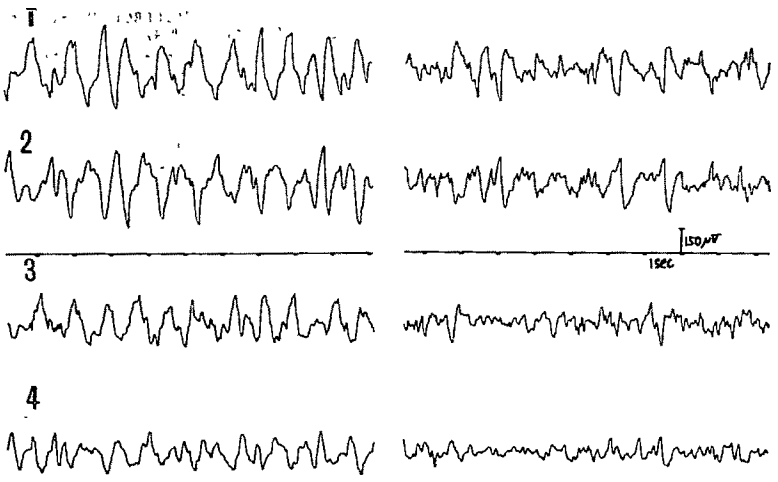


Fig. 2. The effect of infusion of a 10 per cent solution of glycerol by way of the right internal carotid artery on the bipolar electroencephalogram of the hypoglycaemic rabbit. The recordings are from the following electrode pairs (top to bottom): No. 1, left frontal–left parietal; No. 2, left parietal–left occipital; No. 3, right frontal–right parietal; No. 4, right parietal–right occipital. The recordings on the left were made 1 min before the start of the infusion and those on the right were made after 5 min of infusion at the rate of 0·2 ml./min. The decrease in number and amplitude of the high voltage, slow waves on the right (ipsilateral) side after infusion is quite definite

the normal fast activity (4–5 c/s, corresponding to alpha waves of human EEG) and the appearance of spindles (2·5–4 c/s, corresponding to theta spindles of human EEG) and high-voltage, slow waves (0·5–1·5 c/s). During this period when there were definite EEG signs of hypoglycaemia, infusions were made of either glucose or glycerol solutions by way either of the internal carotid artery or intravenously.

Infusion of a 10 per cent glucose solution in 0·45 per cent sodium chloride at the rate of 0·2 ml./min by way of the internal carotid artery was followed by a reversal of the EEG pattern toward normal within 1 min of the start of the infusion. In twenty-three of thirty-one such infusions, there was definite ipsilateral improvement of the EEG and the average amount of glucose required to produce this improvement was 14 mg. The nature of the effects on the EEG are the same as those shown in Fig. 2. When the glucose was administered intravenously, improvement in the EEG was always bilateral, and occurred 2–3 min after the start of the infusion when about 40 mg of glucose had been administered.

Infusion of a 10 per cent glycerol solution in 0·45 per cent sodium chloride at the rate of 0·2 ml./min was followed by ipsilateral improvement of the EEG of the hypoglycaemic rabbit in seventeen of forty-four cases. This improvement (shown in Fig. 2) first appeared after 4–5 min of infusion when an average of about 105 mg of glycerol had been administered. In four additional cases bilateral improvement in the EEG was seen. When the glycerol solution was administered intravenously no significant change in the EEG of the hypoglycaemic rabbit was seen. The infusion of physiological saline solution at the same rate by way of the internal carotid artery or intravenously had no effect on the EEG.

The unilateral nature of the change in the EEG is fairly good evidence that the change is caused by the agent being infused. The abnormalities of the EEG caused by hypoglycaemia are presumably due to the inadequate energy available to maintain the normal activity of the brain. The reversion of the EEG toward normal, when glycerol is made available to the brain, indicates that glycerol is being metabolized and is providing some of the energy requirement of the brain. It is evident from the results of these *in vivo* experiments and from the *in vitro* experiments described above that glycerol is utilized, but at a considerably slower rate than is glucose. The results of our *in vivo* experiments will be reported in greater detail elsewhere.

One previous report[3] indicated that the intact brain does not utilize glycerol. This was based on the inability of systemically administered glycerol to eliminate the symptoms of cerebral hypoglycaemia in hepatectomized dogs. In view of our results with glycerol solutions administered intravenously, it seems unlikely that such an experiment would detect the relatively slow rate at which glycerol is used by the brain. Elliott[12] in a reference to his unpublished results stated that glycerol is not oxidized by brain slices. This observation is probably based on measurement of oxygen consumption, which might be inadequately sensitive if low concentrations of glycerol were used. Pritchard[13] has shown that glycerol-^{14}C is incorporated into the lipids of brain slices. Although this does not indicate that glycerol can provide energy for the brain, it does demonstrate that brain tissue can metabolize glycerol.

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OPTICAL ISOMERS OF PROPRANOLOL

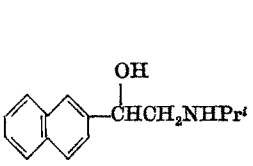
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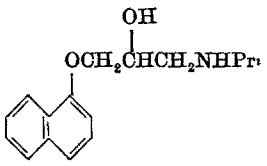
THE β-adrenergic receptor antagonist, pronethalol ('Alderlin'), has anti-arrhythmic properties in experimental animals. It has been shown to prevent or abolish ouabain-induced arrhythmias in guinea-pigs[1], and dogs[2–4], and anaesthetic adrenaline arrhythmias in dogs[2–4]. It has been suggested that some of these effects are not the result of blockade of β-adrenergic receptors[2,4]. (+)-Pronethalol, which has about one-fortieth the β-adrenergic blocking activity of racemic pronethalol[5], has also been shown to be effective in abolishing ouabain and anaesthetic adrenaline arrhythmias in dogs[4].

A new compound, propranolol ('Inderal'), has been described which is at least ten times as active as pronethalol as a β-adrenergic blocking agent[6–8]. This article reports

its resolution, the physical constants, and some of the pharmacological properties of its optical isomers:



Pronethalol
2-Isopropylamino-1-(2-naphthyl)ethanol.



Propranolol
1-Isopropylamino-3-(1-naphthoxy)-2-propanol.

(−)-Di-*O*,*O*-*p*-toluoyltartaric acid[9] was used as a resolving agent. By a tedious fractional crystallization from methanol, the two salts (+)-propranolol hydrogen (−)-di-*O*,*O*-*p*-toluoyltartrate hydrate, m.p. 170° C, $[\alpha]_D^{21}$ −62·0° (*c*, 1·01 in ethanol), and (−)-propranolol hydrogen (−)-di-*O*,*O*-*p*-toluoyltartrate hydrate, m.p. 166°–167° C, $[\alpha]_D^{21}$ −89·0° (*c*, 1·0 in ethanol)[10] were obtained optically pure. Physical data for propranolol and its isomers are given in Table 1.

Table 1. PHYSICAL DATA FOR PROPRANOLOL AND ITS ISOMERS

	(±)-Propranolol m.p. (° C)	(+)-Propranolol m.p. (° C)	(+)-Propranolol $[\alpha]_D^{21}$	(−)-Propranolol m.p. (° C)	(−)-Propranolol $[\alpha]_D^{21}$
Base	96	73	+10·6° (*c*, 1·02)*	73	−10·2° (*c*, 1·02)
Hydrochloride	163–164	192	+22·2° (*c*, 0·99)	192	−22·7° (*c*, 1·0)

* Ethanol was used as solvent.

Pharmacological studies. (Doses are all expressed in terms of the salt used. Propranolol and its isomers were used as the hydrochloride.) Observations were made in cats anaesthetized with chloralose 80 mg/kg given intravenously. Spontaneous breathing was assisted by the insertion of a tracheal cannula. Heart rate was recorded by means of a cardiotachometer triggered by the cardiac *QRS* voltage. Femoral veins were cannulated for the injection of drugs. Increases in heart rate were produced by the intravenous infusion of isoprenaline at 0·2 μg/kg/min for 10 min before and 30 min after commencing the intravenous infusion of (+)- or (−)-propranolol. It has previously been shown that the intravenous infusion of propranolol reduces or abolishes the increase in heart rate produced by isoprenaline[7]. The effect of the intravenous infusion of (+)-propranolol at 20 and 100 μg/kg/min and of (−)-propranolol at 0·5, 1·0 and 5·0 μg/kg on the isoprenaline tachycardia are given in Table 2. These are the averaged results from groups of cats. Table 2 also contains previously published results for propranolol[7]. The administration of (+)-propranolol at 20 μg/kg/min had no effect on the isoprenaline tachycardia but at 100 μg/kg/min reduced the isoprenaline response slightly. The infusion of (−)-propranolol at 1 μg/kg/min reduced the isoprenaline tachycardia while the larger dose almost completely abolished it. These results indicate that the (−)-isomer is more active than racemic propranolol in blocking the isoprenaline tachycardia. The (+)-isomer had little effect on the isoprenaline tachycardia and has probably less than a hundredth the activity of racemic propranolol.

Further observations have been made with racemic propranolol and (+)-propranolol in dogs anaesthetized by the intravenous injection of thialbarbitone, 30 mg/kg. followed by chloralose, 50 mg/kg. Cardiac contractile force was measured by suturing a strain gauge arch on the right ventricle. Arterial pressure was recorded from a cannula in the left femoral artery with an inductive type differential pressure transducer, and heart rate by means of a cardiotachometer. Drugs were injected through catheters in the femoral veins. Isoprenaline, 0·4 μg/kg, was administered every 10 min and the maximum changes in heart rate, cardiac contractile force and diastolic arterial pressure were recorded. When control responses to isoprenaline had been obtained, 0·01 mg/kg racemic propranolol or (+)-propranolol was injected intravenously and 3 min later the isoprenaline administration was repeated. Geometrically increasing doses of racemic propranolol or (+)-propranolol were given at 10 min intervals followed each time by isoprenaline, 0·4 μg/kg. The effects of these two compounds on the increases in heart rate and in cardiac contractile force and on the fall in diastolic pressure produced by isoprenaline are shown in Fig. 1. These are the averaged results from three dogs for propranolol and two for (+)-propranolol.

Propranolol reduced the increase in heart rate, the fall in diastolic pressure and the increase in cardiac contractile force produced by the intravenous injection of isoprenaline. A total cumulative dose of 0·31 mg/kg propranolol abolished these responses. Much larger doses of (+)-propranolol were required to reduce the responses to isoprenaline; a total cumulative dose of 10·23 mg/kg reduced the responses to the same extent as a total dose of 0·15 mg/kg racemic propranolol. Consequently (+)-propranolol has about one-sixtieth the activity of racemic propranolol in blocking these effects of β-receptor activation. In these experiments propranolol produced a slight reduction in resting heart rate; the comparable blocking doses of (−)-propranolol produced a greater bradycardia and had a negative inotropic action.

Table 2. EFFECT OF PROPRANOLOL AND ITS ISOMERS ON THE ISOPRENALINE TACHYCARDIA EXPRESSED IN BEATS/MIN IN ANAESTHETIZED CATS; AVERAGED RESULTS FROM GROUPS OF CATS

Compound	Dose (μg/kg/min)	No. of cats	Isoprenaline tachycardia Control	Isoprenaline tachycardia Treated
(±)-Propranolol	1	3	30	19
	5	3	49	12
	25	3	54	7
(+)-Propranolol	20	4	61	63
	100	3	67	50
(−)-Propranolol	0·5	3	38	34
	1·0	4	42	10
	5·0	3	59	5

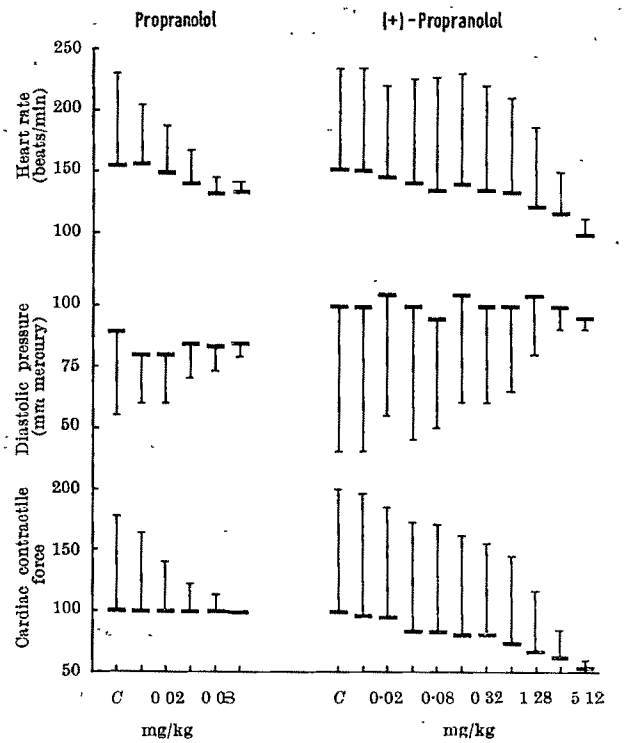
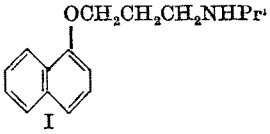


Fig. 1. Observations in dogs anaesthetized with thialbarbitone and chloralose. Heart rate, beats/min, femoral diastolic arterial pressure, mm mercury, and right ventricular contractile force, expressed as percentage of initial control value, were recorded. Resting level of each parameter is represented by (−) and the maximum response to isoprenaline (0·4 μg/kg) by (−). The effect of increasing doses of propranolol and (+)-propranolol on resting levels and on the responses to isoprenaline are shown, averaged results from three and two dogs, respectively.

Effect on experimental cardiac arrhythmias. The effects of propranolol and its isomers have been studied on halothane-adrenaline and on ouabain arrhythmias in anaesthetized cats. Multifocal ventricular extrasystoles were produced in cats anaesthetized with chloralose and spontaneously breathing oxygen and 1 per cent halothane, by the intravenous injection of adrenaline 1–5 μg/kg. When control responses to adrenaline had been obtained, propranolol or one of its isomers, 0·1 mg/kg, was injected intravenously, and 5 min later the administration of adrenaline was repeated. Propranolol and (−)-propranolol, 0·1 mg/kg, prevented the development of arrhythmias; it was necessary to increase the dose of (+)-propranolol to 1·0 mg/kg before it prevented the development of the adrenaline-induced arrhythmias.

Ouabain, 60–110 μg/kg, was injected intravenously in divided doses into cats, anaesthetized with pentobarbitone and artificially respired, to produce a ventricular tachycardia. When this arrhythmia was established, propranolol or one of its isomers was infused intravenously at 2 mg/kg/min until the return of sinus rhythm or death of the animal. Propranolol was administered to nine cats; there was a return to sinus rhythm in seven animals after the intravenous infusion of a mean amount of 10·3 ± 2·3 mg/kg; the remaining two cats died from ventricular fibrillation. (+)-Propranolol abolished the ouabain-induced arrhythmia with a return to sinus rhythm in nine out of nine cats after the administration of a mean dose of 8·4 ± 2·0 mg/kg. (−)-Propranolol was given to six cats; in one animal there was a return to sinus rhythm after 4·0 mg/kg; in the remaining five cats the arrhythmia was unaffected by (−)-propranolol which was given until death occurred from ventricular fibrillation. In these five cats death occurred after the intravenous infusion of a mean amount of 16·4 ± 2·1 mg/kg (−)-propranolol.

These experiments in anaesthetized dogs and cats show that the (−)-isomer of propranolol is 60–100 times more active than the (+)-isomer in blocking the inotropic, chronotropic and vasodepressor actions of isoprenaline. A similar ratio of activity was found for the isomers of pronethalol[5]. In man[11] the (−)-isomer of isoprenaline was about fifty times more active than the (+)-isomer in stimulating β-adrenergic receptors, while in animals the ratio was about 600 : 1 (ref. 12). These variations in activity of the two isomers may result from slight contamination of the (+)-isomer with the (−)-isomer. This may account for the β-blocking activity of high doses of (+)-propranolol; it may, however, be the result of low specific activity of the compound itself. Support for the latter view is gained from the observation that the compound 1-(3-isopropylaminopropoxy)naphthalene (I), which lacks the side-chain hydroxyl group of propranolol and which has no asymmetric centre, is about as active as (+)-propranolol in blocking isoprenaline-induced tachycardia. Ariens has shown[13] that the β-mimetic activities of (+)-noradrenaline and (+)-adrenaline, the isomers with least activity on β-receptors, are about the same as those of the corresponding compounds which lack the side-chain hydroxyl group.



I

Propranolol and its (+)-isomer are equally effective in abolishing ouabain-induced arrhythmias while the (−)-isomer had little effect. These results suggest that the action of propranolol in these arrhythmias does not result from blockade of β-adrenergic receptors and support Lucchesi's observations with pronethalol. The compound I does not abolish ouabain-induced arrhythmias. This highlights not only the need for the side-chain hydroxyl group of (+)-propranolol for the effect on ouabain-induced arrhythmias, but also the requirement that the steric arrangement at the asymmetric centre should be the mirror image of that required for maximum β-blocking activity. Propranolol and its (−)-isomer are equally effective in preventing halothane–adrenaline arrhythmias and are at least ten times more active than (+)-propranolol. The protective action of propranolol in these arrhythmias probably results from blockade of β-adrenergic receptors. These observations do not agree with those of Lucchesi[4], who showed that pronethalol and its (+)-isomer were equally effective in preventing anaesthetic adrenaline arrhythmias.

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VARIABLE SIZE OF THE SEPTAL NUCLEI IN MAN

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THE septal nuclei, which form part of the limbic system, are discrete, well-developed nuclear masses in lower forms but become increasingly smaller and ill-defined in the ascending mammalian scale. They are included in the so-called 'septal area' lying anterior and lateral to the descending fibres of the fornix. A large-celled medial and a small-celled lateral or principal septal nucleus can be distinguished, the latter being continuous posteriorly with nucleus septo-fimbrialis. While both the medial and the lateral nucleus are associated with fibres of the precommissural fornix, nucleus septo-fimbrialis is related to fibres of the postcommissural fornix[1]. Microscopically, the septal nuclei are composed of neurones similar in type to the 'dominant elements in the corpus striatum'[2].

In man, the septal nuclei are small and ill-defined and a division into a medial and a lateral nucleus is often difficult[3] as is delimitation from some of the adjacent

structures. In his cyto-architectonic investigation of this region in man, Brockhaus[4] distinguishes between a principal nucleus, his nucleus septoventricularis, and smaller nuclei septofornicatus, interfornicatus and parvocellularis; together he designates them 'nuclei septi proprius'. Over their whole antero-posterior extent these nuclei are demarcated medially by descending fibres of the fornix and laterally by the ventricular wall.

In the course of our investigations on the central nervous system in cases of Kuru, a neurological disease confined to the Fore people and their close neighbours in the Highlands of New Guinea[5], we were impressed by the relatively large size of the septal nuclei in these brains. In all the eleven cases investigated so far the septal nuclei not only had an unusually long antero-posterior extent, but they also protruded markedly into the medial wall of the lateral ventricle (Fig. 1), causing a surface configuration reminiscent of that produced by the caudate nucleus on the opposite side. A similar phenomenon has not previously been observed in any of the brains dealt with in this laboratory. Microscopically, the nuclei appeared normal and showed none of the pathological changes associated with Kuru[6,7]. They were composed of the typical, irregularly shaped neurones (as described by Cajal[2]) interspersed with thin fascicles of myelinated fibres.

The septal nuclei were measured in eight cases of Kuru and in seven normal cases used as controls, and matched, as far as possible, for age and sex. A further three controls of an older age group are included. Controls 3 and 4 are taken from African negroes and the remainder from Europeans. The area measured corresponds approximately to Brockhaus's[4] 'nuclei septi proprius' with the exception of the most anterior tip which is so diffusely arranged that it does not lend itself to measurement.

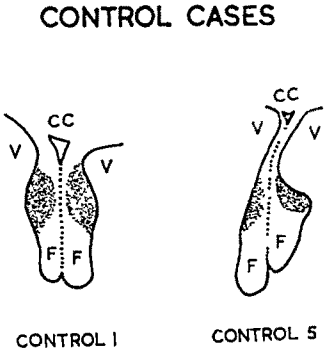


Fig. 1. Projection drawings of representative examples of the septal nuclei (stippled area) taken at a coronal level about 0 75 mm behind the descending columns of the fornix. Note the difference in size and shape between the nuclei of the control and Kuru cases; the latter are much larger and protrude markedly into the wall of the lateral ventricle. CC, Corpus callosum, F, fornix; V, lateral ventricle

The processing of all but two of the brains followed standardized procedures in order to obtain comparable coronal levels and to minimize a variation in shrinkage during dehydration and subsequent embedding in celloidin. The measurements were obtained from projection diagrams drawn at intervals of 0·375 mm from serial sections through the septal nuclei, stained by the method of Nissl. (The outlines of the nuclei were checked under the microscope.) At each level, the area of the nucleus was measured planimetrically and the reading was converted into mm². With a known interval of 0·375 mm between each level the final volume was calculated in mm³ by simple addition of the separate areas multiplied by 0·375.

Table 1

Case No.	Sex	Age at death	Volume of septal nuclei in mm³ left	right	Brain weight‡ (g)
Kuru 1	M	10	57·3	46·5	1025
Kuru 2	F	11	—	41·8	900
Kuru 3	M	11	43·0*	—	983
Kuru 4	F	12	64 0	71·0	873
Kuru 5	F	12	—	54·5*	905
Kuru 6	F	16	38·0*	—	1080
Kuru 7	F	29	46·8*	45·0*	1035
Kuru 8	M	30	—	43·0*	—
Control 1	F	11	16·7	16·5	1350
Control 2	F	15	22·0	25·0	1245
Control 3	M	25	12·0	16·0	1380
Control 4	F	32	12·0	16·0	1320
Control 5	M	40	12·0	13·5	1330
Control 6	F	45	13·5	14·6	1360
Control 7	F	49	14·6	16·0	1460
Control 8	F	53	14·0	13·5	1350
Control 9	F	61	9·4	13·5	—
Control 10	M	69	12·4†	16·5	1370

* Kuru cases 5 and 7 went through a process of double embedding which causes a higher degree of shrinkage; Kuru cases 3, 6 and 8 were damaged at post-mortem—small portions of the septum were missing. The absolute measurements for these five cases should therefore be higher.

† The average volume in the control group amounts to 13·9 mm³ on the left and 16 1 mm³ on the right side. Similar figures would be representative of the large numbers of other European brains seen in this department.

‡ The low brain weight in the cases of Kuru was not due to atrophy. These were all small but perfectly normal-looking brains. Kuru cases 1, 2, 3, 4 and 6 had 10–20 g of tissue removed for virus isolation investigations before fixation and weighing.

A comparison between Kuru cases 1–6 and control cases 1 and 2 shows that the volume of the septal nuclei is, in every instance, considerably larger in the Kuru brains than in the controls. Likewise, Kuru cases 7 and 8 have measurements far exceeding those of control cases 3–10 (this includes the two African negroes). If, in addition, the smallness of the brain in the cases of Kuru, as compared with the controls, is taken into account and thus the size of the septal nuclei is seen in proportion to the total size of the brain, then the difference becomes even more marked.

It is of interest to note that a certain variation also exists between the measurements within the control group and that the two children especially tend to have larger septal nuclei than the adults (though a further correlation with age does not seem obvious within the adult group). However, even the largest volume of 25·0 mm³ (control case 2, right) is far exceeded by the lowest absolute measurement of 41·8 mm³ in Kuru case 2.

The present are preliminary investigations; an investigation of the septal nuclei on a larger scale is planned especially to include the brains of natives belonging to the East New Guinea Highlands stock who did not suffer from Kuru and from other linguistic groups, in particular in Lowland regions of New Guinea and in other parts of Australasia. Apart from the septal nuclei, it is intended

to investigate: (1) the size of the massa intermedia of the thalamus which is large in all cases of Kuru, but, though it may be large in an occasional control, is absent in up to 27·9 per cent of normal brains[8]; (2) the occurrence of a substantial accessory paraventricular nucleus in the hypothalamus which was present in four out of seven cases of Kuru but in none of the ten controls; and (3) the shape of the frontal poles which tend to be rather pointed with a concave orbital surface.

It remains to be seen whether such unusual features are found only in the brains of Kuru patients or whether they are a characteristic of the Melanesian population as a whole.

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PRODUCTION OF ANTIBODY AGAINST "AUSTRALIA ANTIGEN" IN RABBITS

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TRANSFUSED haemophilia patients often have an isoprecipitin in their sera which reacts with an antigen not found in the sera of normal individuals in the United States but not uncommon in the sera of patients with leukaemia (10 per cent)[1] and mongolism (28 per cent)[2] patients. It is also found in the sera of 5 per cent or more of several populations outside the United States[1]. The antigen has not been identified with any of the known serum proteins or cell constituents and, since the original studies were done using the serum of an Australian Aborigine, this "new" isoantigen has tentatively been called Australia antigen.

It has been taken as a working hypothesis that the isoprecipitin is present in the blood of the haemophilia patients as a result of the transfusions the patients have received. The high frequency (29 per cent) of antibody against Australia antigen in haemophilia patients as opposed to the antibody frequency in other transfused patients (approximately 3 per cent) is thought to be due to the fact that the former receive large amounts of fresh frozen plasma. It is postulated that freezing results in the partial denaturation of the plasma with a resulting revelation of an ordinarily occult Australia antigen specificity site. Other evidence consistent with the hypothesis that the isoprecipitin is an antibody is (1) its identification as an IgG immunoglobulin, and (2) the fact that it forms specific precipitins by double diffusion in agar gel in the same manner as other antigen–antibody systems. It was felt, however, that this did not constitute firm proof that the anti-Australia antigen isoprecipitin was indeed a specific antibody. In the present article, experiments are described in which rabbits were immunized with the whole serum containing Australia antigen. Specific antiserum against Australia antigen was produced after absorption with the sera of normal persons which did not contain Australia antigen. The results provide clear support for the antibody hypothesis. This technique also provides a more reliable source of antisera than that available from sick transfused patients.

Blood was collected by venepuncture and allowed to clot at room temperature for 4 h and at 4° C overnight. The serum was separated from the red blood cell clot by centrifugation and stored in glass containers at −20° C until ready for use. The immunizations were performed within 1 month of blood collection. Specimens from three different individuals were used in the experiments. (1) C. F., a 54-year-old white male with chronic reticuloendotheliosis of 2 years duration. This serum contains Australia antigen. (2) C. De S., a 60-year-old white female with chronic lymphocytic leukaemia of 5 years duration. This serum contains Australia antigen. (3) L. M., a normal blood donor. This serum does not contain Australia antigen and is used as a control.

Absorptions were performed with a pool of two sera from normal white United States blood donors. We are indebted to Dr. R. Donato of the Jeanes Hospital, Philadelphia, for these samples. Male rabbits (Laboratory Animals Corp., Plainfield, New Jersey) weighing 2·7–3·2 kg at the beginning of the experiment were used for the immunizations. None showed any signs of illness. The sera of all of the rabbits were tested for the presence of human serum protein antibodies before the immunizations were begun, but none was found.

Rabbits 1 and 2 were immunized with reticuloendotheliosis serum C. F.; rabbits 3 and 4 with leukaemia serum C. De S.; and rabbits 5 and 6 with normal serum L. M., using the schedule of Hirschfeld[3]. Briefly, this consists of a first intramuscular immunization with alum-precipitated serum followed by a similar immunization 2 weeks later. 10 days after the second immunization, 1 ml. of serum was injected intraperitoneally. After a 7–10-day rest period, the rabbits were bled and the antiserum used in the experiments (see Table 1, "after 6 weeks"). After a 2-week rest, the immunization schedule was repeated (Table 1, "after 14 weeks"). The antisera produced contained antibodies against normal serum proteins as well as Australia antigen. In order to produce antisera specific for Australia antigen, the sera from each of the rabbits were absorbed in a test-tube using the pool of two normal sera described above. To establish the proper ratio of antiserum to absorbing sera, various concentrations were tried until the minimum amount of "normal" serum (not containing Australia antigen) necessary to absorb the antibodies against the normal non-Australia antigen proteins was determined. The technique eventually adopted after these experiments consisted of the following: two parts of rabbit serum was mixed with one part of the pool of absorbing (Australia antigen negative) serum and the resulting mixtures incubated at 4° C for 48 h. The precipitate was then centrifuged and the supernatant used in the diffusion experiments. For comparison with the antisera produced in the rabbits, the serum of a haemophilia patient containing antibody against Australia antigen was used.

In some of the experiments, absorption was carried out by the "in basin" technique. In this the antiserum well was first filled with the absorbing serum and after it had diffused into the gel (approximately 30 min), the antiserum to be absorbed was added. Double diffusion in agar gel, using the Ouchterlony micro-pattern, was carried out by a method previously described[4]. Double diffusion in agar tubes was done by the method of Preer and Preer[5]. We are indebted to Dr. Anne S Jones for performing these experiments.

Table 1. DEVELOPMENT OF ANTIBODY AGAINST AUSTRALIA ANTIGEN IN RABBIT NO 4 AFTER 6 AND 14 WEEKS OF THE IMMUNIZATION PROGRAMME (The Table gives the reactions with forty sera known to contain Australia antigen)

	Total	Positive	Percentage positive
After 6 weeks	40	30	75
After 14 weeks	40	39	98

After the 6-week immunization period, the absorbed antiserum produced in rabbits 3 and 4 (which had been immunized with the serum of patient C. De S.) reacted to form what appeared to be a single precipitin band with the sera of normal individuals and patients with leukaemia known to contain Australia antigen, but not with the serum of patients or normals in which Australia antigen had never been found. 75 per cent of the known Australia antigen positive individuals gave a detectable precipitin reaction with the rabbit antiserum (Table 1). After the 14-week immunization period, 98 per cent of the known Australia antigen sera gave a detectable reaction (Table 1). The precipitin lines stained only faintly with Sudan black but gave an intense stain with azo carmine. These were the same staining characteristics exhibited by the human Australia antigen iso-precipitins.

The precipitin line formed against the rabbit antiserum gave a reaction of identity with the precipitin line formed against the human antiserum (Fig. 1). A well-defined single band was also seen using the Preer tube technique (Fig. 2).

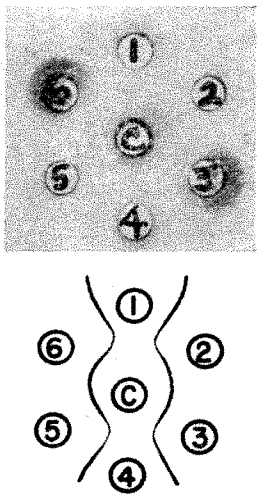


Fig. 1. Photograph and diagram of an experiment designed to show that human serum containing Australia antigen can absorb the antibody against Australia antigen developed in rabbits, and that serum which does not contain Australia antigen does not do so. The following sera are placed in the indicated wells: (*C*) serum from a leukaemia patient which contains Australia antigen; (1) Rabbit-anti-Australia antigen (Rabbit No. 4) absorbed with Australia antigen positive sera from two patients with Down's syndrome (Mongolism). The antibody is absorbed and no precipitin is seen between the central and No. 1 wells; (2) Rabbit anti-Australia antigen serum (rabbit No. 4) absorbed with Australia antigen negative sera from a normal person. The antibody is not absorbed and a precipitin line is seen; (3) Serum from a patient with haemophilia with an antibody against Australia antigen. There is a reaction of identity between this and the rabbit line; (4) same as 1; (5) same as 2; (6) same as 3. The lines between 1 and 2, 3 and 4, 4 and 5, and 6 and 1, are the reactions between the anti-Australia antigen and the Australia antigen in the excess absorbing sera

In another experiment, all the wells contained the same sera except the central well which contained an Australia antigen negative serum. In this case, there were no reactions between the central and peripheral wells

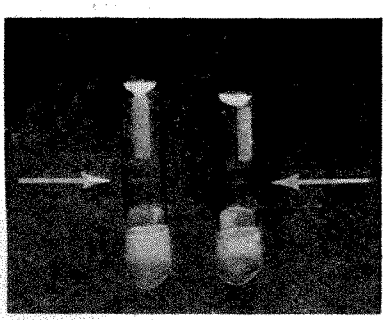


Fig. 2. Double diffusion in agar gel using the Preer tube technique (ref. 5). The reaction of two sera containing Australia antigen with rabbit anti-human Australia antigen is shown

Table 2. PATTERN OF REACTIONS WITH RABBIT ANTI-AUSTRALIA ANTIGEN SERUM AND HUMAN ANTI-AUSTRALIA ANTIGEN SERUM IN THREE POPULATIONS (Antisera from human and rabbit are tested with the same panel of Australia antigen positive and negative human sera)

Antiserum reaction	Normal (Utirik Atoll*) Human +	Normal (Utirik Atoll*) Human −	Down's syndrome Human +	Down's syndrome Human −	Leukaemia Human +	Leukaemia Human −
Rabbit +	15	0	19	0	4	0
Rabbit −	0	96	0	56	1	78
Total	11		75		83	

* U.S. Trust Territory of the Pacific Islands.

After absorption with an Australia antigen positive serum (using the "in basin" method) the rabbit antisera did not give a precipitin reaction. Absorption with an Australia antigen negative serum has no effect on precipitin formation (Fig. 1).

In order to compare the specificity of the rabbit anti-Australia antiserum with that found in the human haemophilia patients, both were tested side by side with the sera from members of three populations in which Australia antigen is relatively common (Table 2). There was very nearly complete agreement, with the exception of a single patient with leukaemia who reacted with the human antiserum, but not with the rabbit antiserum. This is the same serum which did not react with the "after 14 weeks" antiserum (Table 1). There is reason to believe that this patient has an unusual Australia antigen and this interesting possibility is being investigated. There is, in fact, evidence that there is more than one Australia antigen specificity and this will be described elsewhere.

When the rabbit antiserum produced in the rabbit (No. 6) which had been immunized with the normal serum (L. M.) was absorbed and tested in the same manner as the other antisera, it gave no precipitin line with the sera containing Australia antigen, or with other sera. One of the control rabbits (No. 5) died before the end of the immunization schedule.

An observation presumably unrelated to Australia antigen was made using the antisera produced in rabbits 1 and 2. Rabbits 1 and 2 produced a strong antibody against a blood protein present in individuals with and without disease which was different to the antibody against Australia antigen. Using immunoelectrophoresis and commercial specific human antisera, it was shown that this was an antibody against soluble fibrinogen[6,7]. These rabbits also produced a weak antibody against Australia antigen after 6 weeks, which increased in intensity after 14 weeks.

The production of the specific anti-Australia antigen antibody in rabbits by immunization with serum containing Australia antigen shows that the precipitation obtained with haemophilia sera is indeed caused by an antibody and lends considerable support to the hypothesis that this antibody is a result of immunization by transfusion with human plasma containing Australia antigen. It also adds support to the impression (gained from the high frequency of antibody formers among the transfused haemophilia patients) that Australia antigen is a "good" antigen, since there was no difficulty in developing antibodies in all the rabbits immunized after a relatively short period of immunization.

This method of producing antisera will make available much larger amounts than was possible from human sources and should help advance the studies on the significance of the Australia isoantigen system.

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CELLULAR TRANSMISSION OF LYMPHOSARCOMA IN DOGS

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ALTHOUGH leukaemias and lymphosarcomata of fowls and rodents can often be transferred easily by inoculating cells into the same species, cellular transfer of the disease in domesticated animals has proved difficult. Rosenberger[1] has reported producing leucosis in cattle by inoculation of blood, milk, urine, faeces and suspensions of leucotic tumours as well as cell-free material, but details are not given. Hofland, Thorell and Winqvist[2] reported successful transfer to one of two very young calves inoculated with 10^8 leucotic cells after an interval of 4 years. Using a presumably cell-free extract of a lymphomatous node from a cat, Jarrett *et al.*[3] have recently reported the appearance of the disease in all of four kittens inoculated at birth. Nielsen and Cole[4] were unsuccessful in transferring canine lymphoma by the inoculation of cells into irradiated pups. This is a report of the cellular transfer of lymphosarcoma to three irradiated puppies. One clear case appeared 26 days and the second 90 days after the inoculation of cells from lymphosarcomatous dogs. The third animal developed the disease 44 days after the inoculation of a canine lymphosarcoma cell line, *LS* No. 30, which had been in tissue culture for almost 1 year. In previous attempts, thirty-five adult dogs inoculated with lymphosarcoma cells failed to develop the disease.

Three dogs (Table 1) with advanced lymphosarcoma provided the starting material. The first two were treated intermittently with *cyclo*phosphamide in an effort to control their disease. Cell suspensions were prepared either from surgically removed spleen or from lymph nodes on the day the pups were born. After mincing the tissue, the cells were freed by blending at a low speed in 1 per cent heparinized saline. The cells were sedimented by centrifugation at 1,000 r.p.m., re-suspended in balanced saline, and counted in a haemocytometer.

Filtrates were made in the following manner: lymphomatous tissue was weighed, ground in a mortar with sand, and sufficient Eagle's basal medium[5] was added to make a 20 per cent extract. After spinning at 4,000 r.p.m. for 15 min to sediment the coarse particles, the decanted fluid was again spun at 6,500 r.p.m. for 30 min before passage through a 3-μ 'Millipore' filter. The filtrate was then passed through a 0·65-μ, 0·4-μ and finally twice through 0·22-μ 'Millipore' filters.

The *LS* No. 30 cell line was developed from the spleen of an 11-year-old male dog with advanced lymphosarcoma after splenectomy in July, 1964. The trypsinized cells were implanted in milk dilution bottles in Eagle's basic media with 10 per cent agammaglobulinaemic calf serum. In one month the cells had grown out sufficiently to be trypsinized. The cultures then began to grow rapidly, so that they are now subcultured at 3-day intervals. The cells are neither epithelial nor fibroblastic in appearance but resemble those sometimes called reticular. Small, round cells and both multi- and mono-nucleated giant cells are seen. The cells inoculated into dog M.B. were those from the thirty-fifth to thirty-seventh transfers and were prepared by freeing the cells from the glass by trypsinizing and scraping. After centrifugation they were re-suspended in a small amount of medium and counted in a haemocytometer.

All recipients were mongrel new-born dogs of unknown origin. As soon as possible after birth they were given total-body irradiation delivered by a 180 kV dual tube X-ray machine, 25 m.amp, *TSD* 40 cm, at a dose rate of 85·4 r./min (air). Litters 1 and 3 received 85·4 r. immediately after birth, while litter 2 received 128 r. 24 h after birth. The pups were inoculated subcutaneously in the nape of the neck and intramuscularly in the leg 10–24 h after irradiation. Repeated inoculations were given at weekly intervals as outlined in Table 1. Fresh cell suspensions made from biopsy specimens were used except for three animals in litter 1 which received the original suspension after storage in the refrigerator for 1 week. Counts with trypan blue showed that the cells were 65 per cent viable. The *LS* No. 30 cells were administered immediately after trypsinization. Cell filtrates were kept frozen at −80° C during the interval between the inoculations.

As shown in Table 1, lymph nodes became palpable and visibly enlarged 26–58 days after inoculation. The first to appear were the mammary or peripenal nodes, followed by suprascapular, popliteal and cervical. The later appearance of the nodes in litter 2 may be a result of the smaller numbers of cells inoculated or of the fact that they were inoculated 1 day later than the other litters. Biopsies were done on all animals and have been reported as showing hyperplasia, except in the case of

Table 1

Donors of malignant lymphoma material	Recipients	Sex	Material injected	Amount	Appearance of lymph nodes	Death	Confirmed diagnosis of lymphosarcoma
	Litter 1 (born April 1, 1965)						
King, 7-year-old male collie	D.B.	F	Spleen suspension	1×10^9 (2×)	20 days	6 weeks	+
	*D*1	F	,, ,,	,,	26 days		
	*D*2	M	,, ,,	,,	,,		+
	*D*3	M	Cell filtrate ,,	4 ml. (3×)	,,		
	M.B.	F	Tissue culture line *LS* No. 30 cells	7, 10, 5 × 10^6 (3×)	,,	12 weeks	+
	Litter 2 (born April 25, 1965)						
Brandy, 4-year-old female boxer	1*FT*	F	Lymph node suspension	2·5 × 10^8(2×) 1·3 × 10^9(2×)	58 days		
	2*FB*	F	,,	,,	,,		
	3*FBW*	F	,, ,,	,,	,,		
	4*MBW*	M	Cell filtrate	4 ml. (4×)	,,		
	Control	M			,,		
	Litter 3 (born May 18, 1965)						
Betty, 5-year-old female mongrel	1*A*	F	Tissue culture line *LS* No. 30	17, 9, 18 × 10^6	50 days		
	2*B*	F	Spleen and lymph node suspension	1 × 10^9 (4×)	36 days	38 days	
	3*B*	M	,,	,,	35 days	35 days	
	4*C*	F	,,	,,	36 days	38 days	
	5*C*	F	,,	,,	40 days	42 days	
	Control	M					

Litters 1 and 3 irradiated immediately after birth (85·4 r. total body irradiation).
Litter 2 irradiated 24 h after birth (128 r. total body irradiation).



Fig. 1. Suprascapular lymph nodes removed from dog M.B. at autopsy (actual size)

D.B., where the nodes showed lymphosarcoma at 26 days, and M.B., which had one negative biopsy at 26 days, but the next specimen from which showed the beginnings of lymphosarcoma at 44 days. Similarly, dog *D*2 showed no lymphosarcoma in a biopsy taken at 26 days but was reported positive for the disease in a specimen taken at 90 days. The litters are now 3·5, 3 and 2·5 months of age and still have palpable nodes which have not increased in size for the past month. Four dogs in litter 3 died of inter-current infection and although abnormal lymphocytes were seen, a positive diagnosis of lymphosarcoma could not be made.

Recipient D.B. died at 6 weeks of age with generalized lymphosarcoma involving the spleen and lymph nodes. A cell suspension containing $1{\cdot}3 \times 10^9$ lymph node cells from this animal was immediately administered to two new-born irradiated puppies. One died of malnutrition 2 days later and the other died in 14 days of generalized lymphosarcoma, thus achieving the second passage. Cell suspensions from this dog were processed as before and inoculated into three irradiated new-born puppies. Two of these died of inter-current infection and the one remaining pup shows no sign of disease at 50 days of age.

In the cell filtrate recipients *D*3 and M.B.W., lymph nodes appeared as stated in Table 1 and are still present. Biopsy of these lymph nodes showed hyperplasia with an increased number of reticulum cells, but the appearance at this time is not that of a lymph node malignancy.

We believe the following factors are important in the cellular transmission of this disease: (1) Timing of irradiation of new-born pups: irradiation should be given as soon as possible after birth in order to prevent further development of immunological competence. (2) Amount of radiation: total-body irradiation with 85–128 r. (in air) has been sufficient. No haematological changes were encountered at this dose. Puppies are very susceptible to pneumonia and doses which cause leukopenia could be deleterious. (3) Amount of material: this seemed to be a very important factor. When we injected the second litter with little material, lymph node enlargement was temporary and disappeared in a few weeks. After the last two injections of large quantities of cells the lymph nodes reappeared.

In summary, cellular transmission of canine lymphosarcoma has been accomplished by injecting material from adult dogs and a tissue culture line into irradiated new-born puppies. Lymphadenopathy is present in puppies which received cell-free filtrate and their lymph node architecture is abnormal, but the disease has not yet unequivocally appeared.

Since the preparation of this manuscript, Dr. Charles Rickard of the New York State Veterinary College at Cornell has informed us that he, too, has been successful in transferring lymphosarcoma to new-born puppies with cell suspensions.

We wish to thank Mr. Alan Kaplan for his technical assistance.

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TRANSFORMATIONS IN HAMSTER CELLS MEDIATED BY MYCOPLASMAS

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RECENT investigations have shown that mycoplasmas mimic viruses in many of their activities[1]. In tissue culture, for example, mycoplasmas may establish inapparent infections or cause complete cytolysis. This article describes a further similarity. Like polyoma and Rous sarcoma viruses[2,3], certain mycoplasmas have been shown to be capable of mediating changes in the *BHK*21 line of hamster fibroblasts such that the cells will form colonies in agar suspension culture and also grow on glass with altered colonial morphology.

The *BHK*21 cells used in this investigation were low-passage clone 13 cells cultured on glass according to methods previously described[4]. They had been carefully examined for mycoplasma by ourselves and others[5,6]. All tests were negative. Mycoplasma cultures were obtained through the courtesy of Dr. Ruth Lemcke, Lister Institute of Preventive Medicine, London, Dr. D. Taylor-Robinson, Medical Research Council Common Cold Research Unit, Salisbury, and Dr. R. Fallon, Ruchill Hospital, Glasgow, as broth cultures and frozen-dried broth cultures. They were routinely passaged in Difco PPLO broth containing 20 per cent unheated horse serum (Burroughs-Wellcome) and 10 per cent yeast extract. Cells were infected in suspension by shaking 10^6 cells in 1–2 ml. of medium containing a 1 in 10 dilution of a broth culture of mycoplasmas at 37° C for 1 h. Controls consisted of cells shaken with medium plus uninoculated broth and also mycoplasma broth cultures heated at 56° C for 1 h.

Agar suspension cultures were prepared as previously described[2]. Individual cultures contained 10^4–10^5 cells incorporated in top layers of 0·25 per cent or 0·33 per cent agar medium and were incubated at 37° C for 10 days to 6 weeks in a humidified carbon dioxide incubator. Cultures were examined after 10 days, then at weekly intervals for the appearance of macroscopic colonies (> 0·5 mm). Colonies were removed from the agar with finely drawn Pasteur pipettes and transferred to small bottles with 1 ml. of tissue culture medium. After pipetting the colony in the medium to free it from the agar and break it up, the cells attached to the glass and grew out during the course of the following week. The culture was then dispersed with trypsin and passed or plated to produce discrete colonies in Petri dishes.

In the first series of experiments carried out by one of us (I. M.), cells were infected with *Mycoplasma hominis* type 1 (*H* 34) and plated in 0·33 per cent agar. The controls produced colonies at a frequency of 0·005 per cent of the cells plated. The infected cells formed colonies with an efficiency of 0·4 per cent. When colonies were picked from the plates seeded with infected cells and grown on glass, a remarkable range of altered cells was obtained. Multinucleated giant cells, epithelioid cells,

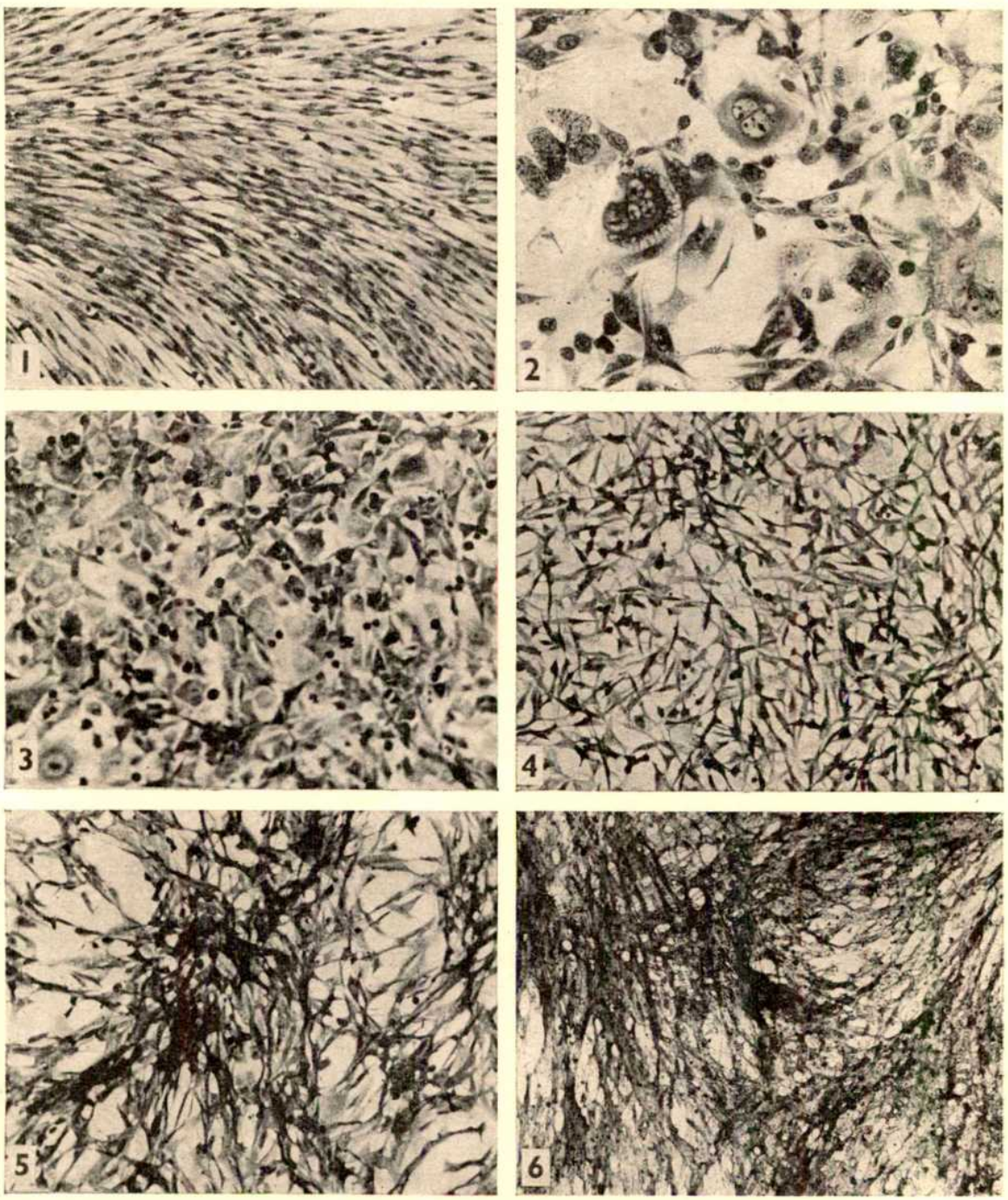


Fig. 1. Uninfected *BHK*21/13 cells. (× 75)

Figs. 2–4. *BHK*21/13 cells following infection with *M. hominis* type 1 (*H*34). Note: Giant cells in Fig. 2, epithelioid cells in Fig. 3, and the random arrangement of the cells in Fig. 4. (× 75)

Figs. 5 and 6. *BHK*21/13 cells following infection with *M. fermentans*. (× 75)

round cells, and long spindle-shaped cells growing in disarray like polyoma transformed cells, were all seen. (See Figs. 1–4.)

Mycoplasmas were regularly isolated from these cultures on agar medium. When the cells were plated to form colonies in Petri dishes and left for more than 7 days without medium changes, the cells in the centre of the colonies degenerated and came off the glass leaving a ring of viable cells. The cytopathogenic changes in such cultures could be delayed by changing the medium each day. In many cultures left for more than 6 or 7 days without medium changes, corynebacteria appeared in association with the cells. The relationship between these bacteria and the mycoplasmas is not known. Similar associations have been previously described[7,8]. No other tissue cultures carried in the laboratory at the same time and cultured in the same medium were contaminated with corynebacteria, and they could not be detected in the broth cultures of mycoplasma by plating on agar media.

Cultures derived from seven colonies with different morphologies, and in culture for 3 weeks since infection, were grown for 5 days in medium containing 500 μg 'Kanamycin', 50 μg 'Novobiocin' and 5 μg 'Aureomycin'/ml. The medium was changed each day. On the sixth day the medium was changed to one without the additional antibiotics and medium changes continued each day for a further 5 days. The cells of each clone were subcultured with trypsin three to four times during the course of this treatment. Each subculture received one-twentieth of the cells in the previous pass. At the end of this period no mycoplasmas or bacteria could be detected in the cells. The cells had in all cases retained their altered growth characteristics on glass and when two lines of cells with fibroblastic and epithelioid morphologies were cultured in agar they plated with efficiencies of 0·3 and 0·7 per cent respectively.

In the second series of experiments (carried out by one of us (W. R.)), *BHK*21/13 cells (obtained from Dr. W. I. H. Shedden, Department of Virology, University of Birmingham, and free from detectable mycoplasmas) were infected with the strains of mycoplasmas shown in Table 1 and plated in 0·25 per cent and 0·33 per cent agar medium. It will be seen from Table 1 that colonies appeared in all cases except with *M. salivarium* and the control cells. With the higher concentrations of agar, no colonies were obtained with cells infected with *M. pneumoniae*. As in the first experiment the cells picked from these colonies were initially morphologically altered but in all cases except cells derived from the *M. fermenians* infection, the cells gradually returned to the fibroblastic shape and the parallel alignment of uninfected *BHK*21 cells.

Table 1. Colony Formation by *BHK*21/13 Cells in Agar Suspension Culture following Infection with Various Mycoplasmas

Mycoplasma	No. of colonies in 0·25 per cent agar (10^5 cells plated)	No. of colonies in 0·33 per cent agar (5×10^4 cells plated)
*M. fermentans PG*18	20	2
M. hominis I *PG*21	31	4
M. hominis II *PG*27	50	6
M. orale I *CH*19299	5	1
M. orale II *CH*20247	17	9
M. pneumoniae Eaton *CH*	10	0
*M. salivarium PG*20	0	0
None	0	0

Cells cultured from agar colonies obtained after infection with *M. fermentans* were initially very elongated in appearance, but after further sub-cultivation they assumed the appearance shown in Figs. 5 and 6. The cells pile up on each other at random, unlike their uninfected precursors, which grow in parallel array (Fig. 1). In very dense cultures the cells eventually assume some degree of parallel orientation. The cells have retained this characteristic morphology and growth pattern for fifty generations. They grow as rapidly as *BHK*21 cells but produce more acid in the medium. Their plating efficiency on glass is 40 per cent—more than twice that of uninfected *BHK*21 cells. Many attempts have been made to recover mycoplasmas from the cells both by direct plating of tissue culture media and cells on agar media and after pre-incubation in broth. No positive isolations have been obtained. After growing the cells in medium containing 50 μg 'Aureomycin' and 50 μg 'Kanamycin' per ml. for 3 days, the medium being changed every day, the cells retained their characteristic morphology.

Cells transformed by *M. fermentans* and cells derived from a colony obtained from *M. hominis* II infection (with unaltered morphology on glass and containing no demonstrable mycoplasmas) were grown in medium containing aureomycin and kanamycin, as already described, and then plated in agar suspension culture. The cells plated with efficiencies of 3 per cent and 0·2 per cent respectively, that is, 150 and 10 times higher than the colony-forming efficiency obtained after the initial infection of the cells.

The experiments indicate that some mycoplasmas, like some oncogenic viruses, are capable of mediating inheritable changes in cells *in vitro*. It seems unlikely that our results can be explained on the basis of selection of pre-existing cells. However, it is possible that mycoplasma infection of some cells stimulates them to grow in agar or acts as a 'co-carcinogen' for some other undefined factor in our system.

It has been reported that cells infected with mycoplasmas may show chromosome abnormalities[9,10]. Mycoplasmas are known to be capable of cleaving thymidine to thymine and deoxyribose[11], and Randall *et al.*[12] have found that in HeLa cells infected with mycoplasmas the host-cell DNA is unstable as detected by incorporation of tritium- and carbon-14-labelled thymidine into DNA and there subsequent release into the medium as acid-soluble radioactivity. The effects reported here may well be the manifestation of a permanent change in the genetic apparatus of the cell induced directly by the mycoplasmas.

Our observations suggest a possible mechanism for the 'spontaneous' conversion of euploid cell lines into aneuploid lines capable of continuous propagation *in vitro*. As we have noted here some of our *BHK*21 cultures, apparently transformed by mycoplasmas, no longer release mycoplasmas in detectable numbers. Perhaps mycoplasmas, like Rous sarcoma virus[13], can exist in 'defective' forms as an obligate cell parasite.

In our hands, *BHK*21 cells in agar suspension culture have a very low plating efficiency. It is possible that the high plating efficiencies of *BHK*21 cells in agar reported by some workers[14,15] may have been due to mycoplasma infection of the cells.

So far, investigations designed to test the oncogenic activity of mycoplasmas in new-born hamsters have yielded negative results[16,17]. Work is in progress to test the transplantability of the *BHK*21 cells transformed by mycoplasmas and also the effect of mycoplasmas in other cell systems.

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LETTERS TO THE EDITOR

ASTRONOMY

Relation between the Red-shifts of Quasi-stellar Objects and their Radio and Optical Magnitudes

TOGETHER with E. M. Burbidge and C. R. Lynds, we have recently given a table of more than forty quasi-stellar objects which includes red-shifts, optical magnitudes, and line spectrum data[1]. Radio fluxes are available for nearly all these objects. Provided we restrict ourselves to objects with flux values $> 8 \times 10^{-26}$ W m^{-2} (c/s)$^{-1}$ at 178 Mc/s, the list is sufficiently complete (over the portion of the sky available to Palomar, Lick and Kitt Peak, the observatories from which the red-shift measurements have been made) for approximate number counts to be made. There are thirty such objects. It happens that $30 = 2 + 2^2 + 2^3 + 2^4$, which provides the following simple way of making a number count. Write $N(S)$ for the number of objects with flux density $\geq S$ and define S_1, S_2, S_3, S_4 to be the smallest values of S such that $N(S_1) = 2$, $N(S_2) = 2 + 2^2$, $N(S_3) = 2 + 2^2 + 2^3$, $N(S_4) = 30$. This permits four points to be plotted in the log N–log S plane. These points are shown in Fig. 1. Joining them gives a slope close to $-1{\cdot}5$, not quite as steep as the value of $-1{\cdot}85$ obtained from the counting of all radio sources, and not as steep as the value of about $-2{\cdot}2$ obtained by Véron[2] for all quasi-stellar objects contributing to the 3C catalogue. However, the slope of the points in Fig. 1 is evidently influenced by the two lower points which must be subject to a considerable statistical scatter. So no undue significance should be attached to the particular value of the slope which these points happen to give. What does appear to be significant, however, is that even this small sample of quasi-stellar objects shows the usual relation between N and S, $NS^{3/2}$ approximately constant. The result $NS^{3/2}$ approximately constant has always been interpreted in terms of distances and volumes—for example, for a Euclidean universe and for objects all with the same intrinsic emission, $N \propto r^3$, $S \propto r^{-2}$ (r being the distance). A similar interpretation for our sample of quasi-stellar objects requires the objects with smaller S to be at the greater distance, in any event statistically. Objects with $S \approx S_4$ should be statistically farther away than objects with $S \approx S_3$, and so on. If we make the assumption that the red-shifts z of the quasi-stellar spectra arise from the cosmological expansion of the universe, then small S must be correlated with large z. In Fig. 2 it is shown emphatically that this is not so. Thus if we adopt the usual distance-volume interpretation of the result $NS^{3/2} \approx$ constant, we must conclude that the red-shifts have nothing to do with distances.

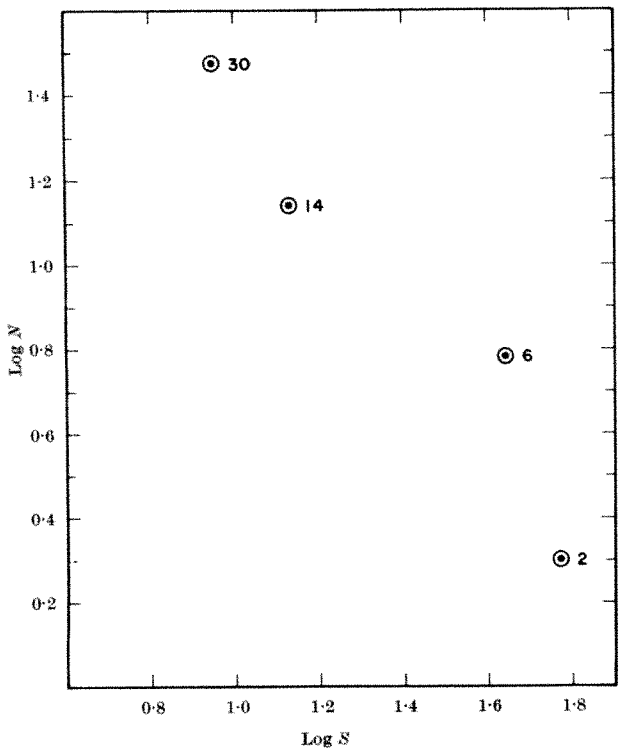


Fig. 1. Log N–log S plot for thirty quasi-stellar objects with radio fluxes $> 8 \times 10^{-26}$ W m^{-2} (c/s)$^{-1}$

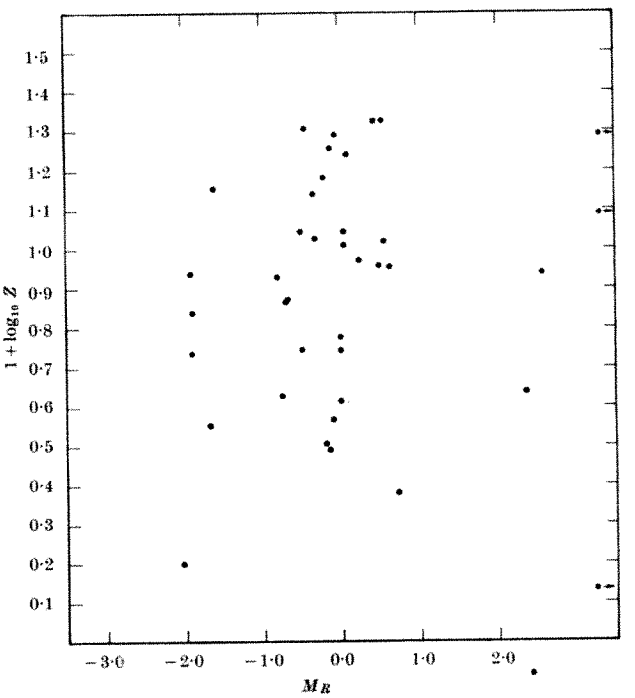


Fig. 2. Red-shift versus radio magnitude for quasi-stellar objects used in Fig. 1, together with several objects having smaller values of S. ($S = 10$ equals $M_R = 0{\cdot}00$)

In Fig. 3 we show log z plotted against visual optical magnitudes, M_v. This is for the full list of quasi-stellar objects[1]. These objects are indicated by filled circles. The open circles show the positions of N-type galaxies. The scatter in the points of Fig. 3 is large compared with the difference between M_v and the bolometric magnitude $M_{\text{Bol.}}$ the latter difference usually lying within $\pm 0{\cdot}3$ magnitudes. To sufficient accuracy, Fig. 3 can therefore be taken as representing the log z–$M_{\text{Bol.}}$ plane. The

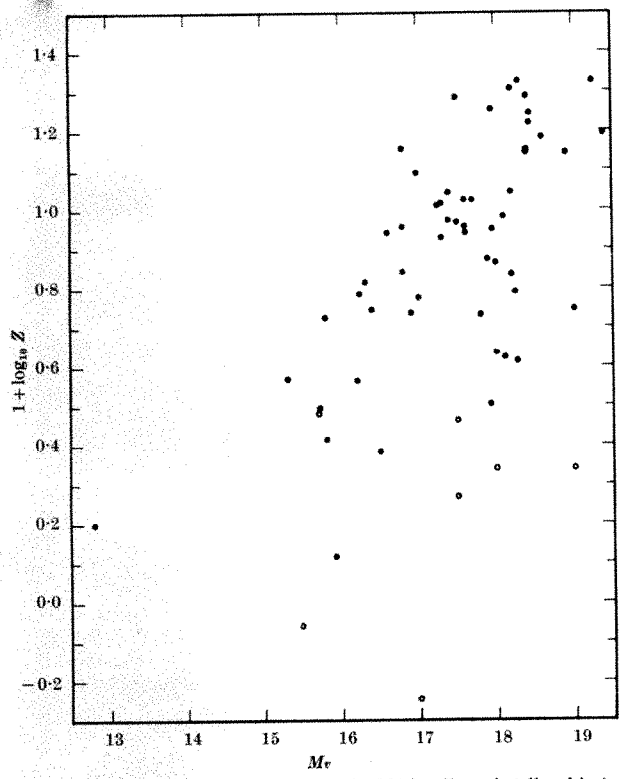


Fig. 3. Red-shift versus optical magnitude (M_v) for all quasi-stellar objects and *N*-type galaxies for which data are available. The list is compiled from published data from the observers working at Kitt Peak Observatory, Lick Observatory and Palomar Observatory. A large proportion of the objects are given in ref. 1. Others have been obtained from a list compiled by Burbidge, E. M., Lynds, C. R., and Schmidt, M., at the International Astronomical Union symposium in Erevan in May 1966

scatter is evidently so large that nothing useful, from a cosmological point of view, can be inferred. Indeed, as new red-shift values have become increasingly available, the plot of the observed quasi-stellar objects has assumed more and more the aspects of a scatter diagram. This can be seen by comparing Fig. 3 with a similar plot given by Sandage[3] about a year ago.

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Origin of the Lunar Maria

THE hypothesis proposed by Kopal[1] concerning the origin of the lunar maria is very interesting. However, it is important to note that the distribution of lunar craters on the Earth-turned hemisphere is far from uniform. There are significant, well-marked chains approximately aligned with the central meridian as seen from Earth; in the west (classical sense) the chain to which Vendelinus and Langrenus belong; near the centre, the Walter and the Ptolemaeus chains; in the east, the Grimaldi chain, and so on. The suggestion by Baldwin[2], according to which the alignments are not real and are due to lighting effects, does not fit the facts. I have discussed this matter elsewhere[3,4]. Particularly important, however, is the conclusion that the well-formed maria fit in to the general pattern; it is difficult to avoid the conclusion that, for example, the Mare Crisium is simply a large member of the Vendelinus chain. To introduce a separate origin for the maria presupposes that they are of a nature basically different from that of the craters.

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GEOLOGY

Age Determinations on Granites, Pegmatites and Veins from the Kibaran Belt of Central and Northern Katanga (Congo)

THE Kibaran–Burundi–Karagwe-Ankolean fold belt is one of the major geological features of eastern central Africa. It is perhaps best known as the seat of important tin and niobium–tantalum mineralizations, occurring in association with post-tectonic granites, pegmatites and veins[1,2]. In the Kibaran portion of the belt, geochronological studies have been carried out by the rubidium–strontium method on tectonically deformed granites, on non-deformed granites, and on pegmatites and veins, from four granitic batholiths: Bia Mountains, Kibara Mountains, Bukena-Manono and Mwanza.

The oldest granitic rocks recognized in both field and petrographic studies are pre- or early-tectonic granites emplaced in Kibaran metasedimentary rocks. The granites were modified during the Kibaran orogeny and are, at present, gneissic biotite granites with or without prominent microcline phenocrysts. The porphyric variety exists in all four of the massifs studied. The age of this type of granite from the Mwanza massif (Table 1, Nos. 1, 2, 4–6) is 1,310 ± 40 m.y., yielded by the closely fitting isochron of Fig. 1, with an initial strontium-87/strontium-86 ratio of $0{\cdot}708_5$. The apparent age of a non-porphyric whole-rock sample from the Bia Mountains (Table 1, No. 7) falls on the same isochron and, if the same initial ratio is assumed, is therefore the same age. It is note-

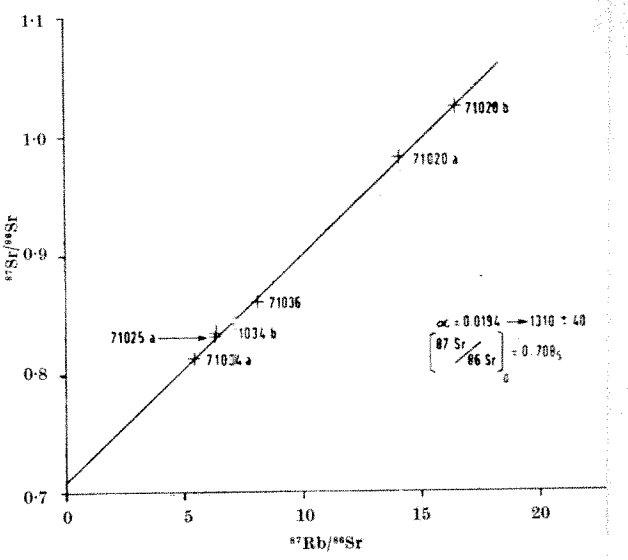


Fig. 1. Pre- or early tectonic granites from Mwanza massif. (No. 71025*a* is from Mt. Bakalenge, Bia massif)

Table 1

No.	Specimen No.	Mineral and rock type	Location	Rubidium (p.p.m.)	Strontium (p.p.m.)	Radiogenic strontium (p.p.m.)	Radiogenic strontium-87 / Total strontium-87 (%)	$^{87}Sr/^{86}Sr$	$^{86}Rb/^{86}Sr$	Apparent age or age (m.y.)†
1	71034*a*	Whole-rock, porphyric gneissic granite	Mwanza massif 26° 30′ E—7° 54′ S	218	11·6	1·18	12·8	0·813	5·42	1,310 ± 40*
2	71034*b*	Microcline, *d*°	*d*°	396	18·1	2·21	15·0	0·834	6·37	1,310 ± 40*
3	71034*c*	Biotite, *d*°	*d*°	940	17·9	3·48	73·8	2·703	152	885 ± 27
4	71036	Whole rock, *d*°	*d*° 26° 35′ E—7° 51′ S	290	5·8	1·76	16·6	0·860	8·11	1,310 ± 40*
5	71020*a*	Whole rock, *d*°	*d*° 26° 32′ E—7° 49′ S	293	6·3	1·59	22·7	0·982	14·25	1,310 ± 40*
6	71020*b*	Microcline, *d*°	*d*°	376	66·2	2·04	30·8	1·025	16·5	1,310 ± 40*
7	71025*a*	Whole rock, gneissic granite	Mt. Bakalenge, Bia massif 25° 53′ E—9° 43′ S	278	127	1·51	14·7	0·831	6·36	1,310 ± 40*
8	71025*b*	Biotite	*d*°	1,116	7·27	3·88	88·5	6·170	445	830 ± 25
9	71002	Whole rock, syntectonic gneissic granite	Bukena massif 27° 19′ E—7° 40′ S	265	105	1·33	15·5	0·844	7·35	1,210 } 1,240 ± 70
10	71002*b*	Microcline, *d*°	*d*°	436	191	2·35	15·1	0·835	6·62	1,280
11	71012	Whole rock, post-tectonic muscovite tin granite	Bia massif 26° 15′ E—9° 28′ S	369	71·6	1·56	23·9	0·942	14·9	1,010 ± 50
12	71004*a*	Whole rock, *d*°	Bukena massif 27° 11′ E—7° 43′ S	840	34·2	3·34	58·5	1·700	71·2	945 } 925 ± 20
13	71004*b*	Muscovite, *d*°	*d*°	1,709	2·47	6·50	96·4	23·13	1,670	905
14	71044*a*	Whole rock, *d*°	Mwanza massif 26° 32′—7° 54′	909	112	3·76	32·6	1·075	23·5	985 } 985 ± 20
15	71044*b*	Muscovite, *d*°	*d*°	1,155	28·0	4·79	71·2	2·460	120	985
16	2053	Muscovite, post-tectonic, pegmatite	Shienzi, Bia massif 26° 11′ E—9° 25′ S	1,847	6·6	7·48	94·2	12·31	809	965 ± 29
17	2052	Microcline, *d*°	Sofwe, Bia massif 26° 07′ E—9° 35′ S	1,165	28·6	4·61	70	2·36	118	945 ± 28
18	3117	Lepidolite, *d*°	Manono, Bukena-Manono massif 27° 23′ E—7° 20′ S	12,603		48·6	99			920 } (ref. 3) 930 ± 28
19	3117*b*	Lepidolite, *d*°	*d*°, *d*°	15,040	10·0	59·6	99			945
20	5825	Microcline, *d*°	*d*°, *d*°	3,380	13·0	12·9	93·4	10·78	749	905 ± 27
21	5826	Microcline (crushed), *d*°	*d*°, *d*°	4,008	12·0	14·7	94·6	13·17	963	875 ± 26
22	3124	Muscovite, *d*°	*d*°, *d*°	5,070	4·18	18·7	98·5	46·5	3,500	880 ± 26
23	3131	Muscovite, *d*°	*d*°, *d*°	7,410	0·10	27·2	99·7	264	20,300	875 ± 26

$\lambda^{87}Rb = 1{\cdot}47 \times 10^{-11}$ y^{-1}. $[^{87}Sr/^{86}Sr]_0 = 0{\cdot}709$.
* Ages obtained by isochron (Fig. 1).
† All ages under 1,000 m.y. are rounded off to the nearest 5 m.y.; those above 1,000 m.y., to the nearest 10 m.y.

worthy that rubidium–strontium (Table 1, Nos. 3 and 8) and potassium–argon (905 ± 40 m.y.)[4] dating of biotites from this type of granite gives apparent ages which are as young as or younger than those of minerals from post-tectonic pegmatites (Table 1, Nos. 16–23). This is a clear case of 'rejuvenation' of biotites from a geochronological point of view, and it is not caused by a superimposed ulterior orogeny, which is entirely non-existent in this area.

During the same deep-seated phase which produced the aforementioned gneisses, granitization occurred, forming rocks which are now gneissic biotite granites, with some muscovite. This syntectonic granitization is, on geological and petrographical grounds, somewhat younger than the pre- or early-tectonic granites mentioned earlier; it is, however, still inadequately dated. Apparent ages of one whole rock and one microcline from a specimen from the Bukena massif are shown in Table 1, Nos. 9 and 10. Assuming an initial strontium-87/strontium-86 ratio of 0·709, the apparent age of this granitization is 1,240 ± 70 m.y.

Post-tectonic manifestations comprise various types of granites, pegmatites and veins. Of the various granites, only specimens belonging to one type, the so-called 'tin-granite', equigranular and rich in muscovite, have been dated (Table 1, Nos. 11, 12 and 14). Fig. 2 concerns these three specimens from the Bia, Bukena-Manono and Mwanza massifs and also a supplementary specimen from Kivu, further north in the same belt[5]. There is no line which fits all four points, or for that matter the three points representing whole rock analyses from the Katanga granites, even when the margins of error are taken into account. The situation is not improved when the data for two muscovites (Table 1, Nos. 13 and 15) are projected on Fig. 2. The six points fall in the vicinity of an 'isochron' of 965 m.y. with an initial strontium-87/strontium-86 ratio of $0{\cdot}708_5$, but it is thought that the lack of a close fit simply reflects, as was expected, the fact that there is no reason or necessity why these granites from four different massifs should have precisely the same age, although they are approximately contemporaneous. The results (Nos. 11–15), listed in Table 1, assuming an initial ratio of 0·709, can thus only be considered as apparent ages despite the excellent agreement between whole rock-muscovite pairs (Nos. 12–15).

As has already been found for a northern portion of the same belt, in Kivu[6], the ages and apparent ages of pegmatites and veins fall into two groups. This is the result

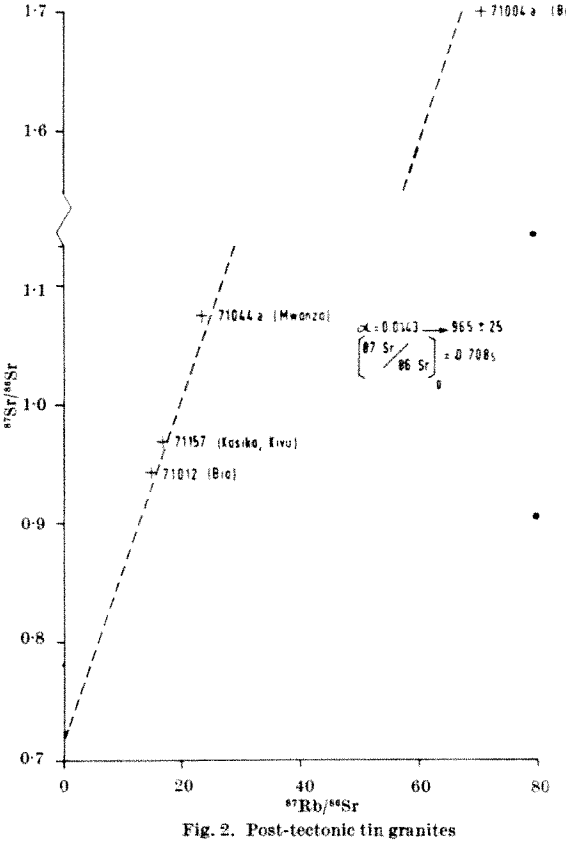


Fig. 2. Post-tectonic tin granites

of geochronological work and was not suspected in the field. The older group is represented by three concordant apparent ages for priorite from a vein cutting through the Kibara massif[7–9]. The results, recalculated with more recent constants[10], are given here and concur at about 1,104 m.y.

t (lead-207/lead-206) = 1,054 ± 50 m.y.; t (lead-206/uranium-238) = 1,133 ± 30 m.y.; t (lead-207/uranium-235) = 1,106 ± 30 m.y.

This is in keeping with the concordant apparent ages obtained by rubidium–strontium analyses of muscovite and microcline from Lugusha 1 pegmatite, Kivu[6]. Assuming an initial strontium-87/strontium-86 ratio of 0·709, these give an age of 1,120 ± 30 m.y. This older group is not usually tin-bearing.

A younger, generally tin-bearing group (Table 1, Nos. 16–23) comprises apparent ages of 965 ± 29 m.y. and 945 ± 28 m.y. for pegmatite minerals from the Bia massif, the tin granite of which has an apparent age of 1,010 ± 50 m.y.

The large pegmatite body of Manono mine, which belongs to, or is linked with, the Bukena massif, the tin granite of which is about 925 m.y. old, carries minerals of apparent ages between 930 ± 28 m.y. and 905 ± 27 m.y. (Table 1, Nos. 18–20). Other minerals from the same pegmatite (Nos. 21–23) have apparent ages which may be too young as one of these is from a demonstrably crushed microcline (No. 21) and the others, on muscovite, are similar both to the apparent age of the mylonitized microcline and to those of some of the rejuvenated biotites.

All these results are from rocks which are geographically and structurally linked to the Kibaran belt. Similar rocks with similar ages occur occasionally as post-tectonic features in older belts but never in the younger, neighbouring Katangan[11]. This important spread of ages characterizes successive events of the history of the Kibaran fold belt. Further work is needed in order to date other events, not mentioned here, and to obtain more definitive ages for some of the events which are at present characterized by apparent ages only.

The majority of the age determinations were made in the Service de Géologie et Géochimie Nucléaires, University of Brussels, under the general direction of Prof. E. Picciotto. Some mass-spectrographic measurements were made in the Laboratorio di Geologia Nucleare, University of Pisa, with the kind permission of Prof. E. Tongiorgi. Petrographical studies and mineral separation were carried out in the Geological Department, Musée Royal de l'Afrique Centrale, Tervuren. This work was supported by the Institut Interuniversitaire des Sciences Nucléaires (Belgium) and by the Fonds de la Recherche Scientifique Fondamentale Collective (Belgium).

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Sedimentation in Subsiding Basins

BARRELL'S[1] concept of base-level control is a well-known theory to explain sedimentation in subsiding basins. In its original definition the base level is a critical depth below which wave action ceases and therefore no erosion occurs. According to Barrell, sedimentation builds up to this level and then erosion keeps the water depth constant. Further sedimentation can only occur if the basin subsides. This mechanism is certainly applicable to some environments; however, other environments, in particular some limestone formations, show little or no evidence of extensive erosion but are nevertheless sediments which have formed at relatively constant water depth. Such equilibrium conditions can be obtained by making certain reasonable assumptions.

The amount of sedimentation, S, is a function of Q, the quantity supplied per unit time, R, the receptor value, D, the dispersal, and M, the type of material[2]. The effect of a base-level control can be obtained by assuming Q and R constant and making D proportional to the water depth w (M is ignored for simplicity) (see Fig. 1). This may be expressed as:

$$\frac{dD}{dt} = D_0 + aw(t)$$

$$D_{(t)} = Qt - (w + w_0) \qquad (1)$$

Assuming $D = 0$ at $w = w_0$, $t = 0$, we find the solution:

$$w(t) = \frac{Q - D_0}{a} + \left(w_0 - \frac{Q - D_0}{a}\right) e^{-at} \qquad (2)$$

The various constants have to be chosen in such a way that deposition does in fact occur, that is, $\frac{Q - D_0}{a} \leqslant w_0$ and $D_0 \leqslant aw_0$ ensures that the dispersal does not exceed the assumed constant Q.

We obtain the case for the lowest permissible dispersal rates by setting $\frac{dD}{dt} = 0$ at $t = 0$ and the solution then simplifies to:

$$w = \left(\frac{Q}{a} + w_0\right) - \frac{Q}{a} e^{-at} \qquad (3)$$

under the condition that $\frac{Q}{a} \leqslant -w_0$.

The system approaches a constant water depth of $\frac{Q}{a} + w_0$, which is determined by the quantity of sediment introduced and the constant 'a' which may be regarded as typical for the environment. If any crustal movement should occur, this will enter as its derivative and is thus exactly equivalent to Q with opposite sign.

For non-clastic sediments and some limestone accumulations, the model can be slightly modified. We assume the sedimentation rate to be directly proportional to the water depth and in addition consider a crustal movement $c(t)$ causing subsidence (see Fig. 2). Thus:

$$\frac{dS}{dt} = aw(t)$$

$$S(t) = c(t) - w(t) \qquad (4)$$

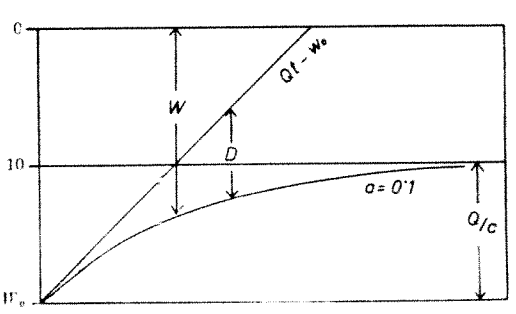


Fig. 1

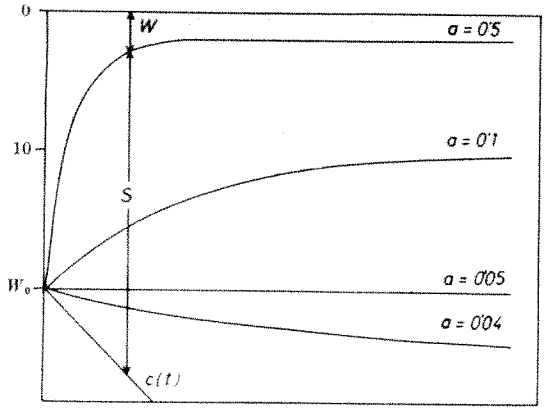


Fig. 2

If we assume $\dot{c}(t) = c$ (that is, constant sinking) the solution has the same form as equation (2). An equilibrium surface is established at a depth $\frac{c}{a}$ which is determined by the rate of sinking and an environmental constant 'a'. It is interesting to note that this equilibrium will always be obtained as long as sedimentation exists. In the example (Fig. 2), an initial water depth of 20 m is assumed and the constant rate of subsidence is 1 m per unit time. The effect of different values of 'a' is shown. Naturally, if $\frac{c}{a} = w_0$ no change of water-level occurs; for $\frac{c}{a} < w_0$ the cumulative curve of sediment laid down in time is convex; for $\frac{c}{a} > w_0$ the curve becomes concave; such features can be checked from the stratigraphical record. The assumption (4) seems reasonable as a first approximation for the behaviour of sediments in shallow basins, but this can be elaborated. Thus, it should be possible to introduce ecological depth distributions of lime producing organisms or other physical or chemical factors. One would then use a numerical or graphical method to solve the resulting differential equations.

It is not claimed that the outlined procedure can solve geological problems in quantitative terms, but it is hoped that it contributes to the clearer understanding of the 'subsidence–sedimentation' relationship.

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PHYSICS

A Precision Femtofarad Capacitor

A CAPACITOR has been investigated which consists of six spheres, a, b, c, d, e and f, each with its centre on an axis of an orthogonal co-ordinate system and equidistant from the origin (see Fig. 1). The capacitance is measured between any two opposite spheres, while the remaining four and the shield are always at ground potential. This makes a three-terminal capacitor; the third terminal is the ground to which the shield and the non-active spheres are connected. This sphere configuration, which has three-dimensional symmetry, is somewhat related to the four-cylinder system having two-dimensional symmetry, described by Thompson and Lampard[1].

The mean value:

$$\frac{C_{ab} + C_{cd} + C_{ef}}{3}$$

of the cross-capacitance values of the three pairs (a–b, c–d and e–f) of opposite spheres exhibits several useful characteristics. The dimensional asymmetries of the configuration are reflected in the mean value to a much lesser degree and, at certain values of sphere spacing, the capacitance is invariant to the first order with sphere diameter and sphere spacing. These properties make it possible to achieve a precise capacitor with moderate requirements on the dimensional tolerances, which is stable in the presence of external disturbances.

The dependence of capacitance (which is in the order of a few to a few tens femtofarad; 1 femtofarad = 10^{-15} farad) on sphere spacing and diameter is shown in Fig. 2. The capacitance increases linearly with sphere spacing when the spacing is small, but as the spacing increases new effects become prominent. At very large spacing, the capacitance decreases, since the field is nearly the same as between two spheres only. In the intermediate region, there is a reversal of the capacitance versus spacing curve which approximates a parabola quite well. At the vertex, for the spacing X_S, the capacitance is invariant with spacing to the first order.

Inspection of the family of curves of Fig. 2 shows that each ascending branch of a parabola is intersected just before the vertex by an ascending branch of a parabola of the next larger sphere size. The triangle formed, for example, by the intersection of the parabolas of the 1-in. (2·54 cm), 15/16-in. (2·38 cm) and 7/8-in. (2·22 cm) diameter spheres is of interest. To the right of this triangle, the capacitance increases with sphere diameter and to the left it decreases. It can be demonstrated that at a spacing X_D, approximately in the centre of this triangle, the capacitance is invariant to the first order with sphere diameter for the 15/16-in. sphere capacitor system.

For every system there is a spacing X_S and a spacing X_D at which invariance with spacing or diameter, respectively, is realized. It can be shown that the two values are related by:

$$X_D = X_S - \frac{ap}{b}$$

where a and b are constants which are determined by the character of the parabola family and p is the semilatus rectum.

From the foregoing discussion, it follows that:

$$\text{for } X < X_D: \frac{dC}{dX} > 0; \frac{dC}{dD} < 0$$

and

$$\text{for } X > X_S: \frac{dC}{dX} < 0; \frac{dC}{dD} > 0$$

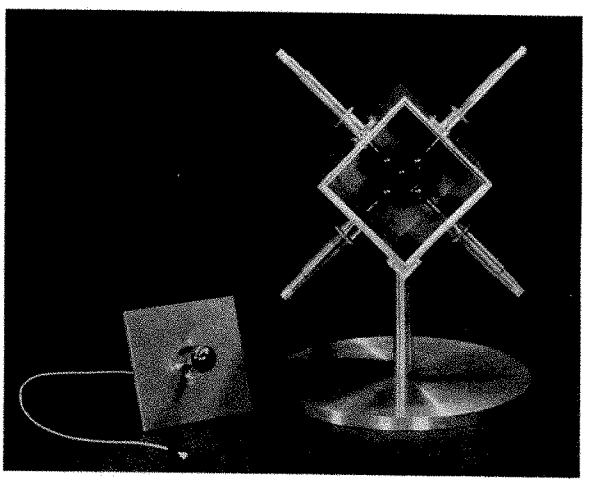


Fig. 1. Experimental unit constructed to permit wide range adjustment of the centre-to-centre sphere spacing. The spacing was varied by pulling out the spring-loaded sphere supporting rods through the mounting tubes outside the shielding cube, and inserting sets of 1/16-in. spacers

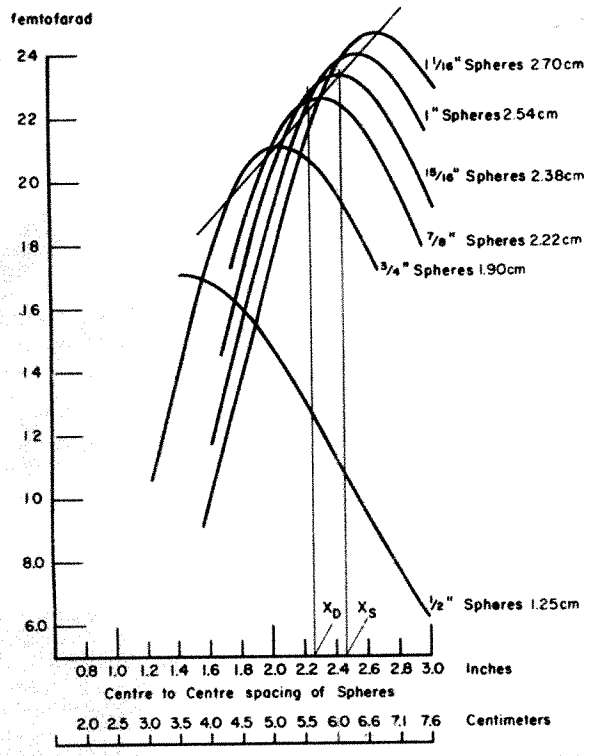


Fig. 2. Capacitance of the unit shown in Fig. 1, as a function of the centre-to-centre spacing of opposite spheres with sphere diameter as parameter

where C is the capacitance, X is the sphere spacing, and D is the sphere diameter.

Thus, for values of spacing smaller than X_D and slightly larger than X_S (the latter being preferred) effective temperature compensation can be achieved by the simple procedure of spacing adjustment. This has been confirmed by temperature tests where positive, negative and nearly zero temperature coefficients have been obtained, depending on the value of sphere spacing.

I believe that this capacitor could be reproduced with a precision in the order of ten parts per million. The spheres are available with a diameter and sphericity tolerance of two parts per million (commercial ball bearings), and the construction of the shield is not too critical. Subsequent adjustment to even closer tolerances could easily be accomplished with a screw penetrating through the shield and influencing the external field of the spheres, which represents a very small fraction of the internal field.

The promising characteristics of this capacitor suggest applications as a reference standard in the femtofarad range. If the value of capacitance could be computed from the dimensions, it would be worth while to consider the application of the capacitor for a primary standard.

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Structure of the Cubic Phase of Lipid–Water Systems

IN the course of the structure analysis of the mesomorphic phases occurring in lipid–water systems[1–4] we have observed an optically isotropic phase, the X-ray reflexions of which can be indexed on a face-centred cubic lattice[2,3]. We assumed that its structure consists of spherical particles, packed in a face-centred cubic lattice. We further postulated that the spheres consist of micelles containing lipid[2,3]; but we pointed out that the alternative model, formed by water spheres embedded in a hydrocarbon matrix, could not be discarded solely on the basis of the X-ray data[5]. We intend to show in this communication that the latter structure is in fact more satisfactory, when other lines of evidence are taken into account.

The cubic phase is generally observed over a narrow range of concentration, between the lamellar (or neat) and the hexagonal (or middle) phases[3]. The essential properties of these two phases are summarized here as a basis for the discussion following[2,4]. The lamellar phase is formed by an alternating sequence of lipid and water leaflets; the hydrophilic groups of the lipid molecules are located on the interface. The hexagonal phase is a two-dimensional hexagonal array of rigid rods; in the case of soaps and detergents the rods contain the hydrocarbon chains, are covered by the hydrophilic groups of the lipid molecules and are separated from each other by water[2–4]. In all these structures (lamellar, cubic, hexagonal) the hydrocarbon chains take up a disordered, liquid-like conformation[2,4]. The lattice parameters are given by the X-ray experiments; when in addition the concentration and the specific volumes of lipid and water are known, various structural parameters can be determined, namely, the thickness of the lipid and of the water layers for the lamellar phase, the diameter of the cylinders for the hexagonal phase (assuming that the rods are circular cylinders) and the diameter of the spheres for the cubic phase. Furthermore, it is possible to calculate for all the phases the average area, S, available to one hydrophilic group on the lipid–water interface.

This type of analysis was recently extended by Clunie, Corkill and Goodman[6] to the system (*N*,*N*,*N*-trimethylamino dodecanoimide)–water. The phases found at room temperature, in the order of increasing concentration, are: hexagonal, cubic, lamellar (named by these authors, middle, viscous isotropic, neat). For each phase the various structure parameters were determined, assuming that the hexagonal phase is formed by circular cylinders, and that the cubic phase contains lipid spheres. Clunie *et al.* discuss these parameters, and point out that the area S in the cubic phase is larger than in both the lamellar and the hexagonal phases (compare S_I, S_H and S_L in Table 1). This anomaly was in fact present in our own data[3] (see Table 1), but we overlooked its significance at the time of our first report. We know now, in the light of the analysis of a large number of lipid–water systems[3,4,7], that such an erratic fluctuation is an exception to the rule that, as the lipid concentration increases, S decreases or remains constant, but never increases. In order to reconcile these conflicting results the structure of either the hexagonal or the cubic phase must be revised.

Clunie *et al.*[6] chose to reconsider the hexagonal phase. They put forward a structure formed by linear aggregates of identical spheres, arranged in a two-dimension hexagonal lattice. With this model in mind, and taking into account the various experimental results, they calculate the radius of the spheres and the area S : S, in this case, decreases linearly with increasing lipid concentration[6]. But there are two objections to this model. The first is based on symmetry considerations. There is ample

Table 1

	C_{12}K (ref. 3)	C_{14}K (ref. 3)	C_{16}K (ref. 3)	C_{12}TAB (ref. 8)	C_{16}TAB (ref. 3)	TAC$_{12}$I (ref. 6)	
T	100	100	100	70	70	20	(°C)
$\bar{v}$	0·99	1·01	1·03	0·997	1·027	1·08	(cm³ g⁻¹)
S_H hexagonal	52	54	54	58	57	54	(Å²)
S_L lamellar	37	39	41	39	40	38	(Å²)
c_v cubic	0·64	0·62	0·62	0·82	0·80	0·70	
a cubic	55·6	54·1	70·4	54·4	69·8	58·6	(Å)
R_I cubic I	18·7	21·4	23·4	19·9	25·3	21·0	(Å)
S_I cubic I	63	63	65	77	73	69	(Å²)
R_w cubic II	15·5	18·1	19·9	12·0	15·9	15·0	(Å)
S_{II} cubic II	43	~5		28	29	39	(Å²)

C_nK: $CH_3.(CH_2)_{n-2}.COOK$; C_nTAB: $CH_3.(CH_2)_{n-3}.CN^+(CH_3)_3$; TAC$_{12}$I: $CH_3.(CH_2)_{10}.CON^-.N^+(CH_3)_3$. $\bar{v}$, specific volume of the lipid. S_H, S for the hexagonal phase at concentrations near the cubic phase. S_L, S for the lamellar phase at concentrations near the cubic phase. c_v, specific volume fraction (lipid). a, side of the face-centred cubic cell. R_I, radius of the lipid spheres, for cubic I. R_w radius of the water spheres, for cubic II.

evidence, resulting from the liquid-like conformation of the paraffin chains and from the polymorphism of the mesomorphic phases, that both the aqueous and the paraffinic structural elements are devoid of internal rigidity[3,4]. The shape of the structure elements must therefore be defined by the lattice interactions or, in other words, their symmetry must not be lower than the lattice symmetry. This rule appears to be obeyed by all the phases[3,4]: it is not clear why the hexagonal phase should be an exception. The second objection is founded on X-ray diffraction arguments. Clunie *et al.* explain the absence of non-equatorial reflexions by assuming that the strings of spheres are randomly displaced parallel to their neighbours. This hypothesis appears quite unrealistic in the light of the result that the distance between the rod axis becomes very close to the diameter of the spheres (at $c_g = 0{\cdot}63$ the diameter is 41·5 Å, the distance 41·6 Å, see Figs. 7 and 8 in Clunie *et al.*[6]).

Most of the difficulties are removed if the cylindrical shape of the rods in the hexagonal phase is preserved, and if the alternative structure of the cubic phase is taken into consideration, that is, if it is assumed that the spherical particles contain the water, and that the hydrocarbon chains fill the gap between the spheres. We call this structure cubic II; the previous one cubic I.

For the saturated potassium soaps, as well as for the detergent investigated by Clunie *et al.*[6], the area S in cubic II is intermediate between the lamellar and the hexagonal phases (compare S_{II}, S_L and S_H in Table 1): furthermore the sharp jump from S_L to S_I is smoothed out for cubic II.

The case of the cationic detergents (C_{12} TAB and C_{16} TAB) is somewhat anomalous, since S is either too large, for cubic I, or too small, for cubic II (compare S_I, S_{II}, S_L and S_H in Table 1). This anomaly could perhaps be explained by the symmetry of the model. Indeed the shape of the particles is bound to conform to the symmetry of the lattice, but need not be spherical, as was assumed in the calculation for S_I and S_{II}. Any departure from the spherical shape increases S, and, for cubic II, improves the agreement with the other phases; for cubic I the discordance is aggravated by increasing S_I (Table 1).

The very high viscosity of the cubic phase can be explained by the presence, in cubic II, of a paraffin matrix; this matrix is likely to determine the rheological properties of the whole structure. The highly viscous matrix also explains the frequent formation of fairly large crystals in the cubic phase, unlike the hexagonal and lamellar phases, in which such crystals are never observed. In these latter phases the probable cause of the lower viscosity is sliding in the water matrix.

Finally, it should be pointed out that another mesomorphic phase, namely, the hexagonal phase of phospholipid–water systems, contains water 'micelles' embedded in a hydrocarbon matrix[4,7].

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CRYSTALLOGRAPHY

Order in Alkali Feldspars

IT has long been assumed that the degree of ordering between silicon and aluminium in the tetrahedral sites of feldspars is related to the conditions of formation of the minerals. A direct test of this idea has, up to the present, been hampered by the lack of an easy method of measuring the degree of order in a genetically related suite of feldspars. This communication describes a new method which, while applicable to all alkali feldspars, should be particularly useful for monoclinic potassium feldspars for which there is at present no generally accepted method of determining the degree of aluminium/silicon order short of a full-scale structural analysis.

Methods at present available for estimation of the degree of aluminium/silicon order are: (*a*) Measurement of the obliquity using 131 and $1\bar{3}1$ reflexions[1]. This is applicable only to geometrically triclinic varieties. (*b*) Measurement of $2V$ (refs. 2 and 3). Finney and Bailey express reservations as to the accuracy of this method because of the unknown effect of sodium content on $2V$. It is hoped to show in a later publication that $2V$ does provide a good measure of order provided suitable allowance is made for any sodium present in the feldspar under investigation. (*c*) Measurement of the partial birefringence ("b" $-\alpha$)[2,4]. This, so far as I am aware, has not so far been applied in practice. A correction for sodium content may be necessary, but the method should be much less sensitive to sodium than (*b*).

The present proposal is to use the ratio c^*/b^* as a direct measure of the degree of order of an alkali feldspar as defined by Mackenzie and Smith[8]. Fig. 1 shows this ratio plotted against the degree of order for all samples the structures of which have been published and Table 1 gives the data on which the figures are based.

It will be noticed that the curve is relatively insensitive to variation in sodium although there is a suggestion that the sodium feldspars may in fact lie on a curve which is slightly displaced from that of the potassium feldspars. No attempt has been made in the present curve to correct for the sodium content of mixed feldspars, although the amount of sodium present does vary slightly among the specimens used.

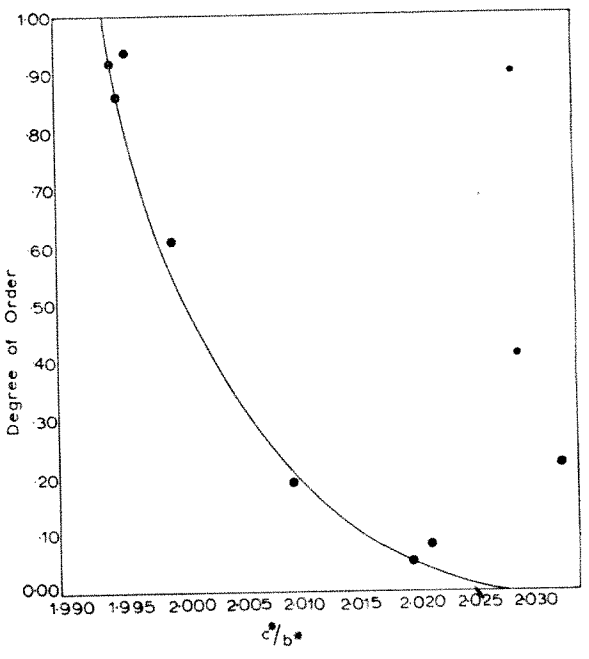


Fig. 1

Table 1

Specimen	Degree of order %	c^*/b^*	Reference
Pellotsalo	92	1·9948	Brown, B. E., and Bailey, S. W., *Acta Crystallog.*, **17**, 1391 (1964).
Pontiskalk	86	1·9952	Finney, J. J., and Bailey, S. W., *Zeit. für Kristallog.*, **119**, 413 (1963).
Spencer *U*	61	1·9999	Bailey, S. W., and Taylor, W. H., *Acta Crystallog.*, **8**, 621 (1955).
Spencer *C*	19	2·0104	Jones, J. B., and Taylor, W. H., *Acta Crystallog.*, **14**, 443 (1961).
Spencer *C* (sanidinized)	5	2·0205	Ribbe, P. H., *Acta Crystallog.*, **16**, 426 (1963).
Low albite	94	1·9962	Ribbe, P. H., Ferguson, R. B., and Taylor, W. H., *Norsk. Geol. Tidsskr.*, **42**, 152 (1962).
High albite	8	2·0219	Ferguson, R. B., Traill, R. J., and Taylor, W. H., *Acta Crystallog.*, **11**, 331 (1958).

Unpublished data on Spencer's specimen *B* (ref. 5) and on another adularia at present under study in this laboratory indicate that they also lie on the present curve. The degrees of order of the specimens investigated by Hewlett[4] and Spencer[6] deduced from the graph agree well with those deduced from measurement of $2V$ (after correction to $2V$ for sodium content has been applied).

The ratio c^*/b^* is amenable to routine measurement. The values used in Table 1 were obtained on single crystals, but for most monoclinic samples the ratio of $2 \times d_{040}/d_{002}$ may be obtained with sufficient accuracy from a diffractometer trace, preferably using an internal standard. In fact, the measurements of Hewlett[4] are of this type.

At $c^*/b^* \sim 2{\cdot}010$, the 002 and 040 peaks become unresolvable, but it is probable that the lines 003 and 060 (see the pattern for high albite[7]) can be used instead. A better procedure is to use all the low-angle lines to obtain accurate values of c^* and b^* with the aid of a computer.

It should be noted in particular that this curve provides an X-ray powder method for distinguishing orthoclase from sanidine. It is proposed that a value of $c^*/b^* = 2{\cdot}016$ (corresponding to $2V \sim 20°$ and degree of order $\sim 0{\cdot}1$) be used as the dividing line between orthoclase and sanidine.

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Opal Genesis

EARLIER work on the structure of opal[1] has shown that precious opal, and probably most 'amorphous' opals with the notable exception of hyalites, are composed of silica spheres 1500–3000 Å in diameter. It was further suggested that the presence of these spheres may reflect a genetic difference from the majority of crystalline opals, which have a different gross structure.

Subsequent laboratory work has indicated one possible way in which spherical colloidal micelles of silica can form from natural minerals. Figs. 1 and 2 show aggregates of silica spheres which formed as a result of leaving flakes of biotite in cold, 10 per cent sulphuric acid for several months. The acid gradually dissolved all cations except silicon, leaving a white, fluffy residue which preserved the original flaky habit of the mica. However, in order to produce the spheres, extensive rearrangement on a submicroscopic scale has taken place. Some silica will have

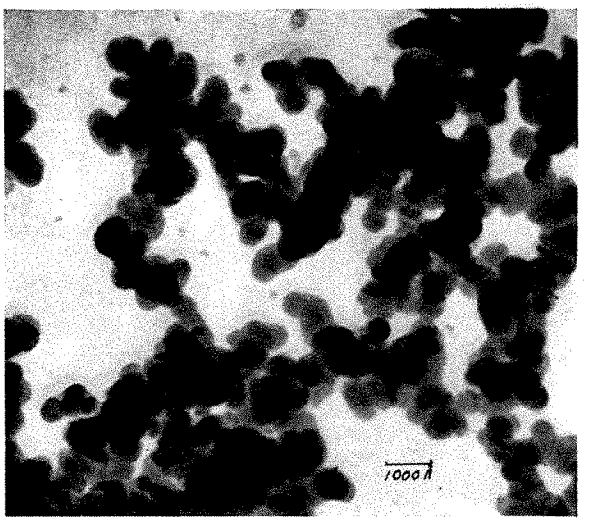


Fig. 1. Transmission electron micrograph of silica residue from the decomposition of biotite



Fig. 2. Electron micrograph of platinum-shadowed aggregate of the silica particles of Fig. 1

dissolved, at least to the extent of the solubility of amorphous silica (approximately 100 p.p.m.)[2], but it is evident that continuous re-precipitation and solution must have taken place between the silica released from the biotite and the solution. It is therefore probable that, as free silica is liberated from the mineral, it forms a supersaturated solution, which rapidly forms a sol. Silica micelles formed in this way normally assume a spherical shape[3]. The high concentration of electrolytes in the immediate neighbourhood of the biotite flakes could then cause the flocculation of the micelles into aggregates such as those of Figs 1 and 2. Under natural weathering conditions with water movement, such micelles could be transported as a sol and may form larger particles by surface growth[4] or aggregation[5].

Biotite is not the most likely source of secondary silica for the formation of most opal deposits. Other minerals (kaolinite, muscovite, montmorillonite, feldspar, chlorite, pyroxene) appeared to be stable when boiled for 2 h with strong acid, but have not yet been subjected to long-term attack by dilute acid. Silicates such as feldspars break down under hydrothermal or natural weathering conditions, and at least in some cases free amorphous or cristobalitic silica is formed, and may be found in the clay deposits so formed[6]. It is therefore possible that a process similar to this may play an important part over long periods of time in the formation of opal deposits. In view of these results, it is probable that silica will dissolve to supersaturation in some cases and will subse-

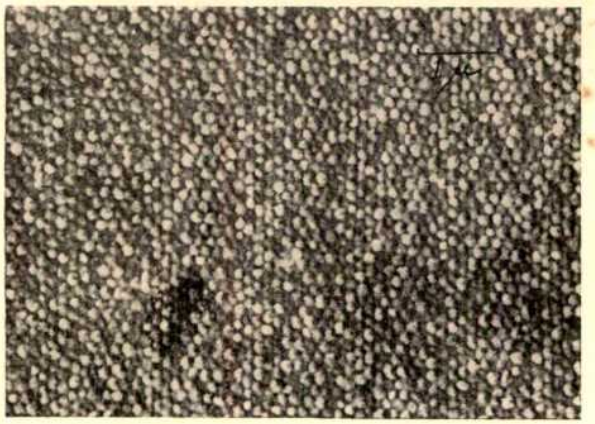


Fig. 3. Electron micrograph of lightly etched surface of a 'crystalline' precious opal from Tintenbar, New South Wales. Surface etched with hydrofluoric acid. (Carbon replica, chromium-shadowed)

quently form colloidal spheres capable of enlargement, flocculation, and deposition as opaline silica gel in the presence of a sufficient concentration of electrolytes.

Silicification of certain horizons is characteristic of many Australian lateritic profiles[7], and the major opal deposits are associated with such profiles. It is noteworthy, therefore, that abundant sulphates occur in the environment of opal deposition. Examples of this are the occurrence of gypsum at Andamooka, South Australia; gypsum and alunite at Coober Pedy, South Australia; gypsum and glauberite at White Cliffs, New South Wales; and alunite at Lightning Ridge, New South Wales.

These observations do not imply that all precious opals are formed by weathering processes. Precious opal from Tintenbar, New South Wales, occurs in amygdales in decomposed basaltic rocks, which may imply a hydrothermal or deuteric origin[8]. This material shows the characteristic regular particulate structure[1] (Fig. 3) of a precious opal, but is exceptional in that it is moderately crystalline (disordered tridymite-cristobalite). Other samples of precious opal associated with volcanic rocks, however (as at Rocky Bridge, New South Wales; Springsure, Queensland), yield only the customary near-amorphous type of diffraction pattern.

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CHEMISTRY

Differential Thermal Analysis of Thallous Azide

IN their review of inorganic azides, Evans, Yoffe and Gray[1] note that the crystal structure of thallium azide is body-centred tetragonal and that the compound melts with decomposition at about 334° C. On differential thermal analysis, a solid-state transition is shown before

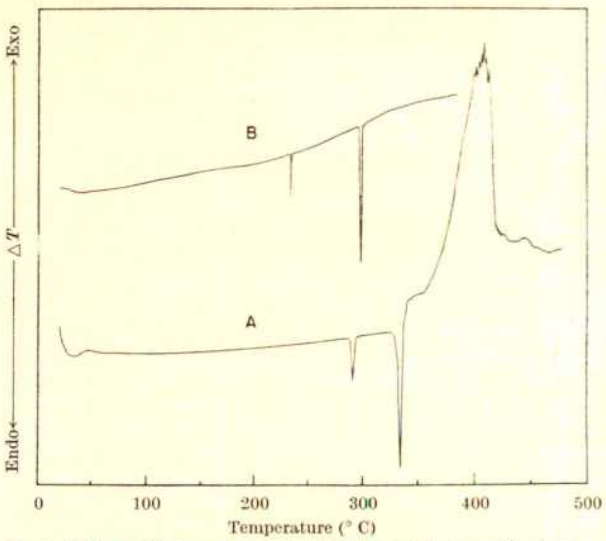


Fig. 1. Differential thermal analysis of thallium azide (*A*) and its decomposition product (*B*)

the fusion process. As shown in Fig. 1*A* this transition has a maximum peak temperature at 291° C. If the sample is allowed to undergo a cooling cycle before decomposition sets in, then the transition is seen to be reversible. Fig. 1*A*, however, takes the sample through the decomposition process. The exothermic peak is caused by oxidation of the azide group to molecular nitrogen. Thallium metal is the other product of the thermal decomposition and Fig. 1*B* shows the hexagonal–cubic transition near 234° C, previously reported by Mazières[2], followed by melting at 299° C.

The DuPont 900 differential thermal analyser, provided with an aluminium heating block and chromel alumel thermocouples, was used to obtain the thermogram. Approximately 3 mg of azide sample, contained in a glass capillary tube, was run in air against glass beads as a reference material. The heating rate was 20° C/min. The sample of thallous azide powder was prepared by precipitation from a mixture of ethanolic solutions of thallous acetate and sodium azide.

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Radiolysis of Nitroalkanes

FEW investigations of the radiolysis of nitroalkanes have been reported[1] and, apart from the use of tetranitromethane as a scavenger in aqueous solutions[2], no systematic study of their radiation chemistry has been made. The liquids are highly polar and ionic reactions might be expected to play a significant part in the decomposition processes. In order to obtain information on this problem we have examined the radiation chemistry of nitromethane, nitroethane, 1- and 2-nitropropanes, the first in considerable detail. Some of the salient features of this investigation are reported here.

Nitromethane was prepared by the reaction of monochloroacetic acid and sodium nitrite and purified by fractional distillation. Nitroethane and the nitropropanes were purified by fractional distillation under reduced pressure after alkali and acid washing. The irradiations were carried out with γ-rays from a cobalt-60 source using dose rates between 0·5 and $2{\cdot}5 \times 10^4$ rads h^{-1}. Higher dose rates up

to 7.5×10^6 rads M^{-1} (mean values) were obtained by means of a linear accelerator at the Atomic Energy Research Establishment, Wantage. Degassed samples were irradiated in break-seal tubes. Gaseous products were separated at $-196°$, $-80°$ and in some cases also at $-120°$ C and analysed by gas chromatography and mass spectrometry. Liquid products were analysed by gas chromatography, infra-red and ultra-violet spectroscopy, and chemical methods (Table 1). The solid residue left after low-temperature distillation was analysed chemically and by ultra-violet absorption spectroscopy in solution.

The production of water in the case of nitromethane was shown by the Carl Fischer reagent and the solid residue was found to contain the *cis* dimer of nitrosomethane. It is possible that the *trans* form could have been carried over with substrate during distillation, but this was not substantiated.

Some of the products quoted were a result of reactions occurring a considerable time after the initial irradiation. The yield of gaseous products from irradiated nitromethane was drastically reduced if after a short irradiation, such as that obtained by using a linear accelerator, the samples were immediately quenched in liquid nitrogen until analysis. Under these conditions $G(N_2)$, 0·19; $G(CO)$, 0·27; $G(NO)$, 0·38; $G(CH_4)$, 0·08; $G(H_2)$, 0·21; $G(CO_2)$, 0·31; $G(N_2O)$, 0·35; $G(CH_3ONO)$, 1·58; $G(C_2H_6)$, 0·09; $G(HCHO)$, 0·32; $G(cis\text{-}(CH_2NO)_2)$, 0·43. It was noted that the decrease in gaseous products was accompanied by an increase in the yield of *cis*-nitrosomethane dimer. Allowing the sample to stand at room temperature for varying lengths of time increased the yields of nitrogen, carbon monoxide, carbon dioxide and nitrous oxide up to the limiting values quoted in Table 1. The post-irradiation reaction effects were complete after 5 h at room temperature.

The addition of iodine as a radical scavenger had no detectable effect on the amount of gaseous products. Nitrous oxide, a potential electron scavenger, slightly increased the nitrogen and carbon monoxide yields.

Table 1. PRODUCTS FOUND (APPROXIMATE VALUES)

(1) From nitromethane, $G(N_2)$, 0·86; $G(CO)$, 0·39; $G(NO)$, 0·34; $G(CH_4)$, 0·095; $G(H_2)$, 0·22; $G(CO_2)$, 0·68; $G(N_2O)$, 0·66; $G(CH_3ONO)$, 1·15; $G(HCHO)$, 0·26; $G(cis\text{-}(CH_2NO)_2)$, 0·22. Small amounts of dimethyl ether, methyl nitrite, formaldoxime, water and methanol were also detected.

(2) From nitroethane, $G(N_2)$, 0·50; $G(CO)$, 0·094; $G(NO)$, 0·08; $G(CH_4)$, 0·02; $G(H_2)$, 0·08; $G(CO_2)$, 0·12; $G(N_2O)$, 0·73; $G(C_2H_4)$, 0·78; $G(C_2H_6)$, 0·18. Ethyl nitrite and acetaldehyde were also detected.

(3) From 1-nitropropane, $G(N_2)$, 0·74; $G(CO)$, 0·08; $G(NO)$, 0·35; $G(CH_4)$, 0·02; $G(H_2)$, 0·02; $G(CO_2)$, 0·31; $G(N_2O)$, 0·71; $G(C_2H_6)$, 0·54; $G(C_2H_6)$, 0·08; $G(C_3H_4)$, 0·06. Propyl nitrite and propionaldehyde were also present.

(4) From 2-nitropropane, $G(N_2)$, 1·48; $G(CO)$, 0·04; $G(NO)$, 0·21; $G(CH_4)$, 0·03; $G(H_2)$, 0·15; $G(CO_2)$, 0·13; $G(N_2O)$, 0·07; $G(C_2H_6)$, 1·31; $G(C_3H_8)$, 0·10. Isopropyl nitrite and acetone were also present.

An examination of the decomposition of pure nitromethane was made by the technique of pulse radiolysis using the apparatus developed by Dr. J. P. Keene of the Christie Hospital and Holt Radium Institute, Manchester[3]. Two absorbing species were found, the spectra of which showed an absorption which increased from 6000 Å as the absorbing wave-length decreased. One of the species decayed by a second-order process, the other seemed to be relatively stable and increased with increasing dose rate and repeated pulsing. The identity of these species has not been definitely established, but it is tentatively suggested that the long-lived material is monomeric nitrosomethane and the shorter lived species is $CH_2NO^-_2$ which is removed by a bimolecular acid-base reaction. Owing to the acidic nature of the solvent, the lifetime of the analogue of the hydrated electron in an aqueous medium would be very short-lived.

No simple scheme is capable of explaining the observations and it is not possible to discuss adequately the mechanism here. The nature of the products can be explained in terms of a free radical scheme involving initial production of an alkyl radical and nitrogen dioxide such as that advanced for the photolysis of nitromethane[4]. This is certainly an oversimplification and it is significant that the proportions of products vary in the photochemical and radiation systems. For example, the low yields of paraffins suggest that primary processes other than homolytic fission are important. Ionic processes which occur in the mass spectrometer[5] are feasible in the radiolysis of nitromethane; in particular:

$$\begin{aligned} CH_3NO^+_2 &\rightarrow CH_3O + NO^+ \\ &\rightarrow CH^+_3 + NO_2 \\ &\rightarrow CH_3 + NO^+_2 \end{aligned}$$

Other reactions possible are:

$$\begin{aligned} CH_3NO^-_2 &\rightarrow CH_3 + NO^-_2 \\ &\rightarrow CH_3^- + NO_2 \\ &\rightarrow CH_3NO + O^- \\ &\rightarrow CH_3NO^- + O \end{aligned}$$

Certainly all the products found can be explained in terms of reactions involving these intermediates.

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Improvements in Sensitivities with Flame Ionization Detectors

COMMUNICATIONS following our letter "Safety in Air Separation Plants—Determination of Atmospheric Pollutants by Gas Chromatography"[1] have indicated that some difficulty has been found in obtaining the quoted sensitivities. In order to obtain these detection levels, certain precautions are necessary which are not commonly adopted when using flame ionization detectors.

The use of oxygen in place of the more widely used air for combustion purposes provides about a five- to ten-fold increase in sensitivity, but of at least equal importance is the carrier and combustion gas purity. The carrier gas used throughout our experiments was helium, purified by the titanium–hopcalite–molecular sieve train described by Berry[2], but it should be noted that less expensive argon may be similarly purified. The combustion gases were passed through 12 ft. (× 0·25 in. o.d.) columns of Davison's silica gel, re-activated at regular intervals. The *in situ* generation of electrolytic hydrogen would be a preferred method[3]. Finally, the signal co-axial connecting cable between the detector and amplifier must be kept scrupulously clean, with all insulators having a resistance in excess of 100 M.ohm. In the present case it was found necessary to clean the signal cable thoroughly every 24 h.

We believe that a more rigorous purification of the combustion gases, in conjunction with improved precautions in order to maintain a high insulation, will improve sensitivity levels even further.

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Oxidation of Gaseous Formaldehyde

IN a recent communication to *Nature* on this subject, J. M. Thomas and I[1] described some experimental results which showed that in a 'matured' quartz vessel at 320° C direct oxidation of formaldehyde by oxygen to hydrogen peroxide occurred to the very considerable extent of 70 per cent:

$$HCHO + O_2 = H_2O_2 + CO \quad (1)$$

In a reaction vessel newly treated with nitric acid or hydrofluoric acid, however, the production of hydrogen peroxide was minimal and not greater than 2 per cent, the main products being water and carbon monoxide:

$$HCHO + \tfrac{1}{2}O_2 = H_2O + CO \quad (2)$$

During a recent discussion in Moscow it was pointed out to me that very similar results had already been reported by Markewich and Filippova[2] and by Markewich and Pecherskaya[3] between 1957 and 1961, in which they showed that reaction (1) occurs to the extent of about 27 per cent in glass or quartz vessels "matured" after treatment with chromic acid, while with vessels treated with a 2 per cent solution of potassium borate ($K_2B_4O_7$), the reaction takes the second course represented by equation (2). Markewich and Pecherskaya[3] also proposed a chain reaction similar to the one which we proposed.

There cannot be the slightest doubt that full priority accrues to Markewich *et al.*, and I wish to express our regret to them for failing to acquaint ourselves with their work and, as a consequence, omitting reference to their publications.

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Neutralization of Hydronium Ions in the Radiolysis of Water Vapour

WHEN water vapour is irradiated, the main primary positive ion is H_2O^+ and it may be assumed that the reaction:

$$H_2O^+ + H_2O \rightarrow H_3O^+ + OH \quad (1)$$

($k_1 = 1{\cdot}26 \times 10^{-9}$ cm^3 molecules^{-1} sec^{-1}) will result in the formation of hydronium ions[1]. It has been suggested[2,3] that neutralization of the hydronium ion by recombination with electrons can lead to the formation of two hydrogen atoms per ion:

$$H_3O^+ + e^- \rightarrow 2H + OH \quad (2)$$

but there has been no evidence available to decide between this process and the alternative:

$$H_3O^+ + e^- \rightarrow H + H_2O \quad (3)$$

giving one hydrogen atom per ion. In an investigation of the radiolysis of mixtures of deuterium oxide and propane, we have obtained results which have a bearing on this question.

Mixtures, prepared by distilling weighed, deaerated deuterium oxide (99·8 per cent) samples and known volumes of propane (Phillips Research Grade) into 'Pyrex' vessels (400 ml.), were irradiated with cobalt-60 γ-rays at 137°. The nitrous oxide dosimeter was used (assuming $G(N_2) = 12{\cdot}0$) and the dose-rates in deuterium oxide and propane calculated assuming stopping powers per molecule relative to nitrous oxide of 0·46 and 1·25, respectively.

Fig. 1 shows $G(HD)_t$ (molecules of deuterium hydride per 100 eV absorbed by the mixture of deuterium oxide and propane) as a function of the fraction of energy absorbed in deuterium oxide and in propane, for mixtures with propane concentrations from 2 to 98 mole per cent $G(HD)_w = 8{\cdot}0 \pm 0{\cdot}5$ and $G(HD)_p = 4{\cdot}6 \pm 0{\cdot}4$ (molecules per 100 eV absorbed in deuterium oxide and propane, respectively) are obtained by extrapolation to zero concentrations of propane and deuterium oxide. The results are consistent with the assumption that $G(HD)_w$ and $G(HD)_p$ are constant over the whole range of concentration (Fig. 1).

The observed $G(HD)_w = 8{\cdot}0 \pm 0{\cdot}5$ is in reasonable agreement with the hydrogen atom yield from water and deuterium oxide ($G_H = G_D = 7{\cdot}5 \pm 0{\cdot}7$) obtained by Baxendale and Gilbert[4,5] using methanol as scavenger. Hydrogen was also formed from mixtures of deuterium oxide and propane, $G(H_2)_p = 6{\cdot}0 \pm 0{\cdot}5$ being independent of the concentration of propane.

The observed $G(HD)_p$ cannot be explained by assuming that the exchange reaction:

$$H + D_2O \rightarrow D + HDO \quad (4)$$

occurs since the propane would compete with deuterium oxide for any hydrogen atoms formed from propane and this would be expected to result in a dependence of $G(HD)_p$ and of $G(H_2)_p$ on the concentration of propane. Charge-transfer, from the hydrocarbon positive ions to deuterium oxide, may also be excluded, on energetic grounds.

The observed $G(HD)_p$ may be interpreted in terms of proton-transfer from hydrocarbon positive ions to deuterium oxide:

$$RH^+ + D_2O \rightarrow R + HD_2O^+ \quad (5)$$

where RH^+ represents the positive ion or ions from propane which undergo proton-transfer. The main positive ions may be assumed to be $C_3H_8^+$ and $C_3H_7^+$, since ions of smaller mass can undergo hydride ion transfer with propane[6,7]. The proton transfer reaction (5), where $RH^+ = C_3H_8^+$, has been observed in the mass-spectrometer[1,8], and is reported to have a rate constant of about 2×10^{-9} cm^3 molecules^{-1} sec^{-1} (ref. 8). Proton transfer from $C_3H_7^+$ to deuterium oxide is exothermic if the proton affinity of deuterium oxide is similar to that of water ($\geqslant 154$ kcal/mole^{-1}) (ref. 9).

It may be assumed that equilibrium:

$$HD_2O^+ + D_2O \rightleftharpoons D_3O^+ + HDO \quad (6)$$

will be established rapidly and that the majority of positive ions undergoing neutralization will be the species D_3O^+. (There is evidence that the hydronium ions will be clustered at the pressures used, since at pressures as

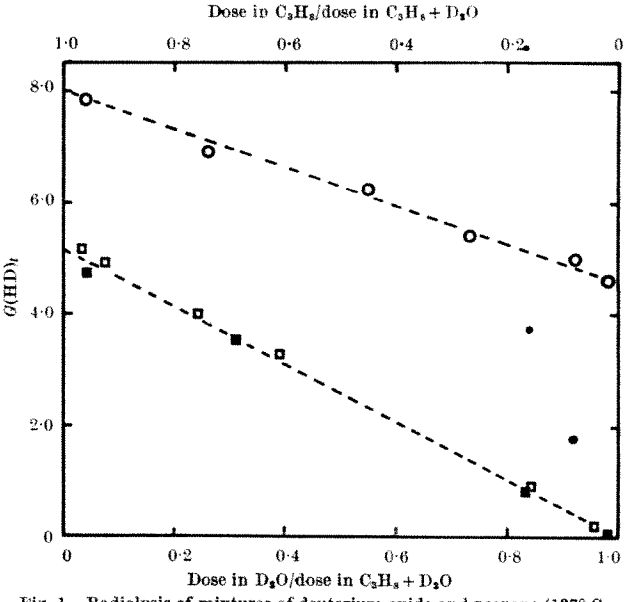


Fig. 1. Radiolysis of mixtures of deuterium oxide and propane (137° C, density 0·8–2·7 g l.). ○, $G(HD)_t$ (molecules per 100 eV absorbed in deuterium oxide and propane mixture); □, $G(HD)_t$ in presence of nitrous oxide (2·0 mole per cent); ■, $G(HD)_t$ in presence of sulphur hexafluoride (0·3 mole per cent)

low as 0·5 torr the ions in α-irradiated water vapour have been shown to be $H_3O^+(H_2O)_n$ where n is between 1 and 7 (ref. 10). For convenience, the hydronium ion is written here as D_3O^+.)

Deuterium atoms formed as a result of neutralization of D_3O^+ will be scavenged by propane according to:

$$D + C_3H_8 \rightarrow C_3H_7 + HD \quad (7)$$

and therefore measured as deuterium hydride.

The observed $G(HD)_p = 4{\cdot}6 \pm 0{\cdot}4$ is close to the total yield of positive ions from propane, $G(RH^+)_p = 4{\cdot}3$, calculated from $W(C_3H_8) = 23{\cdot}4$ eV (ref. 11). It seems reasonable to conclude, from the present data, that all the positive ions from $C_3H_n^+$ undergo the proton-transfer reaction (5) and that neutralization of D_3O^+ occurs according to:

$$D_3O^+ + e^- \rightarrow D + D_2O \quad (8)$$

These results suggest, therefore, that in the radiolysis of water vapour, neutralization of hydronium ions by electrons results in the formation of only one hydrogen atom per ion.

The results obtained when mixtures of deuterium oxide and propane were irradiated in the presence of nitrous oxide or sulphur hexafluoride show that, under these conditions, $G(HD)_w = 5{\cdot}0 \pm 0{\cdot}5$ and $G(HD)_p = 0$ (Fig. 1). At the concentrations used, nitrous oxide and sulphur hexafluoride cannot compete with reaction (7) for deuterium atoms. These scavengers have, however, been shown to be capable of reacting with electrons in irradiated gases to form negative ions[12], and their effect on the yields of deuterium hydride from deuterium oxide and propane may be attributed to a change in the process by which D_3O^+ is neutralized. It appears, therefore, that deuterium atoms are not formed in the neutralization process when D_3O^+ is neutralized by the negative ions from nitrous oxide or sulphur hexafluoride. It follows that $G(HD)_w = 5{\cdot}0 \pm 0{\cdot}5$, observed in the presence of electron scavengers, must be a result of the formation of deuterium atoms by processes other than D_3O^+ neutralization. The primary ionization process:

$$D_2O \rightsquigarrow OD^+ + D + e^- \quad (9)$$

which contributes about 18 per cent of the positive ions in the mass spectrum (see ref. 3) could give a $G(D)$ of about 0·6. The only other source of deuterium atoms envisaged is the decomposition of excited deuterium oxide molecules:

$$D_2O \rightsquigarrow D_2O^* \rightarrow D + OD \quad (10)$$

and the results suggest, therefore, that this process gives $G(D)$ between 4 and 5.

We thank Prof. J. J. Weiss for his interest and Dr. G. Scholes for helpful discussions. We also thank the Atomic Energy Research Establishment, Harwell, for financial support.

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Chemical Group Separation in Radioactivation Analysis

The technique of detection and measurement of low concentrations of impurities by means of radioactivation analysis is now well established and the principles involved are well described in the literature[1,2]. For a large number of elements the technique is extremely sensitive and the sensitivity is limited only by the natural background radioactivity and the intensity of the neutron flux available. Some limitation on sensitivity is imposed by the neutron absorption characteristics of the material being analysed and the distance from the neutron source to the place of analysis. These limitations, however, are not generally serious. The work presented here describes the use of the foregoing analytical technique in conjunction with a chemical group separation. This procedure has been applied to the analysis of mineral specimens[3] and appeared suitable for application to the analysis of single crystal specimens of germanium, in order to obtain information about the concentration of several possible impurities in the samples.

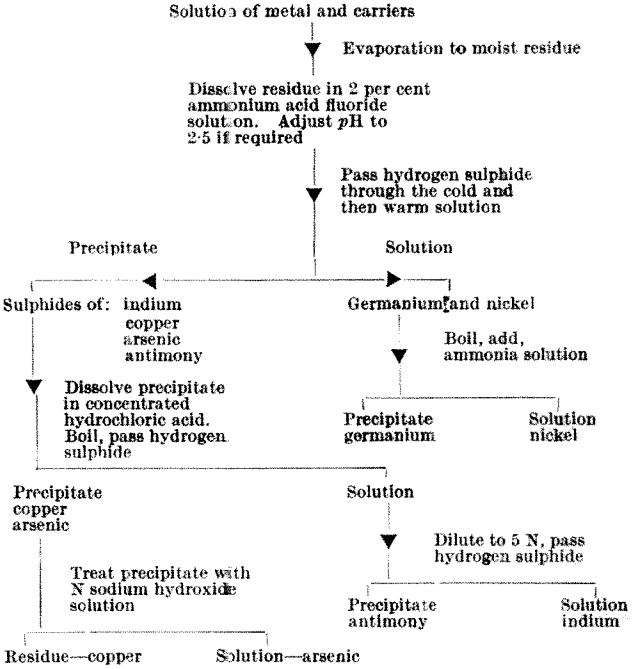


Fig. 1

The germanium samples, plus standard solutions of the impurities of interest, were irradiated together in a neutron flux of approximately 10^{12} n/cm²/sec. The period of irradiation was 6 days. On completion of the irradiation the germanium samples were rapidly dissolved in an equal volume mixture of 48 per cent hydrofluoric acid and fuming nitric acid, along with known weights of carriers for the impurities involved. The solution was then evaporated almost to dryness and the residue dissolved in 2 per cent ammonium acid fluoride solution. The group separation shown in Fig. 1 was applied and the carriers obtained as shown. The scheme depends for the separation of the major part of the germanium matrix on the fact that germanium is complexed in fluoride solution and is not readily precipitated as a sulphide under these conditions. The pH value used ensures the precipitation of the indium as a sulphide.

The separation and further purification of each carrier were performed in order of increasing length of half-life. Samples of the standards were also prepared in the same order. The activity of the carriers was then compared with the activity of known amounts of the standards, using a 1-in. end window Geiger–Müller tube and associ-

Table 1

Specimen	Elements determined	Concentration (w/w)
1	Indium	$1{\cdot}0 \times 10^{-8}$
	Copper	$< 1{\cdot}15 \times 10^{-7}$
2	Indium	$3{\cdot}5 \times 10^{-7}$
	Copper	$< 7{\cdot}5 \times 10^{-8}$
3	Indium	$8{\cdot}2 \times 10^{-9}$
	Copper	$< 3{\cdot}9 \times 10^{-6}$
4	Indium	$2{\cdot}6 \times 10^{-8}$
	Copper	$< 1{\cdot}0 \times 10^{-7}$
	Antimony	$< 3{\cdot}49 \times 10^{-8}$
5	Indium	$9{\cdot}3 \times 10^{-8}$
	Copper	$< 5{\cdot}2 \times 10^{-8}$
	Antimony	$< 3{\cdot}1 \times 10^{-8}$

ated counting equipment. From this comparison the impurity concentration was obtained. The presence of the correct isotope was confirmed by half-life measurements. The analysis of germanium samples for indium, copper and antimony was carried out, and some results are given in Table 1.

It is concluded that the application of a suitable chemical group separation in conjunction with radioactivation analysis can provide useful information about the extent of several impurities in a given specimen and increases the versatility of this method of analysis.

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BIOCHEMISTRY

Volatile Constituents of Coffee-V

PREVIOUSLY[1–4], we described the isolation of an 'aroma complex' from roasted coffee and the methods used for the separation and identification of some of its constituents.

Interest in the chemistry of coffee has now considerably increased[5], and this suggests that progress in this field might perhaps be favoured by the publication of an additional list of volatile compounds the presence of which in roasted coffee has been conclusively established in this laboratory.

Table 1. SOME VOLATILE CONSTITUENTS OF COFFEE

Formula	Name of compound	Formula	Name of compound
H_2S	Hydrogen sulphide	C_4H_5N	Pyrrole
		$C_4H_6O_2$	Diacetyl
CH_2O	Formaldehyde	$C_4H_6O_2$	Butyrolactone
		$C_4H_6O_2$	Crotonic acid
CH_2O_2	Formic acid		
		C_4H_8O	i-Butanal
CH_4O	Methanol	C_4H_8O	Butanone
CH_4S	Methyl mercaptan	$C_4H_8O_2$	i-Butyric acid
		$C_4H_8O_2$	Acetoin
C_2H_4O	Acetaldehyde	$C_4H_8O_2$	Ethyl acetate
$C_2H_4O_2$	Acetic acid	$C_5H_4O_2$	Furfural
$C_2H_4O_2$	Methyl formate		
		C_5H_5N	Pyridine
C_2H_6O	Ethanol		
		C_5H_5NO	2-Pyrrolaldehyde
C_2H_6S	Dimethyl sulphide		
		C_5H_6O	2-Methylfuran
C_3H_6O	Propanal		
C_3H_6O	Acetone	$C_5H_6O_2$	Furfuryl alcohol
$C_3H_6O_2$	Propionic acid	$C_5H_6N_2$	2-Methylpyrazine
$C_3H_6O_2$	Acetol		
$C_3H_6O_2$	Methyl acetate	C_5H_7N	*N*-Methylpyrrole
C_4H_4O	Furan	C_5H_8	Isoprene
		C_5H_8O	*cyclo*Pentanone
C_4H_4S	Thiophene		
		$C_5H_8O_2$	Pentane-2,3-dione
$C_4H_4O_2$	Crotonolactone	$C_5H_8O_2$	2-Methyltetrahydrofuran-3-one
$C_4H_4N_2$	Pyrazine	$C_5H_8O_2$	Senecioic acid

Table 1 (*continued*). SOME VOLATILE CONSTITUENTS OF COFFEE

Formula	Name of compound	Formula	Name of compound
$C_5H_8O_3$	Acetol acetate	$C_7H_7NO_2$	Methyl nicotinate
$C_5H_{10}O$	2-Methylbutanal	C_7H_8	Toluene
$C_5H_{10}O_2$	2-Hydroxy-3-pentanone	C_7H_8O	*m*-Creosol
$C_5H_{10}O_2$	3-Hydroxy-2-pentanone		
$C_5H_{10}O_2$	i-Valeric acid	$C_7H_8O_2$	Guaiacol
$C_5H_{10}O_2$	2-Methylbutyric acid	$C_7H_8O_2$	5-Methyl-2-acetylfuran
C_6H_6O	Phenol	$C_7H_8O_3$	2-Furfuryl acetate
		$C_7H_8O_3$	Methyl ethyl maleic anhydride
$C_6H_6O_2$	5-Methylfurfural		
$C_6H_6O_2$	2-Acetylfuran		
		C_7H_9NO	2-Propionylpyrrole
$C_6H_6O_3$	2-Furfuryl formate	C_7H_9NO	*N*-Methyl-2-acetylpyrrole
$C_6H_6O_3$	Dimethylmaleic anhydride	C_7H_9NO	*N*-Methyl-5-methyl-pyrrole-2-aldehyde
$C_6H_6O_3$	Maltol		
C_6H_6SO	2-Acetylthiophene	$C_7H_{10}O_2$	3,4-Dimethyl*cyclo*pentane-1,2-dione
C_6H_7N	3-Methylpyridine	$C_7H_{10}O_2$	3,5-Dimethyl*cyclo*pentane-1,2-dione
C_6H_7NO	2-Acetylpyrrole	$C_7H_{10}O_2$	3-Ethyl*cyclo*pentane-1,2-dione
C_6H_7NO	*N*-Methylpyrrole-2-aldehyde	$C_7H_{10}O_2$	3-Methyl*cyclo*hexane-1,2-dione
C_6H_7NO	5-Methylpyrrole-2-aldehyde		
		$C_8H_8O_2$	2,3-Dihydroxyaceto-phenone
C_6H_8O	2,5-Dimethylfuran		
		$C_8H_8O_3$	1-(2′-Furyl)-butane-4,2-dione
$C_6H_8O_2$	3-Methyl*cyclo*pentane-1,2-dione	$C_8H_8O_3$	1-[(5′-Methyl)-2′-furyl]-propane-1,2-dione
$C_6H_8N_2$	2,3-Dimethylpyrazine		
$C_6H_8N_2$	2,5-Dimethylpyrazine		
$C_6H_8N_2$	2,6-Dimethylpyrazine	$C_9H_8O_2$	2,2′-Difurylmethane
C_6H_8OS	2-Furfuryl methyl sulphide	C_9H_9NO	*N*-Furfuryl-2-pyrrole
		$C_9H_{10}O_2$	4-Vinylguaiacol
$C_7H_6O_3$	1-(2′-Furyl)-propane-1,2-dione	$C_9H_{12}O_2$	4-Ethylguaiacol

Table 1 lists fifty-four new identifications together with the thirty-one previously reported. All compounds were isolated in pure form by repeated vapour phase chromatography, and their identities were determined by comparison of their properties with those of authentic samples (retention times on two columns, infra-red spectra and, in a few cases, mass spectra).

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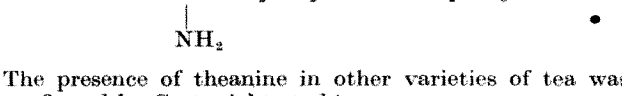
Fundamental Research Department
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Linden, New Jersey.

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Tea as a Source of Urinary Ethylamine

ETHYLAMINE is a normal constituent of human urine[1,2], but it is not found in rat urine. This suggested that urinary ethylamine might be a product of metabolism of a component of food or beverages.

In 1950, Sakato[3] isolated an amino-acid, L-theanine, from Japanese tea leaves and identified it as *N*-ethyl-γ-glutamine:



The presence of theanine in other varieties of tea was confirmed by Cartwright *et al.*[4].

A concentrated extract was prepared by shaking 10 g of tea leaves with 50 ml. of boiling water. Four albino rats (Wistar, 200–260 g; Oxoid diet) were placed in metabolic cages and 24 h urine was collected before and after oral administration of 5 ml. of tea-extract. Samples of urine, containing 1 mg creatinine, were taken and

dinitrophenyl derivatives of amines (DNP-amines) were isolated as described before, except that the preliminary step for the removal of ammonia was omitted[5]. DNP-amines were extracted with *cyclo*hexane and, after evaporation of the *cyclo*hexane, the residue in each case was dissolved in a small volume of ethanol. Amounts, equivalent to 400 μg creatinine, were analysed by reversed-phase chromatography on paraffin-treated paper[1]. Fig. 1 shows the result of the experiment on one of the rats. Identical results were found with the remaining three rats. It is seen that ethylamine appears in rat urine after oral administration of tea-extract. (Tea-extract was processed in the same way as the urine specimens and it was confirmed that ethylamine was not produced during the analytical procedure.)

Experiments, carried out in this laboratory, showed that ingestion of neomycin by human subjects did not result in marked variation in the level of ethylamine in urine. This finding suggested that the formation of ethylamine was not dependent on bacterial action in the intestine. However, the breakdown of theanine in the digestive tract by other processes, not dependent on bacteria, could not be excluded.

In order to ascertain whether hydrolysis of theanine took place in tissues, four albino rats were placed in metabolic cages and 24 h urine was collected before and after subcutaneous injections of 3 ml. of tea-extract, prepared in physiological saline. DNP-amines were isolated and analysed as mentioned before. Results were similar to those obtained when tea-extract was given orally (Fig. 1). Thus, ethylamine resulted from hydrolysis of theanine, presumably as a result of the amino-acid–amidase activity of tissues[6].

In another experiment, 24 h urine was collected from a normal human subject. After abstaining from tea for 72 h, urine was collected during the next 24 h. Finally, after the usual intake of tea in the course of the next 72 h, 24 h urine was again collected. DNP-amines were isolated from samples of urine containing 4·5 mg creatinine.

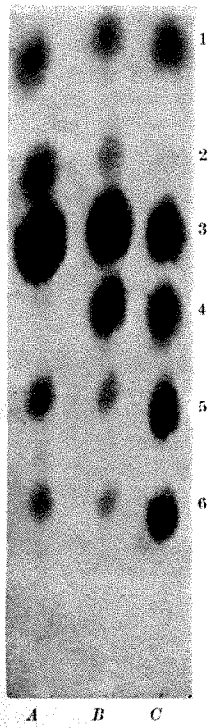


Fig. 1. Reversed-phase chromatography of DNP-amines isolated from rat urine: *A*, from basal urine; *B*, from urine after tea-extract, given orally; *C*, mixture of authentic DNP-amines. Numbers represent DNP-derivatives of: 1, ammonia; 2, methylamine; 3, dimethylamine; 4, ethylamine; 5, pyrrolidine; 6, piperidine. DNP-ethylamine is absent from *A* but is present in *B*. DNP-ethylamine and the corresponding spot in *B* show similar reddish-brown fluorescence in ultra-violet light

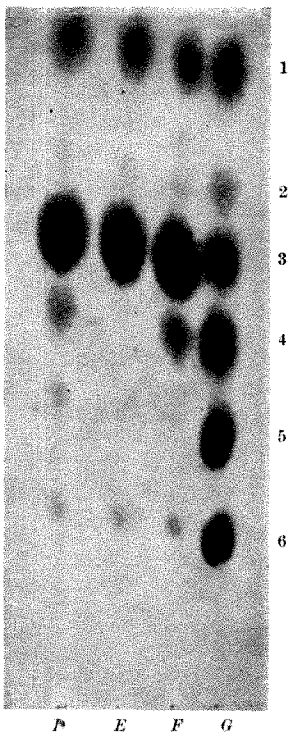


Fig. 2. Reversed-phase chromatography of DNP-amines isolated from human urine: *D*, from basal urine (tea taken); *E*, from urine, after 72 h without tea; *F*, from urine, 72 h after usual intake of tea; *G*, mixture of DNP-amines. Numbers represent DNP-derivatives of: 1, ammonia; 2, methylamine; 3, dimethylamine; 4, ethylamine; 5, pyrrolidine; 6, piperidine. Ethylamine disappears from urine (*E*) when tea is excluded

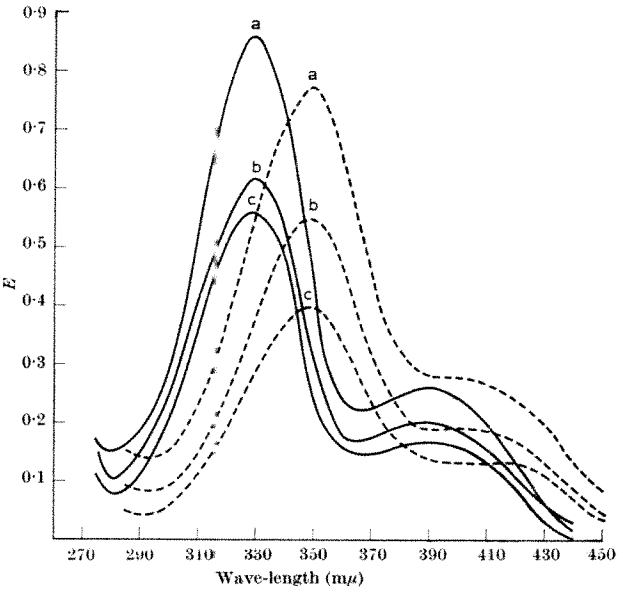


Fig. 3. Absorption spectra of: *a*, DNP-amine isolated from hydrolysate of tea-extract; *b*, authentic DNP-ethylamine; *c*, DNP-amine from rat urine, after giving tea-extract. ——, In *cyclo*hexane; - - - -, in monochlorobenzene

Portions of the final extract of DNP-amines in ethanol, equivalent to 1·6 mg creatinine, were chromatographed on paraffin-treated paper. Results shown in Fig. 2 demonstrate that ethylamine is found in urine only if tea is taken.

A small volume of tea-extract was refluxed with 5 N hydrochloric acid for 2 h and the hydrolysate was then brought to *p*H 7. The DNP-amine isolated from the mixture was chromatographically identical with authentic DNP-ethylamine and showed the same characteristic fluorescence. The DNP-amine from the hydrolysate,

authentic DNP-ethylamine and the spot that appeared in rat urine, after giving tea-extract (Fig. 1), were cut out from the chromatograms and were eluted with acetone. After evaporation of the acetone, the residue in each case was dissolved in *cyclo*hexane and the absorption spectra were determined. Finally, the *cyclo*hexane was evaporated and, using monochlorobenzene as the solvent, absorption spectra were noted again. As shown in Fig. 3, the three derivatives have similar absorption spectra in the two solvents, with a shift in the wave-length of maximum absorption from 330 mμ to 350 mμ.

It is concluded from these experiments that ethylamine found in human urine originates from tea.

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A Sensitive Bioassay for Gibberellins based on Retardation of Leaf Senescence in *Rumex obtusifolius* (L.)

THE senescence and yellowing of detached leaves and leaf disks of many plants are retarded by low concentrations of kinetin. Gibberellic acid (GA) and indolyl-3-acetic acid (IAA) may also induce a similar response, depending on the age of the leaf and the species. Thus, mature leaves of dandelion (*Taraxacum officinale*) respond to low concentrations of kinetin and GA, but scarcely at all to IAA; whereas in banana (*Musa cavendishii* Lamb.) all three types of hormone are effective in retarding leaf senescence. Leaves of the broad-leaved dock (*Rumex obtusifolius*) are unusual in responding only to gibberellin, and this has been made the basis of the bioassay technique described in this report.

Leaves are taken from clonally propagated dock plants grown in pots in a glasshouse. The oldest leaves still retaining a uniform colour are used. After detachment from the plant the leaves are left with their petioles in water for 24 h in the dark at room temperature before use. 7 mm diameter disks are cut from the leaves, avoiding the main veins, and these are placed, abaxial surface downwards, on 2·5 cm diameter disks of filter paper moistened with 0·3 ml. of test solution in small glass dishes. Four leaf disks, each from a separate leaf, are placed on each filter paper disk, and each treatment is normally replicated four times. The small glass dishes containing the disks are placed in Petri dishes lined with moist filter paper and incubated at 25° C in darkness. When the chlorophyll has almost disappeared from the control disks—normally after 4 or 5 days—the four leaf disks from each replicate are placed overnight in 6 ml. of methanol or acetone, to extract the chlorophyll, and the optical density of the solution at 665 mμ (chlorophyll *a*) is measured on a spectrophotometer. The chlorophyll content is expressed as a percentage of that of the control.

Table 1

$A_3 = 100$	$A_2 = 0{\cdot}63$
$A_1 = 12$	$A_6 = 0{\cdot}32$
$A_7 = 4$	$A_9 = 0{\cdot}10$
$A_4 = 1{\cdot}5$	$A_8 = 0{\cdot}04$
$A_5 = 1{\cdot}5$	$A_{10} = 0{\cdot}04$
	$A_{12} = 0{\cdot}02$

The great sensitivity of the *Rumex* leaf senescence test to gibberellic acid is shown in Fig. 1, in which the dosage/response curve is compared with those of two other standard bioassay methods, the oat mesocotyl test and the lettuce hypocotyl test. The lower limit of the *Rumex* test is of the order of 0·00001 μg GA per ml., which is about the same as that of the barley endosperm test[1], and the useful upper limit about 0·1 μg per ml. The test has been successfully used for the detection and measurement of naturally occurring gibberellins in extracts of apple seeds after chromatography. Kinetin and IAA at concentrations of up to 6 μg per ml. produce no response in this test. Glucose and fructose up to 5 per cent, potassium nitrate and potassium chloride up to M/8, and adenine at 100 μg per ml. are also inactive.

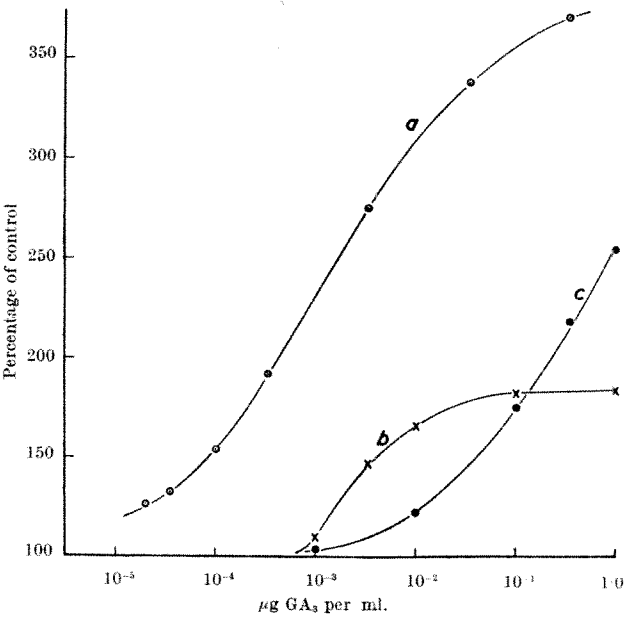


Fig. 1. Relationship between concentration of gibberellic acid and response, measured as per cent of control, in (*a*) *Rumex* leaf senescence test, (*b*) oat mesocotyl test (var. 'Brighton'), and (*c*) lettuce hypocotyl test (var. 'Grand Rapids')

The activities of other gibberellins in this test are considerably lower than that of gibberellic acid, but most are sufficiently active to make the test of value for routine detection and bioassay work. Table 1 shows the relative activities of different gibberellins (kindly supplied by Dr. J. MacMillan, Dr. D. Broadbent, Mr. J. B. Blundell and Dr. G. A. D. Jackson), based on the concentrations required to produce a 200 per cent response.

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Flavoprotein in the Egg of the Silkworm *Bombyx mori*

THE eggs of a developing silkworm of Nippon 124 × China 124 strain were crushed at regular intervals directly on to Whatman No. 1 paper and chromatographed in one or two dimensions. The solvents used were 2,4-lutidine–*n*-butanol–water (4 : 1 : 5), *n*-butanol–acetic acid–water (4 : 1 : 5), 5 per cent sodium citrate and *n*-propanol–1 per cent ammonia (2 : 1). From the results it was demonstrated that riboflavin and a large quantity of flavin mononucleotide (FMN) were contained in the eggs throughout their development, but flavin adenine dinucleotide (FAD) was detected only in the egg on the day before hatching and in the newly hatched larva.

To investigate more closely the changes in flavin during development, further investigations of the quantitative differences of the flavoprotein have been carried out to

determine the protein and flavin content of each fraction by centrifugation. The eggs of silkworms of three strains, Daizo, China 108 and Nippon 124 × China 124, were used, either in the diapause stage or in the late postdiapause stage (1 day before hatching). All fractions of eggs were prepared at 4° C. The eggs were weighed and crushed with a mortar in 0·3 M sucrose solution, containing 10^{-3} M EDTA and 10^{-3} M phosphate buffer at *p*H 7·3. After removal of the chorion membrane by filtration through a layer of gauze, the brei was homogenized with a 'Teflon' homogenizer in 10 volumes of 0·3 M sucrose solution and centrifuged at 800*g* for 10 min to remove nuclei and cell debris. The precipitant was again homogenized and centrifuged. The supernatant obtained was added to the original and the combined supernatant was then centrifuged at 10,000*g* for 15 min. A fluff-layer containing Chino's 'lipid-rich granules'[1] was carefully gathered from the surface of the supernatant solution and washed with 0·3 M sucrose solution. This constituted the fluff fraction. The supernatant was again centrifuged at 10,000*g* for 20 min and the pellet was added to the previously centrifuged pellet. The combined pellet was washed with 0·3 M sucrose solution. These pellets contained mitochondria and pigment granules and constituted the mitochondrial fraction. Microsomes were sedimented from the mitochondrial supernatant by centrifugation at 105,000*g* for 30 min. Both pellets were combined and washed with 0·3 M sucrose solution. These pellets comprised the microsomal fraction. The microsomal supernatant fluid was brought to 80 per cent saturation with solid ammonium sulphate. After being stirred and allowed to stand for 15 min, proteins were sedimented by a 20-min centrifugation at 0° C and 10,000*g*. The supernatant was used as supernatant for the non-protein fraction. The sedimented proteins were dissolved in a small amount of water and re-precipitated with ammonium sulphate. This made up the supernatant of the protein-fraction. The pellet of each fraction was dissolved in water or suspended in it by homogenization.

The protein content in each fraction was determined by Lowley's method[2]. The intensity of the blue pigment resulting from addition of phenol reagent was determined by colorimetry using a 500-mμ filter. Bovine serum albumin was used as a standard protein.

The quantitative estimation of flavin was carried out as follows: 2 ml. of water suspension or solution of each fraction was boiled at 80° C for 15 min, cooled to room temperature, and filtered. The precipitant on the filter-paper was washed with 1 ml. of water at 80° C. 1 ml. of 2 per cent acetic acid was added to the filtrate and it was extracted three times with 3 ml. of water-saturated chloroform. To 2 ml. of the aqueous solution, 1 ml. of 1 N sodium hydroxide was added, and the mixture was exposed under ultra-violet light at 20° C for 30 min. The solution was acidified with 0·25 ml. of glacial acetic acid, treated with potassium permanganate and later with hydrogen peroxide. Thereafter it was extracted with 5 ml. of water-saturated chloroform by shaking vigorously for 3 min. The chloroform extract was determined by fluorometry and estimated in terms of riboflavin values.

The results are summarized in Table 1. No significant differences were found between the three strains. 'Content recovered' was calculated by adding the values of protein or flavin content of the fraction containing nuclei and cell debris to the sum of the values of the other fractions. The total flavin content of a silkworm egg was 7·5 μg/g egg in diapause and 6·0 μg/g egg in late postdiapause. This shows that total flavin content tends to become slightly reduced when hatched and would seem to correspond to the results of Bodine *et al.*[3] that flavin content tends to decrease during the embryonic development of grasshopper eggs. As shown in the non-protein fraction supernatant, the silkworm egg had a rather low free flavin content, which was probably to be regarded as a riboflavin content. The values of 'protein content' of this fraction seemed to represent the values of free aromatic substances in egg such as tyrosine and dihydroxyphenylalanine. The flavin content of the other fractions, except for the non-protein supernatant fraction, probably indicated the content of protein-bound flavin. The flavin content and the protein content of the protein fraction supernatant showed the highest values of the five fractions, but before hatching it was found that its flavin content decreased markedly. This flavin was analysed by paper chromatography and identified as FMN. The FMN detected qualitatively in silkworm eggs seems to be derived, in large part, from the flavoprotein of this fraction. Before hatching, on the other hand, the flavin contents of both the mitochondrial and the microsomal fractions approximately doubled. A marked quantitative increase of protein was also observed, especially in the late postdiapause mitochondrial fraction. In view of the evidence it may be suggested that proteins are plentifully synthesized in this stage in order to form the normal respiratory system in the larval stage. In fluff fraction there were almost no quantitative changes in protein and flavin from the diapause to the late postdiapause stage.

Table 1. DISTRIBUTION OF PROTEIN AND FLAVIN CONTENT IN SUBCELLULAR FRACTIONS OF SILKWORM EGGS IN DIAPAUSE AND IN LATE POSTDIAPAUSE (1 DAY BEFORE HATCHING)

Strain	Subcellular fraction	Protein				Flavin			
		Diapause egg		Late postdiapause egg		Diapause egg		Late postdiapause egg	
		Total protein (μg/g)	Fraction of total protein (%)	Total protein (μg/g)	Fraction of total protein (%)	Total flavin (μg/g)	Fraction of total flavin (%)	Total flavin (μg/g)	Fraction of total flavin (%)
Daizo	Fluff	2,300	1·7	2,200	1 8	0 4	5 6	0·3	4·9
	Mitochondrial	2,500	1·9	9,000	7 4	0·3	4 2	0 8	13·1
	Microsomal	3,300	2·5	5,100	4 2	0 3	4 2	0 8	13·1
	Supernatant, non-protein	3,600	2·7	2,700	2·2	0 8	11·1	0·7	11·5
	Supernatant, protein	92,000	68·7	77,000	63·2	4 5	62 5	2 6	42 6
	Whole homogenate	134,000	100·0	122,000	100 0	7 2	100·0	6 1	100·0
	Content recovered	126,700	94 5	117,000	95 8	7·6	105·5	6 3	103 1
China 108	Fluff	4,000	3·1	3,100	2 6	0 7	9 2	0·5	8 3
	Mitochondrial	4,000	3·1	10,000	8·5	0 4	5·3	0·8	13·3
	Microsomal	3,200	2·4	5,100	4·3	0·2	2 6	0 8	13·3
	Supernatant, non-protein	3,300	2·5	3,000	2·5	0·6	7·9	0·6	10 0
	Supernatant, protein	79,000	60 2	70,000	59·3	4 0	52·7	2·3	38 3
	Whole homogenate	131,000	100 0	118,000	100·0	7 6	100 0	6·0	100 0
	Content recovered	119,500	91 3	111,200	94 4	7·4	97 3	6·1	101 7
Nippon 124 × China 124	Fluff	1,600	1·2	2,400	2 1	0 3	3 9	0·3	4 7
	Mitochondrial	1,500	1 1	8,100	7·1	0·2	2·6	0·9	14·1
	Microsomal	3,300	2·5	3,900	3 4	0·5	6·5	0 8	12·5
	Supernatant, non-protein	3,100	2·4	1,500	1 3	0·7	9·1	0·4	6 3
	Supernatant, protein	86,000	65 2	73,000	63 5	4 7	61·0	3·0	46·8
	Whole homogenate	132,000	100 0	115,000	100·0	7 7	100 0	6 4	100·0
	Content recovered	120,500	91 3	109,900	95·5	7 9	102 6	6 6	103 1

The fact that the soluble fraction in the diapause silkworm egg contained large amounts of FMN-containing flavoprotein is of interest in view of Chino's report[1] that, during embryonal diapause in the silkworm, cytochrome *c* becomes undetectable, while the soluble fraction shows high NADH- and NADPH-cytochrome *c* reductase activity. It has recently been demonstrated that NADH-cytochrome *c* reductase in mammalian mitochondria is a degradation product of the NADH dehydrogenase of the respiratory chain, the prosthetic group of which is also FMN[4]. In silkworm eggs it is known that the catalase activity, which is indispensable to flavin-respiration, increases during diapause[5].

It may therefore be assumed that the flavoprotein which we have detected in the supernatant fraction plays an important part in respiration during diapause. The occurrence of FAD when the eggs hatch suggests the possibility of the formation and arrangement of a normal respiratory system containing cytochrome *c*, which takes the place of this flavin-respiration during diapause.

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Inhibition of Pollen Respiration by Oligomycin

EARLIER experiments showed that respiration of germinating pollen was characterized by an initial high rate prior to pollen tube initiation, a subsequent lower rate during which tube growth began, and a second high rate associated with extensive tube growth[1]. DNP (2,4-dinitrophenol) stimulated oxygen uptake during each of these three phases of respiration. It was postulated that respiration was dependent on oxidative phosphorylation and that the observed respiratory pattern reflected various rates of utilization and resynthesis of energy-rich phosphate compounds[1].

This report presents additional evidence that respiration of germinating pollen is regulated by phosphorylative processes. Oligomycin inhibited pollen respiration, and the inhibition was reversed with DNP.

Oligomycin inhibits oxidative phosphorylation in isolated mitochondria with an accompanying inhibition of respiration[2-6]. Electron transport is not blocked directly because the respiratory inhibition is abolished by uncoupling agents such as DNP[5,6]. Hence, oligomycin acts between the DNP-sensitive step and the terminal reaction in which ATP is formed. In animal mitochondria it seems to prevent the formation of an energy-rich phosphorylated intermediate[2]. However, in plant mitochondria, oligomycin may allow formation of a phosphorylated intermediate but prevent transfer of phosphate to a phosphate-collecting enzyme which forms ATP[4]. In either instance oligomycin can be used to determine whether electron transport is tightly coupled to ATP synthesis.

Several workers have studied the effect of oligomycin on respiration of intact cells. Oligomycin inhibited endogenous respiration 80 per cent in Yoshida ascites cells, preventing the respiratory stimulation observed on adding glucose to uninhibited cells[7]. Oligomycin blocked ATP synthesis since glucose is rapidly phosphorylated by ascites cells, and respiration is stimulated by the ADP produced[8]. Oligomycin also inhibited respiration 22 to 25 per cent in slices of rat liver[9], brain[10] and kidney[11]. Aerobic glycolysis of kidney slices was stimulated to a rate similar to that of anaerobic glycolysis[11]. Oligomycin is reported to inhibit ion uptake by plant roots without affecting respiration[12].

The present investigation was undertaken using procedures similar to those described previously[1]. Oxygen uptake was measured manometrically, and pollen was incubated in a culture medium containing 0·29 M sucrose, 1·27 mM calcium nitrate, 0·16 mM H_3BO_3, 0·99 mM potassium nitrate, 3·0 mM KH_2PO_4 and 10 μg/ml. tetracycline, *p*H 5·2. Pollen tube initiation began after about 30 min of incubation, and 90 per cent germination was reached after about 2 h. The control flasks exhibited the same respiratory pattern reported earlier[1], and DNP stimulated oxygen uptake (Fig. 1). Oligomycin caused 54 per cent inhibition of the initial high rate of respiration, and the inhibition was maintained until DNP was added after 41 min of incubation. Several concentrations of oligomycin (0·5, 1·0 and 2·0 μg/flask) caused the same degree of inhibition. DNP reversed the inhibition; respiration increased 3·2-fold to a rate similar to that obtained by adding DNP to pollen in the absence of oligomycin (Fig. 1). It is of interest that this stimulation is greater than those reported (1·8–2·4-fold) when DNP was added to isolated plant mitochondria inhibited by oligomycin[5,6]. Oligomycin also inhibited respiration of growing pollen tubes, but the DNP stimulation was not as great (Fig. 2). Respiration was inhibited immediately after tipping in oligomycin.

These results could indicate (1) inhibition of reactions utilizing ATP or (2) inhibition of ATP synthesis within the mitochondria. In the former possibility oligomycin would not necessarily penetrate the cell since active transport might be inhibited at the cell membrane just as ion uptake by roots seems to be inhibited[12]. Respiration would be inhibited by accumulation of ATP and depletion of ADP. In the second case ATP would probably decrease, the effect actually observed in rat kidney slices[11].

DNP is thought to stimulate glycolysis in plant tissue by lowering the ATP/ADP ratio[13,14]. This causes an increased respiratory quotient (R.Q.) because pyruvate accumulates and is partly decarboxylated to acetaldehyde and ethanol[13,14]. An increased R.Q. would also be expected if oligomycin inhibited ATP synthesis in germinating pollen. Accordingly, pollen was incubated with oligomycin

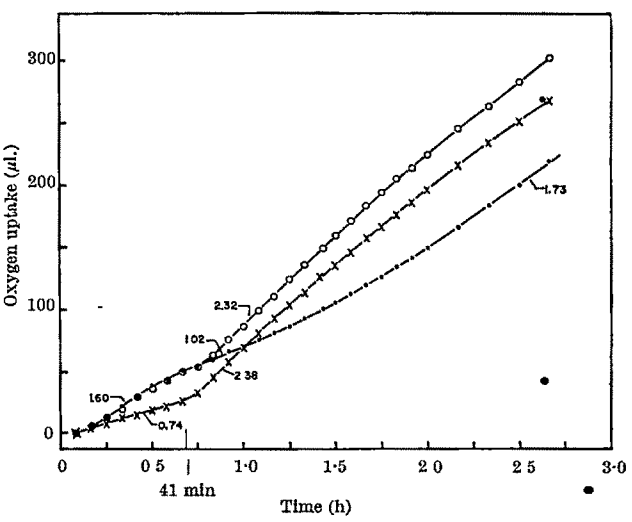


Fig. 1. Inhibition of pollen respiration by oligomycin and reversal of inhibition with DNP. Each point is the average oxygen uptake at 30° C of duplicate Warburg flasks. The numbers on the graph represent rates of oxygen uptake in μl./min. The main compartment of each control flask contained 1·4 ml. culture medium plus 7·0 mg pollen, and 0·3 ml. culture medium was placed in the side arm. Other flasks had the same contents plus the following additives. DNP treatment: sufficient DNP in the side arms to give 10 μM after tipping. Oligomycin treatment: 0·5 μg oligomycin in the main compartment, and sufficient DNP in the side arm to give 10 μM. Contents of all side arms were tipped after 41 min of incubation. ●, Control; ○, 10 μM DNP added at 41 min; ×, 0·5 μg oligomycin added at 0 min +10 μM DNP added at 41 min

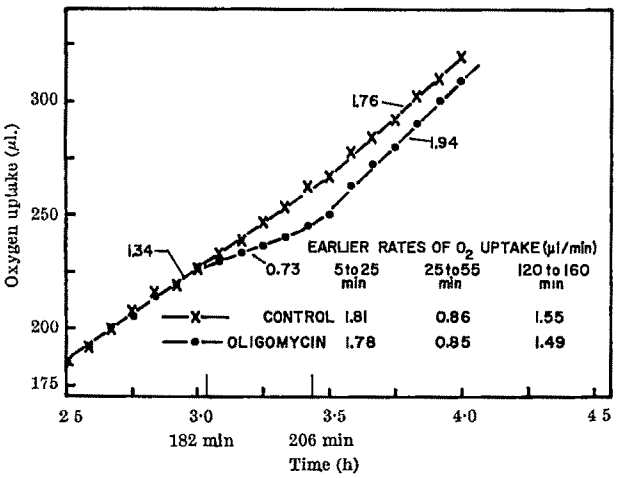


Fig. 2. Effect of oligomycin on respiration of growing pollen tubes. Conditions were as given in Fig. 1 with the following differences Double side arm flasks were used. The main compartments of all flasks contained 1·1 ml. culture medium plus 7·0 mg pollen, and all side arms contained 0·3 ml. culture medium. In the oligomycin treatment the first side arms contained 0·5 μg oligomycin. The second side arms of all flasks contained sufficient DNP to give 10 μM after tipping. First side arms were tipped at 182 min; second side arms were tipped at 206 min

or DNP, and the R.Q. was determined according to Gregory and Winter[15]. Oligomycin inhibited oxygen uptake but stimulated carbon dioxide output to give a 3·3-fold increase in R.Q. (Table 1). These results support the hypothesis that oxidative phosphorylation was blocked and that ATP utilization continued, causing decreased ATP and increased ADP.

Table 1. RESPIRATORY QUOTIENTS OF POLLEN TREATED WITH OLIGOMYCIN AND DNP

Treatment	O_2 (μl.)	CO_2 (μl.)	R.Q.
Control	35·4	38·3	1·08
Oligomycin, 0·36 μg/ml.	15·6	55·4	3·57
DNP, 10 μM	63·8	76·8	1·20

Conditions were as given in Fig. 1 except that the main compartment of each flask contained 10 mg pollen and 1·4 ml. culture medium which lacked KH_2PO_4. Oligomycin and DNP were present initially. CO_2 and O_2 refer to μl. absorbed or evolved during the period from 10 to 30 min after beginning the experiment.

Thus, oligomycin shows promise for use with intact plant cells to determine whether mitochondrial electron transport is tightly coupled to phosphorylative processes. This method may prove especially useful when the nature of the plant tissue causes difficulty in isolating tightly coupled mitochondria. An example is the acid tissue of fruits[16]. Oligomycin may be useful in studies of senescence to determine whether mitochondria remain tightly coupled in ageing cells.

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Conversion of Carbon-14-labelled Orotic Acid into Pyrimidine Nucleotides by Chloroplasts

SINCE it gives rise to 5′phospho oritidine which is rapidly transformed into pyrimidine nucleotides[1], orotic acid is useful in the investigation of nucleic acid biosynthesis. It seems to accumulate temporarily in plants before it is utilized as a precursor of the nucleotides[2].

In the present work, we have attempted to follow the transformations of ^{14}C-labelled orotic acid (specific activity, 5·2 mc./m.mole) in various chloroplast fractions obtained from young leaves of *Bryophyllum daigremontianum* Berger.

The chloroplasts were isolated either in aqueous or in organic media. The isolation technique in the aqueous medium consisted in a series of centrifugations alternately at 1,000*g* (5 min) and at 50*g* (20 sec) in 0·05 M triethanolamine, 0·3 M saccharose buffer. The *p*H of this buffer was 10·1 for the grinding of the leaves, which contain large quantities of organic acids, and for the first centrifugation. Later centrifugations were carried out at *p*H 7·6.

The separation technique in organic medium (mixtures of hexane and carbon tetrachloride) and the nucleic acid extraction and assay procedure were described earlier[3]. Labelled products (free nucleotides extracted in 5 per cent trichloroacetic acid at 0° C, and RNA nucleotides) were analysed by spectrophotometry, electrophoresis (*p*H 3·6 formate buffer, 1 h 30 min, 26 V/cm) or by paper chromatography (isopropanol–hydrochloric acid–water, 680:176:144).

Chloroplasts isolated in the aqueous medium slowly incorporate orotic acid into free nucleotides or into RNA. Taking as an index of RNA-synthetic activity the difference between the free-nucleotide content of chloroplasts heated to 100° C and those not so heated, it is seen (Fig. 1) that this activity is higher (by 40 per cent) in chloroplastic fractions isolated in organic media than in aqueous media. This difference cannot be explained by the activity of thermostable hydrolases. This is shown in Fig. 2, where the smaller differences are found in the fractions containing RNase-rich nuclear and cytoplasmic contaminants. In the course of the fractionation in the aqueous medium an important part of the intermediary or polymerization enzymes was lost in the supernatant through solubilization. The same is true for the free nucleotides as is shown in Fig. 1.

The ^{14}C-labelled orotic acid incorporation experiments in chloroplasts isolated in organic medium were carried

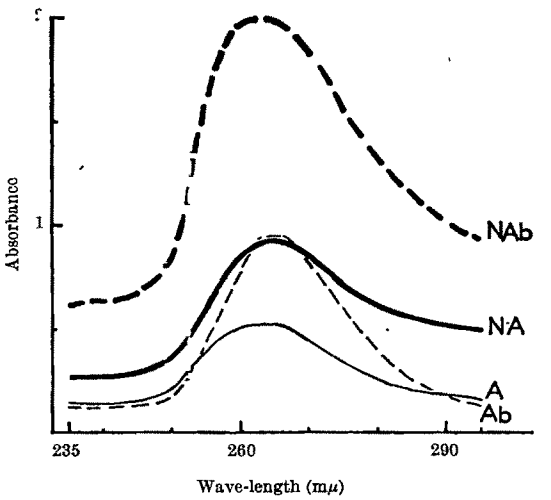


Fig. 1. Absorption spectrum of the free nucleotides of the chloroplast fraction. Each fraction containing the same amount of chlorophyll and incubated under identical conditions is extracted at 0° C by 5 per cent trichloroacetic acid. *NA*, Chloroplasts isolated in organic medium; *NAb*, chloroplasts isolated in organic medium and boiled for 5 min prior to incubation; *A*, chloroplasts isolated in aqueous medium; *Ab*, chloroplasts isolated in aqueous medium and boiled for 5 min prior to incubation

out on the fractions described in Table 1. From 100 to 200 mg of dry matter was used in each experiment. As shown in Table 2, incorporation into the soluble nucleotides is more marked in the various contaminated fractions than in our purest fraction (C_4).

Table 1. PROPERTIES OF THE VARIOUS CELLULAR FRACTIONS ISOLATED FROM LEAVES OF *Bryophyllum daigremontianum*

Fraction	Density (d)	Chlorophyll / Protein nitrogen	Appearance under light and electron microscopes
C_1	$d > 1{\cdot}46$	0 40	Chloroplasts contaminated by unbroken cells, starch grains and nuclei
C_2	$1{\cdot}46 > d > 1{\cdot}44$	0·45	Chloroplasts contaminated by cell walls, cytoplasmic material and a few nuclei
C_3	$1{\cdot}44 > d > 1{\cdot}42$	0 58	Chloroplasts contaminated by nuclei and nuclei fragments
C_4	$1{\cdot}42 > d > 1{\cdot}40$	0 65	Chloroplasts apparently without any impurity

Table 2 RADIOACTIVITY OF THE SOLUBLE NUCLEOTIDES AND OF RNA IN THE VARIOUS CELLULAR FRACTIONS

(C.p.m./g dry matter)

	C_1	C_2	C_3	C_4
Nucleotides	22,000	31,000	40,000	24,000
RNA	1,000	830	1,600	36,000

Each flask contained the following reaction mixture (4 ml) : 2 μM magnesium chloride, 0·2 mM (*p*H 7·5) triethanolamine buffer, 1·2 mM saccharose, 1 0 μM orotic acid (5 μc.), 0 6 μM phospho-ribosylpyrophosphate, 1 2 μM ATP; incubation at 25° C during 45 min at 22,000 lux; C_1, C_2, C_3 and C_4 are the cellular fractions as described in Table 1.

Table 3. RADIOACTIVITY OF PYRIMIDINE NUCLEOTIDES IN THE VARIOUS CELLULAR FRACTIONS

Fraction	Nucleotides C p m./mg dry matter Uridylic acid	Cytidylic acid
C_1	19,000	2,300
C_2	29,000	2,300
C_3	27,000	12,700
C_4	22,000	2,200

C_1, C_2, C_3 and C_4 are the cellular fractions described in Table 1.

Uridylic acid is about ten times more labelled than cytidylic acid in fractions C_1, C_2 and C_4 (Table 3). The relatively high labelling of cytidylic acid in fraction C_3, heavily contaminated by nuclear material, could be due to a higher activity of UTP-aminase catalysing the reaction: UTP→CTP.

In the C_4 fraction more than 50 per cent of the total radioactivity is found in RNA (Table 2) showing the presence in chloroplasts of a very active RNA-polymerase (Fig. 3). Uridylic acid isolated from the hydrolysate of this RNA is twice as labelled as cytidylic acid.

In the C_1, C_2 and C_3 fractions, the RNases may have hydrolysed the newly formed RNA[4], thus liberating the pyrimidine nucleotides found in large amounts in the trichloroacetic acid extract of the contaminated fractions. Although the reaction mixture contains only one tri-

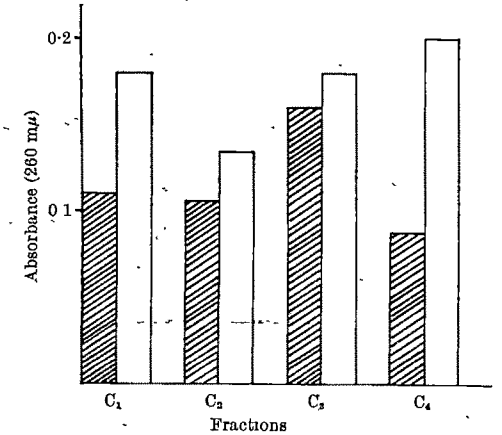


Fig 2 Absorption at $\lambda = 260$ mμ of the free nucleotides contained in mg of dry matter of each fraction isolated in organic medium after incubation under identical conditions (Table 2) Hatched areas, chloroplasts, non-hatched areas, chloroplasts boiled for 5 min prior to incubation. C_1, C_2, C_3 and C_4 are the cellular fractions described in Table 1

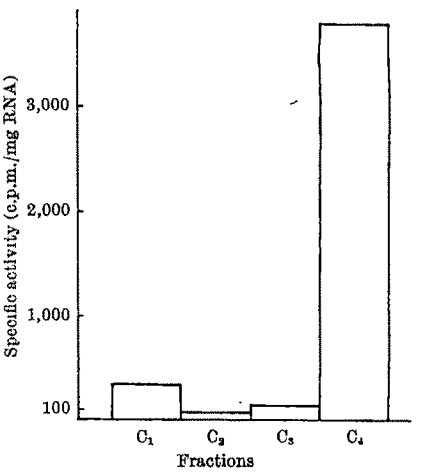


Fig. 3. Radiospecific activity of RNA in the various cellular fractions. C_1, C_2, C_3 and C_4 as described in Table 1

phosphonucleotide (ATP), the absence of one or several factors required for synthetic activity—that is, DNA and the four triphosphonucleotides—does not explain the low polymerization activity of the C_1, C_2 and C_3 fractions.

These fractions have been shown[3] indeed to have a high DNA content and the separation technique used to obtain them ensures the preservation of the nucleotides in the fractionation sediments.

These nucleotides are very largely lost when the chloroplasts are isolated in aqueous media, and it is for this reason that it is necessary to add them back to the reaction mixture[5–7].

The high RNA-synthesis activity in the chloroplast fraction may be another argument for the presence of DNA in plasts. It is probable that this DNA was used by the RNA-polymerase as a 'primer' in the chloroplastic fraction (C_4).

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Effect of Quinidine and Related Compounds on Uptake and Release of Serotonin by Blood Platelets

BLOOD platelets have the ability both *in vivo*[1] and *in vitro*[2] to take up serotonin. It has been shown that *in vitro* this phenomenon may be depressed by certain drugs, such as imipramine[3], chlorpromazine[4], reserpine[5,6] and the cardiac glycosides[7].

In this communication we describe the results of experiments to test the effect of quinidine and related compounds on the *in vitro* uptake of serotonin by human platelets as contained in platelet-rich plasma.

Blood was taken from normal healthy donors, using 3·1 per cent sodium citrate as anticoagulant, and platelet-rich plasma was obtained by centrifugation, using an International refrigerated centrifuge (Model *PR*-2), at 225*g* for 15 min. The platelet count in this plasma varied between 400 and 450,000 per cm; ^{14}C-labelled serotonin

was used in these investigations and was made up in saline to a final concentration of 50 μg/ml. The quinidine solutions used were freshly prepared saline solutions of pure quinidine sulphate. The experimental system consisted of 1·6 ml. platelet-rich plasma and 0·2 m of quinidine or control solution (saline) and 0·2 ml. of radioactive serotonin (containing 10 μg serotonin). This mixture was incubated at 37° C for varying periods of time (15–150 min). At the end of the period of incubation, the tubes were immersed in iced water for 10 min and then the platelet button was obtained by centrifugation for 60 min at 2,000*g*. The platelet button was washed once with saline, then 2 ml. of distilled water was added. By rapidly freezing and thawing the mixture four times, the platelets were disrupted and the platelet debris deposited by centrifugation. Then 1·5 ml. of the supernatant was placed in an aluminium planchet, dried and counted in a gas flow counter (Model 821*B* flow counter, Baird Atomic, Inc., Cambridge, Mass.). The amount of radioactivity in the sample which was incubated for 120 min with saline was taken at 100 per cent and the other values expressed as a percentage of this.

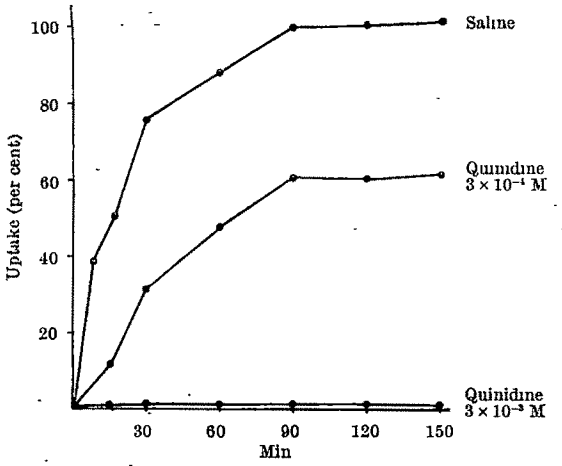


Fig. 1. The *in vitro* uptake of ^{14}C-labelled serotonin by normal human platelets was depressed by the presence of quinidine in the suspending medium

Fig. 1 shows the uptake of serotonin by platelet-rich plasma when incubated with saline and 2 concentrations of quinidine. The uptake of serotonin by platelets as measured by the present experimental model reached a maximum at 90–150 min, and incubation for periods up to 480 min did not result in an appreciable increment in uptake. The inhibiting effect of quinidine used at a concentration of 3×10^{-3} M was immediate and an effect could be seen after 5 min. With a concentration of less than 3×10^{-4} M the degree of inhibition was lessened and if used at concentrations of less than 3×10^{-5} M no significant inhibition occurred.

The addition of the solutions of quinidine did not alter the *p*H of the test mixture which lay in the range of 7·2 to 7·4. Using this experimental model the inhibiting effect of digitonin on the serotonin uptake by platelets was confirmed[7], and it was also shown that quinine, mepacrine and chloraquine, like quinidine, caused severe impairment of serotonin uptake by platelets. These results are set out in Table 1.

Table 1

	Saline	Digitonin 1×10^{-4} M	Quinine 3×10^{-3} M	Mepacrine 4×10^{-3} M	Chloraquine 3×10^{-3} M	Quinidine 3×10^{-3} M
Percentage of amount of serotonin taken up by control after 120 min incubation	100	39	4·5	1	6	3

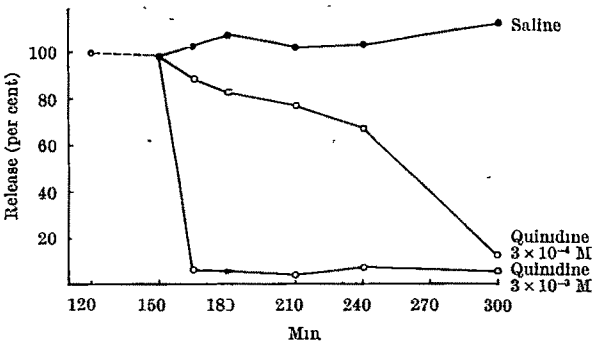


Fig. 2. The addition of quinidine to human blood platelets previously incubated with ^{14}C-labelled serotonin accelerated the release of serotonin from the platelets

Fig. 2 illustrates the result of an experiment in which quinidine and saline were added to platelets which had been previously incubated with radioactive serotonin for 150 min. It shows that following the addition of quinidine in concentrations capable of inhibiting the serotonin uptake, there was an immediate and marked release of serotonin from the platelets.

These experiments therefore, show that quinidine not only inhibits the ability of platelets to take up serotonin but that it also impairs their ability to retain it. The exact mechanism by which platelets take up serotonin and bind it is not clear. Born, Ingram and Stacey[8] showed that in normal platelets the amount of serotonin was related to their content of adenosine triphosphate. Weissbach *et al.*[7] studied the uptake of serotonin by platelets at ranging levels of *p*H. They showed that in systems in which the *p*H was 8·0 diffusion mechanism predominated, whereas at *p*H 5·7 an active transport mechanism was responsible for the uptake. This active transport mechanism functioned when platelets were suspended in saline but was enhanced by the addition of phosphate or potassium. The inhibiting effect of cardiac glycosides and reserpine was maximal in systems in which the active transport mechanism was predominant.

In the present work, in which platelets were suspended in plasma and the *p*H was between 7·2 and 7·4, the uptake of the serotonin by the platelets reached a maximum after 90–150 min, and the pattern of uptake suggested that, in the system used, the uptake was due mainly to an active transport process[7].

The mode of action of quinidine and related compounds, while likely to be similar to that of the cardiac glycosides, that is, depressing mostly the active transport of serotonin by the platelets, has not yet been completely elucidated.

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Metabolism of Alpha-carotene

In the course of an earlier investigation into the utilization of carotene by the rat as a source of vitamin A, it was noted that there was a consistent difference between the absorption spectra of the liver non-saponifiable fractions from those rats fed vitamin A and those fed a mixture of 90 per cent beta- and 10 per cent alpha-carotene[1]. This difference was in the magnitude of the absorbance at 311 mμ as compared with that at 326 mμ. The alpha-ionone analogue of vitamin A was afterwards synthesized[2] and the absorption maxima were found to be at 311 mμ ($E^{1\%}_{1cm}$, 1,650) and 325 mμ ($E^{1\%}_{1cm}$, 1,500). This alpha-vitamin A was found to be biologically inactive but was found to be stored in the liver in the same way as vitamin A (ref. 3).

The purpose of the investigation reported here was to ascertain whether the abnormalities in the absorption spectra noted with rats fed a mixture of alpha- and beta-carotene could be the result of the deposition of alpha-vitamin A in the liver.

Female Sprague–Dawley rats with nursing litters were placed on a purified diet when the young were about 10 days old. Their dietary materials were so chosen that the basic ration was nutritionally complete except for the essential fatty acids and the fat-soluble vitamins. The fat-soluble vitamins were given dropwise by mouth, one drop twice each week. The weekly supplement contained 50 U.S.P. units of vitamin A, 100 U.S.P. units of vitamin D and 3·0 mg of alpha-tocopherol. This level of vitamin A intake allowed normal growth but did not result in any measurable liver storage of the vitamin. Half the animals were allowed to become depleted in the essential fatty acids, while cottonseed oil was added to the basal diet of the remainder of the animals in sufficient quantity to satisfy the requirement for the essential fatty acids. Each group was divided into three parts and the diets were supplemented with either vitamin A, beta-carotene or alpha-carotene when deficiency symptoms appeared in the animals depleted in essential fatty acids. After a 10-day supplementation period, during which the animals received 100 g of alpha- or beta-carotene, or 250 U.S.P. units of vitamin A per day, the animals were killed. The livers were removed, and the non-saponifiable extracts were dissolved in *cyclo*hexane. The absorption spectrum was determined for each solution, and a sample was then exposed to ultra-violet light for a period sufficiently long to effect the complete destruction of any photosensitive material. This was presumed to include both vitamin A and alpha-vitamin A. A second absorption spectrum was obtained over the same spectral range. The difference between the two curves obtained was thought to give a truer picture of the vitamin A content of the extracts, since the non-specific absorption is not uniform over the spectral range investigated.

The alpha- and beta-carotene were examined spectrophotometrically to make certain that they were not mixtures of the alpha and beta forms.

A comparison of typical absorption spectra of the photosensitive materials stored in the livers, and extracted with the non-saponifiable fraction, is shown in Fig. 1. These three animals were females fed on diet I. As can be seen in the figure, the absorption spectra show no significant difference when the animals are fed vitamin A or beta-carotene. There was no reason to expect that there would be any difference. However, when the same comparison is made with the extracts from animals fed vitamin A or alpha-carotene, the results are manifestly

Table 1

Diet I	Sucrose	75 parts
	Casein (vitamin-test)	20
	Salts (U.S.P. XIV)	1
	Vitamin mixture (water soluble)	1
	Hydrogenated cottonseed oil	3
Diet II	Diet I plus 1 part of Wesson oil	
Sample supplement		
	α-tocopherol	1 mg
	β-carotene	100 g
	Diet I	1 g

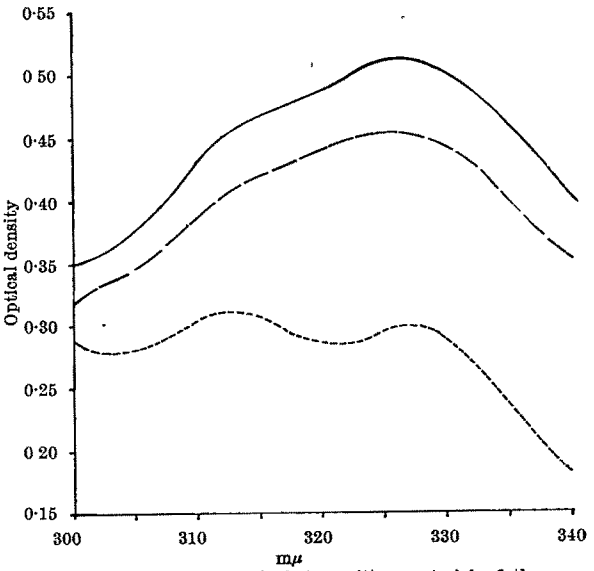


Fig. 1. Absorption spectra of photosensitive material of the non-saponifiable liver extract. (*a*) Vitamin A-fed; ———. (*b*) β-carotene fed; — — —. (*c*) α-carotene; - - -.

different. It is true that the material deposited in the liver with alpha-carotene feeding is not all alpha-vitamin A. The ratio E_{311}/E_{326} found for the pure compound was 1·10 (ref. 2), while for the curve shown in Fig. 1 the ratio is 1·02. It is obvious that the liver extract from the animal fed alpha-carotene contained more alpha-vitamin A than true vitamin A since the corresponding ratio for the vitamin A fed animal is 0·86, and this same value was found for the natural vitamin A concentrate used as a supplement.

When the quantities of vitamin A and alpha-vitamin A are calculated from the data shown in the figure using the $E^{1\%}_{1cm}$ values at 311 mμ and 326 mμ for the pure compounds, it is found that the liver contained 29 g of vitamin A and 67 g of alpha-vitamin A. Since the animal gained weight during the supplementation period, it seems safe to assume that some vitamin A was utilized for growth. If this allowance is made, the quantity of alpha-vitamin A is approximately equal to the quantity of vitamin A which must have been produced.

The feeding of alpha-carotene resulted, then, in the formation of a different end-product than did the feeding of either vitamin A or beta-carotene.

The results of this investigation do not unequivocally support either the theory of oxidation or hydrolysis at the central double bond of the carotene molecule[4,5] or the proposed mechanism of an oxidative process beginning at one end of the molecule[6]. What is demonstrated is that whatever the mechanism it very probably results in the formation of two products—vitamin A and alpha-vitamin A—and the storage of both in the liver of the test animal.

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Urea Cycle Enzyme Activities of Liver of the African Lion

In the course of a continuous survey[1,2] on the occurrence and levels of activity of ornithine–urea cycle[3] enzymes, an unexpected opportunity was afforded for quantitative measurement of these activities in the instance of the African lion, *Felis leo*. At the Vilas Park Zoo in Madison (Wisconsin) the director, Mr. Dan Watson, decided that an infirm male lion about twelve years old had to be destroyed. The lion exhibited progressive difficulty in its ability to leap to a platform some 2 m high in the cage; this created a feeding problem and difficulty in moving the animal to an outside exercise yard.

The lion was killed and, after about 15 min, portions of liver and kidney cortex were removed from the animal, placed on ice, and taken to the laboratory for enzymatic assays. The preparation of the homogenate of liver with the aid of cetyltrimethylammonium bromide and the relevant assay procedures were essentially the same as reported elsewhere[4]. Arginase, however, was determined on a diluted (liver) as well as on an undiluted (kidney cortex) aliquot of 10 per cent (w/v) whole (that is, uncentrifuged) homogenates. Final reactant concentrations for the arginase assay were: 0·085 M L-arginine; 0·0005 M Mn^{++}; final *p*H 9·5. All assays were conducted at 38° C.

Table 1. Urea Cycle Enzymes of *Felis leo* ♂ Liver

Enzyme + (synonym)	I.U.B.* No.	μmoles/ min/g liver wet wt.†	μmoles/ min/mg protein†
Carbamate kinase (= carbamoylphosphate synthetase)	2.7.2.5	10·5 ± 0·6	0·130 ± 0·007
Ornithine carbamoyltransferase (= ornithine transcarbamylase)	2.1.3.3	248	3·07
Argininosuccinate synthetase (= condensing enzyme)	6.3.4.5	1·75‡	0·0240‡
Argininosuccinate lysase (= argininosuccinase; cleavage enzyme)	4.3.2.1		
Arginase	3.5.3.1	658 ± 31	5·13 ± 0·24

Assays performed at 38° C.
* International Union of Biochemistry Enzyme Commission Numbers (1964)
† Including mean deviation of individual assays where applicable.
‡ Reflects activity of the two enzymes in unison ('arginine synthetase'—see text).

The results of the assays are given in Table 1. The enzymes of the cycle were present in liver, and the levels found were of the same general magnitude as previously observed with liver of numerous other mammals[1,2]. The synthesis and cleavage of argininosuccinate are evidenced by the assay for 'arginine synthetase'[4,5] which exhibited an activity of 1·75 μmoles of product (arginine, measured as urea) per min per g liver wet weight. This activity probably reflects the rate by which argininosuccinic acid is synthesized by the enzyme, argininosuccinate synthetase (originally—condensing enzyme).

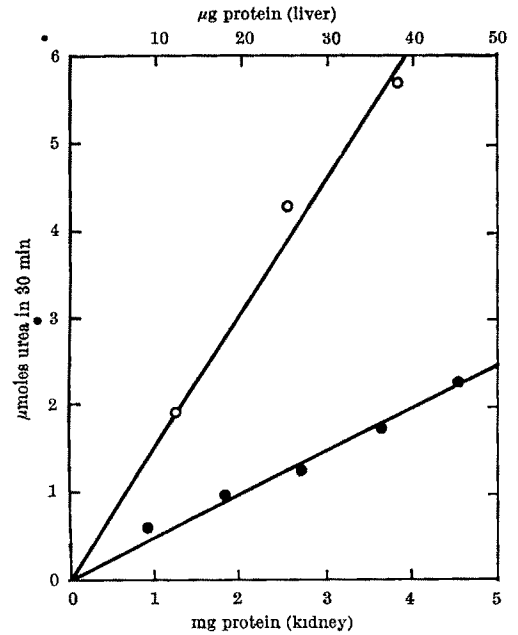


Fig. 1. Arginase of lion liver and kidney cortex. Urea production versus protein concentration. Conditions as in text. ○, Liver; ●, kidney

In addition to the liver assays, arginase was determined on the piece of kidney cortex tissue taken at the same time as the liver. A plot of activity versus protein concentration for arginase of the two tissues is shown in Fig. 1. A value (mean, and mean deviation) of 1·56 ± 0·15 μmoles per min per g wet weight was observed with kidney cortex in contrast to a much higher value of 658 ± 31 for liver. The lower value found for arginase of kidney is in keeping with previous observations[6–9] on the relative levels of this enzyme in these organs in other mammals.

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Identification of the Stimulatory Factors in Citrus Molasses for Carotenogenesis in *Blakeslea trispora*

Citrus molasses and other citrus by-products stimulate carotenogenesis by mated cultures of *Blakeslea trispora*[1]. However, the substances in citrus by-products that stimulate carotene production in the fermentation process remain unknown. We attempted to determine the nature of the enhancing agent present in one of these by-products, citrus molasses.

Because organic acids are known to stimulate carotenogenesis by micro-organisms, we precipitated these compounds from citrus molasses by addition of an 8 per cent (w/v) aqueous solution of lead acetate[2]. The lead salts were treated with hydrogen sulphide to convert them into water-soluble free organic acids. Insoluble lead sulphide was removed by filtration and the aqueous acid solution evaporated to dryness at 55° C in a flash evaporator. The dry residue was redissolved in distilled water and used for fermentation experiments. Methods for conducting the fermentation have been described[1]. After separation of organic acids, the residual citrus molasses were treated with hydrogen sulphide to precipitate excess lead ions. Lead sulphide was removed by filtration, the residual solution evaporated to dryness *in vacuo*, and the residue redissolved in water to give the previous volume of citrus molasses.

Paper chromatography of the acid fraction (*n*-butanol–formic acid–water; 4 : 1·5 : 1) revealed the presence of citric acid as the major component plus a small amount of malic acid and a trace of a third unknown acid having a R_F similar to that of gluconic or 2-ketogluconic acid. Estimation of the total acidity of the organic acid fraction from citrus molasses (titration with 0·1 *N* sodium hydroxide) showed that 5 ml. of molasses contained 0·074 g of organic acids calculated as anhydrous citric acid.

Both citric and malic acid were added to fermentations to determine if these acids stimulated carotenogenesis. In addition, the residue of the molasses after precipitation of acids and also the carbohydrate components of the residue were tested. Approximately 43 per cent carbohydrate remained in the citrus molasses residue after organic acid separation. The carbohydrates were about 50 per cent sucrose and 50 per cent reducing sugars.

Table 1 shows that the combination of organic acid fraction or citric acid with the molasses residue or sucrose resulted in yields approximating that given by unfractionated citrus molasses. Either glucose or fructose at a concentration of 1·5 per cent could substitute for sucrose, but at higher concentrations this resulted in inhibition of carotenogenesis. Malic, gluconic, and 2-ketogluconic acid could not replace citric acid and gave only slight stimulation.

Table 1. STIMULATION OF CAROTENOGENESIS BY CITRUS MOLASSES AND VARIOUS FRACTIONS

Adjuvant* Type	Amount (per cent)	Dry mycelium g/100 ml.	Carotene yield In solids mg/g	Per volume mg/100 ml.
None	—	4 79	9 9	46 6
Citrus molasses	5 0	6 72	17·3	115 0
Organic acid fraction†	2 0	4·67	17·3	80·7
Citric acid	0 075	4 77	16 7	79·2
Sucrose	3 0	5 45	14 9	80·4
Glucose	1·5	7·43	7 2	53 5
Glucose	3 0	7 72	5·9	45·9
Fructose	3 0	7 54	5 4	41·0
Organic acids + residue‡	2 + 5	6·02	17·3	103·9
Citric acid + residue	0 075 + 5	6 31	16 4	103 1
Citric acid + sucrose	0·075 + 3	4 93	20 4	100 2
Citric acid + glucose	0 075 + 1 5	6 73	14·4	96·2
Citric acid + glucose	0 075 + 3	7 41	6 9	50 7
Citric acid + fructose	0·075 + 3	7 56	6 3	47 7
Organic acids + sucrose	2 + 3	5 65	21 1	117·6

* In addition to the adjuvant, the fermentation medium contained cotton-seed embryo meal, 5 per cent; corn, 2·5 per cent; cotton-seed oil, 5 per cent, deodorized kerosene, 5 per cent; non-ionic detergent, 0·12 per cent, thiamine hydrochloride, 0 2 mg per cent; tap water; *p*H not adjusted

† One ml. of the organic acid fraction was equivalent to 2 5 ml of citrus molasses

‡ Residue is the aqueous fraction remaining after separation of organic acids from citrus molasses; 1 ml residue was equivalent to 1 ml citrus molasses.

Inhibition of metabolic processes by glucose is well documented[3,4] and has become known as the "glucose effect". Neidhart and Magasanik[5,6] formulated the concept that the glucose effect represented catabolite repression; that is, "Catabolites formed rapidly from glucose accumulate in the cell and repress the formation of enzymes whose activity would augment the already large intracellular pools of these compounds". However, glucose inhibition of carotenogenesis does not appear to be the result of catabolic inhibition as defined by Neidhart and Magasanik since carotene biosynthesis is an anabolic process.

Two hypotheses may be advanced to explain the present data. (*a*) Part of the biosynthetic pathway of carotene synthesis may be under repressor control[7]. If a potential internal inducer is used in biosynthetic processes that also involve catabolic products produced from a readily metabolized substrate such as glucose, then insufficient internal inducer is available to act on the repressed carotene pathway. When the carbon source is a less readily metabolized compound such as sucrose, the metabolite pool decreases and the internal inducer becomes available to de-repress carotenoid synthesis. (*b*) The second hypothesis assumes that the biosynthetic pathway of carotene synthesis is under continuous influence of an internal inducer but that in the presence of a readily metabolizable carbon source such as glucose, sufficient carbon is available to produce an "internal inducer-destroying enzyme" and the system becomes repressed. If sucrose were a less readily available carbon source because of slower uptake, or because of lower turnover number or concentration of invertase or sucrose phosphorylase, insufficient carbon would be available for synthesis of the enzyme. These hypotheses will be investigated.

The function of citric acid in enhancing yields is probably non-specific and would appear to be that of a precursor to early metabolites in the carotene biosynthetic sequence.

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'Endogenous' Alcohol in Body Fluids

THE question as to whether or not ethanol is a normal constituent of body fluids has attracted the attention of many workers, and their findings have been ably reviewed by Harger and Forney[1] and Ward Smith[2].

Many methods used have been of doubtful accuracy and/or specificity at the levels of ethanol found, and the method of Curry, Walker and Simpson[3] has been modified to study the problem.

A Pye 104 gas–liquid chromatograph, fitted with a flame ionization detector and a *PEG* 400 on 'Celite' column, is used with an amplifier alternation setting of 20. 10 μl. of a 1 + 5 dilution of the sample (blood or urine) with a 0·25 mg per cent solution of *n*-propanol in water is injected.

Due to baseline instability, an integrator is not used as in the unmodified method, and quantitation is achieved by the ratios of the peak heights of the ethanol and *n*-propanol peaks: this means that each individual operator must establish his own factor.

The peak height ratios are linear against ethanol concentrations over the range 0·05–1·0 mg per cent, and it is possible to detect with certainty the presence of 0·03 mg per cent of ethanol. The accuracy found varies from ±5 per cent at the 1 mg per cent level to ±25 per cent at the 0·05 mg per cent level.

Preliminary results of a survey of the occurrence of ethanol in the blood and urine of normal persons who have not taken alcohol indicate that the amounts present are less than 0·1 mg per cent.

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BIOLOGY

Genetical Homogeneity and the Stability of the Mating-type Factors of 'Fairy Rings' of *Marasmius oreades*

EXAMINATIONS have been made of the distribution of multiple-allelomorphic mating-type factors (m.t.f.) carried by basidiospores and produced by adjacent carpophores of higher fungi. The spatial distribution patterns found have been interpreted to provide evidence concerning the disposition of the mycelium giving rise to the sporophores and its genetical homogeneity or heterogeneity[1].

Many fungi give rise to a 'fairy ring' type of growth. It is generally assumed that such a mycelium has a central

Table 1. NUMBER OF CARPOPHORES INVESTIGATED AND THE DISTRIBUTION OF THE RINGS GIVING RISE TO THEM, THEIR SIZE, MATING-TYPE FACTORS AND ESTIMATES OF THEIR AGES

Group	Ring No.	No. of carpophores	M.t.f. No.	M.t.f. Designation	Radius of ring (m)	Estimated age (nearest whole year) min.	mean	max
	2	9	2	*A1A2*	1·5240	31	67	201
	4	10	2	*A1A2*	3·1623	64	140	416
A	6	5	2	*A5A6*	3·0480	63	135	401
	8	6	2	*A1A2*	3·2639	68	144	430
	1	8	2	*A3A4*	3·5052	71	155	461
B	5	7	2	*A3A6*	2·2733	46	101	299
	9	12	2	*A3A6*	3·8700	77	169	501
C	7	7	2	*A1A2*	2·0955	42	93	276

origin and shows outward, radial marginal growth associated with the presumed death of the central older region[2].

We have used these techniques to study the genetical condition of the mycelium of rings of *Marasmius oreades*. Presumptive evidence concerning the stability of mating-type factors, in a natural environment, has also been obtained.

Many single-basidiospore isolations were made from carpophores disposed around eight rings at Embleton Golf Course (*NU* 245225) just north of Dunstanburgh Castle, Northumberland. Their mating types were determined; data are recorded in Table 1. Group *A* is in the centre of the area, group *B* at the southern end, and group *C* at the northern end (about 1 km from group *B*). No measurements could be made of the rates of growth of the rings because of inevitable interference with the golf course, but estimates have been based on previous studies with this species in England[3]. The mean rate was 22·6 cm/year; the highest and lowest annual rates ever recorded were 49·5 (ref. 4) and 7·6 cm respectively. These values have been used to estimate the minimum, mean and maximum ages of each ring.

The results show that *M. oreades* has a bipolar mating system and that no ring has more than 2 mating-type factors. This is good presumptive evidence that the mycelium of each ring is genetically homogeneous and, therefore, that each originated from a single dicaryotic mycelium. Had their origins been genetically heterogeneous, sectors giving rise to carpophores of different mating-type factor constitution might have been expected in the rings.

Such a situation might also be expected to arise (*a*) if a mating-type factor had undergone mutation either to a new effective allelomorph or to one lacking mating-type specificity; (*b*) if a suppressor or modifier mutation affecting the action of a mating-type factor had arisen; (*c*) if a new mating-type factor had arisen as a result of somatic recombination in hyphae of the perennial mycelium. Mutations of types (*a*) and (*b*) have been recorded from *Coprinus lagopus* and *Schizophyllum commune*[5] and recombinational events of type (*c*) have been recorded for the latter fungus[6]; both are diaphoromictic but tetrapolar, not bipolar, in their mating systems.

The absence of sectors of different mating-type factor constitution provides presumptive evidence for genetical stability of the mating-type factors of *M. oreades* for at least 30 years (the youngest estimated age) or even for about 500 years although the most likely estimates are of the order of 100–150 years (Table 1).

The inability of new effective mating-type factors, or more recently the sub-units of which some are composed[5], to arise spontaneously or to be induced, in the laboratory, has been known to be a characteristic feature of diaphoromictic forms since such mating systems were discovered nearly 50 years ago. However, evidence for mutation or stability in natural conditions has been lacking and this is true also for somatic recombination which, so far, is a laboratory phenomenon. It might have been supposed that a bulky perennial mycelium, such as that of *M. oreades*, would have provided an ideal situation in which mutation and/or recombination would have occurred and been detectable. The apparent absence of their effects suggests either that fungal mating-type factors are remarkably stable over long periods and somatic recombination involving them exceedingly rare, or else that, in nature at least, selection against the consequences of such events is very high. This seems improbable in view of the large numbers of mating-type factors found and estimated to occur in many higher basidiomycetes[7]. Experimental evidence also suggests that the occurrence of genetically heterogeneous, single mycelia is perfectly possible[1] so that the possibility of selection against such mycelia formed naturally seems to have no obvious basis. The mode of origin of new mating-type factors in higher fungi continues to be a most perplexing problem.

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Wheat Root Exudate and Early Stages of Infection by *Helminthosporium sativum* Pamm., King and Bakke

THE observations of Flentje *et al.*[1,2] on *Corticium solani* led them to the hypothesis that the morphology of hyphal infection structures at the host surface may depend on a chemical stimulus contained specifically in host root exudate and not only on the physical stimulus of contact[3]. Evidence produced recently by de Silva and Wood[4] refutes this hypothesis. Working again with *C. solani* and the closely allied *C. praticola* they report that infection structures can be induced both with and without root exudate in a non-specific manner. Clearly this topic needs further examination, especially with other fungal genera. One recent report of another source of chemical stimulus supports Flentje's hypothesis. Guttation fluid, according to Endo *et al.*[5,6], stimulates the formation of infection structures of *Helminthosporium sorkinianum* on plastic slides, these structures being rare or absent in tap water and distilled water controls.

During work in progress at Leeds on the effect of various factors of the root environment on the germination of fungi causing diseases of cereals, an opportunity was taken to make detailed microscopical investigations of the effect of sterile root exudate obtained from wheat plants, on the early stages of infection by spores of *Helminthosporium sativum* Pamm., King and Bakke. Using wheat, variety Nord Desprez, root exudate was obtained by means of the Buxton[7] technique, concentrated to one-tenth of its original volume in vacuum and stored at −10° C. The characteristic feature of the germinating spores of this fungus on the intact wheat coleoptile is the profuse branching seen before penetration (Fig. 1*a*). Spores germinate readily in distilled water but afterwards show only one or two branches after 12 h compared with twenty or more usually seen after a similar time on the host surface. To examine the relationship of root exudate to this characteristic profuse branching, spores were germinated by placing them, dry, on the surface of small pieces of boiled and washed 'Cellophane' placed on top of exudate in small glass cylinders (5 mm in diameter) and enclosing the cylinder in a Petri dish with a piece of moist filter paper. Control cylinders contained distilled water. The microscopical examination of the 'Cellophane' over root exudate after 12 h and later showed profusely branched structures virtually indistinguishable from those obtained on the intact host (Fig. 1*b*). Branching of hyphae was infrequent on the 'Cellophane' over distilled water (Fig. 1*c*).

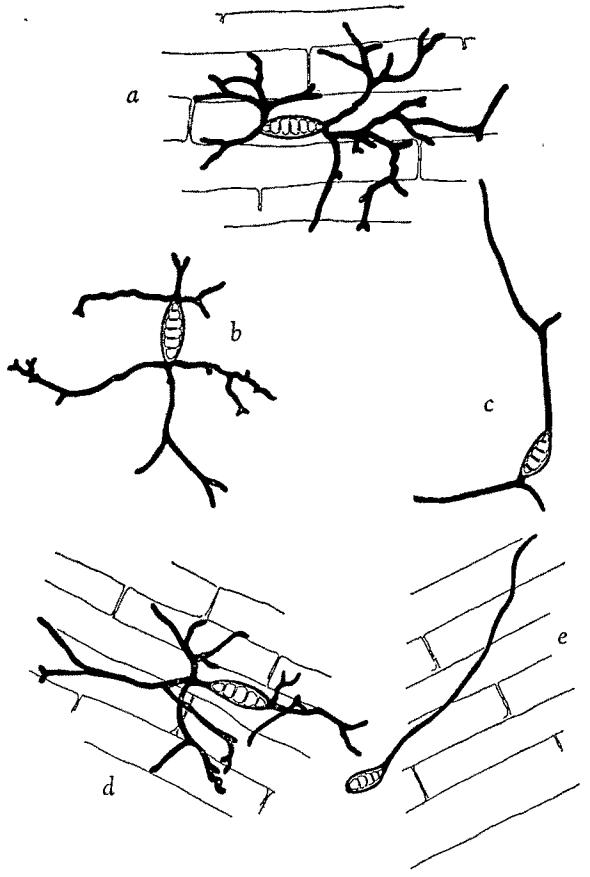


Fig. 1 Growth from a conidium of *H. sativum* after 12 h at 22° C. (*a*) On living wheat coleoptile; (*b*) on 'Cellophane', over exudate, (*c*) same over water; (*d*) on killed epidermis over exudate; (*e*) same over water. (× 172)

These experiments were then repeated using epidermal strips[8] killed by fixation in ethyl alcohol. These pieces of epidermal strips again gave branched hyphal structures (Fig. 1*d*) on the root exudate cylinders similar to those seen on the living host and on 'Cellophane' in contact with exudate. Branching was a rare occurrence on the epidermal strips over distilled water (Fig. 1*e*).

These results are reported here because this is a record of the formation of an essential pre-penetration structure induced by root exudate, and also the technique used here may not only be of value in examining the pathogenicity of similar fungi but could also be used to illuminate our picture of the mechanism of hyphal branching[9] in fungi.

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Primordial Germ Cells and Fecundity

THE origin of the definitive germ cells of vertebrates from the primordial germ cells of the embryonic period is now widely accepted and has been supported in the case of a few species[1–4] by quantitative observations, which make it unnecessary to postulate the neo-formation of germ cells from the somatic elements of the so-called 'germinal epithelium'. In the lamprey, the history of the germ line has been followed from the first appearance of the primordial germ cells in the yolky endoderm[5], through their migration to the genital ridge and subsequent proliferation in the indifferent gonad, to the period of sex differentiation. At this time, in the female ammocoete larva, the stock of definitive oocytes is established[4,6]. These oocytes, or such of them as survive, are finally matured and deposited as eggs several years later, in the single short spawning season that immediately precedes the end of adult life.

Among the various species of lampreys there are wide differences in fecundity, ranging from the non-parasitic brook lampreys which produce 1,000–3,000 eggs to the large anadromous, parasitic sea lampreys which may contain up to 260,000 eggs[7–9]. These wide differences in fecundity offer an opportunity to test the possibility that species differences in primordial germ cell numbers might show some correlation with the differences in the numbers of definitive germ cells characteristic of the parasitic and non-parasitic forms. This possibility is suggested by the observations of Mintz[10] and Mintz and Russell[11] on a sterile strain of mutant mice, in which complete sterility or greatly reduced fecundity was associated with either the complete absence of or a marked reduction in the numbers of primordial germ cells. On the other hand, Bounoure *et al.*[12] and Blackler and Fischberg[13] have found some evidence for regulation in the germ line, whereby after the experimental destruction of germinal material, those germ cells that survive may show an increased tendency towards proliferation.

In comparing the fecundity of the various species of lampreys it is important to distinguish between the actual fecundity of the adult female and the potential fecundity of the ammocoete larva, as represented by its oocyte stock. Particularly in the brook lamprey, but also to a lesser extent in the landlocked form of the sea lamprey, the oocyte potential of the larval ovary is considerably higher than that of the adult female and a large proportion of these oocytes either undergo atresia during metamorphosis or fail to develop in the later stages of oocyte growth that precede sexual maturity. In contrast, it is believed that in the river lamprey, *Lampetra fluviatilis*, losses of oocytes through these causes are relatively unimportant and that the oocyte stock of the larval gonad is little inferior to that of the adult ovary[7,8,14]. A further factor that must be taken into account is the extent of germ cell degeneration before and during sex differentiation. In the ammocoete of the landlocked sea lamprey it has been estimated that after proliferation, during the first 2 or 3 years of the larval period, up to 500,000 germ cells may be present, but that up to 70 per cent of these may undergo degeneration, mainly in the course of the meiotic prophase[4]. In the undifferentiated gonads of *L. planeri*, it is thought that there may be up to 30,000 germ cells and that a similar proportion are lost by degeneration. The enormous differences in the fecundity of these two species (Table 1) have therefore been attributed to differences in the proliferation of the germ cells, rather than to differences in the proportion which degenerate.

Counts have been made of the total numbers of primordial germ cells in *L. planeri*, *L. fluviatilis* and the landlocked *Petromyzon marinus* at the pro-ammocoete stage, after the yolk had been completely absorbed from the gut. At this stage, migration of germ cells to the genital anlage had generally been completed and, although ectopic germ cells have been reported[5,6], the proportion which fail to reach the gonad site is probably quite small. Mitotic germ cells have never been observed at this stage and division is thought not to occur until some months later. In common with other vertebrate species, a wide range of variability was encountered in germ cell numbers, and this was more pronounced in the case of *L. planeri*. The

Table 1. FECUNDITY AND PRIMORDIAL GERM CELL NUMBERS IN THREE SPECIES OF LAMPREYS

Species	Fecundity: Adult egg numbers	Fecundity: Larval oocyte numbers	Fecundity: Estimated total germ cell numbers	Counts of primordial germ cells: Number of animals	Counts of primordial germ cells: Mean and S.E.	Counts of primordial germ cells: Range
L. planeri	1,000–3,000	5,000–10,000	30,000	44	51·4 ± 3·3	10–94
L. fluviatilis	11,000–26,000	7,000–42,000	—	13	26·5 ± 4·2	7–49
P. marinus (landlocked form)	21,000–107,000	114,000–165,000	500,000	7	54·1 ± 5·6	27–69

mean germ cell number for this species (51) was rather higher than the figures given by Beard[15] and Okkelberg[5] for the primordial germ cells of brook lampreys. In spite of its greater fecundity, the river lamprey, *L. fluviatilis*, gave significantly lower counts than *L. planeri* (Table 1) and a similar trend was also observed in some preliminary observations made during the preceding year. Counts on the larvae of *P. marinus* showed no significant differences compared to either of the other two species. There are, therefore, no grounds for believing that the number of primordial germ cells bears any direct relationship to the number of definitive germ cells characteristic of the three species, although the possibility cannot be excluded that within a particular species the wide range of observed germ cell numbers might have some influence on the fecundity of individual animals.

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Dwarf Eggs and the Timing of Ovulation in the Domestic Fowl

THE production of eggs considerably smaller than the normal has been reported in various birds[1] and has been well documented for the domestic fowl[2]. Such eggs have been described as "witch eggs", "cock eggs", "dwarf eggs" and "wind eggs"—the latter term also being applied to infertile eggs[3]. It was known as early as the seventeenth century[3] that the yolks of such eggs were frequently absent, but that there was a central nucleus around which albumin was secreted. In an investigation of 236 dwarf eggs, Pearl and Curtis[2] reported that 35 per cent of all dwarf eggs were without a yolk, 55 per cent contained free yolk and 15 per cent had a complete yolk.

During recent observations on the time of oviposition in a group of hens, five were observed to lay dwarf eggs. The degree of calcification varied from a soft-shelled to a normal-shelled egg. On opening, the eggs were free from yolk but possessed a nucleus of solid material around which albumin had collected. No eggs weighed more then 25 g.

The eggs were not laid at any particular point in the clutch, and on examination of the time of lay it was found that this time was very close to that expected for the laying of an egg following a normal oviposition. This is illustrated by the data in Tables 1 and 2. In Table 1 is given the actual time of oviposition of all eggs including the dwarf eggs in each clutch, and in Table 2 is given the period of "lag"[4] found and expected between the laying of the dwarf eggs and the preceding and succeeding oviposition. The time of "expected lag" was calculated on the basis of a normal ovulation occurring. It will be seen that the production of the dwarf egg in no way altered the timing of the subsequent ovulation.

The normally low incidence of dwarf eggs, less than 0·1 per cent[2], has not in the past permitted many investigations to be made as to their cause. Pearl and Curtis[2], from an examination of autopsied hens, concluded that the eggs were probably formed as a result of an ovulation occurring at the normal time but depositing the yolk in the body cavity with debris entering the oviduct. This would appear to be a reasonable explanation both for their production and for their oviposition at the expected time.

Table 1. TIME OF OVIPOSITION OF DWARF EGGS IN CLUTCHES LAID BY FIVE HENS

Bird No.	Time of oviposition of follicle (h): C_1	C_2	C_3	C_4	C_5	C_6	C_7
1	—	11·15	13·15	14·00	[17·30]		
2	—	—	11·30	12·00	13·15	[14·00]	16·45
3	—	[09·00]	11·30	16·00	16·30	—	—
4_1*	08·00	[09·30]	11·00	12·15	16·00		
4_2*	08·00	09·15	[10·30]	12·30			
5	10·30	[13·30]					

The nomenclature C_1, C_2, etc., is that used by Fraps (ref. 4) and designates the first, second, etc., ovum in the clutch. A dash indicates a missing time. Dwarf eggs are enclosed in a panel.
* This bird laid a dwarf egg in two successive clutches recorded here as 4_1 and 4_2.

Table 2. CALCULATED AND OBSERVED LAG IN THE PRODUCTION OF DWARF EGGS

Bird No.	Lag between dwarf egg and previous egg: Found	Lag between dwarf egg and previous egg: Expected	Lag between dwarf egg and succeeding egg: Found	Lag between dwarf egg and succeeding egg: Expected
1	27·5	26·25		
2	26·25	25·0	26·75	25·75
3			25·5	25·5
4_1	25·5	25·5	26·5	25·75
4_2	25·25	25·25	28·0	27·75
5			27·5	27·5

The term "lag" is used as described by Fraps (ref. 4) and denotes the time in hours between one oviposition and the next.

Present theories for the control of ovulation in the fowl[5] place varying emphasis on the role of the oviducal egg, which has been ascribed a major[6] and a minor[5] role in controlling the time of ovulation. If dwarf eggs are produced without an accompanying ovulation, their appearance at the expected point in the clutch might be taken as support for the hypothesis ascribing a major role to the oviducal egg. On the other hand, if they are accompanied by an ovulation into the body cavity no major significance can be attached to their appearance.

A search of the literature has not revealed any further data on the precise time of production of dwarf eggs, and it is hoped that this communication will encourage the collection and publication of further data relating to the time of oviposition and the origin of these relatively rare curiosities.

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Haemoglobin in a Marine Nematode

ALTHOUGH haemoglobin is present in numerous parasitic nematodes, it has never been recorded from a free-living form[1]. Even the recent demonstration of its presence in the free-living egg-laying female of *Mermis subnigrescens*[2] scarcely affects the generalization, for *Mermis* is parasitic in insects for most of its life-history. However, we have now found that it is present in abundance in the purely marine form *Enoplus brevis* and, to a lesser extent, in *E. communis*.

The haemoglobin of *Mermis* was present in the so-called "chromotrope". Since haemoglobin had not hitherto been implicated as a sensory pigment, the finding, in our view, cast doubt on the function hitherto attributed to this organ[3]. In addition to experimentation with *Mermis* itself, it seemed worthwhile to examine the pigments found in the eye-spots which are a not uncommon feature of certain marine nematodes. In all the examples examined, mostly Enoploidea, the eye-spot pigments have been found to be other than haemoglobin. However, during the course of our searches, we found specimens of a haemoglobin containing enoplid in the estuarine mud of Budle Bay, Northumberland; we are now satisfied that these are specimens of *Enoplus brevis* Bastian. As shown by spectroscopic and histochemical test, the haemoglobin is present in the pharynx of both male and female, and in the "copulatory muscles" of the male (Fig. 1); it is also present, in smaller quantity, in hypodermal chord material along the length of the animal.

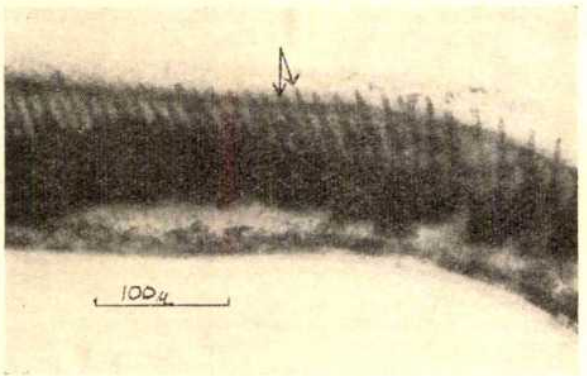


Fig. 1. Benzidine test on posterior end of male *E. brevis*. Haemoglobin is present in the series of "copulatory muscles" (arrows) which show heavy black

E. brevis has frequently been recorded from various localities[4] and it is, at first, puzzling that the presence of haemoglobin in such quantity should have gone undetected. Presumably, most other workers have only examined fixed material. We have confirmed that *E. brevis*, taken from two local habitats from which it has previously been recorded, have haemoglobin, distributed in the same way.

The haemoglobin in *E. communis* is present in much smaller quantity and, in this form, it occurs solely at intervals along the length of the hypodermal chords. Since it is present in this position also in *E. brevis*, and the tissue in which it is found in *Mermis* is hypodermal chordal in nature[5], the pigment may prove to be much more widely distributed in free-living forms.

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ENTOMOLOGY

Chemosterilization of *Aedes aegypti* (L.) by Pupal Treatment

THE concept of insect control through the liberation of sterile males[1] proved successful in the eradication of the screw-worm fly (*Cochliomyia hominivorax*) from the south-eastern United States[2]. Following this success, in which sterility was induced by γ-irradiation, interest was taken in the possibility of inducing sexual sterility by chemical means[3]. Screening of chemicals yielded several substances with a prospective sterilizing capacity[4,5], notably alkylating agents incorporating the aziridine moiety. In general they are less injurious to insects than radiation[6,7], and treatment of insects with chemicals is in certain respects more convenient than the procedures of irradiation.

In the first of the experiments carried out on chemosterilization of mosquitoes[8], the fertility of *Aedes aegypti* and *Anopheles quadrimaculatus* was reduced by feeding adults on the alkylating agents 'Tepa', aphomide or apholate dissolved in honey; other workers successfully sterilized both sexes by tarsal contact[9,10]. Sterilization of several mosquito species has also been obtained by immersion of larvae in solutions of the order of 10 p.p.m. of various aziridine compounds[11–13].

For practical applications of chemosterilization against natural mosquito populations, it seems logical to focus attention on sterilization of the mosquitoes in the aquatic stages. This is due to the fact that the principle of sterilization as a control method depends on sterilization of male insects and adult male mosquitoes cannot be readily found in nature, only females of troublesome or disease-carrying species being common as they come to feed, or at rest in the precincts of their hosts. By treatment of aquatic stages in their natural breeding sites, both sexes would be exposed to sterilization and the sterile males at emergence immediately available to produce sterile inseminations in females of other broods which had not been exposed. An alternative, and more acceptable, approach is to avoid dispersal of these mutagenic chemicals in the natural environment by treating the male insects in the laboratory and later releasing them at selected points in the area to be controlled so that they may disperse into the wild population to mate with normal wild females.

Although treatments of male insects for their subsequent release have been made by larval exposure, little attention has been given to the effectiveness of treatment of mosquitoes at the pupal stage, and this communication directs attention to certain advantages which pupal treatment appears to offer.

The toxic and sterilizing dosages of alkylating agents for mosquito larvae do not differ greatly, so that the dose required to ensure induction of complete sterility in the resultant adults also incurs mortality[9,12,14]. Investigations carried out in this laboratory on the sterilization of *Aedes aegypti* with the chemosterilant 'Thiotepa' (*tris* (1-aziridinyl) phosphine sulphide) show that this difficulty is avoided when the pupal stage is treated. Preliminary tests using aqueous solutions of this alkylating agent indicated that, although fertility was unaffected in adults which emerged from pupae subjected to doses of the same order as those which sterilized but also caused partial mortality of larvae, pupal mortality was negligible. Further experiments were conducted to determine what dosage pupae would tolerate and if sterility could be obtained with sub-lethal treatments. Immersion of pupae in 'Thiotepa' solutions as strong as 500 p.p.m. or more for 24 h proved highly satisfactory in inducing complete and permanent sterility in both sexes. It was also repeatedly found that this was far below the toxic dose for pupae, which could tolerate immersion in concentrations as high as 2,000 p.p.m. for 24 h. Adults

obtained following this treatment also survive well, there being no increase above control mortality at emergence nor during the first 2 weeks of adult life. In further tests pupal treatment at as low a concentration as 100 p.p.m. for 24 h rendered more than 90 per cent of 200 males completely infertile, with an average of only 6·5 per cent of eggs hatching in the ovipositions of normal females with which the incompletely sterilized males were mated; total sterility was obtained in all resultant males after pupal immersion for 28 h. The same pupal treatment was less efficient in reducing the fertility of twenty emergent females, an average of 50·6 per cent of eggs laid being viable following crossing with normal males. This comparative tolerance by the females may well be simply a function of their greater size. However, a heavier dosage to female pupae, immersion in 'Thiotepa' at 500 p.p.m. for 24 h, gave better results, causing considerable inhibition of ovarian development. This was yet more pronounced at still higher concentrations, immersion of female pupae for 24 h in 1,000 p.p.m., that is a 0·001 per cent solution, suppressing ovarian development in the emergent female at a point earlier than the usual resting stage characteristic of the unfed, newly emerged, normal mosquito. Before these treated females could oviposit, three blood meals were necessary and, even then, the few eggs obtained were spherical and failed to hatch, while others did not tan.

In tests of pupae at different ages, using a dosage of 2 h in 'Thiotepa' at 200 p.p.m. as inadequate for complete sterilization and therefore suitable to compare the responses of pupal batches of different ages, no major differences in their susceptibility with age were observed, other than extreme sensitivity within 1 h of either ecdysis.

In ten tests of the ability of males, treated as pupae, to compete with normal males for copulation with normal females, equal numbers of untreated males and of sterilized males exposed as pupae to the highly sterilizing doses of 100 p.p.m. or 200 p.p.m. for 24 h (none of the males being more than 10 days old) were allowed to compete for copulation with half their combined number of virgin females. At least half the females which were inseminated consistently proved infertile. When treated and normal males were only 2–4 days old, the totals of sterile : fertile matings were 62 : 35, while in experiments comparing 14–16-day-old sterilized males in competition with normal males of the same age totals of sterile : fertile matings were 18 : 38. Thus, treated males appear to be more competitive when young.

Throughout all experiments with pupal-treated females no change occurred in the initial level of sterility during the course of sequential ovarian cycles and, more important, the pupal-treated males showed no recovery of fertility with time as tested in repeated crosses with normal female mates. This is to be expected as sterilization in the pupal stage precedes full differentiation of the gonads, making recovery of fertility in relation to differential sensitivity of stages in gametogenesis highly improbable.

In conclusion, administration of sterilizing alkylating agents such as 'Thiotepa' to mosquitoes at the pupal stage seems a particularly promising method of dosage, the pupae tolerating well immersions for 24 h in concentrations which ensure complete sterility of adults of both sexes. Emergent adults survive well for at least 2 weeks and the sexual vigour of young sterilized males is good, with evidence for some enhanced ability over normal young males in mating with females. Thus, pupal treatment may well be of value as a means of treating male mosquitoes under controlled laboratory conditions for subsequent mass release after emergence, but the possibility of dispersion of the treated pupae themselves into the natural environment, either in artificial containers or placed in natural breeding places, seems also to be a feasible approach to distributing sterile males in the environment of a wild population. Apart from efficacy and convenience, the distribution in this way of pupae after treatment eliminates problems of toxicity to man and wild life.

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CYTOLOGY

A Penetrated Human Ovum studied by Electron Microscopy

UNTIL the present time, only two unequivocally penetrated human ova have been recovered and studied morphologically. Both of these were in the pronuclear stage. The first was recovered by Khvatov in 1959[1], and the second was recently described by Dickmann, Clewe, Bonney and Noyes[2]. These ova were studied by light and phase microscopy. A penetrated human ovum in the pronuclear stage has been recovered in our laboratory and studied by electron microscopy.

The ovum was obtained from a 33-year-old woman (D. E.), who had a vaginal hysterectomy for symptomatic pelvic relaxation. A bilateral salphingectomy was also performed. The patient had sexual intercourse approximately 26 h before the operation. The two oviducts were flushed with Krebs–Ringer phosphate solution from the utero-tubal junction to the fimbriated end. An ovum was collected and immediately fixed with cold 1 per cent buffered osmium tetroxide. The specimen was embedded in 'Epon' and serially sectioned. Thin sections were stained with lead hydroxide.

The ovum was surrounded by the zona pellucida from which it was separated by a 7-μ wide perivitelline space. A few corona radiata cells were still present around the zona pellucida. In addition, two spermatozoa were observed within the zona. Three polar bodies were present in the perivitelline space. Two of them, conceivably representing a divided first polar body, were located adjacent to each other. In addition to cytoplasmic organelles, they showed naked clumps of chromatin and microtubules of the meiotic spindle. The second polar body was located at a considerable distance from the first two, outside the opposite hemisphere of the ovum. It had recently separated from the egg, to which

it was still connected by a stretched cytoplasmic bridge. It contained a spherical mass of chromatin which was surrounded by a nuclear envelope.

Two large, spheroidal pronuclei (Fig. 1) were observed in the cortical region of the ovum close to the second polar body. They showed highly hydrated chromatin and dense prominent nucleoli. The pronuclei were completely surrounded by a double membrane envelope. The tail of the penetrated sperm was located very close to one of the two pronuclei (Fig. 2).

It would seem that the fine structural characteristics of the fertilized human ovum are markedly similar to those of the fertilized rabbit[3] and monkey ovum[4]. Further detailed investigations are in progress.

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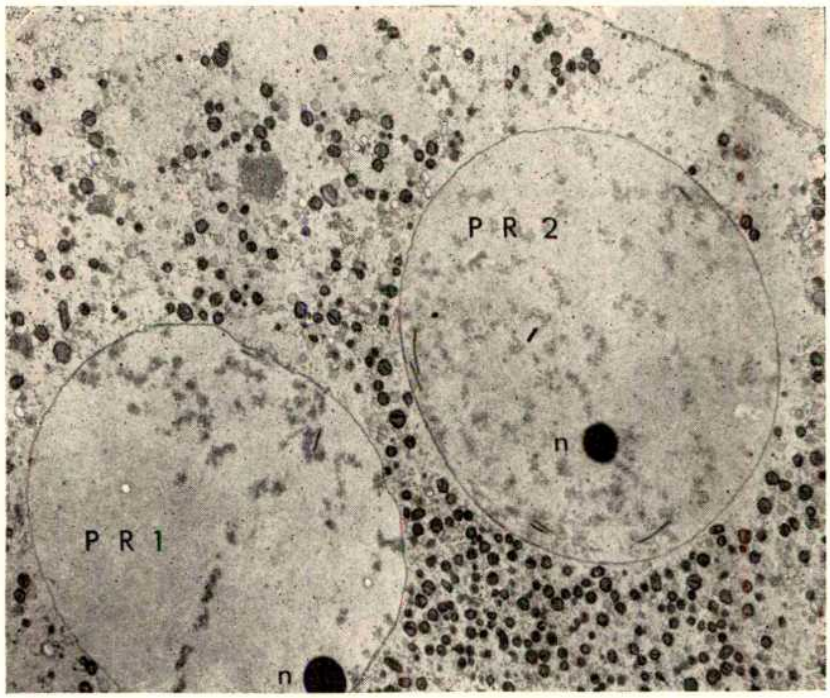


Fig. 1. Portion of human fertilized ovum. Two pronuclei (PR 1 and PR 2) are observed in the cortical cytoplasm of the ovum. Note the hydrated chromatin and the dense nucleoli (*n*). (×3,000)

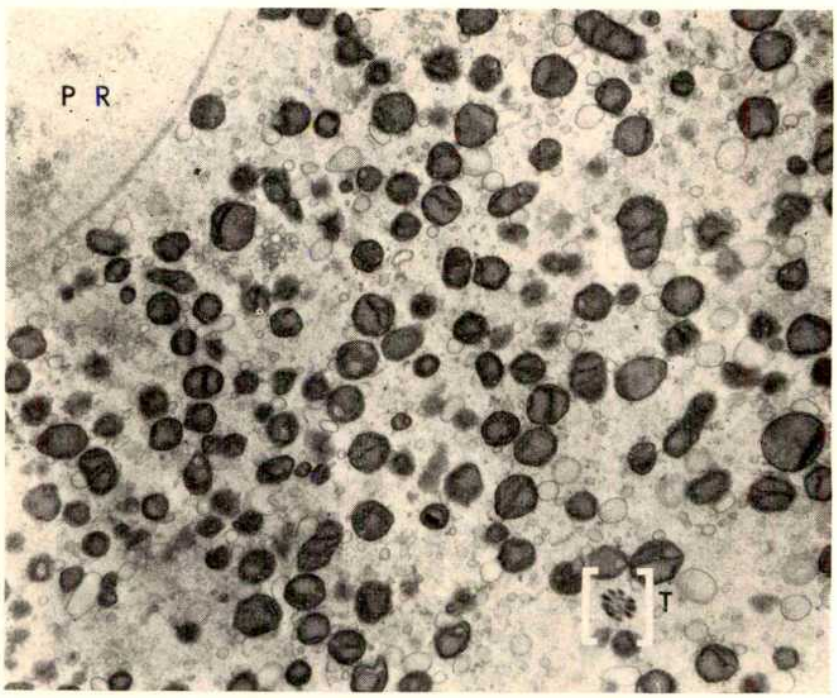


Fig. 2. The tail (*T*) of the penetrated sperm located close to one of the two pronuclei (PR). (×10,000)

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A Diploid *Gymnocarpium dryopteris* (L.) Newman in Alaska

THE fern species *Gymnocarpium dryopteris* (L.) Newman is widely distributed over the northern continents, being found in Europe, Asia, North America and Greenland. The taxonomic status of this species has been uncertain for a long time. It was first named *Polypodium dryopteris* by Linneus (1753). Bory (1824) included it in the genus *Lastrea*, and even Copeland, as late as 1947, preferred to keep it there[1]. Chromosome investigations carried out on the Pteridophyta during the past 15 years have shown, however, that the genus *Lastrea* Bory consists of many different basic chromosome numbers: $n = 30$ (*Lastrea phegopteris* e.g.); $n = 40$ (*Lastrea dryopteris* e.g.); $n = 27$, 30, 31, 34, 35 and 36 (*Lastrea thelypteris* group, and others)[2,3]. On this account the genus *Lastrea* Bory has recently been divided into many different genera, such as *Phegopteris* (Fée 1850), *Gymnocarpium* (Newman 1851), and *Thelypteris* (Schmidel 1762).

The chromosome number of *Gymnocarpium dryopteris* (L.) Newman (= *Dryopteris linnaeana* C.Chr. 1906) has been reported by many cytologists to be $n = 80$ (refs. 4–7).

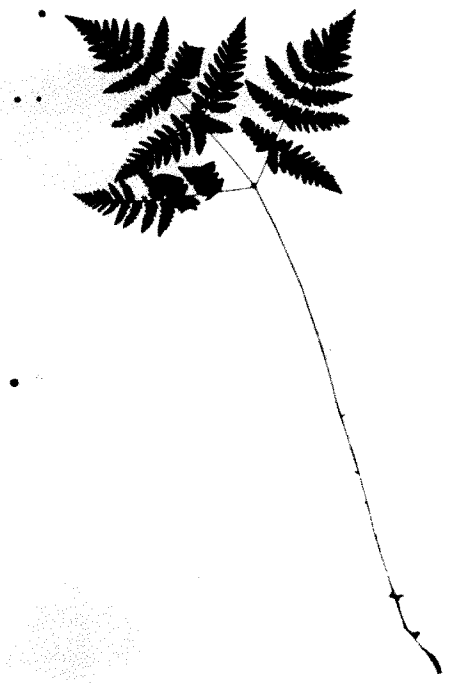


Fig. 1. Photocopy of the diploid *Gymnocarpium dryopteris* (L.) Newman found in Alaska (one-third natural size)

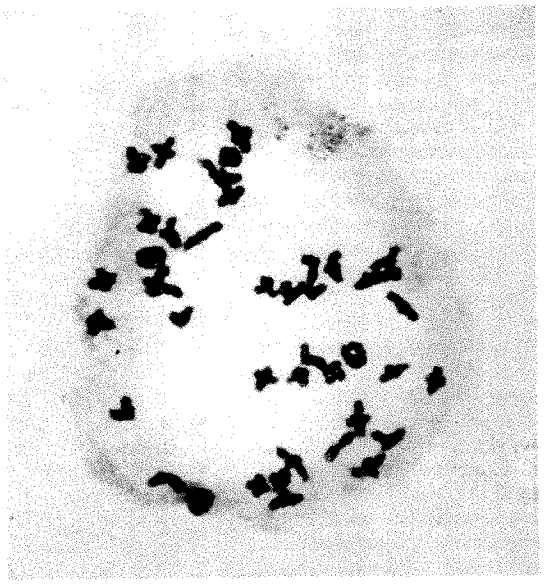


Fig. 2. Chromosomes of the diploid *Gymnocarpium dryopteris* (L.) Newman at diakinesis, $n = 40$. Acetic–iron–haematoxylin squash. (× *c.* 1,500)

The only information about the diploid *Gymnocarpium dryopteris* from Washington, United States, was reported, as a personal communication from Wagner, by Fabbri (1963) (ref. 3).

In June 1964 I had an opportunity to collect some plant material from Alaska for cytological investigations. The fern species *Gymnocarpium dryopteris* (L.) Newman was collected from coastal forest in the Auke region about 20 miles north-west of Juneau. Young sporangia were fixed in acetic alcohol (1 : 3) and stored for almost a year in the fixative in a refrigerator (−10° C) before the preparations were made. After squashing on slides, sporangia were stained for some minutes in acetic–iron–haematoxylin solution.

Forty bivalents were counted at diakinesis and the first meiotic metaphase. Three or four of them were regularly found to be ring bivalents. The chromosome number, $n = 40$, observed now is diploid compared with the tetraploid counts, $n = 80$, made previously. The diploid species of *Gymnocarpium dryopteris* (L.) Newman obviously has a north-western distribution in North America, as is also indicated by the diploid species found in Washington, United States, as mentioned earlier[3]. In the central and eastern parts of North America, as well as in Europe, only the tetraploid number, $n = 80$, was known before[2,3,7].

The interesting hybrid of *Gymnocarpium dryopteris* and *Gymnocarpium robertianum*, with 120 chromosomes, reported by Wagner[7] is possibly a hybrid between the diploid *G. dryopteris* and the tetraploid *G. robertianum*. *Gymnocarpium dryopteris* × *G. robertianum* hybrids were also found in Sweden, Finland and Russian Karelia[8], but they have not been investigated cytologically.

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PHARMACOLOGY

Effect of Reserpine on the Reactivity of Atrial and Ventricular Pacemakers to Quinidine

AN antagonism of the cardiac actions of quinidine by exogenous catecholamines has been demonstrated[1–3]. It is likely, therefore, that cardiac catecholamines also antagonize the depressant action of quinidine. To test this possibility, the effect of reserpine pretreatment on the action of quinidine in isolated cat hearts with complete heart block was investigated.

Cats were rendered unconscious with electric shock. The heart was removed and perfused according to the method of Langendorff with Krebs–Henseleit[4] solution, bubbled with 95 per cent oxygen and 5 per cent carbon dioxide. The perfusion pressure was kept constant at 52 cm water; the temperature of the perfusion fluid was maintained at 37° C by appropriate thermoregulation of the water jacket. Complete heart block was produced by using a modification of the method described by Grumbach[5]. The right atrium was opened to visualize the bundle of His, and complete heart block was produced by ligating the bundle.

Quinidine was perfused in the concentrations of 2, 10 and 50 μg/ml. The quantities used are expressed in terms of the free base. Reserpine, 5 mg/kg, was administered intraperitoneally 24–30 h before the experiment.

Table 1. EFFECT OF RESERPINE ON QUINIDINE DEPRESSION OF ATRIAL AND VENTRICULAR PACEMAKERS

Quinidine concentration (μg/ml.)	Average maximum depression of rate (percentage of depression ± *S.E.*) Atrium		Ventricle	
	Non-reserpinized	Reserpinized	Non-reserpinized	Reserpinized
2	13 ± 5·8 (5)*	57 ± 10·8‡ (7)	9·4 ± 4·4 (5)	33·3 ± 11·8 (7)
10	73·9 ± 3·2 (10)	87·4 ± 4·9 (7)	8·4 ± 3·3 (10)	52·4 ± 7·3‡ (7)
50	53·0 ± 6·5† (7)	93·4 ± 6·4 (6)	80·3 ± 5·9 (7)	91·3 ± 8·2 (6)

* The numbers in parenthesis represent the number of experiments.
† It was not possible to observe further depression since five of seven preparations developed rhythm disturbance after this point.
‡ Significantly different from non-reserpinized group ($P < 0·05$).

After reserpine, the reactivity of the atrial and ventricular pacemakers to quinidine was increased. The results are summarized in Table 1. The average atrial and ventricular rates before quinidine was administered were, in non-reserpinized hearts, 113 ± *S.E.* 3·8 beats/min and 49 ± *S.E.* 2·4 beats/min respectively, and, in the reserpinized hearts, 119 ± *S.E.* 5·3 beats/min and 46 ± *S.E.* 1·6 beats/min respectively. In non-reserpinized preparations the decrease in atrial and ventricular rates produced by 2 μg/ml. and the decrease in the ventricular rate caused by 10 μg/ml. were not significantly different from that which occurs normally during the perfusion of Krebs–Henseleit solution ($P > 0·05$). Therefore, what appears to be a quinidine effect on rate at these concentrations actually represents the spontaneous decay of the preparation.

While it required the perfusion of 10 μg/ml. of quinidine to alter the rate of the atrial pacemaker in the non-reserpinized preparations, after reserpine pretreatment, 2 μg/ml. was sufficient to produce significant depression ($P < 0·05$). However, the effect of quinidine when it was perfused at 10 μg/ml. on the atrial pacemaker was similar in reserpinized and non-reserpinized hearts. It was not possible to observe the full effects of quinidine perfused in a concentration of 50 μg/ml., since rhythm disturbances developed in five of seven preparations within 30 min of perfusion. Consequently, the effect of reserpine on the reactivity of the atrium to quinidine perfused at this concentration could not be determined.

Whereas it required 50 μg/ml. of quinidine to depress the ventricular pacemaker in the non-reserpinized preparations, after reserpine it required only 10 μg/ml. to reduce significantly the ventricular rate ($P < 0·05$). It might appear that 2 μg/ml. of quinidine also reduced the ventricular rate in the reserpinized preparations, but the responses at this concentration were quite variable and were not significantly different from those produced in the non-reserpinized hearts ($0·10 > P > 0·05$). Reserpine pretreatment did not affect the depression of the ventricle produced by the perfusion of 50 μg/ml. of quinidine. Thus, the effect of reserpine pretreatment on the reactivity of the atrial and ventricular pacemakers to quinidine was evident only when lower concentrations of quinidine were perfused.

After reserpinization, the recovery of the atrial and ventricular pacemakers from quinidine depression was not so marked as in the non-reserpinized preparations (Table 2). After perfusion with 10 μg/ml. was terminated, it required about twice the time for the activity of the atrial pacemaker to recover to approximately the same degree as in the non-reserpinized preparations. Furthermore, the degree to which the atrial pacemaker recovered from 50 μg/ml. of quinidine was considerably less and the time to maximum recovery was almost doubled. While the recovery of the ventricular pacemaker from quinidine depression proceeded to almost the same extent as in the non-reserpinized preparations, after reserpine the time to maximum recovery was doubled.

Although reserpine decreased the incidence of rhythm disturbances occurring when quinidine was perfused at 50 μg/ml., the total incidence of atrial and ventricular rhythm disturbances caused by quinidine was not affected by reserpine pretreatment. Thus, atrial arrhythmias occurred in eight of twenty-two non-reserpinized hearts and in six of twenty reserpinized preparations (χ square test; $0·30 < P < 0·50$). Ventricular arrhythmias occurred in five of twenty-two non-reserpinized hearts, while they occurred in two of twenty reserpinized preparations (χ square test; $0·10 < P < 0·20$). These results confirm those previously reported by Roberts *et al.*[6], who showed that reduction in sympathetic activity in unanaesthetized dogs with complete heart block did not alter the arrhythmogenic effects of quinidine.

It is clear from the data that, after reserpine, the isolated heart became more sensitive to quinidine depression. The action of reserpine causing greater sensitivity of the heart to quinidine is probably related to its catecholamine-depleting effect. On acute denervation, it is unlikely that the heart loses all its catecholamines. It is possible, therefore, that the residual catecholamine activity is of sufficient magnitude to antagonize, at least in part,

Table 2. EFFECT OF RESERPINE ON RECOVERY OF ATRIAL AND VENTRICULAR PACEMAKERS FROM QUINIDINE DEPRESSION

Quinidine concentration (μg/ml.)	Non-reserpinized: No. of exp.	Average control rate just before quinidine (beats/min)	Average maximum recovery (percentage control)	Average time to maximum recovery (min)	Reserpinized: No. of exp.	Average control rate just before quinidine (beats/min)	Average maximum recovery (percentage control)	Average time to maximum recovery (min)
				Atrium				
2	—	—	—	—	7	106	55	30
10	10	110	63	25	7	119	69	45
50	7	112	68	25	6	104	43	45
				Ventricle				
10	—	—	—	—	7	41	76	60
50	7	53	92	30	6	44	91	60

Recovery from quinidine depression was followed for 1 h after perfusion of the drug was terminated.

quinidine depression. Consequently, depletion of catecholamines by reserpine would permit quinidine depression to develop more readily. It may also be argued that reserpine, like quinidine, is a cardiac depressant[7] and that against a background of reserpine-induced depression, the effect of quinidine would be enhanced. However, a direct depressant action by reserpine seems unlikely since the rate and stability of isolated reserpinized hearts did not differ from those of non-reserpinized preparations[8]. Furthermore, unlike quinidine, reserpine pretreatment does not affect the rate of rise or the overshoot of atrial action potentials[9].

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Tumour-inducing Effect of Hydrazine in Mice

AFTER the discovery of the experimental tumour-inducing effect of isonicotinic acid hydrazide[1] several later investigations verified that this compound showed carcinogenic effects in white mice of different inbred strains when administered intraperitoneally or orally over a long period[2–8]. According to Mori *et al.*[2], the carbamyl group is responsible for the cancerogenic effect of isonicotinic acid hydrazide. On the other hand, Biancifiori *et al.*[3] are of the opinion that the hydrazine radical[10] detached from the compound during the metabolic process plays a part in producing tumours. They found in their experiments that the frequency of tumours in mice of *BALB/c* strain caused by isonicotinic acid hydrazide or hydrazine sulphate was very high, while isonicotinic acid alone showed no carcinogenic effect.

In our own experiments we administered hydrazine to white mice having an average weight of 20 g. Hydrazine (NH_2–NH_2) is easily soluble in alcohol and water. It is a bivalent weak base; in a watery solution mono- or bivalent mono- or dihydrate develops. Each injection contained 0·5 mg hydrazine in a 0·5 ml. physiological saline solution. We treated thirty male and thirty female mice intraperitoneally, at the beginning of the experiment on each second day, and later on each third day. 16 injections were given in all over a period of 46 days. Altogether, 400 mg/kg body weight of hydrazine entered the organism of the experimental animals.

Treatment with hydrazine caused the development of focal liver necroses in some of the animals; twenty-six animals died as a result of severe liver damage in the first period of the experiment. The liver-damaging effect of drugs containing the hydrazine radical is a well-known clinical phenomenon[11–13]. Thirteen of the thirty-four mice surviving the treatment developed tumours and myeloid leukaemias between days 100 and 313 reckoned from the beginning of the experiment. The tumours appeared in the mediastinum and showed a local invasion, sometimes provoking metastases in the lungs, pleura and liver. Histologically the tumours proved to be reticular-cell sarcomata. In the cases of myeloid leukaemia, enlargement of the liver, spleen and lymph nodes was to be seen; histologically, there was infiltration of these organs as well as of the bone marrow by immature myeloid cells. As controls, we used sixty white mice of the same pedigree and age; there was only one case of spontaneous thymic lymphoma in them during a year's observation.

The hepatotoxic effect of hydrazine has been known for some time, but there were no communications on its cancerogenic effect. The investigations carried out with isonicotinic acid hydrazide directed attention to this property of hydrazine. The further examination of this compound, which has a very simple chemical structure, may reveal other valuable results. The question is of interest also from the point of view of human pathology, since many other drugs also contain hydrazine radicals, which may liberate hydrazine in the course of metabolism. Thus, a thorough investigation of hydrazine may also give us information about the important question of the cancerogenic effect of drugs.

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Effects of 3,4-Dimethoxyphenylethylamine in Man

FOUR laboratories have reported finding 3,4-dimethoxyphenylethylamine (DMPEA) in the urine of schizophrenic patients[1–4]. Evidence for its occurrence in some patients with this disorder seems well established despite a single negative report[5]. As the compound is quite closely related chemically to mescaline, the latter possessing an additional methoxy-group in the 5-position, it is conceivable that DMPEA might have similar activity in humans.

In initial attempts to determine activity in man, conservative oral doses of the free amine were used, beginning with 50 mg and increasing to a dose of 600 mg, without any appreciable clinical effects being noted. Because of the possibility that the free amine might not be absorbed, a bitartrate salt was prepared, and administered in similar fashion with similar lack of effect at doses up to 800 mg. A similar testing procedure was followed with the hydrochloride salt and then a formal investigation was contrived to compare DMPEA with mescaline and placebo under blind controlled conditions.

Thirteen volunteer subjects were studied. Twelve were recovered psychiatric patients, seven with previous diagnoses of schizophrenic reaction, three with depressive reactions and two with psychoneuroses; the other volunteer had no history of emotional disorder. Three trials were conducted at weekly intervals. In the first trial, DMPEA was given on each occasion, nine of the subjects receiving doses of 500 mg, the last three receiving doses of 1,000 mg. Mescaline and placebo were administered in random order over the next two trials; the dose of mescaline was based on 6 mg/kg, the actual ranges being 405–

635 mg. Clinical effects were assessed by observation, by self-report of patients on the Clyde Mood Scale prior to treatment and at two, four and six hours after, and by completion of a symptom-sign check-list after the effects of drugs had worn off. A 24-h urine sample was collected from each subject after each treatment.

In each instance in which mescaline was administered, a rather typical clinical syndrome ensued, but neither DMPEA nor placebo produced any discernible effects. As judged by the symptom-sign check-list, mescaline evoked nausea, dizziness, blurred vision, tension, weakness, drowsiness, perceptual distortions, depersonalization, difficulty in thinking or expression, rapid flow of thoughts, poor memory, and a dream-like state. Only a few symptoms were reported from the DMPEA and placebo trials and these most commonly were weakness, drowsiness, and sadness, possibly attributable to the enforced inactivity of the testing situation. As reported on the Clyde Mood Scale, mescaline produced significant increases in friendliness, sleepiness, and dizziness, and a significant decrease in clear thinking, over several time-periods in each case. A sporadic significant increase in sleepiness was reported from DMPEA after four hours and an increase in aggressiveness from placebo at the same time. Due to the number of comparisons made, at least one significant change might have been expected to occur by chance from treatment. In addition, of the three treatments only mescaline produced pupillary dilation, increased deep tendon reflexes, unsteadiness and tremors, signs characteristic of increased sympathetic stimulation.

Table 1. RECOVERY OF METABOLITES FROM EIGHT OF THIRTEEN SUBJECTS ADMINISTERED DMPEA AND MESCALINE

	Mean percentage recovered per 24-h urine*		
	As DMPEA	As dimethoxyphenyl-acetic acid (DMPAA)	Total
After administration of DMPEA	0·37 (σ = 0·20)	77·1 (σ = 18·5)	77·5
	Percentage recovered as mescaline (TMPEA)	Percentage recovered as trimethoxyphenyl-acetic acid (TMPAA)	Total
After administration of mescaline	23·1 (σ = 8·4)	18·1 (σ = 13·2)	41·2

* Uncorrected for losses from the procedure. Approximate recovery of authentic material added to urine was: DMPEA 74 per cent, TMPEA 73 per cent, DMPAA 98 per cent, TMPAA 98 per cent.

It can be concluded that DMPEA administered orally in doses comparable to those used for mescaline exhibits none of the psychotomimetic effects of the latter drug. This is in contrast to findings from investigations carried out with lower mammals[6,7].

The difference in response of human subjects to mescaline and DMPEA may be related to the relatively complete conversion of DMPEA to its corresponding acid. Mescaline, in contrast, is, in significant amounts, excreted as the unmetabolized amine which is presumably the active form. This can be concluded from the data in Table 1. A detailed report of the metabolic aspect of this investigation is in preparation.

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PATHOLOGY

Teratogenic and Carcinogenic Effects in the Offspring after Single Injection of Ethylnitrosourea to Pregnant Rats

ALKYLNITROSOUREAS give the corresponding alkylating diazoalkanes on heterolysis, and are potent carcinogens, selectively producing malignant tumours in the brain[1] and peripheral nerves[2] of rats. As might be expected, methylnitrosourea is also mutagenic as in *Saccharomyces cerevisiae*[3] and is teratogenic in rats[4]. In teratogenesis investigations the embryos are generally killed and examined immediately after Caesarean delivery. If, however, only slight teratogenic effects are produced to allow the offspring to survive, cancer may also develop later on.

Ethylnitrosourea (ENU; melting point, 103° C) has been used in our experiments on rats of our inbred strain *BD* IX (*CPAH*, agouti). Using intravenous injection, the *L.D.* 50 is 240 mg/kg. At the fifteenth day of pregnancy, three rats were injected with 80 mg ENU/kg bodyweight. Twenty-one young rats were born. All showed malformations of the paws, as shown in Fig. 1. Two of these three litters were lost after 5 days due to agalacty of the mother rats. Five rats of the remaining litter survived and matured under artificial feeding. At the age of 160 days, three of them died with the symptoms of intracranial pressure. Autopsy revealed extensive tumours of the trigeminal nerve (Fig. 2), histologically classified as malignant neurinomata. Additional tumours were observed in two of these rats; one was a polymorphic cell glioma in the right frontal lobe, and the other was a very malignant neurinoma of the left brachial plexus. This was sucessfully transplanted to young *BD* IX rats.

The fourth animal developed paralysis of the left foreleg and the right hind limb. At the age of 165 days it

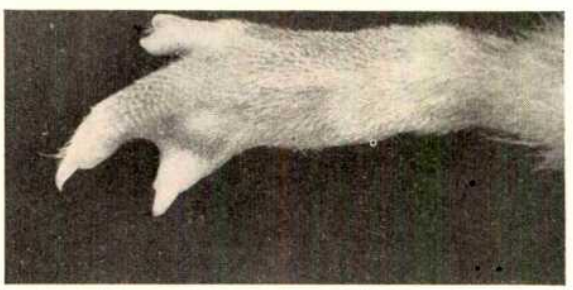


Fig. 1. Oligodactylia and syndactylia in a rat, induced by a single injection of 80 mg ethylnitrosourea per kg at the fifteenth day of pregnancy. (×2·5)

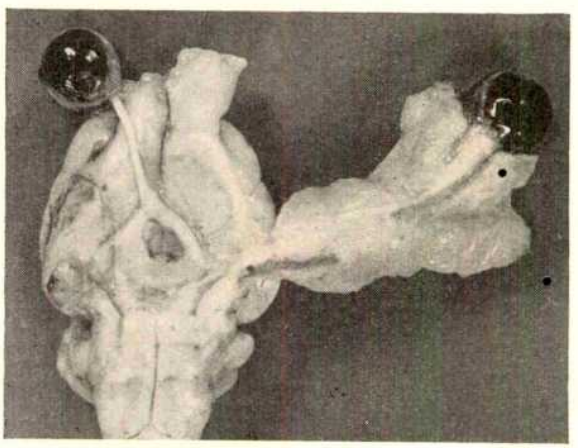


Fig. 2. Malignant neurinoma of the left trigeminal nerve with exophthalmia and rupture of the optic nerve. Ethylnitrosourea, 80 mg/kg, fifteenth day of pregnancy. (×2·5)

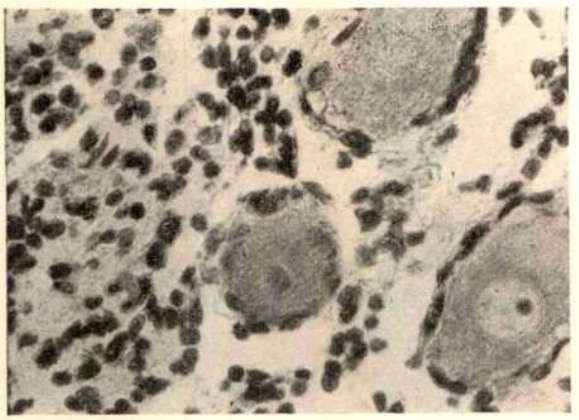


Fig. 3. Section of malignant neurinoma shown in Fig. 2. (×400)

died with neurinomata of the respective brachial and lumbosacral plexuses. The last rat also shows clear symptoms of intracranial tumour development.

Neither neurogenic tumour nor malformation of the paws occurred spontaneously in our strains of rats. The mother animals are still alive and show no sign of tumour formation, which we have found can only be expected after an induction period of more than 360 days[5].

The observations reported here are interesting in the following respects: (1) a single dose of a potent carcinogen given to a pregnant animal can produce teratogenic as well as carcinogenic effects in the offspring, (2) the organotropy of the carcinogen is unchanged, and (3) embryos are more susceptible than adults. The relatively short induction time observed may have some bearing on the important problem of cancer in childhood. Furthermore, it offers a rapid method for use in carcinogenicity tests.

Systematic investigations of these phenomena are under way. Detailed results will be published elsewhere. The work was supported by the Deutsche Forschungsgemeinschaft.

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IMMUNOLOGY

Structural Heterogeneity of L Chains in Antibodies of Restricted Specificity

ZONE electrophoresis suggests that L chains of specific antibodies are less heterogeneous that L chains of whole γ-immunoglobulins (IgG)[1]. It would therefore be interesting to determine whether all L chains of an antibody directed against a single antigenic determinant (and therefore possessing a restricted specificity) have the same primary structure or not. If their primary structure is different, it may be wondered whether their degree of heterogeneity in this respect is the same in antibody as in whole IgG, and whether this heterogeneity is related to antibody specificity. The analysis of the N-terminal amino-acids is a useful preliminary approach to the study of this problem.

IgG were obtained from rabbit sera with allotypic markers, Aa3 Ab4. Sodium sulphate was used to bring about the precipitation (according to Kekwick[2]). IgG were purified by chromatography on DEAE cellulose columns using 0·0175 M sodium phosphate, *p*H 6·3. Reduction and alkylation were performed essentially according to Fleischman[3]. The final concentrations of mercaptoethanol and iodoacetamide were 0·75 M.

After dialysis for 15 h against 6 M urea and 0·05 M formic acid, fractionation was performed on 'Sephadex *G*-100' columns (3 cm × 105 cm) previously equilibrated with this solution. Harris and Knight[4] have shown that the presence or absence of the C-terminal threonines of tobacco mosaic virus (TMV) injected into rabbits determines the appearance of two different antibodies. A fraction of antibody molecules, here called anti-TMV thr$^+$, does not precipitate with TMV which has lost its C-terminal threonine by carboxypeptidase action. Another category of antibody does not precipitate with normal TMV but only with TMV treated with carboxypeptidase (antibody anti-TMV thr$^-$). This has been confirmed recently[5].

After complete absorption of the respective sera with TMV thr$^-$ and TMV thr$^+$, the remaining anti-TMV thr$^+$ and anti-TMV thr$^-$ antibodies were precipitated with the corresponding antigens. Antibodies were extracted by treatment of the immune precipitate at *p*H 1·8 (ref. 6). TMV was removed by ultracentrifugation at 28,000 r.p.m. for 45 min and the antibodies were lyophilized. Rabbits were injected with bovine DNP γ-globulin and the corresponding anti-DNP antibodies were purified by the method of Farah[7]. Dinitrophenylation of IgG and antibodies, hydrolysis and DNP amino-acid extraction were performed according to the method devised by Phillips[8]. DNP amino-acids were identified by two-dimensional thin-layer chromatography on kieselgel *G* according to Stahl's method[9].

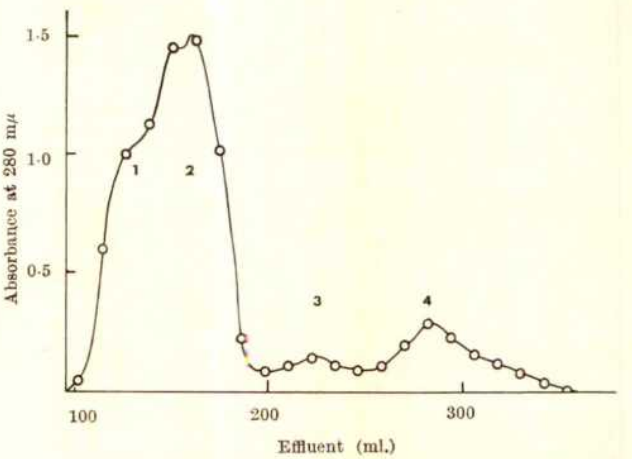


Fig. 1. Fractionation of reduced and alkylated IgG on 'Sephadex *G*-100' in 6 M urea–0·05 M formic acid

Filtration on 'Sephadex' of reduced and alkylated IgG always showed four peaks (Fig. 1). These different peaks were run again on 'Sephadex *G*-100' under the conditions already described. The content of peak 2 did not show detectable N-terminal amino-acids. This peak must therefore have contained almost exclusively H chains. Peak 4, containing the bulk of L chains, was characterized by the presence of alanine and aspartic acid, as previously shown by Fleischman[3]. Other amino-acids were detected and were present in lower but notable concentration (see Fig. 2): glutamic acid, serine, threonine, valine and leucine (or isoleucine). Peaks 1 and 3 had the same N-terminal amino-acids as L chains and, for this reason, would have been composed of incompletely separated L and H chains (peak 1) and aggregated L chains (peak 3).

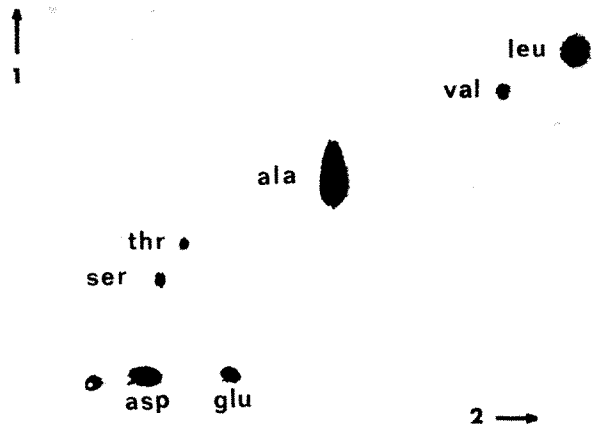


Fig. 2. Two-dimensional chromatogram of the DNP derivatives of the N-terminal amino-acids of IgG. Solvent 1 : toluene–pyridine–ethylene-chlorhydrin–0·8 N ammonia solution (100 + 30 + 60 + 60). Solvent 2: chloroform–benzyl alcohol–glacial acetic acid (70 + 30 + 3)

Total anti-TMV antibody from a single rabbit, homozygous for allotypic markers Aa3 Ab4, has the same seven N-terminal amino-acids in its L chains as whole IgG. The amounts of alanine and aspartic acid we have found per mole of IgG agree with the results of Fleischman[3]. Antibody anti-TMV thr$^+$ from the same rabbit also has seven detectable N-terminal amino-acids, like antibody anti-TMV thr$^-$ and anti-DNP-OH.

These findings concerning antibodies of restricted specificity indicate that the N-terminal amino-acid multiplicity found for whole IgG is not due to contamination, since this multiplicity is also found in that fraction of IgG molecules specifically precipitated by an antigen.

On the other hand, it seems improbable that these N-terminal amino-acids come from partial proteolytic hydrolysis, because H chains treated in the same way as L chains do not reveal detectable N-terminal amino-acids. A more quantitative comparison of the N-terminal amino-acids of antibodies anti-TMV thr$^+$ and anti-TMV thr$^-$ using 1 fluoro, 2-4-dinitrobenzene carbon-14 has been undertaken. The radioactivity of each N-terminal amino-acid of antibody anti-TMV thr$^+$, measured in a scintillation counter[10], is compared with the radioactivity of the corresponding N-terminal amino-acid of anti-TMVthr$^-$. Initial results seem to show that the N-terminal amino-acids are present in equal amounts in the two types of restricted specificity.

These results can be compared with those obtained with Bence-Jones proteins and L chains of myeloma proteins. Ten years ago, Putnam[11] and Vis and Crokaert[12] showed that Bence-Jones proteins synthesized by a single patient are homogeneous and characterized by a single N-terminal amino-acid—though they differ from one patient to another. The amino-acid composition of the N-terminal region of group I Bence-Jones proteins which have been studied differs considerably from one to another[13,14].

It can be concluded that: (1) There are at least as many different L chains in an antibody as N-terminal amino-acids detected. Thus one of the differences between a Bence-Jones protein and an antibody of restricted specificity is the multiplicity of L chains which are involved in antibody formation. (2) The L chains of antibodies of restricted specificity do not seem to be less heterogeneous than L chains of whole IgG with respect to their N-terminal amino-acids. (3) If specificity depends on the primary structure of the H chain involved[15], different L chains can be combined with that particular H chain.

This work was carried out under Euratom-ULB contract.

Note added in proof. The heterogeneity of the N-terminal amino-acids of IgG has since also been pointed out by Utsumi, S., and Karush, F. (*Biochemistry*, 4(9), 1766; 1965).

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Transplantation and Function of a Histo-incompatible Tumour in Thymectomized Rats

NEONATAL thymectomy is known to diminish the ability of rats to react immunologically to some antigens[1–5]; the Jensen sarcoma has been shown to grow faster in thymectomized than in intact Sprague-Dawley rats[6]. In mice, a mammary adenocarcinoma has been transferred into a histo-incompatible host thymectomized before 48 h of age[7]. In the present investigation, thymectomy is shown to alter sufficiently the immunological capacity of the rat so that it will not only accept a histo-incompatible tumour, but the tumour–host relationship which results permits the tumour to produce hormones and the host to respond to these hormones.

The tumour used in the present investigation was chosen because previous attempts had shown that it would not grow in intact Wistar/Furth rats. The tumour is designated 'mammotropic tumour number 4 of Fisher/Furth rats' or *MtT/F4*; it originated in a Fisher/Furth (Fisher/Fu) rat implanted subcutaneously with diethylstilboestrol, and has been carried subcutaneously in this laboratory for over sixty transfer-generations[8]. It has a latent period of approximately three weeks and kills the host after eight to fifteen weeks of progressive local growth. Occasionally, metastases are found in the thorax and/or abdomen. The *MtT/F4* tumour consistently produces strong ACTH and prolactin effects in the host; the adrenal glands are stimulated to approximately ten times normal size and the mammary glands are greatly enlarged and frequently lactate. These effects begin just before, or soon after, the tumour becomes palpable.

The hosts in this investigation were male and female Wistar/Furth (Wistar/Fu) rats thymectomized within 48 h of birth. The *MtT/F4* was transferred when these thymectomized hosts were eight to eleven weeks of age using scissor-minced tumour diluted approximately 1 : 1 with a tissue-culture fluid containing penicillin and streptomycin. Approximately 0·1 c.c. of the tumour-diluent suspension was injected subcutaneously in the intra-scapular area, using a No. 18 needle. Groups receiving the tumour included: thymectomized Wistar/Fu; intact Wistar/Fu; thymectomized Fisher/Fu; and intact Fisher/Fu. Each group was further divided into males and females. In addition, non-tumour-injected controls were observed for each of these groups.

Unlike previous experience with the *MtT/F4*, most of the animals in which the tumour grew had some, and usually extensive, growth of tumour in the thorax; this included both the Wistar/Fu and Fisher/Fu rats. This unusual pattern of growth is probably due to a technical error whereby the tumour was inadvertently introduced directly into the thorax; a subsequent investigation in which the tumour was transplanted as a single small fragment by trocar into the subcutaneous tissues, has resulted in local subcutaneous growth only. For this reason it was not possible to make observations on the latent period or rate of growth in relation to thymectomy.

The tumour killed, or severely debilitated, most of the hosts by extensive intra-thoracic growth, seven to eight weeks after transfer. At this time, all the debilitated animals were killed and it was found that the tumour had taken in 2/5 female and 2/5 male thymectomized Wistar/Fu rats. Ten weeks after transfer of the tumour, five male and five female Wistar/Fu, intact littermates of the thymectomized rats, were killed and none was found to have growth of the tumour; seventeen weeks after the transfer, an additional four male and four female Wistar/Fu, intact littermates, were alive without detectable growth of tumour.

As expected, all five of the intact, and all four of the thymectomized, Fisher/Fu rats had successful growth of the tumour when they died or were killed, seven to eight weeks after the transfer. In all the rats in which the tumour grew, both Wistar/Fu and Fisher/Fu, the adrenals were approximately ten times normal size and the mammary glands were greatly enlarged.

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Effect of *p*H on Human Complement Activity

WHILE investigating the effect of acetylsalicylic acid (aspirin) on complement (*C′*) activity, it was observed that incorporation of 40 mg per cent of the drug in a well-buffered reagent diluent, 0·15 M triethanolamine buffered salt (TBS) solution[1], significantly increased the haemolytic activity of human complement (Hu*C′*)[2]. Since it was also noted that the drug slightly reduced the *p*H of the reaction mixture, the studies were extended to investigate the effect of *p*H change on Hu*C′* activity.

Pooled human serum freshly collected and stored not longer than one month in a mechanically operated freezer (−60° C) provided the source of Hu*C′*. Assays of *C′* were carried out using a precise spectrophotometric method as previously described[3]. Haemolytic activity was expressed as 50 per cent haemolytic units per ml. ($C'H_{50}$/ml.) of serum. TBS, *p*H 7·2, containing magnesium and calcium ions in concentrations optimal for immune haemolysis, was used as the standard reagent diluent. For experiments in which it was desired to alter the *p*H of the system, required volumes of 0·1 N hydrochloric acid or 0·1 N sodium hydroxide were added to a stock concentration of TBS and then diluted so that a constant ionic strength was maintained. The influence of *p*H on the haemolytic activity of Hu*C′* was investigated over the *p*H range 5·55–8·20.

Table 1. EFFECT OF *p*H ON THE HAEMOLYTIC ACTIVITY OF Hu*C′*

*p*H	5·55	6·14	6·53	6·77	7·20	7·55	7·80	8·20
Hu*C′* titres* ($C'H_{50}$/ml.)	<30	88	226	279	239	197	186	166

* Arithmetic means of three independent determinations.

The results of this investigation are summarized in Table 1. Maximum haemolytic activity of Hu*C′* was observed at *p*H 6·77 and not at the *p*H level (7·2) usually used. Adjustment of *p*H to either the acid or alkaline side of *p*H 6·77 resulted in reduced Hu*C′* titres. These observations paralleled the findings of Ibe and Wardlaw[4] in which it was demonstrated that in the case of guinea-pig *C′* (Gp*C′*), maximum haemolytic activity was manifested at *p*H 6·8. Both studies corroborate and extend the observations of Kabat and Mayer[5], who noted that the haemolytic activity of Gp*C′* progressively increased as the *p*H of the system was reduced from 8·52 to 7·15. However, the latter investigators did not present data concerning the effects of lower *p*H values on immune haemolysis. In view of the present findings, consideration should be given to the potential value of conducting tests involving guinea-pig and human *C′* at *p*H 6·8 rather than using the more generally used *p*H 7·2.

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Adenovirus 12 Virion-free 'T' Antigen from Infected Cells inhibited with Cytosine Arabinoside

RAPP *et al.*[1] described a method for producing *SV* 40 'T' antigen free of virion by using cytosine arabinoside (CA) to block the growth of virus in tissue cultures of cercopithecus kidney cells. We found that cytosine arabinoside could also be used to produce virus-free adenovirus 12 'T' antigen in human cell tissue cultures as follows: It was found that the *p*H of the media and the condition of the human cell lines (*HEK*, HeLa or *KB* monolayers) used to produce adenovirus 12 'T' antigens could be best controlled in test-tubes and that when this was done more uniform production of 'T' antigen was obtained. About three hundred test-tubes were used for each experiment. Evenly growing cell monolayers were drained of fluid and washed with Eagle's basal medium containing 4 mM glutamine plus antibiotics. Each culture was then inoculated with 0·2 c.c. virus mixture (virus diluted with Eagle's basal medium containing 4 mM glutamine plus antibiotics) at multiplicities between 1 and

Table 1. HARVEST OF CRUDE ADENOVIRUS-12-INDUCED NON-VIRION 'T' ANTIGENS FROM INFECTED CELLS INHIBITED BY CYTOSINE ARABINOSIDE

HEK cells, 72-h harvests:	Complement-fixed antigen titre: Human*	Broad-reacting hamster‡	Infectivity titre $TCID_{50}$
Cytosine arabinoside inhibited 40× concentrate	0†	32	$<10^{-0.5}$§
Control 40× concentrate	64	32	10^{-2}§

* Human adenovirus antisera, Laboratory for Infectious Diseases (LID) pool 26186, diluted 1–32 (for detecting virion antigen).

† Reciprocal of complement-fixed antigen titre (0 equals negative at a 1 : 2 dilution).

‡ Adenovirus 12 hamster tumour antisera, LID pool 13, diluted 1–20 (for detecting non-virion 'T' antigens (ref. 5)).

§ Twenty-one-day titration in *HEK*, five test-tubes per dilution, tenfold dilutions; undiluted through 10^{-5} with secondary passage of negative materials, undiluted observed for 21 days.

2 $TCID_{50}$ per cell. The cultures were incubated for 1 h at 34° C and then each was fed 1 c.c. of a mixture of Eagle's basal medium + 2 per cent A-γ calf serum + 2 mM glutamine + antibiotics + 10γ cytosine arabinoside. 72 h after infection, the media were drained and the cultures were washed with large quantities of Eagle's basal medium and overlaid with 0·2 c.c. Eagle's basal medium + antibiotics + 4 mM glutamine. The cells were frozen in the test-tubes on a slant, thawed quickly, pooled, homogenized and clarified at 2,000 r.p.m. for 10 min. The resulting material was concentrated forty to fifty times by Kohn's method[2], and three hundred test-tubes yielded 6 c.c. of crude 'T' antigen. A large number of replicate experiments using this method yielded results comparable with those presented in Table 1. Adenovirus 'T' antigen produced in *HEK* monolayers in the presence of cytosine arabinoside had an average complement-fixed antigen titre of 1 : 32, whereas, when produced in HeLa and *KB* cells, the average titre achieved was 1 : 8. In other experiments using cytosine arabinoside, a multiplicity of 10 $TCID_{50}$ adenovirus 12 per cell yielded about the same 'T' antigen titre, but virion antigen was not inhibited and 30-day infectivity titrations in *HEK* cells showed the presence of at least one log of virus.

et al.[1] and Melnick *et al.*[3], which showed that the progression to mature *SV* 40 infectious virion was delayed by IUDR. Rowe *et al.*[4] in their investigations of *E*46+ hybrid virus found that pretreatment of *HEK* cells with a $10^{-4.1}$ M concentration of 5-fluorouracil deoxyriboside (FUDR) did not affect the induction of adenovirus or *SV* 40 'T' antigens but did prevent formation of adenovirus 7 viral antigen. Gilead and Ginsberg[5] described the inhibition of adenovirus type 12 viral proteins and the production of 'T' antigen in *KB* cells treated with 10^{-6} M FUDR.

Because it was likely that the crude preparations of cytosine arabinoside-inhibited adenovirus 12 would contain at least a few infectious particles which could not be demonstrated by routine titration on *HEK* cultures, a further separation of the 'T' complex was carried out using 'Sephadex *G*-200' columns in a Beckman 'Spectrochrom Analyzer'. The 'T' preparations were run, under a constant *p*H of 7·6 and constant conductivity, over 'Sephadex' columns with phosphate-buffered saline. One hundred drops were collected in each of seventy-five test-tubes and the drops were monitored at 220, 260 and 280 mμ simultaneously. Eluents were concentrated and re-tested for infectivity. Eluents from two connected peaks (absorbance at 220 and 280 mμ) were negative for infectivity and again showed no complement-fixing reaction with human adenovirus antisera. These two components were labelled, for convenience, part No. 1 and part No. 2 of the adenovirus 'T' complex and were tested with broad- and narrow-reacting hamster antisera (Table 3). It can be seen in Table 3 that part No. 1 of the 'T' antigen complex[6,7] reacts primarily with broad-reacting antisera and that part No. 2 reacts with both narrow- and broad-reacting antisera. The sensitivity to heat of part No. 1 of the adenovirus 12 'T' complex is illustrated. Investigations of the molecular weights and chemical composition of these components are in progress.

In conclusion, cytosine arabinoside blocked adenovirus 12 virion antigen and permitted 'T' antigen to be produced. Previously described heat-labile and heat-stable

Table 2. COMPARISON OF THE INHIBITORY ACTIVITY OF THREE DNA ANTAGONISTS ON THE DEVELOPMENT OF ADENOVIRUS TYPE 12 VIRION AND NON-VIRION 'T' COMPLEMENT-FIXED ANTIGENS

Complement-fixed antisera:	Adeno 12 10^{0}			adeno 12 10^{-1}			10γ CA + adeno 12 10^{0}			10γ CA + adeno 12 10^{-1}			50γ FU + adeno 12 10^{-1}			100γ FU + adeno 12 10^{-1}†			10γ IUDR-5′P adeno 12 10^{-1}			30γ IUDR-5′P adeno 12 10^{-1}		
(h)	*H*	*A*	*B*	*H*	*A*	*B*	*H*	*A*	*B*	*H*	*A*	*B*	*H*	*A*	*B*	*H*	*A*	*B*	*H*	*A*	*B*	*H*	*A*	*B*
24	0	2*	2	0	0	0	0	2	2	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0
48	>32	4	16	>32	4	16	0	8	8	0	4	8	4	2	8	2	2	4	2	2	8	2	0	2
72	>32	2	16	>32	4	16	0	4	8	0	8	16	16	2	16	4	2	8	>32	2	16	>32	2	4
72 spnt.	4	2	2	4	2	2	4	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

H, Human adenovirus antisera (for detecting virion antigen), LID pool 26184, diluted 1–16; *A*, hamster tumour antisera, pool *A* (for non-virion 'T'), LID pool 12, diluted 1–20; *B*, hamster tumour antisera, pool *B* (for non-virion 'T'), LID pool 11, diluted 1–20.

* Reciprocal of complement-fixed antigen titre (0 equals negative at a 1 : 2 dilution).

† With 250γ FU, all tubes negative for complement fixation.

A preliminary pilot study comparing three different DNA inhibitors was performed (Table 2). The antigen titres are not very high as only five *HEK* monolayer test-tubes were used for each time period and the final concentration of cells to fluid was five times more dilute than in the experiments already described. The twenty-seven sets of five test-tubes were each removed from the incubator after the times shown in Table 2, the supernate removed for separate testing, the cells thoroughly washed, drained, suspended in 1 c.c. of buffer, frozen and thawed three times, pooled and concentrated[2] for complement-fixation testing from 5 c.c. to 0·5 c.c. As can be seen in Table 2, cytosine arabinoside inhibited adenovirus type 12 virion antigen but not 'T' antigen. As fluorouracil (FU) was increased from 50γ to 100γ to 250γ, the complement-fixing titre of the virion antigen and the 'T' antigen were both reduced in a proportionate manner. In the 48-h period, the 10γ and 30γ iododeoxyuridine 5′-phosphate (IUDR-5′P) slowed the formation of the adenovirus antigens; this was analogous with the observations of Rapp

components of the adenovirus 12 'T' complex were shown to be present.

Table 3. REACTIONS TO BROAD- AND NARROW-REACTING ADENOVIRUS 12 HAMSTER TUMOUR ANTISERA BY ADENOVIRUS 12 'T' ANTIGEN COLLECTED AT 72 H FROM CYTOSINE ARABINOSIDE-INHIBITED INFECTION OF HELA CELL CULTURES AND SEPARATED ON 'SEPHADEX *G*-200'

HeLa cells, 72 h separations, 50× concentrate:	Complement-fixing components of adenovirus 12: 'T' antigen complex Broad*	Narrow‡
Isolated 'T' antigen		
Part No. 1	8†	0
Part No. 2	8	8
(Before isolation)	16	8
'T' antigen heated 56° C/30′		
Part No. 1	2	0
Part No. 2	8	8
(Before isolation)	4	4

* Adenovirus 12 hamster tumour antisera, LID pool 13, diluted 1–20 (for detecting non-virion 'T' antigens)—broad-reacting.

† Reciprocal of complement-fixed antigen titre (0 equals negative at 1 : 2 dilution).

‡ Adenovirus 12 hamster tumour antisera, LID pool 15, diluted 1–20 (for detecting non-virion 'T' antigens)—narrow-reacting.

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Significance of a Single-hit Event in the Initiation of Antibody Response

THE origin of the physico-chemical and immunochemical heterogeneity of immunoglobulin molecules has been a perplexing problem for many years. Recently, evidence has been accumulating which points to variability in the expression of antigen-stimulable progenitor cells as the cause of immunoglobulin heterogeneity. Whether or not this is the case—or whether, for example, the variability may result from non-uniformity of polypeptide chain construction by polysomes—can only be decided when methods are available for analysis of immunoglobulins at various times during response by the progeny of a single precursor cell. Before such clonal investigations can be attempted, it is obviously necessary to have some clear notion about the number and frequency of these precursor cells. This communication presents the results of experiments designed to enumerate such cells in spleen cell suspensions from non-primed and primed mice of two different hybrid strains of various age groups.

For our purposes it was desirable to investigate the production of antibodies in a system involving the quantitative transfer of mouse spleen cells. The system consisted of transfer and culture of inocula of dispersed spleen cells in genetically compatible (syngeneic) recipient mice; the latter had been rendered immunologically incompetent beforehand by X-irradiation[1]. In this manner, stimulation of spleen cell inocula with optimum doses of antigen resulted in antibody formation.

The amount of antibody formed in such a system is a linear function of the number of cells transferred[2]. This observation strongly suggests that there are independent immunocompetent 'units' which control antibody formation (a unit could be an 'antibody virus', or a multicellular aggregate involving one competent cell and phagocytes or nurse cells, but is presumably a single competent cell). The probabilities are such that only one unit would be necessary for the initiation of antibody synthesis. Co-operative events among immunocompetent units would lead to non-linear dependence on cell number of antibody formation. The statement that there are independent units that are essential for the initiation of antibody synthesis merely focuses attention on the limiting step, but does not deny the existence of other steps in the process.

If there are independent units that control the production of antibody, it should be possible to reduce the inoculum size so that there is a finite probability of the inoculum containing only one or none of the controlling units. Statistical methods based on the Poisson distribution would be applicable to such an experiment, and:

$$\ln p_0 = -n \qquad (1)$$

where p_0 is the probability of a null response and n is the number of immunological units. The probability of a null response would be 0·37, 0·14 and 0·09 for 1, 2 and 3 units respectively.

In any such experiment a crucial point is the sensitivity of the assay system and the question of the possibility of detecting the output of one unit. In Fig. 1 are shown the results of two experiments in which teased spleen cells were mixed with an optimum proportion of rat red blood cells and a small inoculum was injected into a series of mice. The observed frequencies of the different haemagglutinating antibody titres of sera obtained 7 days later are plotted. The distribution is bimodal and can be separated into a group of non-responders with background titres and a group of responders with titres significantly above background. Although the observed titres depend on the particular technique used (micro-titration versus standard tube titration), the bimodal distribution is a characteristic feature of the results obtained with small inocula.

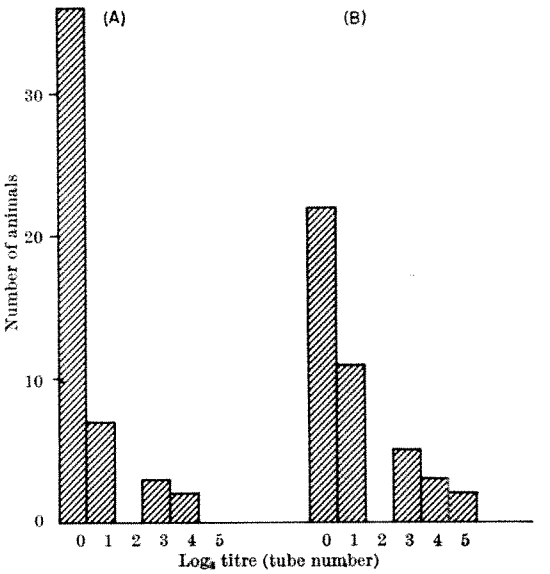


Fig. 1. Number of animals from which sera were obtained 7 days after antigenic stimulation gave haemagglutination titres of 1, 2, 4, etc. *A*, Secondary response by *C*3*H*.*F*1 spleen cells; *B*, primary response by *C*3*H*.*F*1 spleen cells

In a series of experiments using rat red blood cells as the antigen, the frequency of non-responders as a function of inoculum size was determined for several groups of animals. Spleen cells were obtained from non-primed and primed donors. In the latter case the mice were injected with 2×10^6 red blood cells 1–6 months before their spleen cells were collected. The number of units in the spleen cell inoculum has been calculated using the statistical method of Finney[3], and the results are presented in Table 1. The frequency of non-responders as a function of the number of immunological units is plotted in Fig. 2. For graphical simplicity, almost identical points from the experiments have been omitted. The straight line is the theoretical line calculated from equation 1. A comparison of frequencies predicted by equation 1 with observed frequencies gave a χ^2 (ref. 4) of 23·0 with 35 degrees of freedom. The probability of observing this good a fit or better by chance would be 0·05 and it can be concluded that equation (1) adequately represents the data. Because there is no suggestion of a shoulder in the experimental data, it is reasonable to conclude that there are independent units controlling the synthesis of haemagglutinating antibody, regardless of whether the response is primary or secondary. Although it is improbable, one must admit that the statistical evidence could be fortuitous and that the process may be more complicated.

Table 1. STATISTICAL ANALYSIS OF IMMUNOLOGICAL UNITS IN SPLEEN CELL SUSPENSIONS

Exp. No.	No. of mice	Source of cells	Units/10^7 cells	95 per cent confidence limits
1	234	6-month-old non-primed *BC3F1* mice*	2·0	1·6–2·4
2	169	2-month-old non-primed *BC3F1* mice	0·80	0·58–1·10
3	203	6-month-old *BC3F1* mice primed at 1 month of age	4·7	3·6–6·1
4	271	2-month-old *BC3F1* mice primed at 1 month of age	1·8	1·4–2·5
5	100	3-month-old non-primed *C31F1* mice†	0·25	0·16–0·39
6	138	3-month-old *C31F1* mice primed at 2 months of age	4·1	2·9–5·9

* (*C57BL/6 Cum* ♀ × *C3H/Anf Cum* ♂)F_1 = *BC3F1*.
† (*C3H/Anf Cum* ♀ × 101/*Cum* ♂)F_1 = *C31F1*.

Previous data have shown that antibody production is correlated with cellular replication[5–8] and that the mean generation time is 8–9 h[9]. Furthermore, there are about ten generations involved, or a thousand-fold increase in the number of functional cells[10]. Although it remains to be proved, it is very reasonable to assume that the independent immunocompetent unit is the progenitor cell for this replication cycle. It follows then that all the functional cells in this system initiated by a single progenitor cell constitute a functional clone, and according to the Poisson distribution, in the case where only twenty out of two hundred mice respond (10 per cent responders), nineteen of these twenty responders are due to functional cells derived from a single antigen-stimulated progenitor cell.

Recent investigations[11–14] have clearly demonstrated that most functional cells are committed to the exclusive production of one of a number of genetically possible globulin antibodies. However, it is not known when in the differentiation process of immunologically competent cells the decision is made; that is to say, does the decision precede or follow antigenic stimulation? This problem of decision-making at the genetic level, which is the essence of cytodifferentiation, can be directly resolved by assessing the capabilities of functional cells derived from one antigen-stimulated progenitor cell.

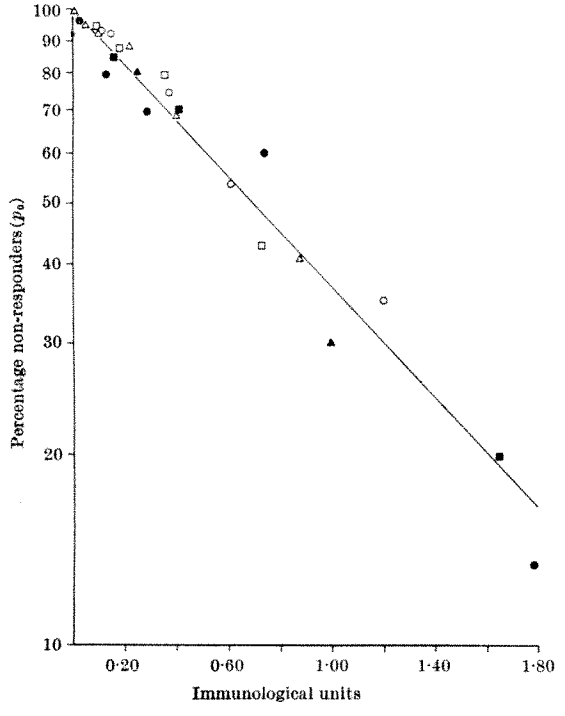


Fig. 2. Frequency of non-responders as a function of immunological units. See Table 1 for experiments: ○, experiment 1; △, experiment 2; □, experiment 3; ●, experiment 4; ▲, experiment 5; ■, experiment 6

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Demonstration of Circulating Antibodies to *Plasmodium falciparum* by Gel-diffusion Techniques

As age advances, Africans resident in hyperendemic regions of The Gambia, West Africa, acquire a marked ability to limit malarial parasitaemia[1]. IgG (7 *S* gamma-globulin) prepared from the sera of adult Gambians exerts a profound anti-plasmodial effect about the time of schizogony when administered to young children experiencing heavy *Plasmodium falciparum* infection[2]. Recently, using antigen preparations of locally acquired *P. falciparum*, we have been able to demonstrate, by gel-diffusion techniques, the existence of precipitating antibody in the sera of Gambians believed to be immune to malaria.

Two antigen samples (*T*1 and *T*2) were prepared by Zuckerman's modification[3] of the technique of Spira and Zuckerman[4] from trophozoites recovered from the blood of heavily infected children. This modification included the preliminary removal of leucocytes by sedimentation of the blood in a solution of dextran. Two further samples, *P*1 and *P*5, have been prepared from asexual parasites of more advanced development (pre-schizonts and schizonts) recovered from densely infected placental blood. The *P*1 antigen was prepared by Zuckerman's modification, as already described. The *P*5 antigen was prepared as follows without attempting to separate the parasitic material from the cellular constituents of placental blood. After mincing, the mixture of blood and placental tissue was suspended in M/15 phosphate buffered saline, *p*H 7·2, and filtered. The filtrate was centrifuged for 15 min at 900*g* and the supernatant discarded. The cellular deposit was washed once with M/15 phosphate buffered saline and recentrifuged at 1,400*g* and the supernatant again discarded. The parasite-rich upper layer of the deposit, visible as a dark band, was removed and resuspended in its own volume of M/150 phosphate buffered saline, *p*H 7·2. This final suspension (containing $1{\cdot}045 \times 10^6$ large parasites/mm^3) was lyophilized and stored *in vacuo* at −20° C. Before it was used, the lyophilized material was reconstituted to half its original volume with distilled water and disintegrated in a Hughes press[5]. The disintegrated material was later centrifuged

at 28,000*g* for 20 min and the resulting supernatant taken as antigen.

A range of sera obtained from Gambian Africans has been tested against the various antigen preparations. One serum (J. S., fluorescent antibody titre (FAT)[6], 1 : 6,400) was concentrated threefold by the globulin precipitation technique developed by Thurston *et al.*[7], and to the same degree following lyophilization and reconstitution with distilled water. This serum and samples from six adults, selected to represent the blood groups A, B and O (T. S., FAT, 1 : 800; A. M., FAT, 1 : 3,200; A. S., FAT, 1 : 400; J. D., FAT, 1 : 3,200; M. S., FAT, 1 : 800; J. B., FAT, 1 : 3,200) from the donor of antigen *P*5 (V. B., FAT, 1 : 800) and from her infant (F. G., FAT, 1 : 25), have been studied (unconcentrated). Sera from non-immune expatriates (I. M., T. P., D. H., P. H. and L. J.), representing the blood groups A, B and O, and all possessing negative fluorescent antibody titres, acted as controls. The Ouchterlony double-diffusion technique[8], using 1·5 per cent special Difco 'Noble-Agar' in 0·3 M phosphate buffer (*p*H 8·0), was used to observe reactions between the various sera and antigen preparations.

Concentrated J. S. serum was tested against all the antigen preparations and against disintegrated erythrocyte stromata prepared from the blood of a non-malarious African (Fig. 1). It yielded three distinct precipitin lines with antigen *P*5, no definite lines with antigen *P*1 (although two of the lines evident against antigen *P*5 were apparently deviated towards the *P*1 region), a faint line with antigen *T*2, but no definite line with antigen *T*1.

Unconcentrated samples of the other immune sera yielded single precipitin lines when tested against schizont antigen *P*5. At a dilution of 1 : 10 only sera J. D. and T. S. were reactive. The following samples of non-immune sera were not reactive: globulin concentrate of I. M., unconcentrated L. J. and D. H., and a 1 : 10 dilution of L. J.

To obviate the possibility that the precipitin lines observed were due to blood group agglutinins of the A, B or O type, three non-immune and three immune sera from donors representing groups A, B and O were studied simultaneously in the same system against schizont antigen *P*5 (Fig. 2). A single line of precipitation was produced against two of the immune sera and two lines against the remaining one. All three non-immune sera failed to react.

As it was known that schizont antigen *P*5 contained disintegrated fragments of blood cells, certain absorption experiments were made. A sample of serum T. S. was absorbed with a third of its volume of erythrocytes obtained from the donor of the relevant placenta. A second sample was absorbed with leucocytes and thrombocytes from the same source. When these absorbed preparations were tested against schizont-antigen *P*5 (Fig. 3), a precipitin line was still produced in each case and fused in a reaction of complete identity with the line produced by the unabsorbed serum. Serum from the donor of the placenta (homologous serum) produced two lines, one of which fused in a reaction of complete identity with the single line that developed against the serum of her infant. The sera of mother and child were taken 6 weeks after delivery.

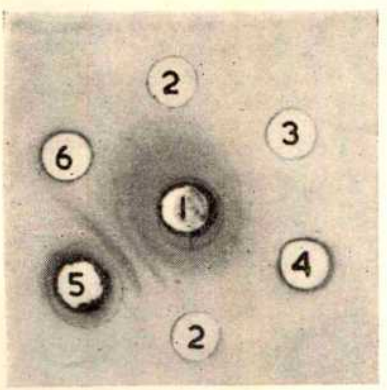


Fig. 1. Gel-diffusion analysis of J. S. serum concentrated by lyophilization and reconstitution (1), with trophozoite antigens *T*1 (3) and *T*2 (4), with schizont antigens *P*1 (6) and *P*5 (5) and with erythrocytic stromata obtained from non-parasitized blood (2). Reaction occurred only against *P*5 (three lines) and *T*2 (one line)

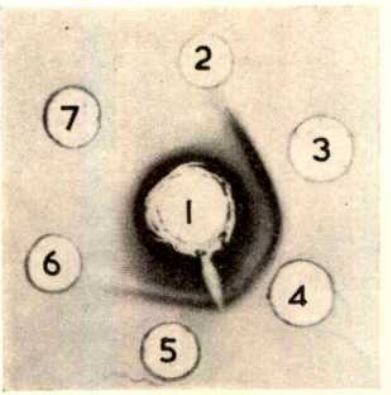


Fig. 2. Gel-diffusion analysis of schizont antigen *P*5 (1) showing the line of precipitate with each of the immune sera T. S. (3), J. S. (4), J. B. (5), representing blood groups A, B and O respectively. Three non-immune sera T. P. (2), P. H. (7) and D. H. (6), representing blood groups B, A and O, respectively, failed to react. (Schizont antigen was obtained from donor of blood group B)

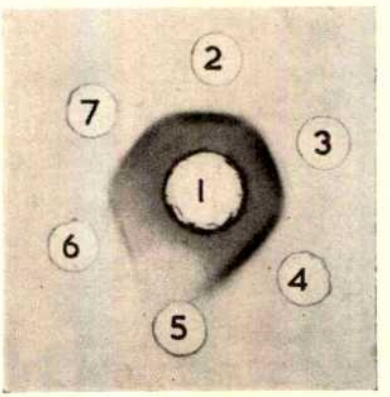


Fig. 3. Gel-diffusion analysis of placental schizont antigen *P*5 (1) showing precipitin lines with unabsorbed T. S. serum (2), the same serum absorbed with erythrocytes of the placental donor (3) and with erythrocytes and leucocytes from the same source (4), the homologous serum (from placental donor) (7), and the serum of her infant (6). No line developed against non-immune serum D. H. (5)

It would seem that the observations described here can be ascribed to the specific interaction of malarial antigen and malarial antibody, and preliminary observations have shown that the reactive material in immune serum migrates in the γ-globulin region. There was no evidence that the naturally occurring isoagglutinins of the serum donors influenced the results. It may be that some unidentified antigen, possibly a virus, reacted with its specific antibody, but this is unlikely as our reactant antigens did not derive from a single source and because the non-immune expatriates studied had encountered, in periods of Gambian residence ranging up to 17 years, many local infections but never malaria.

Several findings seem worthy of comment. First, the positive results obtained with adult sera and with serum from a 6-week-old infant, in whom no evidence of malarial infection could be found, suggested that both actively and passively acquired malarial antibodies can be demonstrated by gel-diffusion techniques. Secondly, the fact that our most reactive antigen derived from plasmodia at a late stage of their asexual development may lend support to the view that the antigenicity of the asexual

parasite may change quantitatively and/or qualitatively as it matures[9–11]. Finally, the apparent lack of correlation between the fluorescent antibody titres of the immune sera tested and their ability to produce lines of precipitation indicates that gel-diffusion and fluorescent techniques may not measure the same antibody.

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HISTOLOGY

A New Method for the Decalcification of Adult Human Enamel

Certain stages of an investigation into the histopathology of adult human fluorosed enamel, which is at present being carried out in this department, have called for the decalcification of thin ground specimens used for light microscopy and microradiography. As none of the previously designed methods for the decalcification of enamel (including the most recently devised one[1]) has proved satisfactory for this purpose, a search was made for a new method.

In the investigations undertaken to obtain a satisfactory routine method for the decalcification of thin ground specimens of adult human enamel, the aim has been primarily to retain as completely as possible the organic matrix. A simultaneous tanning and decalcification of the specimens were thought to offer many advantages; results obtained with a simple watery solution of chromium sulphate have proved particularly promising. The enamel matrix recovered with chromium sulphate decalcification at *p*H 3·5 is well preserved throughout its thickness and shows no separation from underlying dentine. It further shows prismatic markings and cross-striations (Fig. 1) corresponding to the appearances of the non-decalcified section. In polarized light, the matrix is positively birefringent when immersed in water. In inhibition fluids with refractive index 1·48 and 1·62 the same matrix shows a weaker positive birefringence, whereas at refractive index 1·55 the birefringence is very weak. The thickness of the ground specimens used so far has been about 40 microns.

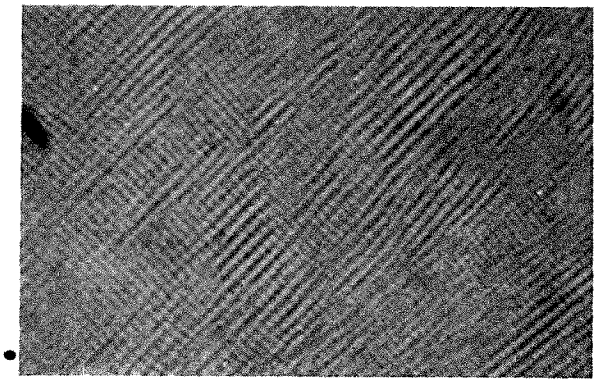


Fig. 1

Further investigation is needed to establish any more general validity of the decalcification procedure. Thus it is desirable to know to what extent the preserved matrix is chemically related to the total organic content of enamel and also to what extent its structure may have been altered as a result of the procedure.

An interesting observation, which might be of special importance, is that decalcification in a chromium sulphate solution of *p*H about 2·8 initially is slower than when a comparable section from the same tooth is decalcified in, for example, the solution suggested by Brain (*p*H about 3·5). Tanning of the matrix seems to have protected the crystals from solution by the acid. The role of the enamel matrix in carious destruction has recently received fresh attention[2], and any possible use of tanning agents as a means of preventing caries should be investigated.

A detailed account of the chromium sulphate procedure is being prepared. Further investigations of the properties of the matrix recovered with chromium sulphate decalcification are also being undertaken.

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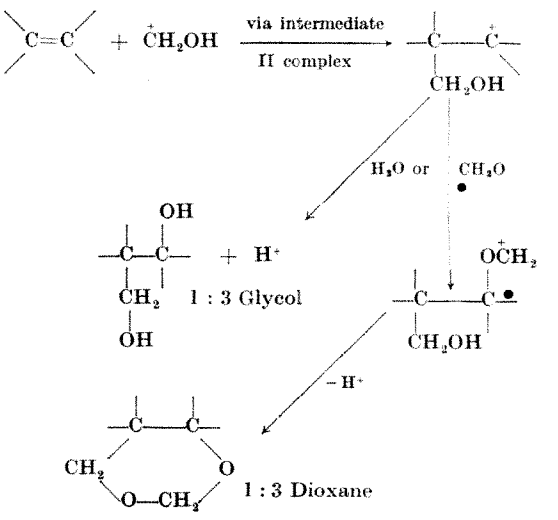
Reaction of Formaldehyde with Unsaturated Fatty Acids during Histological Fixation

Formaldehyde reacts with ethylenic double bonds in the presence of an acid catalyst to give 1 : 3 glycols and 1 : 3 dioxanes (the Prins reaction)[1]. This reaction is well documented[2,3] and the following sequence is generally accepted:

(*a*) Formation of electrophilic species:

$$CH_2{=}O \; + H^+ \rightarrow \; CH_2{=}O^+H \leftrightarrow \dot{C}H_2OH$$

(*b*) Reaction with double bond:



Though the Prins reaction is widely used in synthetic organic chemistry, little attention has been directed to the reaction of formaldehyde with lipids in biological materials. Wolman[4] suggested that formaldehyde may react at the sites of double bonds in fatty acids; he offered little evidence of this, and his suggestion that a side-chain carbonyl group is formed is obviously not in keeping with the accepted modes of reaction of formaldehyde. It is probable that the formaldehyde did react with the unsaturated acids during Wolman's experiments, but following the scheme outlined above.

As most material required for lipid histochemistry is 'fixed' in formaldehyde-containing solutions, it was considered desirable to investigate further the reaction of formaldehyde with lipids, particularly unsaturated fatty acids, both in the pure state and in tissues.

To characterize the products likely to be observed during fixation, unsaturated acids of more than 99 per cent purity were heated at 100° C with a solution of the following composition for up to 8 h:

40 per cent formaldehyde solution	85 parts v/v
Glacial acetic acid	10 parts v/v
Concentrated hydrochloric acid (35 per cent hydrogen chloride in water)	5 parts v/v

The *p*H of solution was 0·5.

New products were detected by gas chromatography of the methyl esters on polyethylene glycol adipate (PEGA) as stationary phase. The apparatus used was a Pye argon chromatograph.

With oleic acid, five new products totalling 34 per cent of the mixture were detected. Their retention times were considerably longer than that of methyl oleate. The retention time of one product was increased on hydrogenation. Table 1 gives representative results. More complex mixtures were obtained from linoleic and linolenic acids.

Table 1. EFFECT OF FORMALDEHYDE ON PURE OLEIC ACID*

Description	Carbon Nos. on PEGA 16·0	18·0	18·3 (oleic acid)	23·35	23·85	24·15	24·5	25·9
Oleic acid, heated 6 h reagent *H*†	0·2		79·0	2·5	1·0	3·9		10·9
Above sample after hydrogenation	0·1	78·9		2·5	0·8		3·3	12·6
Oleic acid 'fixed' for 15 days in 10 per cent formal saline			93·3				1·3	5·4

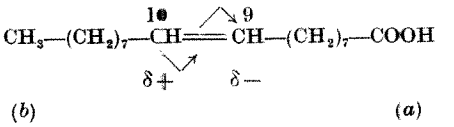
* Expressed as percentage of total.
† *H*, 40 per cent formaldehyde solution, 85 parts v/v; glacial acetic acid, 10 parts v/v; concentrated hydrochloric acid, 5 parts v/v (*p*H 0·5).

At room temperature, reaction was much slower. Acids were stirred with a 4 per cent solution of formaldehyde in 0·9 per cent sodium chloride (routine histological fixative, *p*H 4·05). Only two new products were obtained from oleic acid (see Table 1). The significance of these results is discussed later on.

In order to appraise the true significance of these reactions, the fatty acid patterns of the fractions[5] obtained from tissue lipids before and after fixation have been determined. Table 2 gives figures for the lipids of a sebaceous tumour, both before fixation[6] and after fixation for 3 years.

It was noted that with oleic acid 'fixed' in formal saline at room temperature a compound of carbon No. 24.5 appeared in the reaction products. This does not correspond to any compound appearing in the reaction mixture when oleic acid was heated with formaldehyde, but the reagent used in this case contained acetic acid and hydrochloric acid. The hydrochloric acid, in addition to supplying protons to catalyse the reaction of formaldehyde with the double bond, also catalysed the esterification of hydroxy-groups formed in the reaction.

The number of compounds formed from the 'hot' reaction can be attributed to isomers:



(*b*) (*a*)

Arrows indicate charge distribution due to inductive effect of the carboxyl group (*a*) and methyl group (*b*). Both these effects are very slight, owing to the saturated chains of methylene groups separating the functional groups. However, at room temperature preferential attack by the $^{+}CH_2OH$ electrophile will lead to addition at position 9. At higher temperatures the slight energy barrier to addition at position 10 is overcome. This leads to two slightly different glycols and two slightly different dioxanes.

Table 2 represents the effect of fixation on a biological mixture of lipids, and serves to demonstrate only that a change does occur during fixation. Many compounds were observed with high carbon numbers, but, due to the large number of acids originally present, it is impossible to correlate these with the original reactant. Instead, decrease in concentration of unsaturated acids must be taken as proof of reaction. Table 2 only presents a few of the acids observed, but it does indicate that, during fixation in tissues, the predominant reaction was between free fatty acids and formaldehyde, since the fatty acid composition of this fraction shows the greatest change. Esterified fatty acids do not seem to react so readily. This is not due to any intrinsic difference in reactivity, but to the physical nature of these compounds. Triglycerides and cholesterol esters are 'hydrophobic', and not permeable to aqueous formaldehyde solutions, whereas fatty acids possess a slight solubility in aqueous solutions. Formaldehyde-based fixatives hydrolyse ester linkages slowly; in the presence of phospholipid, which tends to emulsify triglycerides and cholesterol esters, the rate of hydrolysis is greater[7]. Under such conditions, formaldehyde should react more rapidly with these 'hydrophobic' fractions; this point is being investigated.

It is difficult to demonstrate lipids histologically in tissues which have been stored in formaldehyde for more than 4 or 5 years[8]. This is due partly to loss of lipid into

Table 2. EFFECTS OF FIXATION ON FATTY ACIDS OF A SEBACEOUS TUMOUR*

Description	Before / After fixation	Carbon Nos. on PEGA Less than 16·0	16·0	16·3 / 16·4	16·7† / 16·8	16·9 / 17·0	17·3 / 17·4	18·0	18·3 / 18·4	18·9 / 19·1	19·2 / 19·4	More than 19·4
Acid			16:0	16:1		17:0	17:1	18:0	18:1	18:2	18:3	
Cholesterol esters	Before	16·3	11·7	14·5		4·3	7·4	3·6	14·0	0·7	20·6	6·9
	After	27·0	10·2	8·6	0·5	2·9	7·7	3·5	8·9	—	17·4	13·3
Triglycerides	Before	19·0	29·1	6·3	—	5·2	4·8	6·9	22·1	1·2	1·4	3·8
	After	23·1	34·0	4·7	—	3·6	0·7	6·4	17·8	0·1	1·2	6·4
Free fatty acids	Before	20·8	20·8	7·8	—	4·8	8·5	6·2	24·0	1·4	1·7	4·1
	After‡ 1	44·6	15·8	8·2	—	3·0	3·9	5·0	18·3	—	1·2	—
	2	62·4	9·0	5·1	—	1·4	3·2	1·4	5·5	3·4	2·4	6·1

This table represents only acids with 16, 17 or 18 carbon atoms. Acids with carbon Nos. less than 12 are not detectable by this method.
* Expressed as percentage of total.
† This fatty acid did not occur before fixation.
‡ There were two free fatty acid fractions after fixation. Fraction 1 was least polar on thin-layer chromatography.

the fixative, and partly to destruction or modification of lipid so that staining by conventional methods is impossible; if fixation is sufficiently prolonged, many of the unsaturated fatty acids may be polymerized by formation of intermolecular bonds, and converted into a macromolecular network which ceases to behave, either chemically or histologically, as lipid.

These reactions are being investigated further; it is hoped to elucidate the structure of all products, and determine any practical use which the presence of these products in the tissues can be applied to.

One of us (D. J.) acknowledges a personal grant from the British Egg Marketing Board.

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PHYSIOLOGY

Thalamic Inhibitory Interneurone

SINCE the investigations of Renshaw[1] and Eccles *et al.*[2] on the central inhibitory action of antidromic impulses in motoneuronal axons, indirect evidence has accumulated which suggests that a similar mechanism occurs at higher levels of the neuraxis[3]. Physiological identification of neural units responsible for such effects has proved to be technically difficult. Andersen *et al.*[4] recently published records of presumed inhibitory interneurones from the ventrobasal complex of the thalamus.

This communication gives a brief account of neurone recordings during the course of a larger series of investigations on striothalamic pathways in the cat. Further investigation will be described in a later article. The specimen records shown were obtained with a 1-μ tip glass micropipette, filled with 2 M potassium citrate. The micro-electrode lead was fed through cathode followers to d.c.—and a.c.—coupled amplifiers. Photographs were taken of the amplified signals as they were displayed on a dual-beam oscilloscope.

In Fig. 1*A* two free sweeps are shown with two "spontaneously" discharging neurones labelled (*r*) and (*i*). Cell (*r*), of considerably larger amplitude, fired at an average of 50 impulses per sec, while cell (*i*) at times followed, or intermingled with, bursts of spike potentials from cell (*r*). This neurone (*i*) fired at 225 impulses per sec. The responses of these two cells to electrical stimulation were studied in the succeeding frames. *B* shows that cell (*r*) could be triggered at lower gain and sweep speed by a stimulus applied to the nucleus ruber. After this initial spike potential a silent period of 60 msec ensued in association with hyperpolarization of the cell membrane. Thereafter a burst of spike potentials followed riding on the peaks of depolarization. In *C*, a stimulus was applied to the internal capsule. Neither antidromic invasion nor synaptic activation of the cell was attained. The unit responded, however, with a short-latency hyperpolarizing potential terminating in a greater burst of late discharges but similar in temporal sequence to that evoked by rubral stimulation in *B*. Frame *D*, in conjunction with *B*, would appear to characterize unit (*r*) as a thalamo-cortical relay cell. Note that in this frame (*D*) a single shock of opposite

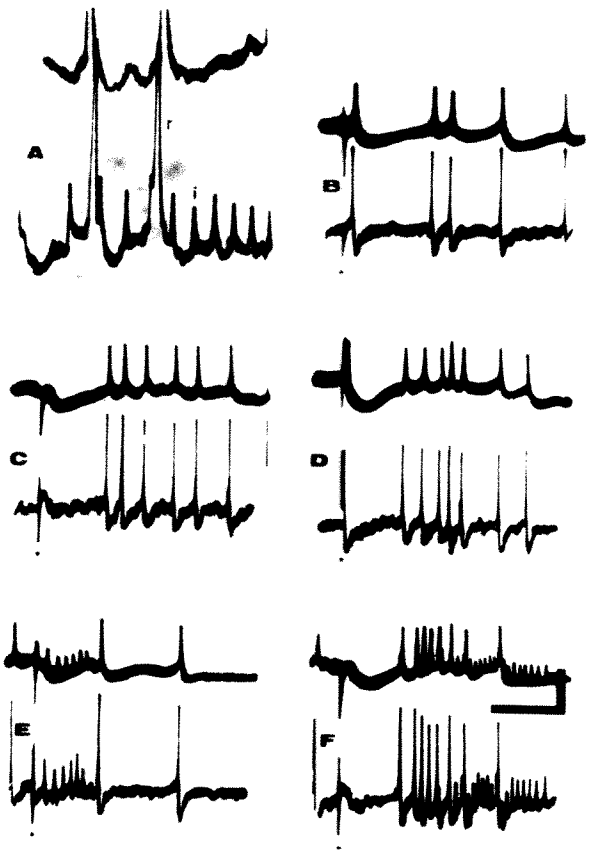


Fig. 1. Illustrating two cells in the nucleus ventralis lateralis of the thalamus firing spontaneously (*A*) and in response to orthodromic rubral (*B*) and antidromic capsular (*C*–*F*) electrical stimulation. Upper trace is d.c. and lower trace is a.c. recording for all frames except *A* in which both traces are d.c. records. Positive is represented as an upward deflexion. Stimulus (*B*–*F*) is marked by a dot. Frames are continuous. Time scale gives 15 msec for *A* and 60 msec for all other records. Potential scale gives 10 mV for all d.c. traces from *B* to *F*. Further description in text

polarity to the internal capsule succeeds in evoking an impulse discharge in less than 1 msec. In *E*, the same stimulus as in *C* failed again to evoke the short latency antidromic spike potential but produced a similar hyperpolarizing potential. In association with the latter, this time the stimulus succeeded in bringing into activity cell (*i*) which is represented in *A* at greater sweep speed and amplification. The hyperpolarizing potential of cell (*r*) and the burst of discharges of cell (*i*) coincided in time and duration. Compare the discharge rate during the following 100 msec in *E* with those of *B*, *C* and *D*. Cell (*i*) already fell within the recording field of the micro-electrode tip at *D*. Observe one single impulse discharge fused with the rising phase of the sixth spike of the relay cell. In *F*, a late high-frequency burst from the relay cell appears to be successfully arrested by a long-lasting (100 msec) barrage of neurone (*i*) discharges. The beginning of activity of cell (*i*) coincides with the slowing down of the relay cell. Toward the middle of the burst of cell (*i*), coinciding with the longest relay cell interspike interval, the rate of discharge was around 300 per sec. The correlation between the frequency of cell (*i*) spike discharges and build-up of the relay cell hyperpolarizing potential can also be observed in this record.

Cell (*i*) would appear to fulfil the criteria postulated in the literature for qualification as an inhibitory interneurone. These illustrations show spike potential characteristics of a thalamic inhibitory interneurone in interaction with a relay cell and its concomitant hyperpolarizing potentials. Judging from the positive polarity of the spike responses of the two cells, it is presumed that the micro-electrode tip was on, or very near, the relay

cell membrane and the interneuronal axon, possibly at the synaptic zone.

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A Simple Catheter for Intermittent Intravenous Anaesthesia in Animal Surgery

RECENT allograft experiments on rabbits in our laboratories prompted the development of a simple and safe anaesthetic technique which could be administered by the surgeon to two animals simultaneously. Inhalation techniques require the almost constant attention of another assistant; parenteral barbiturate administration requires repeated and increasingly difficult intravenous injections for maintenance. The following intravenous catheter and continuous technique were therefore devised, based on the standard technique of pentothal induction of anaesthesia in human surgery.

Two No. 25 gauge needles (Yale hypodermic needle, sterile, disposable, No. 25*G*, 5/8, Becton-Dickinson and Co., Rutherford, New Jersey) and a 12-in. length of polyethylene catheter (Clay-Adams polyethylene tubing, No. *PE*20, New York) are used. The hub is pulled off and discarded from one needle, and the catheter is swedged on to it, allowing most of the needle to protrude. The other end of the catheter is swedged to the other whole needle, or is attached to an adapter (Clay-Adams plastic adapter, No. *A*-1025, size *A*). A 2·5-c.c. syringe containing a 5 per cent solution of thiamylal sodium (Surital sodium, Parke-Davis and Co., Detroit, Michigan) is attached.

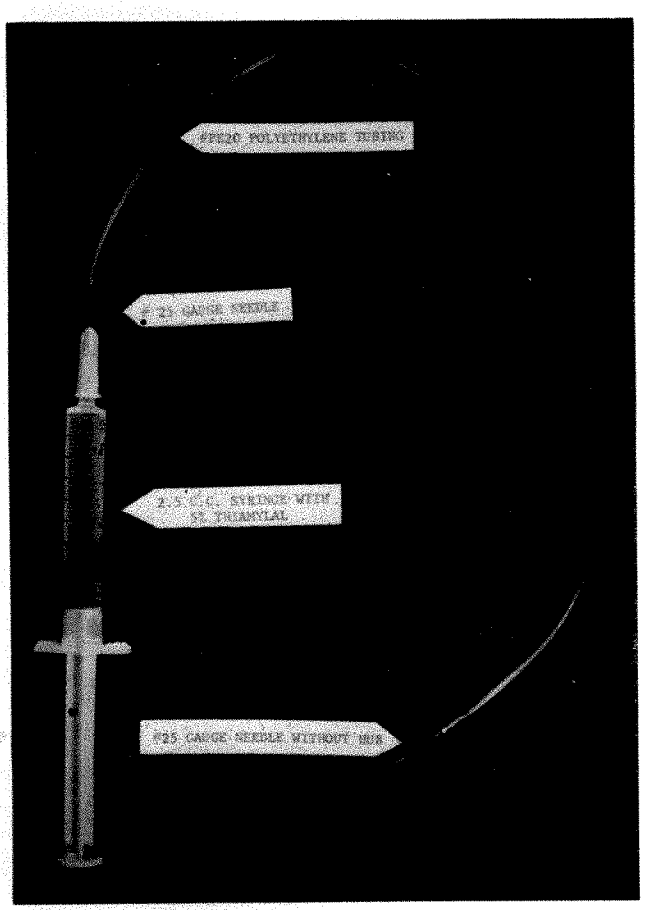


Fig. 1

The distal needle is introduced into an ear vein and taped in place while the rabbit is alert. To induce anaesthesia, 0·5–0·8 c.c. of barbiturate is injected over a period of 30 sec. For maintenance, repeated doses of 0·2 c.c. can be given, usually about every 5–10 min. The taped catheter allows the rabbit's head to move without dislodging the needle; the syringe allows the surgeon himself to maintain satisfactory anaesthetic levels. For sterile procedures, the syringe can be wrapped in a sterile towel or glove in the surgical field.

We have found this technique inexpensive and effective in more than 500 laparotomies and graft exchanges.

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Blood Volume of the Nasal Salt Glands and other Glands and Organs of Sea Gulls

THE ability of an organ to function is dependent on its blood supply. The salt gland operates as a highly efficient sodium pump capable of secreting about 8 sodium/adenonine triphosphate[1,2] and its secretion has a concentration of sodium ranging from 400 to 1,200 m.equiv. sodium chloride/l. (ref. 3). The unique function of the avian salt gland and the lack of information on its blood volume prompted this investigation.

Six sea gulls (Laridae) captured along the northern California coast were used. The gulls were maintained in a screened flight cage and given canned dog food to eat and 3 per cent sodium chloride to drink. Their wing vein was cannulated with a short piece (about 8 in.) of 'Teflon' tubing, A.W.G. No. 22, and 5 ml. of blood withdrawn into a heparinized syringe. The blood was incubated with about 100 μc. of chromium-51-labelled sodium chromate at 25° C for 1 h. The labelled erythrocytes were packed by centrifugation and the supernate discarded. The cells were washed twice in physiological saline by alternately suspending and packing by centrifugation. The erythrocytes were finally resuspended in physiological saline to about their original concentration and injected into the cannulated wing vein. After 15 min a 1-ml. sample of blood was drawn, and the gull was killed by rapidly injecting 2 ml. of 6 per cent pentobarbital sodium. The organs were dissected and care was taken to minimize any contamination of their surfaces with blood or any squeezing of blood from the organ. The haematocrit and radioactivity of triplicate 0·1-ml. portions of blood were determined using a lead-shielded well-scintillation counter, and the radioactivity per unit volume of whole blood calculated. The whole organ or two representative portions were weighed to the nearest mg, the radioactivity measured with a scintillation counter, and the blood volume calculated.

Of the twelve glands and organs examined, the brain had the lowest blood volume per unit weight, while the lung had the greatest (Table 1). Both right and left salt glands had a blood volume of about 25 μl./g, which was comparable with that of the thyroid, adenohypophysis, Harderian gland, pancreas and the pectoralis muscle. However, both the cranial and caudal lobes of the kidney had a blood volume about 4·5 times greater than that of the salt glands. One noticeable reason for this marked

difference in blood volume between these two important excretory organs of the gull was the larger venous pool of blood present in the veins of both kidney lobes. The liver with its large venous channels and sinusoids had the second largest blood volume per unit weight. Many of the blood volumes reported here for the sea gull are comparable with values reported for similar organs in various mammals and fowl[4]. Therefore, the data of the gulls appear to be valid, even though there are gross errors resulting from surface contamination, oozing of blood, and the tendency for venous pooling due to death from a barbiturate.

Table 1. BLOOD VOLUME OF THE NASAL SALT GLANDS AND OTHER GLANDS AND ORGANS FROM SEA GULLS

Organ	μl./g organ ± S.E.
Brain	9·1 ± 1·3
Nasal salt gland, right	25·6 ± 4·6
left	26·5 ± 4·9
Thyroid	28·1 ± 1·7
Adenohypophysis	29·5 ± 1·3
Harderian gland	35·9 ± 5·2
Pancreas	38·2 ± 7·1
Pectoralis muscle	38·5 ± 6·9
Adrenal	62·5 ± 13·6
Lacrimal gland	80·3 ± 15·8
Kidney, cranial lobe	113·1 ± 23·8
caudal lobe	112·0 ± 18·1
Liver	159·9 ± 32·7
Lung	573·6 ± 105·4

Although this report has presented data on the static blood volume of the nasal salt gland, there are no reports dealing with the circulatory rate of this gland. This is mainly because of the difficulty in approaching the blood vessels supplying the gland. Sufficient information, however, is available to estimate the circulatory rate of these glands.

A recent enzymatic study[5] of the salt gland has indicated that the energy yield for the gland is about 148 μmoles adenosine triphosphate/g/min, of which one-third would be required to support a secretory rate of 400 μmoles sodium/g/min. Avian blood contains about 8·3 μmoles glucose/ml., and each μmole glucose yields 38 μmoles adenosine triphosphate. Assuming that 10–25 per cent of the blood glucose is removed by the gland, a blood flow of 1·9–4·7 ml./min/g would be required to supply the total energy for the gland. This estimated flow for the nasal salt gland is comparable with the 4 ml./min/g flow in human and dog kidneys[6]. With a static blood volume of 25 μl./g the blood of the salt gland would be replaced 80–200 times a minute—essentially a total replacement with each cardiac output.

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Potential and Resistance Measurements of Rat Liver Cells *in situ*

ALTHOUGH information is available[1–4] concerning the potentials of liver cells *in situ*, no systematic investigations of the electrophysiological properties of these cells have yet been reported.

In a series of experiments on this topic, we measured the potential by means of intracellular micro-electrodes, drawn by hand, of 'Supremax' glass, and filled with 3 M potassium chloride. The rats weighed 150 g and were anaesthetized with urethane. The abdomen was then opened and the liver immobilized. A plastic ring, 8 mm in diameter, was placed on the liver surface and filled with medium through which the penetrations were made[4]. The reference electrode was situated in the abdomen and a check was made that the resistance between medium and reference electrode was negligible. The standard medium had an ionic composition (in mmoles): sodium chloride, 129; potassium chloride, 5·6; calcium chloride, 2·2; magnesium sulphate, 0·5; sodium orthophosphate, 1·1; sodium bicarbonate, 11·9; and glucose, 10.

When we undertook our experiments, it was already known that liver slices and the liver *in situ* lose about 50 per cent of their potential within 1 h of the death of the animal[1–4]. In our experiments, these results were confirmed. We found a membrane potential of 50·69 ± 1·33 mV (mean ± standard error) in eighty-three measurements on four animals. 30 min after clamping the trachea of the animal the potential had dropped to half its original value. It was now of interest to determine whether this decrease in membrane potential after the death of the animal was characteristic for the membrane of the liver cell or was a general characteristic of all rat cells. To decide this question, simultaneous measurements of the membrane potential of liver cells and of fibres of a superficial abdominal muscle were made before and after the death of the animal. Fig. 1 shows the results of these measurements. While the potential of the liver cells drops within 20 min from 56 mV to 27·5 mV, the potential of the muscle stays constant over the same period. This difference in the time course of changes in the membrane potential of liver and muscle indicates a difference in the membrane properties of both kinds of cells.

Measurements of the membrane resistance were made with double-barrelled micro-electrodes[5], using one barrel as polarizing electrode and the other as recording electrode. The common resistance of both barrels was determined in the bathing solution before every measurement. This was then subtracted from the total measured resistance. The diameter of the rat liver cell was determined from cells in the smear and it was assumed that the cells were spherical with a diameter of 50μ. On the basis of these data, a value of 37 Ωcm² was obtained as a mean for the membrane resistance (R_m) of the rat liver cells. This resistance is much lower than the membrane resistances known from excitable cells. However, there is a good agreement with the value of 30 Ωcm² published for rat thyroid cells *in situ*[6] and the values of 3–10 Ωcm² for glial cells in tissue culture[7]. A rectification of the membrane of the liver cell could not be detected in the current range of ± 10^{-7} amp. With the low membrane resistance found in the rat liver cell, the expected time constant cannot be

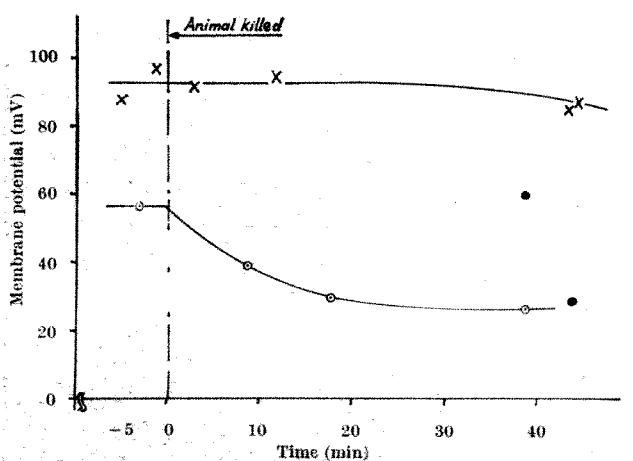


Fig. 1. Simultaneous recording of membrane potentials of the liver (○) and an abdominal muscle (×) of the rat. At the time $t = 0$ the animal was killed by clamping the trachea. Each point is a mean from four to twenty-two measurements

detected with double-barrelled micro-electrodes, even if a compensatory network is used[8]. For this reason, no data about the membrane capacity were obtained.

Preliminary experiments were carried out to determine the relation between the membrane potential and the ionic composition of the bathing solution. It is already known that the potential of the thyroid cells of the rat scarcely responds to an increase in the concentration of external potassium[6]. In the case of liver, increase in $(K)_0$ to 40 mM reduced the membrane potential by 7·2 mV; reduction of the sodium concentration to 70 mM raised the membrane potential by 7·1 mV. Substitution of the chloride by sulphate and reduction of the sodium concentration to 70 mM resulted in a decrease of 1·7 mV. Although the reports of the internal ionic concentration of the rat liver are not consistent, a rough approximation to the measured potential changes could be obtained by assuming the following values for the relative permeability constants in the Goldman equation: P_K, 1; P_{Na}, 0·3; and P_{Cl}, 1·5. These coefficients were calculated by means of the estimated intracellular ion concentrations: (K), 180 mM; (Na), 20 mM; and (Cl), 5 mM. The most impressive feature of these permeability constants is the high sodium permeability. For the cells of the rat thyroid, a similar high value for P_{Na} of 0·12 has been reported[6]. It is possible that the high sodium permeability and the low membrane resistance are characteristics of inexcitable cells.

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Distribution of Colloidal Particles in Extracellular Space and Synaptic Cleft Substance of Mammalian Cerebral Cortex

ADJACENT plasma membranes of neurones and glia in the central nervous system are separated by a 150–200 Å space which has been estimated to represent 3–5 per cent of brain volume[1,2]. Recent electron microscopic investigations using freeze-substitution methods[3] and perfusion with aldehydes[4] indicate that a great variability in the amount of extracellular space can be obtained in central nervous tissue. The functional significance of this intercellular space in the movement of ions and small molecules between vascular and neuronal elements has been a subject of considerable controversy. However, investigations of changes in brain volume leading to cerebral oedema or dehydration have not revealed significant alterations in the dimensions of the intercellular gap at a time when marked changes in glial cell volume can be demonstrated[5]. These and other investigations[6,7] have been viewed as evidence that the extracellular compartment of brain is represented largely by glial elements which are immediately involved in exchange of ions and metabolites between blood and neurones. In contrast to this, investigations of the mechanisms of ionic transport in the leech central nervous system have indicated that changes in spike potentials of neurones subsequent to removal of environmental Na^+ are attributable to rapid diffusion of administered solutions by way of extracellular spaces[8]. No evidence for participation of glial elements in neuronal effects of administered low Na^+ solutions was obtained in these investigations of the leech nerve cord.

We have investigated the functional properties of the extracellular space in the mammalian cerebral cortex by following the sequential passage of saccharated iron oxide particles (about 80–100 Å in diameter) from small sub-pial, intracerebral depots to intravascular locations. Experiments were performed on adult cats under pentobarbital anaesthesia. Quantities of saccharated iron oxide contained in volumes less than 0·1 ml. were injected into the most superficial portions of suprasylvian neocortex at several sites. Samples of tissue at the site of injection or a few millimetres away were removed at different times up to 20 min following injection. Samples were fixed for 1 h with cold 1 per cent osmium tetroxide at *p*H 7·3 buffered with Millonig's solution[9] and embedded in 'Epon 812' (ref. 10).

As might be expected, distortion of tissue and tissue spaces was found at the site of injection of marker particles. However, tissue examined from sites 1–3 mm from points of injection appeared intact in all respects. Elements and interneuronal relationships were entirely comparable with those described in previous electron microscope investigations of adult cat neocortex[11]. In these locations marker particles were found exclusively in the intercellular spaces (Fig. 1) It is of interest that in many instances particles were preferentially located at the synaptic clefts of typical axo-dendritic synapses (Fig. 2). Particles of saccharated iron oxide appeared to concentrate

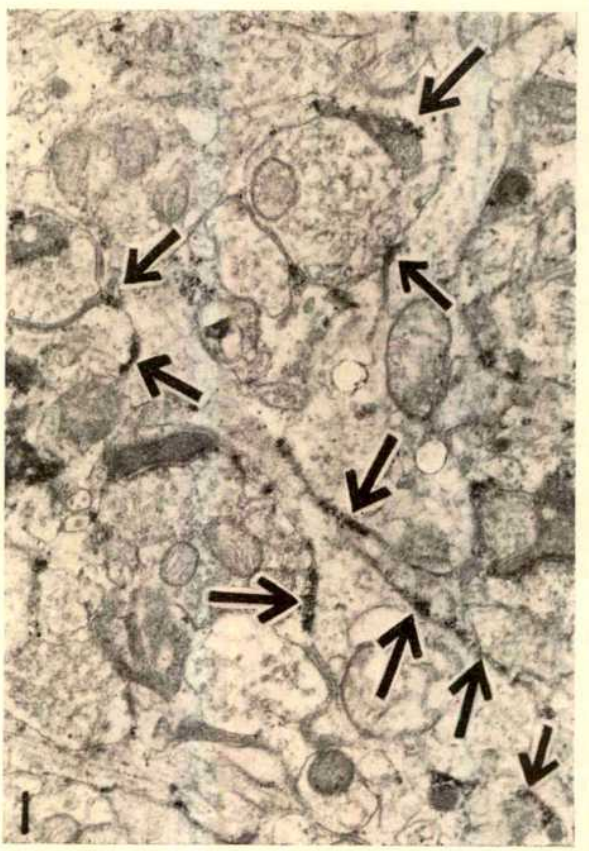


Fig. 1. Electron micrograph of a section of neuropil in the superficial neocortex of the cat. Twenty minutes before fixation, saccharated iron oxide was injected directly into the cortex and formed a small bleb. This tissue was removed about 2 mm from the bleb. Accumulations of marker particles (at arrows) are present in the intercellular spaces of the neuropil. (× *c.* 20,000)

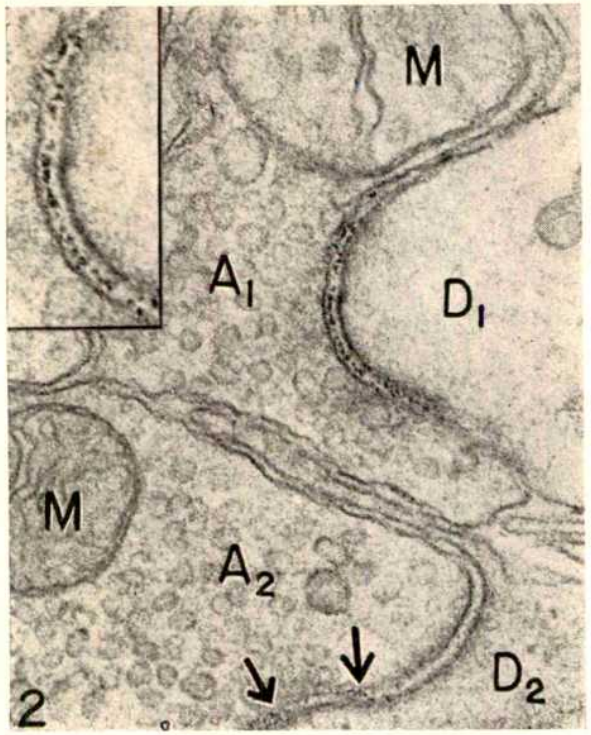


Fig. 2. Electron micrograph of portions of two axo-dendritic synapses (A_1-D_1 and A_2-D_2) taken from a preparation similar to that described in Fig. 1. In many instances marker particles were preferentially located at the synaptic clefts. In a typical axo-dendritic junction formed by axon A_1, filled with synaptic vesicles, and dendrite D_1, the synaptic gap contains particles which are concentrated in linear array. (See higher magnification in the inset on the upper left.) While particles are also present in the synaptic cleft (at arrow) of the junction formed by axon A_2 and dendrite D_2, the linear arrangement is not apparent in this tangential profile. *M*, Mitochondria (× *c.* 56,000, inset = × *c.* 111,000)

in a linear array in the synaptic cleft equidistant from pre- and post-synaptic membranes (Fig. 2 and inset). Within a period of 20 min, marker particles were also observed in intravascular locations. None was noted in the cytoplasm of glial or neural elements during this period.

The results described here provide direct evidence that the extracellular space of the mammalian cerebral cortex is patent to the passage of colloidal particles. Similar findings have been reported in spinal ganglia of the toad[12]. A somewhat different situation is encountered at synaptic sites where marker particles are concentrated by the sub-synaptic cleft substance. Similar adherence of marker particles on to cell surfaces was originally demonstrated with the polysaccharide-rich coated membranes of amoeba[13] and is now recognized to be a general property of cell surfaces that have an 'extraneous coat'[14] or 'glycocalyx'[15]. The synaptic cleft substance at the neuromuscular junction has properties characteristic of mucopolysaccharides—periodic acid–Schiff-positive, diastase-resistant material has been demonstrated there[16]. Furthermore, Birks[17] has observed adherence of thorium dioxide particles to this material. Hess[18] has suggested that periodic acid–Schiff-positive material represents the 'ground substance' or the interstitial component of the central nervous system. Our results do not permit inferences concerning the possible nature of the intercellular material at non-synaptic sites. However, the observations that marker particles adhere to synaptic cleft substance suggest that there is a relatively high concentration of mucopolysaccharide material at these locations in the mammalian brain. If the synaptic cleft substance is indeed rich in mucopolysaccharides, it is reasonable to expect that this material is of functional significance in synaptic transmission. Such specialized sites of glycocalyx formation might provide a suitably structured matrix for effecting cohesion between pre- and post-synaptic membranes[19] as well as provide a basis for immuno-chemical 'sensing' of functionally related synaptic pathways. The presence of mucopolysaccharide material at synaptic sites could explain the affinity of immunologically active substances for synaptic loci in tissue culture preparations of mammalian cerebral cortex[20].

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RADIOBIOLOGY

Induction of Lung Tumours by the Action of Radiation and Urethane

IN a recent paper, Foley and Cole[1] reported some investigations of the incidence of lung tumours in mice exposed to different doses of X-rays, followed by an injection of urethane. They assumed that the lung tumours are induced by the urethane and that the X-rays have an inhibitory effect on the tumour incidence. On this basis they interpreted their results as showing a dependence of this inhibitory action on radiation dose with a threshold of about 300 rads.

It seems to us that these results can also be interpreted in an entirely different way, that is, that the main action of urethane is not to induce tumours but to promote, or to speed up, the appearance of potential tumours induced by radiation or other causes.

Foley and Cole present their results as two sets of data: the mean number of tumours per tumour-bearing animal, and the percentage of animals in which tumours were observed. Actually, these two are not independent but closely related end-points. The number of animals with zero, one, two, etc., tumours follows a Poisson distribution based on the mean number of tumours per animal from the whole population. Using this latter criterion, the results of Foley and Cole were recalculated and are presented in Table 1.

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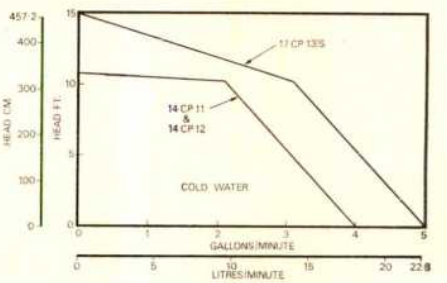
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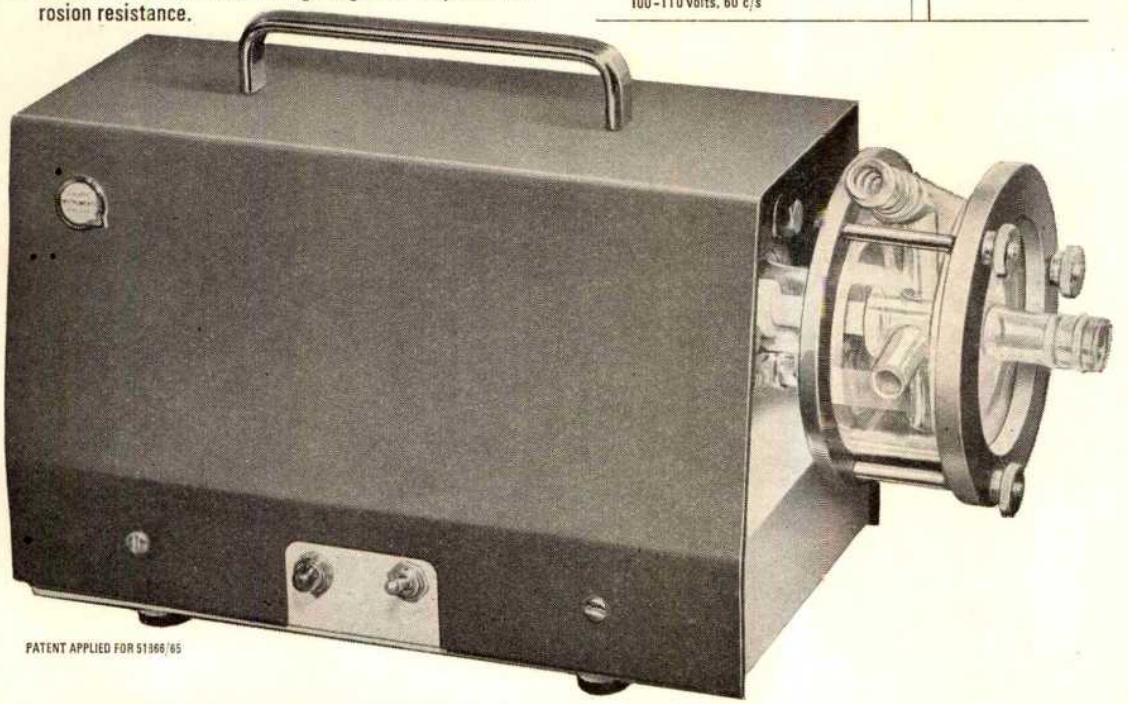
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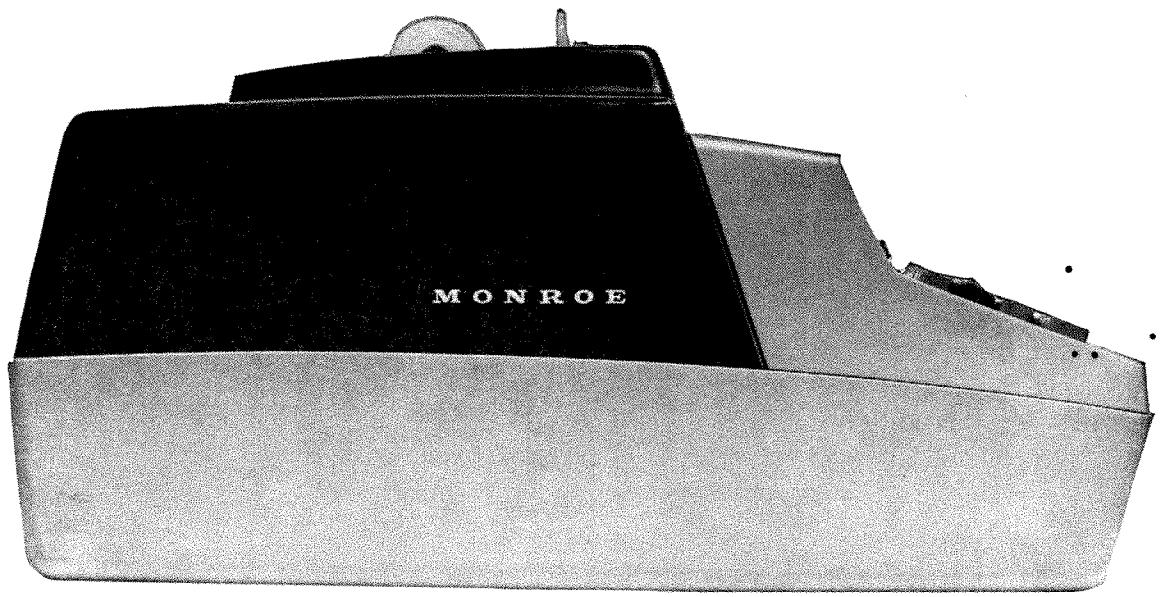
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Table 1. MEAN NUMBER OF TUMOURS PER MOUSE

1 Dose (rads)	2 X	3 $X+U$	4 $\frac{X}{C}$	5 $\frac{X+U}{U}$	6 $\frac{X+U}{X}$
0	0 27	3·1	1	1	11·5
100	(0·11)	3·1	(0 41)	1 0	(28·2)
300	0·30	3·5	1·11	1·13	11 7
500	0·10	1·2	0·37	0·39	12 0
700	0 08	0 8	0 30	0·26	10 0

The second column in Table 1 gives the mean number of tumours in mice which were exposed to X-rays only (X); the first line in this column represents the spontaneous incidence in the control population (C). It will be noted that the value for the incidence after an exposure to 100 rads is comparatively low; considering that this figure is based on the observation of only one tumour, an incidence more than twice this value could easily be within the experimental error. Column 3 gives the tumour incidence in mice which were exposed to X-rays and then injected with urethane ($X + U$); the first line represents the incidence in mice which received urethane alone (U). In columns 4 and 5 we give the ratios $\frac{X}{C}$ and $\frac{X+U}{U}$, and it is seen that, with the foregoing exception, the corresponding values in the two columns are practically identical. The last column shows that the ratio $\frac{X+U}{X}$ is constant within the experimental error, with an average value of 11·3, if the 100-rad data are omitted.

Although the errors in an experiment using a small number of animals are necessarily large, the figures in columns 4 and 5 give an indication of an initial increase in tumour incidence with radiation dose, followed by a decrease. This is as expected from the variation of the incidence of radiation-induced tumours with dose[2]; it shows a "turnover" point at a certain dose, which is the result of the interaction between the processes of tumour induction and cell killing by radiation. Combined with the observation that for each dose the figures in columns 4 and 5 are the same, this may be taken as evidence that in both cases the same potential tumour population was involved, and that the action of urethane was simply to promote the appearance of tumours already induced by radiation or resulting from other causes. The mean value of 11·3 from column 6 is a measure of the promoting action of the urethane; naturally, it depends on the amount of urethane used.

It should be mentioned that a study of the combined action of chemical carcinogens and radiation has been carried out in this laboratory for the past three years[3]. The results of these investigations fit in well with the model proposed here. The possibility that urethane acts as a promoting agent has been discussed before[4], particularly in relation to leukaemogenesis[5].

The accuracy of the experimental data is not sufficient to exclude a direct tumour-inducing action by urethane, and a combination of both types of action is possible. It is hoped that work now in progress will help to clear up this point.

Apart from the fundamental significance of the proposed model, the promoting action of urethane can be utilized to facilitate the study of the effects of radiation on lung tumour incidence, by using urethane as a means of increasing the number of tumours observed.

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Degradation of Ribonucleic Acid in Dilute Solution by Ionizing Radiation

So far, no absolute figure has been available for the sensitivity of ribonucleic acid to ionizing radiation in dilute solution. Ginoza and Norman[1] were able to show that tobacco mosaic virus (TMV) RNA is sensitive to ionizing radiation such that one ionization within its structure renders it inactive. However, their work was not designed to investigate the action of active radicals formed in water by radiation.

This report describes experiments in which the degradation of RNA in dilute solution was observed and the number of breaks produced in a unit of molecular weight 10^6 estimated by comparison with the thermally induced scission of RNA. It is found that 0·8 breaks are produced for each 100 eV released in solution. Direct comparison shows RNA to be about twice as sensitive as DNA to this type of action.

The technique for observing the degradation of RNA was the measurement of the turbidity of the precipitate produced by 10 per cent trichloroacetic acid (TCA). Large hydrogen-bonded macromolecules form a precipitate with 10 per cent TCA and the resulting turbidity develops to a maximum in a matter of a few minutes at 37° C. The amount of undegraded RNA can be measured by observing the turbidity in a simple spectrophotometer at 425 mμ wave-length. Calibration in terms of different concentrations of the original RNA sample makes it possible to convert any figure into concentration of undegraded RNA. In order to estimate the number of scissions produced the thermal degradation of RNA was first measured. This has been investigated by Ginoza, Hoelle, Vessey and Carmack[2]. Using 10^6 as the reference molecular weight of RNA, the number of breaks per unit time can be derived from their data for any temperature. We investigated the loss of TCA precipitate turbidity at 85° C, 100° C and 125° C and, using the figures for RNA scission derived from Ginoza *et al.*, the results given in Fig. 1, relating residual undegraded RNA to number of

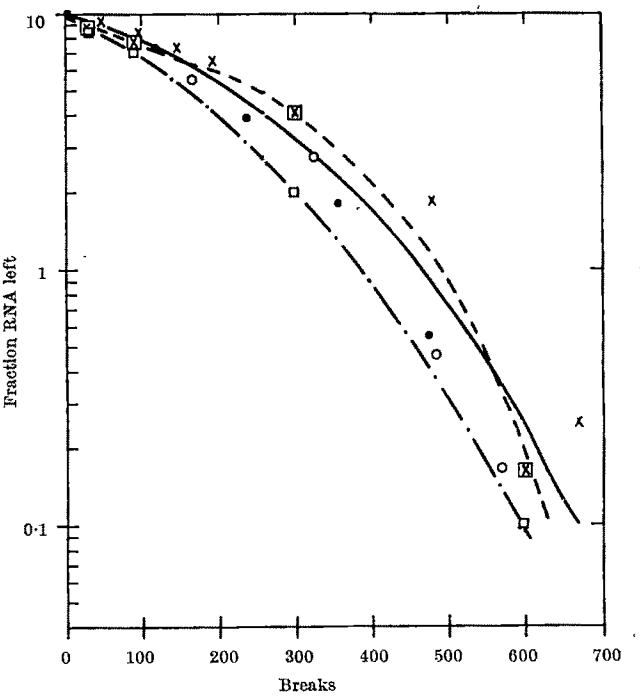


Fig. 1. The effect of heat applied at three different temperatures on the fraction of RNA which is insoluble in trichloroacetic acid. The number of breaks per million molecular weight unit is estimated from Ginoza *et al.* Approximate theoretical values for ten (– – – –) and fifteen (– – ·–) nucleotides remaining in the precipitate are shown for comparison. ×, 85° C, ○, 100° C, ●, 125° C

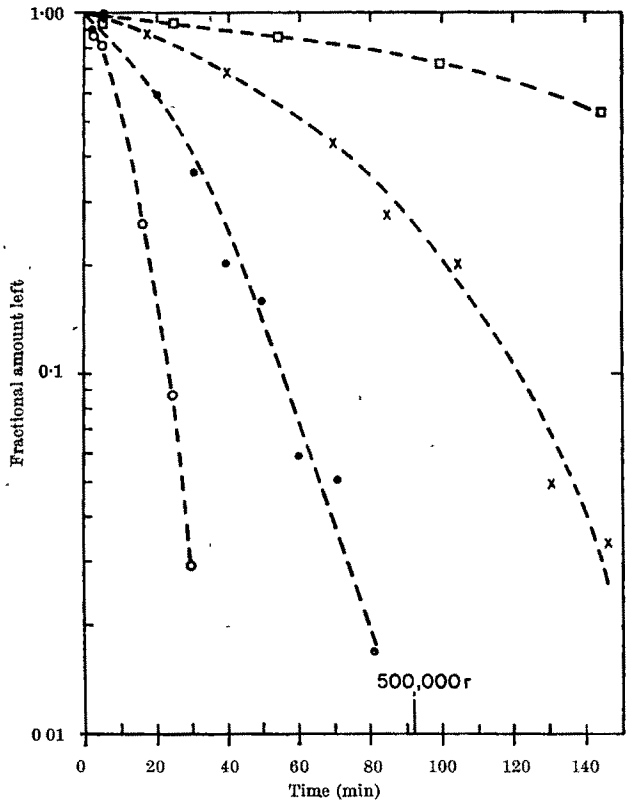


Fig. 2. The effect of exposure to cobalt-60 γ-radiation on RNA in dilute solution. Four concentrations are shown and the fraction left in the TCA precipitate is shown as a function of time in the source. From Fig 1 the breaks produced can be estimated and a yield of breaks per 100-eV radiation energy calculated ○, 0 25 mg/ml., ●, 0 5 mg/ml.; ×, 1·0 mg/ml ; □, 2·0 mg/ml.

breaks, were obtained. An approximate theory for the amount of residual precipitate can be devised as follows.

Let us suppose that the RNA is a long chain of nucleotides, and let us suppose also that the average number of breaks per nucleotide is X. Then if we are to have an intact chain of n nucleotides, starting from one end, we must fail to break the first n nucleotides, for each of which the probability of miss is e^{-x} or a total probability of e^{-nx}, and we must succeed in breaking the $(n + 1)^{th}$. This is a probability of $(1 - e^{-x})$ or, because x is small, X. So both events together have a probability of xe^{-nx}. The weight of precipitate is proportional to n, so the function describing the weight of chains of length n is nxe^{-nx}. If graphs of nXe^{-nx} are plotted versus n, the proportion of chains can be estimated for each value of X. Doing this, and recalling that in Fig. 1 the number of breaks is given for 10^6 molecular weight, so that the actual number of breaks plotted is 3,000 X, we find theoretical curves shown as dotted lines in Fig. 1. The upper curve is for ten nucleotides as the smallest number to yield a precipitate. The lower is for fifteen. It seems likely that ten fits better and it is suggested that TCA precipitates polynucleotides of chain-length exceeding ten.

To observe radiation degradation four concentrations of RNA, 2, 1, 0·5 and 0·25 mg/ml., were exposed to radiation from a cobalt-60 source delivering 5,500 r./min. The fractional amount left in each case after various times of exposure is shown in Fig. 2. From Fig. 1 the number of breaks can be determined. The number of breaks for various doses and various concentrations of RNA is shown in Table 1. The number of breaks produced in a unit of 10^6 molecular weight per 100-eV energy released, the G-value of radiation chemistry, is found to be $0{\cdot}8 \pm 0{\cdot}1$.

In order to observe the effect of this type of radiation on DNA by this method, a comparable experiment was

Table 1. BREAKS INDUCED IN RNA AND DNA IN PHOSPHATE BUFFER, *p*H

Concentration (RNA) (mg/ml.)	Molecules per ml.	Breaks	Dose (r.)	Dose (100 eV/ml.)	G-value
0 25	$1{\cdot}6 \times 10^{14}$	380	$1{\cdot}05 \times 10^5$	$6{\cdot}6 \times 10^{16}$	0·91
			$1{\cdot}07 \times 10^5$	$6{\cdot}72 \times 10^{16}$	0 90
0 50	$3\,2 \times 10^{14}$	380	$2{\cdot}70 \times 10^5$	$1{\cdot}71 \times 10^{17}$	0·72
			$2\,31 \times 10^5$	$1{\cdot}46 \times 10^{17}$	0·84
			$2\,80 \times 10^5$	$1{\cdot}76 \times 10^{17}$	0·70
1·00	$6\,4 \times 10^{14}$	380	$5{\cdot}6 \times 10^5$	$3{\cdot}54 \times 10^{17}$	0·70
			$5{\cdot}05 \times 10^5$	$3{\cdot}54 \times 10^{17}$	0·70
			$6{\cdot}15 \times 10^5$	$3\,86 \times 10^{17}$	0·63
	Average G-value, RNA				0·77
(DNA) 0·30	$1{\cdot}92 \times 10^{14}$	380	$3{\cdot}44 \times 10^5$	$2{\cdot}17 \times 10^{17}$	0·34
	Approximate G-value, DNA				0 34

performed on heat-denatured (and presumably single-stranded) DNA and normal DNA. The results are shown in Fig. 3. For the single-stranded form the behaviour is very much like that of the RNA with the difference that the G-value for a break is less by about a factor of two. The figure is in reasonable agreement with 0·45 for the sugar phosphate rupture obtained by Scholes, Ward and Weiss[3]. The behaviour of double-stranded DNA is somewhat different. There is an initial increase in the amount of precipitate formed up to about 130 breaks. After this, the behaviour is very much like single-stranded DNA. The increase in precipitate is not unexpected, as relatively large pieces of DNA would be expected to be detached from the double-stranded condition and give an increase in the DNA bound to the TCA to form the precipitate.

The foregoing figures were obtained in 0·1 M phosphate buffer, *p*H 7. If similar experiments are conducted in

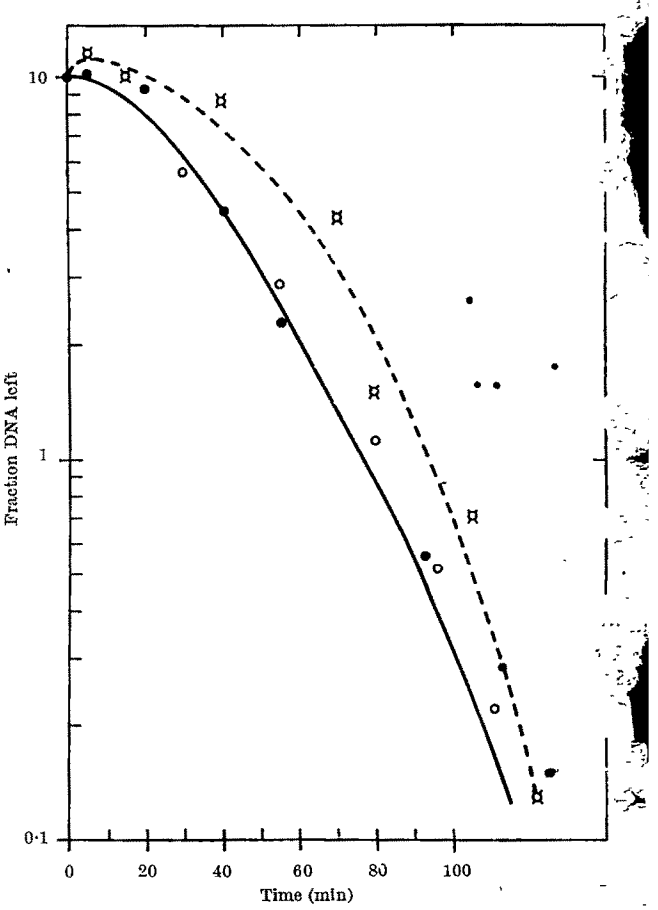


Fig 3. Data similar to Fig. 2 for radiation action on single-stranded and normal DNA. There is a slight increase in precipitate as the random radiation-induced breaks appear in the case of normal DNA but not for the single-stranded DNA. ———, Heat-denatured DNA; – – – – normal DNA

tris(hydroxymethyl)aminomethane-chloride buffer, the *G*-values for both RNA and DNA are reduced by approximately a factor of ten. This is in agreement with the observation by Ginoza[4] of a high X-ray sensitivity for single-stranded virus nucleic acids in phosphate buffer.

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Metabolic Dissociation of the Parent and Daughter Radionuclide Pair, Barium-140 and Lanthanum-140

BARIUM-140, one of the γ-ray emitting isotopes in fall-out, has a physical half-life of 12·8 days. It decays to lanthanum-140 which emits γ-rays and has a physical half-life of 40·3 h. Barium-140 contributes approximately 10 per cent of the total fission product activity at 10 days and 30 days after production. About 12 per cent of the total radioactivity of fission products aged for 10 or 30 days after production is lanthanum-140 (ref. 1). Therefore, barium-140 and its daughter contribute significantly to that portion of the fall-out radiation dose caused by radionuclides of short or intermediate half-lives.

In view of the close proximity of barium and lanthanum in the Periodic Table (barium, Group 2*a*, atomic number 56; lanthanum, 3*b*, atomic number 57), one would reasonably expect similarities in their physicochemical properties and physiochemical behaviour. Therefore, it is generally considered that the distribution of the radionuclides of barium and lanthanum is similar, and the parent radionuclide (barium-140) is present in transient equilibrium with its daughter (lanthanum-140) after administration of barium-140–lanthanum-140. Although there is close resemblance in the excretion, distribution and retention of barium and lanthanum in mammals, quantitative differences have been reported in the metabolism of the radionuclides of barium and lanthanum[2,3]. Therefore, a question arises whether the parent and daughter radionuclides are selectively metabolized by cells and tissues.

A knowledge of the metabolism of the parent (barium-140) in relation to that of the daughter (lanthanum-140) is essential in order to estimate the total internal radiation hazard due to barium-140–lanthanum-140, because the parent and daughter decay at different rates. Furthermore, the photopeak energies of barium-140 (0·304, 0·539 MeV) are so close to those of lanthanum-140 (0·329, 0·489 MeV) that it is not possible to resolve and identify the photopeaks of both parent and daughter isotopes simultaneously by γ-scintillation spectrometry[1]. Lanthanum-140 is measured by its characteristic gamma radiation at 1·6 MeV, which is used to assay barium-140–lanthanum-140 in the present experiments. The physical decay patterns of (1) barium-140–lanthanum-140 excreted in the urine in the first 4 days, and (2) barium-140–lanthanum-140 accumulated by rat tissues (bone, kidney, blood) in 4 days after intraperitoneal administration of barium-140–lanthanum-140 have been determined to evaluate the metabolic dissociation of the parent and daughter radionuclides *in vivo*.

The period of 4 days after the intraperitoneal administration of barium-140–lanthanum-140 was selected for our investigations for the following reasons. The experiments on whole-body retention of barium-140–lanthanum-140 after intraperitoneal injection indicate that: (1) rats excrete no barium-140–lanthanum-140 after the fourth day; (2) the parent and daughter radionuclides are in transient equilibrium in the barium-140–lanthanum-140 which is retained by the animals after the fourth day of administration; and (3) the peak concentration of the gamma activity is found in the bones (critical organ) on the fourth day after the injection of barium-140–lanthanum-140.

White male rats of Sprague–Dawley strain weighing between 317 and 342 g were injected (intraperitoneally) with 1·52 μc./kg of carrier-free barium-140–lanthanum-140 as chlorides in normal saline. The sample of barium-140–lanthanum-140 was aged until transient equilibrium was attained between the parent and daughter radionuclides. At the end of 4 days, the animals were decapitated and the blood was drained into a heparinized beaker. The bones (humerus, ulna, radius, femur, tibia and fibula) and the kidneys from each animal were dissected immediately. The same tissues from all animals were combined and dissolved in a nitric acid–dioxane–water system as described elsewhere[4]. The physical decay patterns of the nuclide pair, in various tissues, and in a standard sample of barium-140–lanthanum-140, administered to the animals were determined simultaneously by standard γ-scintillation spectrometric methods[5]. The gamma activities of the standard sample, as well as the solutions of tissues on various days, were expressed as a percentage of their gamma activities on the first day, the day of death. Each value in Figs. 1 and 2 is obtained from a pooled sample of at least five animals.

The physical decay patterns of the standard sample of barium-140–lanthanum-140, and barium-140–lanthanum-140 accumulated by bone and kidney, are shown in Fig. 1. The decay pattern reported for pure lanthanum-140 is shown in the same figure for comparison. The physical half-life of barium-140–lanthanum-140 found in the kidneys (48 h) is considerably shorter than the 'effective physical half-life' of administered barium-140–lanthanum-140 (14 days). The amount of gamma activity in the blood at the end of the fourth day is very small and has an effective physical half-life of about 48–52 h. The effective physical half-life of barium-140–lanthanum-140 in the bone (15 days) is nearly equal to that of the injected barium-140–lanthanum-140 (14 days). These observations suggest that (1) the parent and daughter radionuclides in the bone are in transient equilibrium and (2) the blood and kidney retain the daughter (lanthanum-140) preferentially.

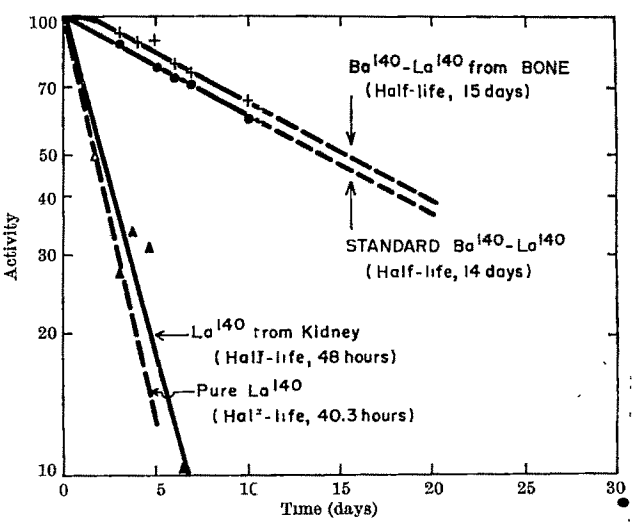


Fig. 1. Decay patterns of barium-140–lanthanum-140 accumulated by rat tissues in four days after intraperitoneal administration. Dose, 1·52 μc./kg

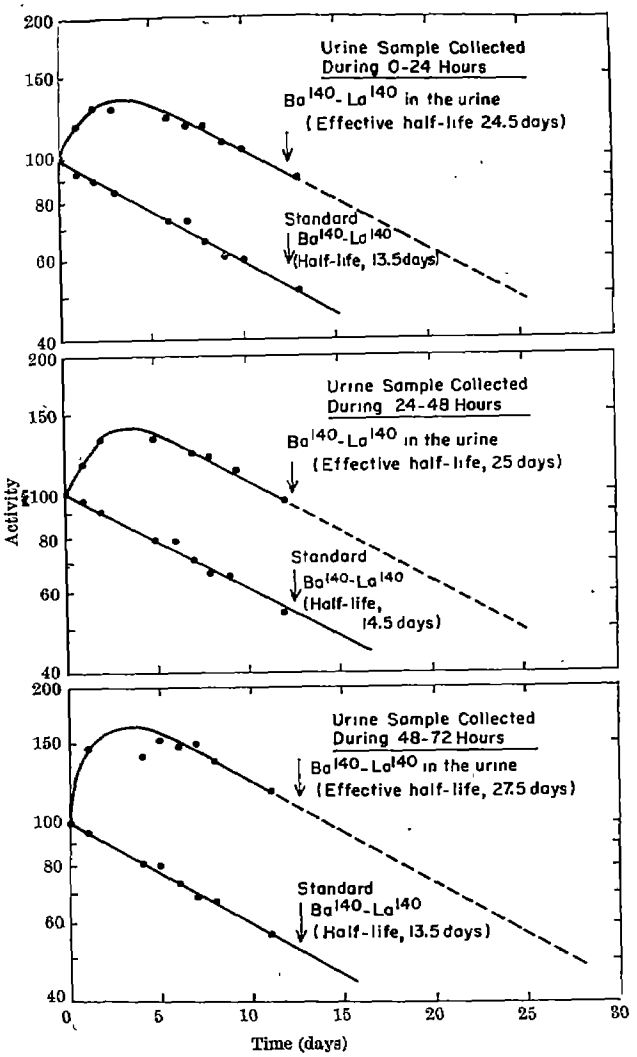


Fig. 2. Decay patterns of barium-140–lanthanum-140 excreted in the urine by the rat after intraperitoneal administration. Dose, 1·52 μc./kg

The decay patterns of barium-140–lanthanum-140 excreted in the urine are plotted in Fig. 2. The effective physical half-lives of barium-140–lanthanum-140 (24·5–27·5 days) in the urine samples collected during 0–24, 24–48, 48–72 h after the administration of the isotopes are longer than the physical half-life of injected barium-140–lanthanum-140. The increase in the activity of barium-140–lanthanum-140 in urine samples during the early period of the decay curve is due to the accumulation of the daughter lanthanum-140 due to the disintegration of the parent barium-140. This behaviour corresponds to the increase in activity of barium-140 separated chemically from the daughter. The amount of barium-140–lanthanum-140 in the urine over the period of 3–4 days is very small and it has an approximate physical half-life of 25–28 days. On the fourth day the gamma activity in the blood is small (see above). These observations suggest that the parent is preferentially eliminated in the urine.

Our results indicate that although barium and lanthanum occupy adjacent positions in the periodic table and resemble each other in many of their physicochemical properties, some rat tissues show differential transport between barium-140 and lanthanum-140. Selective metabolism of closely related parent and daughter radionuclides is an important and interesting technique for the investigations of cell and tissue transport systems.

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Effects of Fast Neutrons on Eye Colour Mutations in *Dahlbominus*

VERY little information is available on the effects of neutrons on mutagenesis in female germ cells. In mice, published work with fast neutrons at high and low intensities has been confined to the irradiation of spermatozoa for the induction of dominant lethals[1], and to spermatogonia for specific locus mutations[2,3]. In insects, however, relative biological effectiveness (RBE) of 2–4 has been demonstrated in *Mormoniella* adult females for recessive eye colour mutants following high doses of fission neutrons[4], and higher values for low doses. Similarly, acute doses of 14 MeV neutrons have an RBE of 1·2 for recessive visible mutations in silkworm oogonia[5], while fission neutrons have RBEs of 2·5–3 (ref. 6). This communication presents data on the mutagenic effects of 14 MeV neutrons as compared to cobalt-60 gamma rays in adult females of *Dahlbominus fuscipennis*.

The numbers of four classes of eye colour mutants (carmine, claret, chestnut and russet) were scored following exposure of *Dahlbominus* adults to cobalt-60 gamma rays and 14·6 MeV neutrons. Unmated females of this species produce haploid male progeny in which all mutations induced in the germ plasm of the mother are expressed in her sons[7,8]. Groups of adult females were irradiated each day in small gelatine capsules, with neutrons and with gamma radiation, at average ages of 12, 60 and 108 h from emergence. Monochromatic neutrons, from the deuterium–tritium reaction, average energy 14·6 MeV, were obtained from a 150 keV accelerator at a dose-rate of approximately 80 rads per min (at 3 cm from the target). The neutron dose was determined from measurements of the sodium-24 activity produced in thin aluminium strips placed in front of and behind the gelatine capsules during the exposures. In converting neutron flux to dose, the hydrogen content of the insects was assumed to be similar to that of soft tissue. The total dose, whether of neutron or of gamma rays, was about 750 rads; for each experiment the gamma-irradiated flies received their exposures as soon as the neutron doses had been measured (that is, 1 h later) so that the gamma dose could be adjusted to the same number of rads. A 1,500-c. cobalt-60 source furnished gamma radiation at 100 r./min., measured with a calibrated '*EIL* Model 37*A*' portable electrometer. Following irradiation, the females were confined in small shell vials with host cocoons, and eye-colour mutants were scored in the male progeny.

The neutron exposures produced about 30 per cent more mutations (see Table 1 and Fig. 1). As the age of the insects at the time of exposure was increased from

Table 1. FREQUENCIES OF EYE COLOUR MUTATION IN *Dahlbominus* ADULTS FOLLOWING IRRADIATION WITH 750 RADS OF GAMMA RAYS (COBALT-60) AND NEUTRON (14·6 MeV) RADIATION

Age (h)	Gamma rays Mutants/ total sons	Mutants/ 10^5 sons	Clusters	Neutrons Mutants/ total sons	Mutants/ 10^5 sons	Clusters	Ratio of effect
12	29/24 948	116	—	52/32 712	159	—	1·4±0·4
60	52/29 176	178	—	69/32 699	211	—	1·2±0·3
108	58/21 753	267	2	99/29 958	330	5	1·2±0·3

12 to 108 h, the frequency of mutations rose about two-fold. This was so for both neutrons and gamma rays, but the relative effectiveness of the neutrons did not change detectably with age. The overlapping of the standard limits for each age group demonstrates that no significant differences were detected between the results with gamma rays and with neutrons. Only one eye colour mutant was found among 19,824 males in non-irradiated controls. "Clusters" of mutant offspring from gametes that had arisen by cell division following a single mutational event were observed in seven separate instances. Each consisted of two eye colour mutants (Table 1). It was interesting that all clusters occurred among the older (108 h) females. Each mutant in the clusters was counted in calculating the mutation frequencies. Sterility levels in the irradiated insects were almost identical with those found in controls (that is, 8·2 per cent unparasitized cocoons with neutron irradiation as compared with 9·3 and 8·1 per cent for gamma irradiation and for controls).

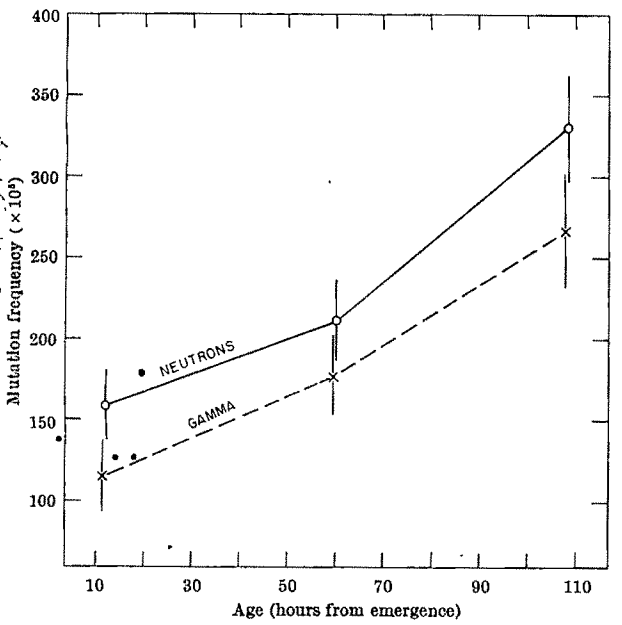


Fig. 1. Frequency for eye colour mutations in *Dahlbominus* oocytes following exposure to 750 rads of 14·6 MeV neutrons and cobalt-60 gamma rays

The ratios for neutron/gamma effects in *Dahlbominus* (1·2 : 1·4) agree with the corresponding RBEs for silkworm oogonia mentioned previously, and also with the value of 1·23 found by Edington and Randolph[9] for sex-linked recessive lethals in *Drosophila* sperm following exposure to 14 MeV neutrons. In *Mormoniella*[4] and silkworm oogonia[9], slightly higher RBEs (2–4) were found for fission neutrons, which have a lower average energy (about 1 MeV) and higher average linear energy transfer. In all these investigations with insects, high dose rates were used, whereas, in mice, RBEs for mutagenesis of 5–6 (ref. 3) and of 19 (ref. 4) have been reported following chronic exposures to fission neutrons.

The increase in both gamma and neutron induced mutation with age from emergence in *Dahlbominus* is probably associated with high sensitivity in mature oocytes.

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VIROLOGY

Phenotypic Mixing between Group *A* Arboviruses

WHEN two different viruses infect the same cell some of the progeny may exhibit phenotypic mixing: that is, the genome of one parent may be enclosed in a coat determined, at least in part, by the genome of the other parent. Thus the phenotype may not correspond to the genotype. This anomaly disappears after one cycle of growth if further mixed infections are avoided. Phenotypic mixing was first detected in bacteriophage[1] and has subsequently been observed in polio viruses[2] and myxoviruses[3]. We shall describe a double mutant of Sindbis virus (a group *A* arbovirus) that can be used in a simple test for phenotypic mixing with other wild-type arboviruses. Such a test for phenotypic mixing may be useful in confirming or suggesting relationships among these viruses. The methods used for the growth and titration of arboviruses in primary chick embryo cultures have been described[4].

A Sindbis virus mutant with high resistance to thermal inactivation was selected by several cycles of alternately heating at 60° C for 5 min and regrowing the survivors. In this way a strain was obtained (designated HR) which, after heating at 60° C for 5 min, yielded 10^5 times more viable virus than the wild type under identical conditions (Fig. 1). This heat-resistant mutant retained the growth temperature requirements of the wild type: both were able to make plaques over the temperature range 27°–40° C. This property of the cloned heat-resistant mutant was next altered by selection of a temperature sensitive conditional-lethal (CL) mutant[5]. The resulting mutant (HR–CL) grew in liquid medium and produced plaques normally at 27° C but was unable to reproduce at 39° C. This double mutant thus multiplied only at low temperatures but produced virus that was very resistant to heating.

The HR–CL mutant was examined for phenotypic mixing with three group *A* arboviruses: wild-type Sindbis, Western equine encephalitis (WEE) and Eastern equine encephalitis (EEE). These three viruses had a similar high sensitivity to heating (Fig. 1) and were able to grow well over the temperature range 27°–39° C (Table 1). Chick embryo fibroblasts in monolayers were infected by adding 25 plaque-forming units (P.F.U.) per cell of HR–CL virus together with 25 P.F.U. per cell of a second virus in a small volume of phosphate-buffered saline. After an adsorption period of 1 h at 0° C, the monolayers were drained, rinsed, and then incubated with fresh medium at 27° C for an additional 8 h to permit growth of both viruses. Since

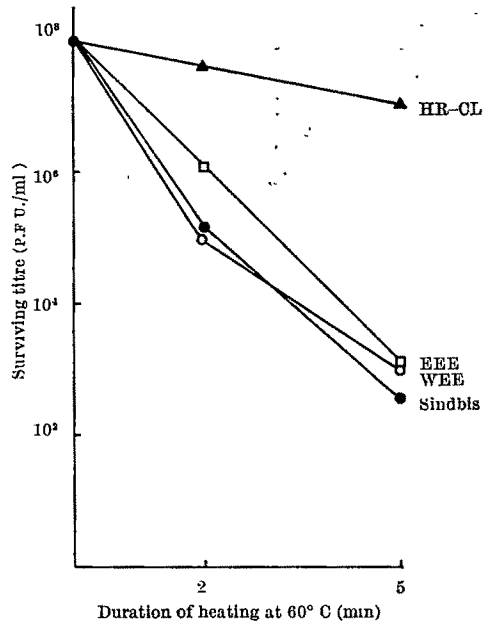


Fig. 1. Heat inactivation of wild-type group *A* arboviruses and heat-resistant mutant of Sindbis (HR–CL). Survival of all viruses was measured by plaque-forming ability at 27° C

viruses of the HR–CL genotype are unable to form plaques at 39° C, the plaques produced at 39° C by fluids collected after mixed infection provide a count of the genomes of the second virus (wild-type Sindbis, EEE, WEE) present. If these genomes were enclosed in their own protein-lipid coats, their survival after 5 min heating at 60° C would be predicted by the curves in Fig. 1. If, however, these viruses included in their coats protein determined by the HR–CL mutant, some protection from heat inactivation should be afforded.

Table 1. PLAQUING EFFICIENCY OF GROUP *A* ARBOVIRUSES AT 27° C AND 39° C

Virus	Titre in P.F.U /ml. with incubation at 27° C	39° C
Sindbis	$1{\cdot}5 \times 10^8$	3×10^8
WEE	3×10^7	3×10^7
EEE	3×10^7	1×10^8
HR	1×10^8	2×10^8
HR–CL	7×10^7	3×10^3

Virus was collected after 9 h growth at 27° C on chick fibroblast monolayers. Plaquing efficiency of the infected fluids was determined by measuring plaque formation at the two incubation temperatures.

Table 2. HEAT RESISTANCE ACQUIRED DURING MIXED INFECTION

Progeny of mixed infection with HR–CL and:	Titre of virus able to make plaques at 39° C	Titre after 5 min at 60° C	Predicted* titre after 5 min at 60° C
Sindbis (wild-type)	$1{\cdot}5 \times 10^8$	7×10^6	5×10^2
WEE (wild-type)	4×10^7	$1{\cdot}5 \times 10^6$	4×10^2
EEE (wild-type)	5×10^7	1×10^6	6×10^2

* Titres are predicted from the heat-inactivation curves of Fig. 1, which also records the heat inactivation characteristics of HR–CL.

Each wild-type arbovirus was grown in mixed infection with HR–CL at 27° C. Viral titres were measured by plaque formation at 39° C and are expressed in P.F.U./ml.

Table 2 records the yield of virus able to make plaques at 39° C, the actual sensitivity of these particles to heating, and the sensitivity expected if these virus particles were phenotypically wild type. Substantial protection was observed, whereas, in contrast, no protection was seen when artificial mixtures of the viruses were examined. (The protected particles have less resistance to heating than the HR–CL parent, as would be predicted from similar experiments with myxoviruses[3].)

The protected virus particles might have been recombinants that had inherited the ability to make plaques at 39° C from one parent, and resistance to heat from the other. To test this possibility, plaques produced by t of these 'protected' particles were cloned and regrow at 27° C. All clones had regained the sensitivity of the wil type virus to heat inactivation. Furthermore, in t mixed infection with HR–CL and EEE, several protecte particles, cloned and regrown, were all serological demonstrated to be EEE. Therefore, we interpret th observed protection as evidence of extensive phenotyp mixing between wild-type arboviruses and the HR–C mutant.

The three viruses used in this experiment are known t be serologically related[6] and undoubtedly have structura features in common. To discover if this was an essenti factor in phenotypic mixing, we then examined a serolog cally and morphologically unrelated virus. Vesicula stomatitis virus (VSV) is at present unclassified[7] and i in gross morphology[8], quite different from Sindbis virus Nonetheless, these viruses have certain characteristi in common. Both are RNA viruses and contain lipid[4] that is probably acquired as they pass through a cellul membrane. Both have been isolated from insects[11], When heat inactivation of VSV grown in mixed infectio with HR–CL was compared with inactivation of VS grown in single infection, no protection was observe (Fig. 2).

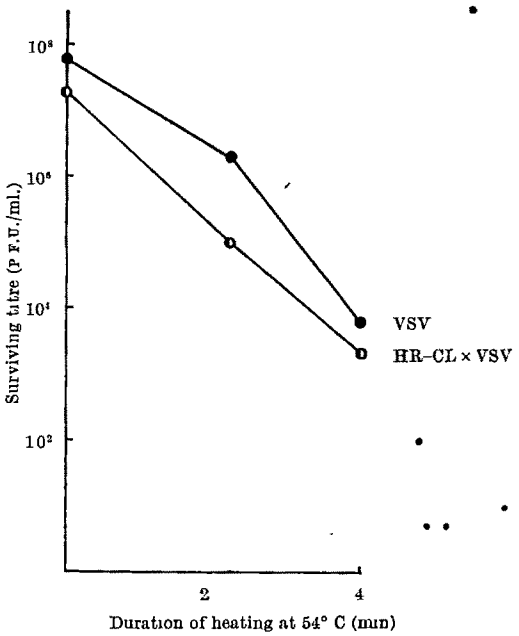


Fig. 2. Heat inactivation of vesicular stomatitis virus (VSV) grown in single infection (●), and grown in mixed infection with HR–CL (○)

It may be that no phenotypic mixing was observe between these two viruses because they are structural so dissimilar that their coat components are not inte changeable. However, this result would also have be obtained if true mixed infections had not been establishe and each cell in the culture was releasing only one speci of virus. This possibility will be tested by examining the progeny of single cells.

We conclude that extensive phenotypic mixing occurs during mixed infections with certain group *A* arboviruses, but does not occur when VSV and a group *A* arbovirus simultaneously infect the same tissue culture. Regardless of its basis, the presence or absence of phenotypic mixing appears to correlate with the serological classification of these viruses. The extent of this correlation is being examined with a greater variety of arboviruses of groups *A*, *B* and *C*.

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VETERINARY SCIENCE

An Epizootic of Equine Sarcoid

JACKSON[1] first recognized and described equine sarcoids, hich are locally aggressive, fibroblastic tumours of quine skin. They are often multiple, frequently recur ollowing surgical excision or radiotherapy, and generally ave fibropapillomatous features. Horses, donkeys and ules are susceptible. Neither a seasonal incidence nor an ge distribution of equine sarcoids has been detected[2]. ackson pointed out that the gross and microscopic ppearance of the tumours, the predilection sites, and the attern of spread to secondary sites on affected animals ere suggestive of viral aetiology. Auto- and homo-ansplantation have been reported[2-6]. Voss recently ported that several tumours were produced in horses ith centrifuged sarcoid extracts which probably were ll-free although they had not been filtered[5]. Intrader-al inoculation of bovine papilloma virus in horses oduced localized, fibroblastic growths similar to equine coid which suggests the interesting possibility of a sal relationship to equine sarcoid[7]. Equine sarcoid has known relationship to Boeck's sarcoid in man.

An epizootic of equine sarcoids occurred in the spring of 35 in a herd in Eastern Washington, United States. Fig. gives details of the important features of this herd from s beginning in 1961 until the end of 1965. The herd

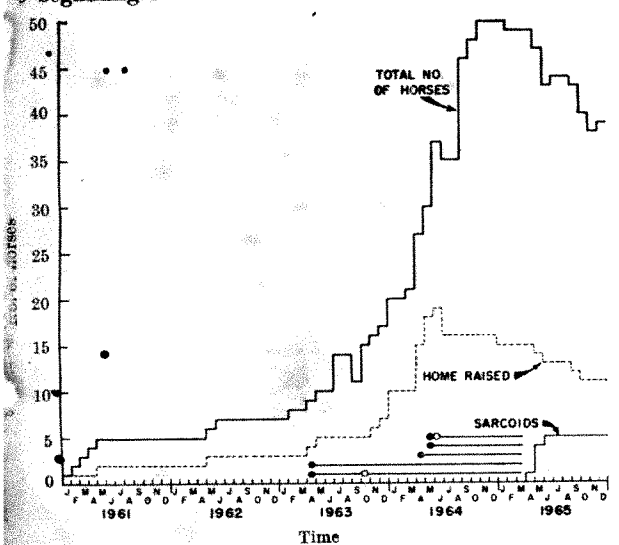


Fig. 1. The occurrence of sarcoids in a herd of horses. The total number of horses at risk for any month is given by the top solid line. The number of these horses born in the herd is designated by the broken line. The number of sarcoid cases is given by the bottom solid line. Horses less than 6 months of age are not included. The solid circles indicate when each of the five affected horses was born. Two of the affected horses were not born in the herd. The dates they were introduced into the herd are designated by open circles

was made up of Appaloosa, Quarter, Pony of the Americas, and mixed breed horses. The entire herd was at risk but was free from sarcoids until April 1965 when one case was observed. There were three additional cases in May and a fifth case in June of that year. All five cases occurred in a six-week period.

One horse was a mixed breed and the other four were Ponies of the Americas. There were four fillies (two yearlings and two 2 year-olds) and one 15-month-old colt. Four cases had solitary tumours (right upper forearm, left foreleg just above the carpus, right distal thigh, or the anterior right stifle). One case had multiple sarcoids located on the right mandible, base of the right ear, and on the right shoulder. The herd was maintained under close observation so that all the cases were detected early and presented for treatment while the tumours were still small (the largest was 3 cm in diameter). The tumours were removed by surgical excision and the diagnosis was confirmed by histopathological examination. Microscopically the tumours appeared to have been adequately excised, and none has recurred.

An estimate of the incidence of equine sarcoids in the Pacific Northwest, based on equine accessions at the Washington State University Veterinary Hospital for a 140-week period, was 0·0049 per hundred per week. The true incidence is undoubtedly lower. The attack rate in this herd during the 6-week outbreak was 1·8 per hundred per week.

The most striking feature of the history of the herd was the addition of a large number of horses 8–12 months before the appearance of equine sarcoids. If it is assumed that equine sarcoids are caused by a virus, the incubation period was probably less than 1 year because three of the affected individuals were yearlings. Sarcoids have been observed in 6-month-old foals[2]. If a sarcoid-evoking virus had been present in the herd early in 1963, one might have expected that the two affected 2-year-olds would have developed sarcoids at an earlier date. Since these two individuals developed tumours at about the same time as the other three, it seems reasonable that they were all infected at about the same time. Thus, we believe that a sarcoic-evoking virus might have been introduced into the herd with the horses added to the herd 8–12 months before the tumours appeared.

There was also a familial pattern in this epizootic as four of the five affected individuals were members of a highly inbred family. The herd husbandry made it difficult to explain this pattern on the basis of environmental factors. The elucidation of a genetic factor would require a prospective study.

While the epizootiologic picture in this herd was compatible with a viral aetiology, it will be necessary to isolate and characterize a sarcoid-evoking agent by appropriate procedures. The relationship of such a virus to bovine papilloma virus should be determined.

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SOIL SCIENCE

Optical Microscopy and Soil Structure

USE of a photometer in conjunction with a polarizing microscope has yielded useful information regarding the structural anisotropy of soil mechanics test specimens.

Kaolin was thoroughly remoulded at 100 per cent moisture content and divided to form three specimens. One specimen was consolidated in a shear box to 4 kg/cm², allowed to rebound to 2 kg/cm², sheared at 1 mm/min and finally unloaded completely. For the second specimen shearing was omitted. The third specimen was left in the remoulded unconsolidated state.

Using a cheese wire, undisturbed samples were cut from the sheared and from the consolidated specimens. Samples were taken from the unconsolidated specimen with a spatula. After impregnation with 'Carbowax 6000', without an intermediate drying stage, thin sections were prepared by grinding and polishing.

Between crossed-polars the thin sections showed a mottled appearance. Fig. 1 is typical. The shades of the light and dark patches changed as the angle between section and polars was altered. A parallel electron-microscopical examination suggested that each patch is a domain of almost parallel particles.

For microphotometry, an auxiliary lens, mounted above the microscope eyepiece, imaged the section in the plane of a variable iris diaphragm. Light passing through the diaphragm was filtered to 5420 Å wave-length, and collected by a photocathode. An auxiliary viewing system made possible both visual control of the measurements and calibration of the diaphragm setting.

The polar diagram (Fig. 2*a*) illustrates a series of measurements made on a circular area of 0·1 mm diameter. The intensity of light transmitted by this area (I) was measured as a function of angle while thin section and microscope stage were rotated together. In the diagram the intensity is plotted as radius at the corresponding angle. The symmetrical four-lobed figure is as might be expected from an anisotropic area.

An anisotropy index (A) has been defined as:

$$A = \frac{I_{max} - I_{min}}{I_{max} + I_{min}}$$

Fig. 2*b* shows the dependence of anisotropy index on radius of area of measurement for observations made on three series of concentric circles. The locations of the observations were judged by visual examination to be typical of the specimens in the unconsolidated and in the consolidated cases. Increases of anisotropy due to consolidation and to shear are in accordance with observations made elsewhere[1–8] and with preliminary electron microscopical observations made on other samples of these specimens.

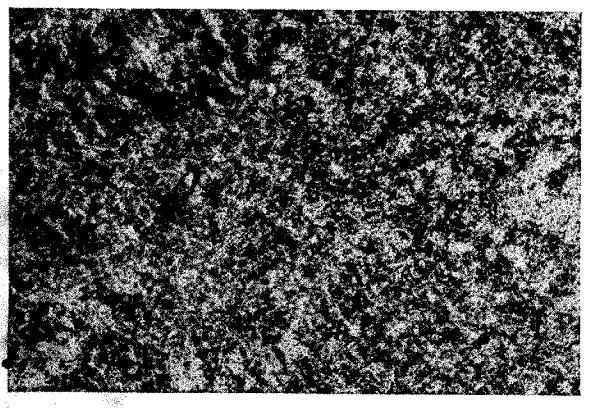


Fig. 1. Remoulded kaolin between crossed-polars. (× *c.* 100)

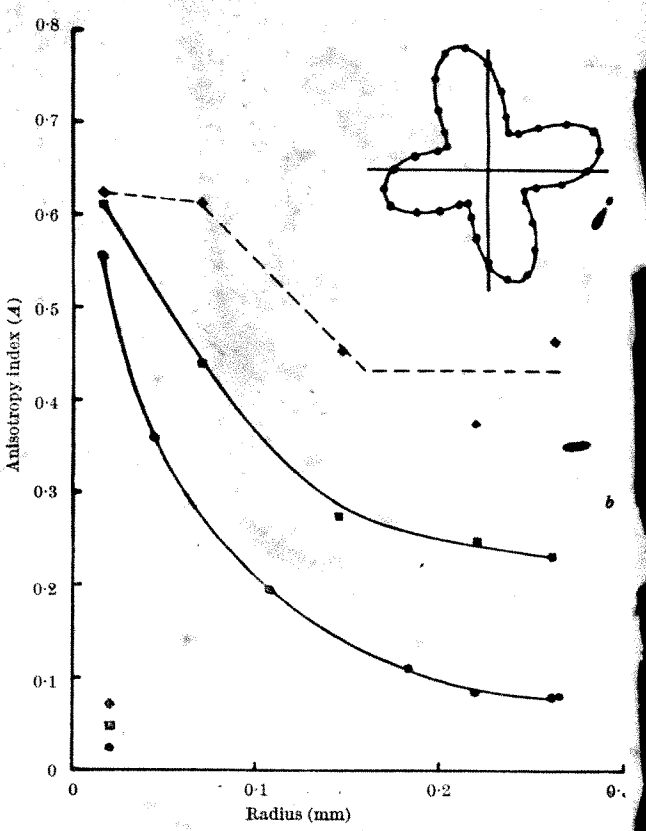


Fig. 2. (*a*) Polar diagram: intensity of light transmitted: inclination of polarizing system. (*b*) Anisotropy index: radius. ●, Unconsolidated; ■, uniaxial consolidation; ◆, sheared

In any interpretation of the results, the nature of the optical phenomena involved, the statistical variation inherent in the specimens, and the thickness of the sections studied must all be considered.

The results available are of sufficient extent only to illustrate the methods. I thank Ernst Leitz *G.m.b.h.* the Soil Mechanics Laboratory of the Department of Engineering, University of Cambridge, and the Nuffield Foundation for their assistance.

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GENERAL

Giorgi: a Proposed New Name for the Kilogramme

THE rapidly increasing acceptance of S.I. units by physicists, other than nuclear physicists, by chemists by electrical engineers, and by mechanical engineers has led to the expression in several quarters of the desirability of a single symbol and a simple name to replace the kilogramme. I suggest the symbol G and the name Giorgi The reasons for this are obvious.

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